THE EFFECT OF CARBON INPUTS ON MICROBIAL COMMUNITY STRUCTURE AND FUNCTION: THE ROLE OF FERMENTATION PROCESSES IN GROUNDWATER

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DEDICATION

This dissertation is dedicated to my loving husband Scott. Without him I would truly not have been able to finish this degree. We met after I had already embarked on this journey, and then married and started a family before I had finished. This degree has been a constant throughout our relationship and I look forward to starting a new chapter in our life together.

ABSTRACT

Carbon inputs to groundwater aquifers include intentional applications, as in bioremediation practices, and unintentional spills. The addition of carbon to an aquifer environment promotes the growth of a diverse and complex microbial community capable of generating several fermentation products, including some regulated compounds and methane, an explosive gas. This dissertation focuses on the fermentative community that develops in response to carbon application in an aquifer environment. Research was conducted to specifically examine 1) how fermentation processes affect partitioning of trichloroethene (TCE), a common groundwater contaminant, 2) the extent that continuous or pulsed carbon inputs affect microbial community structure and function, and 3) how an ethanol-based fuel (E85) stimulates fermentation processes, including methane generation, and the effect of ethanol toxicity on plume longevity.

Remediation of groundwater plume source areas is challenging because lingering contaminants are often present as non-aqueous phase liquid (NAPL) and sorbed mass, and therefore difficult to remove via biodegradation or other commonly used remedial methods. Experimental results indicated that enhanced dissolution of TCE NAPL was possible through the addition and/or subsequent fermentation of a dilute molasses solution. Two mechanisms were responsible for the enhanced dissolution of NAPL; the addition of fresh molasses increased TCE solubility (>200%), thereby increasing the concentration gradient and subsequent mass transfer of NAPL to the dissolved phase, and mixing NAPL with fermented molasses solution significantly increased the surface area of the NAPL through formation of an emulsion, thereby increasing the mass flux of NAPL to the dissolved phase. In addition, the fermented liquid may have also decreased the soil partitioning coefficient (K_d) of TCE, indicating that enhanced transfer of sorbed mass to the aqueous phase could also occur in the presence of fermented molasses. These results can be used to optimize remedial systems to increase NAPL and sorbed-mass dissolution and are therefore important, particularly when bioremediation is used to polish residual source zones.

The addition of organic carbon to a groundwater aquifer for biostimulation purposes promotes the growth of a diverse fermentative community as well as organisms targeted for contaminant degradation. Engineered carbon application systems commonly include either a continuous low dose of carbon, or periodic high doses of carbon. Experimental results indicated that a monthly pulse of a high dose (10% by volume) of molasses generated several fermentation products at high levels following each application, while a continuous feed of low molasses solution (0.4%) reached steady-state in 130 days, after which no further detection of fermentation products occurred. Methane generation in both systems was similar, indicating that methane production was not affected by the carbon addition strategy. Significant shifts in both *Eubacteria* and *Archaea* community structures were observed after carbon introduction, with the greatest changes correlating to the higher concentrations of carbon provided by the pulsed system. The total quantity of bacteria and methanogens was higher along the pulsed-fed column

compared to the continuously-fed system. The continuously-fed column exhibited greater biofouling behavior. Taken together, biofouling did not appear to be a result of biomass quantity, rather a function of community structure. In summary, the method of carbon introduction (pulsed high-dose versus continuous low-dose) can result in significantly different community structures, functions, and densities of indigenous organisms. These data suggest that systems can be engineered to control fermentation product generation and biofouling behavior by manipulating the style of carbon application. Methane, however, will need to be controlled in either system.

A spill of ethanol-based fuel will not only contaminate an aquifer, but will also serve as a food source to stimulate fermentative organisms that can generate potentially regulated compounds and create an environment conducive for production of explosive methane gas. Experimental results indicated that a continuous supply of a dilute ethanol-based fuel (E85) resulted in a profound shift in the community structure of *Eubacteria* and *Archaea* accompanied by the production of volatile fatty acids and butanol, a compound with a groundwater regulatory standard in Minnesota. Data also indicated that dissolved methane was produced at concentrations that could accumulate to an explosive level (>2 mg/L) in headspace. Quantitative polymerase chain reaction (qPCR) data showed a statistically significant increase in methanogenic populations, when compared to a control column. These results strongly correlated to areas of the column containing acetate, a breakdown product of ethanol. Toxicity data indicated that microbial growth was completely inhibited at approximately 6% (vol/vol) ethanol. These results suggest that even though ethanol is readily degradable, the core of an E85 spill may serve as a long-term source of contamination, and subsequent methane production, as it cannot be degraded until significant dilution has occurred.

The research presented in this dissertation shows that the addition and subsequent fermentation of molasses can enhance the mass transfer of TCE, and that the style of carbon application affects the microbial community structure, density of biomass, and subsequent production of fermentation processes. Similarly, an input of E85 will result in the generation of fermentation products, some of which are regulated, and produce methane at levels that can potentially accumulate to explosive levels. This research furthers our understanding of the importance of fermentation processes resulting from carbon inputs to a groundwater environment. These results can be used to optimize bioremediation systems that incorporate carbon addition in order to manage fermentation product formation and biofouling impacts, and to mitigate potential human health hazards stemming from ethanol-based fuel spills through more accurate fate and transport modeling efforts.

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CHAPTER 1 Introduction

"We can use our scientific knowledge to improve and beautify the earth, or we can use it to ...poison the air, corrupt the waters, blacken the face of the country, and harass our souls with loud and discordant noises, or we can use it to mitigate or abolish all these things." - John Burroughs, 1913

Over 70% of our planet's surface is covered with water, and of this, only 2.5% is fresh water. Most fresh water is inaccessible in the form of icecaps, leaving only ~0.007% of the earth's water accessible for human use (U of Mi, 2006). This makes fresh water one of our most precious resources. Most trends in water supply are related to the depletion of local water through increased consumer usage (Hassan, 2004), causing varying degrees of distress. An equally alarming trend is the decrease in overall *usable* fresh water due to factors such as pollution (Hassan, 2004). For example, in the U.S., 80% of water systems use groundwater as a source of drinking water (EPA, 2007). A 2006 report from the United States Geological Survey indicated that almost 20% of the 2,400 domestic wells and 1,100 public wells surveyed were impacted with one or more contaminants (USGS, 2006). The use of aquifers for drinking water is greatly diminished when they become contaminated, and when a drinking water aquifer is impacted, the cost for pre-treatment can drastically increase to meet health-based drinking water standards. The longer it takes to clean up the impacted groundwater plume, the greater the cost of treatment.

Many groundwater contaminants are water immiscible, or hydrophobic. Hydrophobic compounds, such as chlorinated solvents, will partition into several different phases after contacting water. For instance, a spill of pure-phase hydrophobic

compounds will pool together to form a non aqueous phase liquid (NAPL) that slowly dissolves into the water. The slow rate of dissolution causes the NAPL to serve as a long-term source of groundwater contamination. Sorbed mass can also be present, in which spilled compounds adsorb to the surface or absorb into soil particles and slowly dissolve back into the groundwater. Both NAPL and sorbed mass can contribute to a contaminant plume for an indefinite amount of time (estimates run from decades to hundreds of years), as they are present in non-aqueous forms, and therefore unavailable for treatment using cost-effective means.

One common practice to treat chlorinated contaminant plumes is via biostimulation, a technology that delivers substrate to aquifers to promote the *in situ* biological degradation of contaminants. The delivery of substrate promotes the growth of naturally-occurring microorganisms in which certain species can biodegrade the targeted contaminants. Significant research has been conducted on optimizing the growth of these contaminant-degrading species (Fennel et al., 1997; Fennell and Gossett, 1998; Smidt and de Vos, 2004). The function of the remaining community that develops in response to carbon inputs, however, has been generally ignored.

The addition of carbon will promote the growth of a fermentative community capable of producing cosolvents or biosurfactants that can affect the physical and chemical structure of water, resulting in a reduction of surface tension and thereby influencing the behavior of hydrophobic contaminants (Yalkowsky, 1999). In bioremediation practices, this can be a positive attribute, as an enhancement in the mass transfer rate of the targeted contaminants from sorbed or NAPL to groundwater may

occur in these instances, making them accessible for biological degradation and consequently decreasing the overall timeframe for groundwater restoration. Nevertheless, some of the compounds produced during fermentation can be harmful, such as ketones or methane. While simple ketones like acetone can contribute to enhanced mass transfer, they can also be regulated in groundwater. In addition, it is documented that high levels of methane can be produced after carbon has been injected into an aquifer (Suthersan and Payne, 2005) leading to potentially explosive situations. If the production of methane gas is anticipated, it can be easily addressed through the installation of a vapor containment system, thereby preventing methane gas from accumulating into a potential explosion hazard. Nevertheless, with unintended carbon additions, such as in a spill of readily degradable compounds, similar communities could develop and harmful products could be formed. In particular, in the instance of a spill of something like ethanol-based fuels, safeguards may not be in place to address the buildup of explosive gas or of other fermentation byproducts. Understanding the conditions that promote the formation of these regulated and/or hazardous substances will enable better engineering practices.

1.1 Objectives

The objectives of my research, each represented by a separate chapter in this dissertation, were to: 1) establish whether fermentation processes can affect the partitioning behavior of trichloroethene (TCE), and determine by what mechanisms the partitioning behavior is affected, 2) examine whether the outcomes of fermentation, including both product formation and methane generation, can be controlled by changing

the method of addition (low dose, continuously-fed versus low dose, pulsed-fed systems), and to evaluate how these techniques affect microbial community structure, diversity and density, and 3) investigate these processes (fermentation products, community structure and methane generation) as related to a spill of ethanol-based fuel to determine the risks of regulated product formation, methane generation and longevity of the plume as a function of ethanol toxicity. Brief descriptions of each chapter are summarized below.

Chapter 3 Enhanced Dissolution of Trichloroethene: Effect of Carbohydrate Addition and Fermentation Processes

TCE NAPL partitioning experiments were conducted to evaluate whether TCE solubility could be affected by individual low levels of several cosolvents that can be produced during fermentation. Results showed that cosolvent compounds generally need to be present at or exceeding 20% of the solution to have a statistically significant effect on TCE solubility. Field and reactor-fermented molasses samples along with non-fermented molasses solution were also evaluated for TCE solubility increases using NAPL partitioning experiments. Surprisingly, the non-fermented molasses solutions showed the greatest impact by increasing TCE solubility >200%. The field and 10% (by volume) fermented molasses solution (234 days of fermentation) did not have an effect on TCE solubility. The 20% fermented molasses solution (after 239 days of fermentation) did increase TCE solubility, although the enhancement decreased with time. The 20%

fermented molasses solution was analyzed for cosolvents, and data indicated several cosolvent compounds were present, all at individual levels much less than the 20% needed to increase TCE solubility. A solution combining the detected cosolvent concentrations was used in a NAPL partitioning experiment to determine whether a cumulative effect was responsible for the observed increase in TCE solubility. No solubility increase was observed. This, along with the results of the individual partitioning experiments, indicated that fermentation products were not responsible for solubility enhancement. Accordingly, the increased solubility was attributed to the protein fraction of the fresh molasses, which can enhance solubility by altering the properties of water through extensive hydrogen bonding. This was supported by the observed decrease of TCE solubility over time in the 20% fermented reactor, likely a result of the protein degrading over time. This also explains the lack of TCE solubility enhancement in the 10% fermented reactor as the protein had been degraded prior to conducting the partitioning experiments.

While no solubility increases of TCE were observed with the 10% fermented molasses reactor, the mixing NAPL phase surface area was significantly affected, creating a stable (>100 days) microemulsion upon mixing the NPAL and fermented liquid phases. A field sample also formed an emulsion that was slightly less stable than the one formed with the 10% fermented molasses solution, as some breakdown in the emulsion occurred after 67 days. The fresh molasses did not create a stable emulsion. These data suggested that a biosurfactant was formed after substantial molasses fermentation, which influenced the NAPL surface area when physically mixed with the

fermented fluid. The resulting increase in surface area can greatly affect the mass transfer of TCE NAPL into the aqueous phase, as interfacial surface area is a primary variable affecting the mass flux of hydrophobic compounds.

Soil partitioning experiments were conducted to further evaluate the effect of fermented and fresh molasses solutions on sorbed mass. Data indicated that a lower TCE soil partitioning coefficient was obtained when fermented molasses was present, suggesting that fermented molasses can also enhance the dissolution of sorbed TCE mass.

The results of this research showed that enhanced mass transfer of TCE can occur through the addition and subsequent fermentation of a molasses solution. The mechanism of enhancement, however, varies depending on whether the molasses solution is fresh or has been allowed to ferment. Enhanced dissolution of TCE NAPL occurs through an increased concentration gradient in the presence of fresh molasses solution, or through an increased surface area if fermented molasses solution is present. Additionally, evidence suggested that fermented molasses could affect the soil partitioning coefficient of TCE, thereby enhancing the dissolution of sorbed TCE mass. These results can be used to optimize bioremediation systems used to treat residual source zones containing NAPL and sorbed mass.

Chapter 4 Carbon addition strategy affects *Eubacteria* and *Archaea* community structures, function, diversity and density

Three columns were packed with aquifer material and used to evaluate how fermentation product generation, community structure, and density were influenced by the method of carbon introduction. One column was continuously fed a low concentration of molasses (0.44% by volume in minimal groundwater media), a second received a monthly pulse of 10% molasses, and the third served as a control and was fed only minimal groundwater media. *Eubacteria* community significantly shifted as a result of the carbon feed, with the changes in communities steadily growing larger along the carbon gradient. *Archaea* communities were also affected by the carbon feed, with large changes observed between communities along the length of the continuously-fed column, corresponding to the carbon gradient. The communities along the length of the pulsed-fed column exhibited less-significant changes with respect to one another indicating the intermittent presence of high concentrations of carbon created similar communities regardless of position within the column.

The density of organisms varied from the continuously-fed and pulsed-fed columns, with the greatest change in biomass (*Eubacteria* + methanogen) attributed to the increased numbers of methanogens in the pulsed-fed column. An increased density of biomass was observed throughout the pulsed-fed column. The continuously-fed column did not exhibit as great of an increase of biomass, with the exception of the first sampling port location. Greater biofouling, however, was exhibited in the continuously-fed column.

The biofouling, therefore, was not attributed to density of organisms, rather some other factor, such as the production of extracellular polymeric substances (EPS). The continuously-fed system appeared to either select for organisms capable of producing EPS, or created an environment conducive to production of EPS (i.e. biofilm formation).

The generation of fermentation products was observed after each pulsed input of 10% molasses. Acetate was the dominant volatile fatty acid at the beginning of the study, with a shift to butyrate observed towards the end of the study. The continuously-fed system behaved differently, with the production of fermentation products ceasing after Day 125, indicating that the system had reached a pseudo steady-state. Methane generation was similar in both columns suggesting that even though the pulsed column contained higher numbers of methanogens, they did not necessarily correlate to dissolved methane concentrations. These results provide information that could be used to optimize or minimize fermentation, biomass distribution and biofouling by using different methods of carbon addition.

Chapter 5 Impact of an Ethanol-based Fuel on Native Aquifer Community Structure and Methane Production

A column study was used to identify and quantify the fermentation products and associated microbial community changes stemming from the input of a dilute ethanol-based fuel (E85). Acetate, butyrate and butanol were the detected final products of ethanol fermentation. Acetate was the primary fermentation product observed in the

sampling ports located closest to the inlet of the E85-fed column. The presence of butanol, a compound regulated by the Minnesota Department of Health, corresponded to times when the pH of the column decreased below a value of 7. Dissolved methane was observed in all sampling ports approximately 30 days after E85 introduction, and statistically significant numbers of methanogen gene copies (compared to the control) were present in five of six sampling ports, indicating the addition of E85 created an environment conducive to methane production. The number of gene copies was strongly correlated with the presence of acetate (r^2=0.99). A large shift in *Eubacteria* community structure occurred in response to E85 introduction, with the magnitude of the shift corresponding to the presence and concentration of fermentation products. The data, however, showed less of a difference in *Archaea* community structure along the length of the E85-fed column than observed with the *Eubacteria* communities. The shift in the *Archaea* community was attributed to the growth of methanogens.

Microcosms were constructed containing various percentages of ethanol up to 12% (by volume) to test at what level ethanol would be toxic to the complex microbial community present in aquifer material. Low concentrations of acetate were detected in the microcosm containing 6% ethanol, while two orders of magnitude higher concentrations of acetate were present in the 5% ethanol treatment. Elevated concentrations of butyrate and butanol were also present in the 5% ethanol treatment. These data indicate that the microbial population was strongly inhibited when 6% ethanol was present. No fermentation products were detected in microcosms containing >6%

ethanol, indicating complete inhibition of microbial activity and/or toxicity at ethanol concentrations greater than 6%.

The results of this research indicate that the core of an E85 plume may serve as a long-term source of substrated used in various fermentation processes, including methane generation, until it dilutes to a level conducive for microbial growth (<6% ethanol, by volume). Fate and transport of an E85 spill can be more effectively modeled using these data by incorporating an ethanol toxicity threshold. The production of compounds such as acetone and butanol can also be more accurately predicted based on these results, and acetate can be used to predict methane production.

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CHAPTER 2 Literature Review

2.1 Non-Aqueous Phase Liquid (NAPL) Remedial Technologies

A United States Environmental Protection Agency (U.S. EPA) analysis suggested that NAPL is present at approximately 60 percent of Superfund sites where organic chemicals have been detected (U.S. EPA, 1993). NAPL is comprised of water-immiscible organic liquids that slowly partition into the aqueous phase, causing a long-term source of groundwater contamination. The most prevalent type of NAPL in source zones consists of halogenated organic solvents; nevertheless, many sites are impacted with other types of NAPLs, including coal tar, creosotes, polychlorinated biphenyls, and pesticides (U.S. EPA, 2003).

Over the past decade, innovative technologies have emerged to address NAPL source areas *in situ*. One of these technologies is chemical flushing, a remedial method that combines the injection and subsequent extraction of chemicals to solubilize and/or mobilize NAPL (ITRC, 2002). The chemicals are injected in a manner designed to "flood" the NAPL zone, thereby mobilizing or solubilizing the NAPL, which is subsequently extracted and treated *ex situ*. Chemicals used in this process include surfactants and cosolvents (such as alcohols). For surfactants, the process is referred to as Surfactant-Enhanced Aquifer Remediation (SEAR), and for cosolvent application, the process is termed cosolvent flooding (ITRC, 2002).

The use of cosolvents and surfactants to solubilize NAPL has been shown by many authors (Lee and Peters, 2004; Jawitz et al., 2003; Chen and Delfino, 1997;

Bernardez and Ghoshal, 2004; Chu et al., 2005; Cowell et al., 2000; Dulfer et al., 1995). Cosolvents work by reducing the NAPL-water interfacial tension, thereby influencing NAPL through (a) enhancing mobilization of NAPL as a discrete phase, (b) enhancing NAPL solubility in the aqueous phase, and/or (c) altering the NAPL phase volume, density, and viscosity (Lee and Peters, 2004). Surfactants can solubilize hydrophobic compounds several hundred times in excess of their aqueous solubility (Bernardez and Ghoshal, 2004) through the formation of micelles.

2.2 Solubility Enhancement of Hydrophobic Compounds

Cosolvents reduce the polarity of water, and thereby increase the solubility of hydrophobic compounds (Boving and Brusseau, 2000). In addition, cosolvents can also reduce the interfacial tension between water and NAPL, and may also mobilize NAPL. There is not one particular and unique structural feature that characterizes a compound as a cosolvent to enhance solubility. There are, however, several generalizations that can be applied to most cosolvents: they contain a hydrogen bond donor group (i.e. OH, SH, NH or NH_2), and/or a hydrogen bond acceptor group (i.e. ≡N, =N–, =O, =S, –NH–, –O–, –S– or –N<), and usually have three or fewer carbons per each hydrogen bond donor or acceptor group (Yalkowsky, 1999). The hydrogen bond donor or acceptor groups interact strongly with water by disrupting the hydrogen bonding between water molecules, while the small hydrocarbon regions of the compound do not interact strongly with water. The net reaction of these compounds is to create an overall disruption in the hydrogen bonding between water molecules, thereby decreasing the overall polarity of the water.

This in turn increases the solubility potential for non-polar compounds within the aqueous media. Compounds stemming from fermentation processes that may act as cosolvents using the above criteria include: acetone, ethanol, methanol, 2,3-butanediol, and volatile fatty acids in protonated form (lactic, acetic, butyric, succinic, formic, propionic, valeric and caprioc). Other compounds produced during fermentation that fall into similar chemical classes, but do not follow the general criteria above are: methyl ethyl ketone, methyl isobutyl ketone, and butanol. These compounds contain greater than three carbons per hydrogen bond acceptor/donor groups.

Several models have been developed to estimate solubilities of compounds in cosolvent mixtures (Banerjee and Yalkowsky, 1988; Heermann and Powers, 1998). Yalkowsky et al. (1972) described a log-linear relationship for the aqueous solubility of pharmaceutical drugs in aqueous solvent. This was revised by Banerjee and Yalkowsky (1988) when they observed deviations from the log-linear cosolvency relationship in fractions of cosolvent less than 15 to 20 percent, where a linear response was shown instead of the log-linear response. At low cosolvent fractions, the molecules of solvent become partially separated from the water through formation of hydration spheres. At higher fractions, the hydration spheres expand, intersect, and eventually encompass the entire aqueous phase. At these fractions hydration is no longer a dominant process and the hydrophobic compounds have access to both water and cosolvent molecules (Heermann and Powers, 1998).

Interestingly, some of the same compounds that are used for SEAR and cosolvent flooding such as biosurfactants, ethanol, and acetone can be produced naturally via fermentative processes (Madigan and Martinko, 2006; Jones and Woods, 1996; Ren et al., 2007). Claims have been made by practitioners that enhanced solubilization of contaminants has occurred in several biostimulation field studies (Suthersan and Payne, 2005) where carbon substrate was added to promote degradation of hydrophobic compounds, such as TCE. Nevertheless, the extent of solubility enhancement and the compounds responsible for this enhancement has not been determined in controlled studies.

2.3 Fermentation of Carbon

The fermentation of organic carbon, eventually forming methane and carbon dioxide, is a complex process that involves the interactions of four different groups of bacteria, including primary and secondary fermenters, and acetoclastic and hydrogenotrophic methanogens (Schink, 1997). This process involves the conversion of polymers (polysaccharides, proteins, nucleic acids, and lipids) to oligomers and monomers (sugars, amino acids, fatty acids) by extracellular hydrolytic enzymes produced by the "primary" fermenting bacteria. These organisms use the resulting monomers to produce compounds such as fatty acids and alcohols through fermentation (Figure 2.1). Several of these fermentative pathways exist and tend to be described by the products they form, such as ethanol fermentation, lactic acid fermentation, and acetone-butanol fermentation. Some primary fermentation products, such as acetate and

hydrogen, are directly used by methanogenic organisms (both acetoclastic and hydrogenotrophic) to produce methane (Conrad, 1999). Other fermentation products, such as fatty acids containing more than two carbons, alcohols longer than one carbon, and branched-chain and aromatic fatty acids, are used by "secondary" fermenters who convert these compounds to acetate and carbon dioxide, which are then subsequently used by methanogenic organisms to produce methane (Schink, 1997).

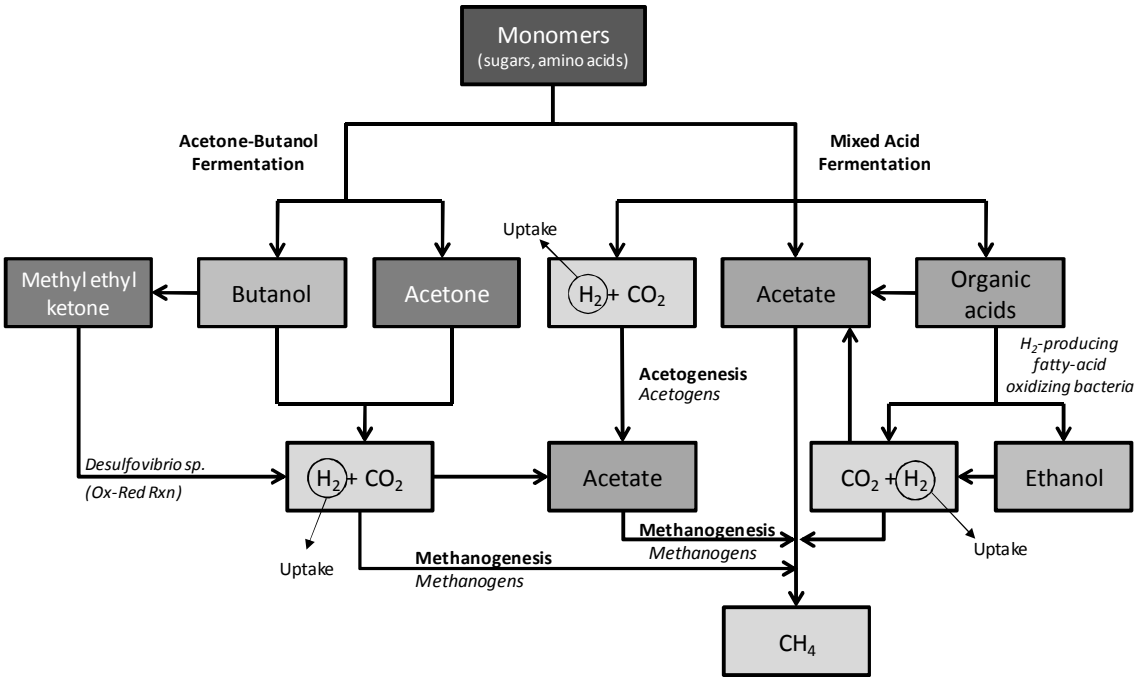

Figure 2.1 Depiction of the various interactions that can occur between different fermentation pathways. Hydrogen uptake can occur by several different organisms including acetogens (shown), methanogens (shown), dehalorespirers, and lithotrophic bacteria . Figure adapted from Payne et al. (2006) and Madigan and Martinko (2006).

Several different types of fermentation processes can occur as a result of carbon addition. The most common types of fermentation reactions produce alcohols, such as butanol and ethanol, and volatile fatty acids, including acetate, propionate, butyrate and lactate (Madigan and Martinko, 2006). In the instance of acidogenic fermentation, which

occurs at a pH of less than 7, three acidogenic fermentation types have been widely documented: (a) butyric acid at a pH of 5.5, (b) propionic acid at pH values greater than 6.0 and (c) ethanol fermentation at pH values less than 4.5 (Ren et al., 2001). Butyric-type fermentation is characterized by the production of butyrate, acetate, carbon dioxide, and hydrogen. Propionic type fermentation generates propionate, acetate and some valerate, but with no significant gas production, and ethanol type fermentation is characterized by the production of ethanol, acetate, hydrogen and carbon dioxide (Ren et al., 2001).

Acetone-butanol (AB) fermentation is another widely studied fermentation pathway where soluble carbohydrates undergo fermentation by *Clostridium* species, resulting in the production of acetone, butanol, ethanol, lactate, acetate, butyrate, and carbon dioxide (Jones and Woods, 1986). Large-scale production of acetone via this pathway was utilized in World War I and the pathway has been well-studied and characterized. AB fermentation takes place in two stages; the first stage involves the production of hydrogen, carbon dioxide, acetate and butyrate during the initial growth phase of *Clostridium*, referred to as the acidogenic phase. This process results in a decrease of the media pH. The metabolism then undergoes a shift to solvent production, termed the solventogenic phase, as the culture enters the stationary growth phase. This phase involves the reassimilation of acids along with the continued consumption of the carbohydrate, normally resulting in an increase in pH. Several factors are involved with the outcome of AB fermentation, with pH playing an important role in product generation (Jones and Woods, 1996). Cultures maintained at neutral pH (> 6.0) have been shown to

produce mainly acids, whereas cultures maintained at a low pH (4.5 to 5.0) have been shown to produce mainly solvents. The range of pH that triggers solvent production varies widely depending on the strain of *Clostridium* and the culture conditions in which it was grown. Carbon limitation has also been shown to be a major factor affecting the outcome of AB fermentation, as studies have shown that only acids were produced when the concentration of the carbon source was limited (Jones and Woods, 1996).

As shown above, several different types of fermentation reactions can occur with the addition of organic carbon. The types of products generated by fermentation processes are directly affected by (a) the type of substrate used for the fermentation, and (b) environmental conditions such as retention time, substrate loading rate, temperature, and pH (Ren et al. (1997). Most studies regarding fermentation have been performed on specific cultures, with the intent of commercial development of certain products. Little research has been performed regarding the outcome of fermentation stemming from organic carbon inputs in remedial applications.

2.4 Fermentation Products and Solubility Enhancement

When cultures are established for solvent production, such as AB or ethanol fermentation, yields of targeted solvent are shown to be less than 2% vol/vol of solvent to media (Jones and Wood, 1997, Ren et al., 2007).The small fractions of individual compounds are well below the observed 15 to 20 percent fraction of cosolvent needed to observe a log response in hydrophobic compound solubility (Banerjee and Yalkowsky,

1988). Nevertheless, the combination of several compounds present at low concentrations may have a synergistic effect in which solubility enhancement will occur.

In addition to cosolvents, biosurfactants can significantly enhance hydrophobic compound solubility. Biosurfactants are compounds that are produced by microorganisms and have been shown to enhance hydrophobic compound desorption and solubility by lowering the surface and interfacial tensions of liquids (Christofi and Ivshina, 2002). Several species of bacteria are capable of producing biosurfactants, including the fermentative species *Clostridium* (Christofi and Ivshina, 2002). *Clostridium pasteurianum* was shown to produce neutral lipids during anaerobic growth, reducing the surface tension of growth media from 72 dynes/cm to approximately 55 dynes/cm (Cooper, 1980). Fatty acids, neutral lipids and phospholipids also serve as biosurfactants (Desai and Benat, 1997). Release of lipids through cell lysis may also contribute to the increased solubility of hydrophobic compounds.

2.5 Methane Generation

Fermentation products such as acetate and hydrogen can be directly used by methanogenic organisms to produce methane (Schink, 1997). Acetoclastic (use of acetate) and hydrogentrophic (coupled hydrogen/carbon dioxide) methanogenesis are the two dominant pathways for methane formation with acetatoclastic methanogenesis responsible for >67% of methane formation in anaerobic systems (Conrad, 1999). Optimization of the growth of bacteria through control of hydrogen thresholds has been the subject of several laboratory studies over the past decade (Yang and McCarty, 1998;

Lee et al., 2004; Ma et al., 2006). Results indicated that the use of relatively low concentrations (<5 mg/L as benzoate) of different substrates (Yang and McCarty, 1998) and methods of hydrogen input (Lee et al., 2004; Ma et al., 2006) are capable of suppressing methanogenesis by maintaining the hydrogen thresholds at a low level. In the case of large-scale bioremediation applications, carbon is typically delivered at high concentrations (>1,000 mg/L as total organic carbon) (Suthersan and Payne, 2005) which is not conducive for control of hydrogen thresholds. As a result, case study data have documented that high levels of methane can be produced after carbon has been injected into an aquifer (Suthersan and Payne, 2005) leading to potentially explosive situations. While the quantity of carbon delivered in these systems is above that which could control hydrogen thresholds, different methods of carbon delivery may affect microbial community structure, thereby impacting the quantity of generated fermentation products, ultimately affecting the production of methane.

2.6 Carbon/Electron Donor Delivery Studies

The cost-effectiveness of any *in situ* remedial technology relies on the distribution of injected fluid, whether it is a surfactant, cosolvent, or carbon amendment. In the instance of bioremediation, the distance a degradable amendment can travel from the point of substrate introduction directly affects the spacing of injection wells. In addition, the frequency of injection also has a significant cost impact, as the more often an amendment requires injection, the more costly a remedial technique can become. Carbon injection systems can also be complicated by biofouling, as supplying too much substrate

will result in significant growth of organisms near the injection point which leads to decreased efficiency of the injection wells (Moretti, 2005). Balancing all these factors is vital to the success of any *in situ* remedial technology. Spatial distribution of substrate has been modeled to determine the optimum delivery method required to achieve large radii of influence when injecting amendment into a groundwater aquifer (Franzen et al., 2004). Results of the numerical model indicated short nutrient pulses followed by long injections of unamended water would achieve greater substrate distribution than continuous injection of nutrient would achieve. The application of this method has also been touted by field practitioners as a way to prevent biomass buildup in the injection zone, thereby minimizing adverse permeability effects resulting from biofouling (ESTCP, 2005). Nevertheless, a column study evaluating the distribution of biomass near the point of substrate introduction using both continuous and pulsed methods for nutrient (carbon and electron acceptor) delivery indicated that larger quantities of biomass developed near the point of introduction using pulsed inputs of nutrient (Peyton, 1996). The above discrepancy may be a function of injection frequency, as field practitioners tend to inject on a less-frequent basis than was used in the laboratory study. For example, the above column study fed acetate for 0.5 hours every 12 hours, whereas pulsed inputs on the field scale tend to inject substrate for hours to days on a monthly basis (Moretti, 2005). Regardless, these results indicate that a different microbial community structure is achieved using different methods of carbon input. No laboratory studies to date have mimicked field conditions using longer intervals between pulses of carbon. Understanding the impact that these different methods of carbon application have on

indigenous microbial community structure and density may allow for better engineering practices to minimize biofouling and methane generation.

2.7 Effect of carbon type and concentration on microbial communities

The impact of different carbon types on microbial communities has been evaluated with respect to biotechnology applications (Temudo et al., 2008), and the denitrification of wastewater (Osaka et al., 2008). Osaka (2008) concluded that environmental factors (i.e. increased salinity) coupled to different substrates (acetate versus methanol) resulted in the development of different microbial communities capable of different nitrate removal efficiencies. This indicates that both environmental conditions and substrate type affect the microbial community stimulated by the carbon input. Temudo (2008) also provided clear evidence that different communities developed when supplied with different types of carbon (glucose, glycerol and xylose) in a continuously stirred tank reactor. Moreover, the addition of a second substrate resulted in the appearance of new organisms when compared to the use of a single substrate. These studies suggest that the diversity of the microbial community increases and a structural shift occurs when new substrates are supplied to an established community. Temudo et al. (2008) also gave evidence for a further community shift that stemmed from an increase in carbon loading, indicating that microbial populations are impacted by different concentrations of the same type of carbon. This provides evidence that high versus low carbon concentrations can have different effects on microbial community structure, which in turn may affect the formation of fermentation products.

2.8 Ethanol-based Fuel

Ethanol, a common component of renewable fuel, can serve as a microbial food source and promote the growth of a broad fermentative community capable of producing several fermentation products including alcohols, ketones and volatile fatty acids (Madigan and Martinko, 2006). The Energy Independence and Security Act of 2007 requires the production of 36 billion gallons of renewable fuel, including ethanol-based fuels, by 2022 (RFA, 2007). Flexible-fuel vehicles (FFV) utilize fuels containing up to 85% ethanol (E85), and over 5 million FFVs are on the road today with automakers slated to produce millions more each year. As a result, over 1,120 retail stations currently offer E85, and increased availability at local filling stations will undoubtedly occur over the next decade because of this initiative. The greater the number of gas stations carrying this fuel, the greater the likelihood of an E85 spill during transportation or storage.

Similar to bioremediation case studies, the addition of ethanol to an aquifer can result in the production of methane. Studies have shown dissolved methane concentrations up to 16 mg/L and the production of methane coinciding with increased numbers of *Archaea*, the domain to which methanogens belong, after addition of ethanol to a pilot scale aquifer tank (Cápiro et al., 2008) and an aquifer (Feris et al., 2008). High levels of ethanol, however, are toxic to microorganisms, with toxicity levels ranging from 6% to 18% (vol/vol) depending on the organism tested (Ulrich, 1999). It is therefore critical to understand how a spill of ethanol-based fuel affects the microbial community

in terms of toxicity effects and methane generation to effectively assess potential risks posed by the fermentation of ethanol.

2.9 References

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CHAPTER 3 Enhanced Dissolution of Trichloroethene: Effect of Carbohydrate Addition and Fermentation Processes

Nelson, D., and Novak, P. (2009) "Enhanced Dissolution of Trichloroethene: Effect of Carbohydrate Addition and Fermentation Processes" *J. Environ. Eng.* September. 861-868

3.1 Introduction

It has been estimated that between 15,000 and 25,000 sites in the U.S. are impacted with dense non-aqueous phase liquid (NAPL) contaminants. If all of these sites were treated by hydraulic containment (i.e., pump and treat), the annual operating costs would range from $2.7 to $4.5 billion dollars over the next three decades (Kavanaugh and Rao 2003). An analysis of 36 sites undergoing source area treatment (bioremediation, chemical oxidation, surfactant/cosolvent flushing, or thermal treatment) indicated that bioremediation was the lowest median cost per volume treatment (McDade et al. 2005). Although using bioremediation as a strategy to treat residual source zones has been declared promising (Akladiss et al. 2005), case study analysis has suggested that these sites will still require further management due to residual lingering NAPL and sorbed contaminant mass (McGuire et al. 2006). It is critical to better understand how to address non-aqueous and sorbed residual mass during site remediation to more effectively promote the clean-up of residual mass areas.

Biostimulation is a technology that delivers substrate to aquifers to promote *in situ* bioremediation of contaminants that serve as electron acceptors (*e.g.*, chlorinated ethenes). The addition of organic carbon to a groundwater aquifer for biostimulation purposes promotes the growth of a diverse fermentative community (Schink 1997) as well as targeted dechlorinating organisms (Cope and Hughes 2001; Yang and McCarty 2002). This community is capable of producing several products, such as alcohols, ketones (Madigan et al. 2006) and biosurfactants (Christofi and Ivshna 2002) as the

organic carbon is consumed. When present in high enough concentrations, these compounds can alter the properties of water (Yalkowsky 1999), and consequently may affect the behavior of NAPL and hydrophobic contaminants. Because fermentation has the potential to enhance the dissolution of sorbed and NAPL mass, the objective of our research was to determine how the partitioning behavior of trichloroethene (TCE), a model NAPL, is affected by both the addition of model fermentation products and the addition and subsequent fermentation of carbon (e.g. molasses). Specifically, we were interested in investigating how both TCE NAPL and sorbed mass dissolution could be affected by fermentation.

The theory of mass flux of hydrophobic compounds from the NAPL to aqueous phase is well documented (Miller et al. 1990) and affected by three primary variables: concentration gradient, mass transfer coefficient, and interfacial surface area. Of these, the two parameters that may feasibly be manipulated in the field are the concentration gradient, and the NAPL interfacial surface area.

Increasing the concentration gradient to enhance mass transfer has been the primary focus of laboratory studies to date, either by decreasing the aqueous concentration through biodegradation (Cope and Hughes 2001; Yang and McCarty 2002) or increasing the solubility limit of the targeted compound via the use of cosolvents or surfactants (Boving and Brusseau 2001; Hood et al. 2007; West and Harwell 1992). Interestingly, some compounds produced during microbial fermentation can act as cosolvents (e.g., lactic and acetic acids, acetone, or ethanol) (Yalkowsky 1999). Nevertheless, fermentation is expected to produce low concentrations of these

compounds (Jones and Woods 1986; Ren et al. 2007) and individually, they are unlikely to cause a significant increase in solubility. It is possible, however, that the combination of several compounds may be sufficient to enhance solubility. Although some preliminary work in this area has been performed (Hood et al. 2007), more detailed and mechanistic studies are warranted.

NAPL surface area is another primary mechanism by which enhanced mass transfer can occur. Indeed, several studies have demonstrated the importance of understanding surface area to better model NAPL mass flux in aquifers (Khachikian and Harmon 2000; Miller et al. 1990; Nambi and Powers 2003). The relationship between concentration gradient and surface area is linear (Miller, 1990); therefore, an increase in surface area will result in an increased concentration gradient. The effect of fermentation products on NAPL surface area, to our knowledge, has not been investigated.

In addition to mass flux, the partitioning of hydrophobic compounds between aqueous and soil systems may be influenced by fermentation products. This partitioning between sorbed and aqueous mass can be described by K_d, an experimentally derived soil distribution coefficient (Watts 1997). K_d is the ratio of sorbed to aqueous phase contaminant, and could be affected by the presence of fermentation products. This has also not been investigated.

3.2 Materials and Methods

Soil and Field Sample Collection

Aquifer material was obtained from the saturated portion of a surficial sand aquifer (Fridley, MN) using a hollow stem auger. The material removed from the site was comprised of 93.8% sand, 1.5% silt, 4.8% clay, and had an average organic matter fraction of 0.25 (by mass). Soil was sieved through a 2-mm mesh to remove large particles prior to laboratory use. Additional field samples were collected from two separate sites impacted with chlorinated solvents and currently undergoing biostimulation using a dilute molasses solution (4% vol/vol). Samples "Field 1" and "Field 2" were collected from sites located in California, near the cities of Los Angeles and Downey, respectively. Field 1 was collected from a monitoring well within the radius of influence of a biostimulation injection well approximately 9 months after the last injection of a 4% (vol/vol) molasses solution. Similarly, Field 2 was also located within the radius of influence of the biostimulation injection well and was collected approximately 14 months after the last injection of a 4% molasses solution.

Soil Aging

Approximately 2-L of soil was aged with TCE by placing soil into a 3-L amber glass container, filling the void spaces and remaining volume of the container with TCE-saturated nanopure water, and sealing the container with a Teflon-lined screw cap to minimize volatilization. The sealed container was placed on a shaker table for

approximately twelve months. The container was periodically removed from the shaker table and hand shaken to ensure that all soil was in contact with the TCE-saturated water.

Reactors

Three separate reactors were constructed for experiments. Two reactors were fed molasses and allowed to ferment for several months for use in partitioning experiments. The third reactor contained a molasses-free media (no carbon amendment), and was considered the control reactor for partitioning experiments. Reactor construction is detailed below.

Two 4-L fill-and-draw reactors were started by adding 400 g of non-aged soil to either (a) minimal groundwater media only (Control reactor), or (b) molasses in minimal groundwater media (10% vol/vol). Wholesome Sweeteners™ dark (blackstrap) molasses (unsulphured with approximately 3% crude protein) was used in reactors and experiments. The minimal groundwater media was adapted from Wilbur et al. (1995) and contained trace nutrients and mineral components consistent with natural groundwater. The media contained the following (per L of distilled water): 20 mg $NaNO_3$, 20 mg $MgSO_4 \cdot 7H_2O$, 0.7 mg $FeCl_2 \cdot 4H_2O$, 95 mg $K_2HPO_4 \cdot 3H_2O$, 40 mg K_2CO_3, 16.3 mg $MgCl_2 \cdot 6H_2O$, 11.8 mg $CaCl_2 \cdot 2H_2O$, 0.05 mg $CoCl_2 \cdot 6H_2O$, 0.05 mg KI, 0.06 mg $MnCl_2 \cdot 4H_2O$, 0.007 $ZnCl_2$, 0.01 mg $NaMoO_4 \cdot 2H_2O$, 0.007 mg H_3BO_3, 0.01 mg $NiCl_2 \cdot 6H_2O$, 0.008 mg $CuCl_2 \cdot 2H_2O$, 40 mg K_2CO_3, 0.4 mg NH_4HCO_3 and 12 mg $NaHCO_3$. Gases resulting from the fermentation process were allowed to escape through

a headspace trap. The reactor pH was maintained circum-neutral to promote biological growth by adjusting with a 10M sodium hydroxide solution as needed.

A 20% (vol/vol) molasses-fed culture was started in a 500-mL batch reactor using approximately 400 g of non-aged soil and 250 mL of 20% molasses in minimal groundwater media. Off-gassing was performed manually by periodically piercing the septa present in the bottle cap. The pH was manually adjusted in the batch reactor to maintain a near-neutral pH in an anaerobic glovebag using a 10M sodium hydroxide solution.

Experimental Procedures

NAPL Partitioning Experiments

Partitioning experiments were conducted in 20-mL vials fitted with Teflon-lined septa sealed with aluminum crimp caps. The partitioning experimental setup was adapted from Hayden et al (1999), with each vial containing the following: 5 acid-washed glass beads to promote mixing (3 mm diameter [Kimble, NJ]), 4-mL of pure-phase TCE (NAPL) and 10-mL of a filtered liquid sample. The filtered liquid samples consisted of minimal groundwater media mixed with varying percentages of individual cosolvents, liquid samples from reactors, liquid field samples, or minimal groundwater media to which a combination of multiple cosolvents was added, as discussed below. Field and reactor samples were filtered through a 0.2-μm polyethersulfone syringe filter (Nalgene, Rochester, NY) prior to being placed in the vials. Each sample was run in triplicate. Vials were placed on a rotator (Glas-Col, Terre Haute, IN) at 50% power for a minimum of 12

hours at room temperature (23ºC) for equilibration. Prior to data collection, the vials were allowed to settle for a minimum of 24 hours after mixing to separate the NAPL from the aqueous samples. Equilibrium was verified through an additional experiment in which samples were mixed for 12 hours, allowed to separate for 24 hours, after which samples were taken daily for 8 days. Equilibrium was obtained by the first sampling point. Two mL of the aqueous phase sample was then transferred to 2-mL liquid autosampler vials using a 5-mL gastight syringe (Hamilton Ind., Reno, NV) and immediately crimped shut. Samples were analyzed for TCE in the aqueous phase using a gas chromatograph equipped with a flame ionization detector (GC/FID).

Soil Partitioning Experiments

Soil partitioning experiments were modeled after those by Pavlostathis and Jaglal (1991) and Xia and Pignatello (2001). Briefly, triplicate vials for each sample were constructed using approximately 9 g of aged soil (containing TCE-saturated water) added to a 7-mL glass vial and sealed with Teflon-lined septa and aluminum crimps. Vials were centrifuged at 1200g for 30 minutes and excess fluid was decanted. Filtered (0.2-µm) reactor and field samples (containing no TCE) were added to each vial; care was taken to minimize the headspace in each vial. Samples added to the vials included field samples, solution from the control reactor, fermented 10% molasses solution and freshly made 10% molasses solution. Vials were crimped shut and placed on a rotator at 50% power for 10 days to allow for equilibration. After 10 days vials were centrifuged and the liquid samples were analyzed for TCE using a GC/FID. Excess sample was then removed and

the soil was extracted by adding 2-mL hexane, mixing on the rotator for 24 hours, centrifuging the vials, and analyzing for TCE using a GC coupled to an electron capture detector (GC/ECD). For calculation purposes, the mass of soil, volume of sample, and hexane added to the vials were each determined gravimetrically.

Analytical Methods

Aqueous phase TCE from partitioning and sorption experiments was analyzed using on-column injection with a 7673 automatic liquid sampler fit with a nanoliter adaptor and connected to a Hewlett Packard (HP) 5820 Series II gas chromatograph equipped with a flame ionization detector. A sample volume of 0.2 μL was directly injected onto a 5-m 0.53-mm diameter deactivated fused silica pre-column connected to a 30-m 0.32-mm (5%-Phenyl)-methylpolysiloxane (HP-5) capillary column (Agilent Technologies, Santa Clara, CA). Helium (99.99%) was used as the carrier gas at a flowrate of 18.2 cm/s. The inlet temperature was set to track 3°C above the oven temperature at all times. The detector was maintained at 300^{o}C. High purity ACS-grade TCE was used to prepare standards. Technical grade TCE (99% pure) was used in all experiments.

TCE extracted with hexane was analyzed using an HP 5890A GC equipped with an electron capture detector and an HP Model 6890 liquid autosampler (1 μL injection volume). An HP-5 column (30-m x 0.53-mm) was used for analysis with a carrier gas (helium 99.99%) flow rate of 10 mL/min. The inlet and detector temperatures were set to 225^{o}C and 300^{o}C, respectively. Extraction efficiencies for TCE in saturated soil averaged

108% with a standard deviation of 7%. Alcohols and ketones from field and reactor samples were analyzed using split/splitless injection with a 7673 automatic liquid sampler fit with a nanoliter adaptor and connected to a Hewlett Packard (HP) 5820 Series II gas chromatograph equipped with a flame ionization detector. A sample volume of 0.2 μL was injected onto a 12-m x 0.25-mm HP-1 column (Agilent Technologies, Santa Clara, CA). Helium (99.99%) was used as the carrier gas at a flowrate of 52.1 cm/s. The inlet and detector temperatures were set to 125°C and 300°C, respectively. High purity ACS-grade alcohols and ketones were used in experiments and to prepare standards.

Volatile fatty acid analysis of field and reactor samples was performed using a high performance liquid chromatograph (Shimadzu LC-19 AT) equipped with a SPD-10A UV-Vis detector (210 nm wavelength) and an Aminex HPX-89H column (Biorad, Hercules, CA). A sample volume of 25 μL was injected with an isocratic mobile phase of 0.005 N H_2SO_4 at a flow rate of 0.6 mL/min. Concentrated acids (85% or higher) were used in experiments and to prepare standards. Standard curves were adjusted to account for purity.

Total organic carbon was analyzed using a HACH High Range (100 to 700 mg/L) Total Organic Carbon Test ‘n Tube Reagent set. An Orion Model 420 digital pH meter equipped with a combination electrode was used for pH measurement. Soil was sent to the University of Minnesota Research Analytical Laboratory for textural analysis via sieving and hydrometer analysis, and fraction of organic matter (f_{oc}) analysis by the Loss on Ignition method.

3.3 Results and Discussion

NAPL Partitioning Experiments

Figure 3.1 shows increases in TCE solubility resulting from the addition of different fractions of selected cosolvents. Cosolvents typically have small hydrocarbon regions and contain either hydrogen bond donor or acceptor groups within their chemical structure. The hydrocarbon regions of these compounds usually have less than three carbons per hydrogen bond donor or acceptor group (Yalkowsky 1999). These small regions, therefore, do not interact strongly with water, which in turn increases the effect of the hydrogen bond disruption by the hydrogen bond donor or acceptor groups within the aqueous media. Accordingly, cosolvents can create an overall disruption in the hydrogen bonding between water molecules, thereby decreasing the overall polarity of the water. This in turn increases the solubility potential for non-polar compounds within the aqueous media. Compounds stemming from fermentation processes that can act as cosolvents include acetone, ethanol, methanol, 2,3-butanediol, and volatile fatty acids in the protonated form (lactic, acetic, butyric, succinic, formic, propionic, valeric and caprioc). Other compounds produced during fermentation that fall into similar chemical classes but are not typically thought of as cosolvents (Yalkowsky 1999) are methyl ethyl ketone, methyl isobutyl ketone, and butanol. All listed compounds were used in partitioning experiments to determine whether they behave as cosolvents when present at low levels (0.2 vol/vol or less).

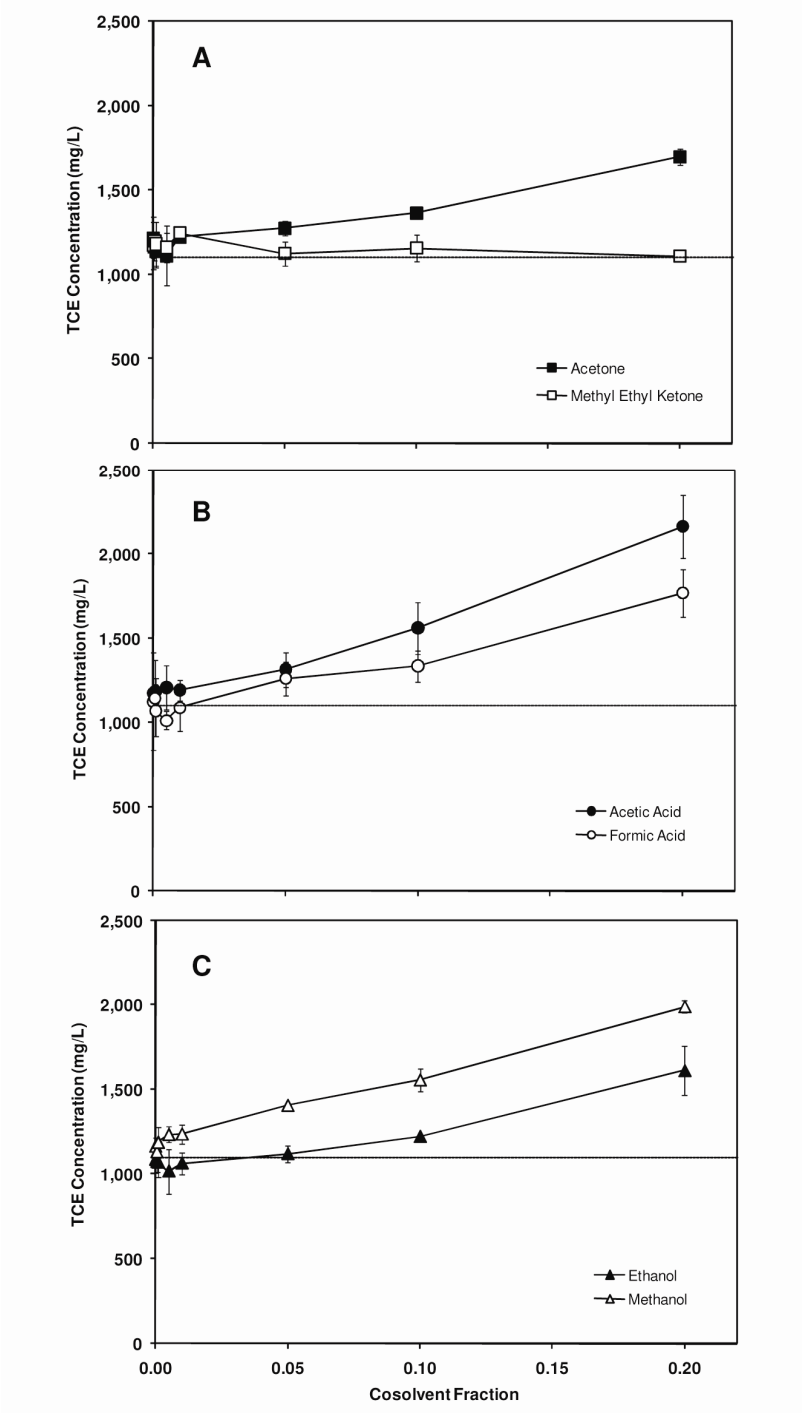
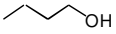
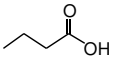
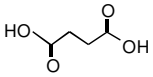
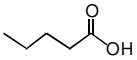
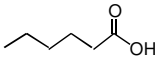


Figure 3.1 TCE solubility in presence of selected (A) ketones, (B) volatile fatty acids, and (C) alcohols. Data points represent average of triplicate runs with error bars showing standard deviation. Dashed line represents TCE solubility (1.1 g/L) in water (Pankow and Cherry, 1996).

Table 3.1 summarizes the results of all tested cosolvents. As shown, cosolvency effects were generally observed at higher fractions (≥ 0.2) with the exception of methanol, which exhibited a statistically significant ($p \leq 0.05$) increase in solubility at a fraction of 0.05. Our results are similar to other published values. For example, Hood et al. (2007) reported a normalized solubility enhancement factor of 1.18 and 1.09 for lactic acid and ethanol, respectively. These values are within 5% of our normalized results. All cosolvents that influenced TCE solubility had three or fewer carbon molecules and contained either one hydrogen bond donor group or one hydrogen bond acceptor group; characteristics typical of cosolvent molecules, as discussed above. Compounds such as 2,3-butanediol, and volatile fatty acids with four of more carbons did not enhance solubility at a fraction of 0.2 or lower.

Table 3.1 Summary of results from 3-phase cosolvent partitioning runs.

Compound	Molecular Formula	Structure	Effect on TCE Solubility ($P \leq 0.05$)[a]	Normalized Solubility Increase[b]
Acetone	C_3H_6O		No change ≤ 10%[c] **Increase 20%**	1.39 (20%)[d]
Methyl Ethyl Ketone (MEK)	C_4H_8O		No change ≤ 20%	None
Methyl Isobutyl Ketone (MIBK)	$C_6H_{12}O$		No change ≤ 2%	None
Methanol	CH_4O		No change ≤ 1% **Increase ≥ 5%**	1.21 (5%) 1.34 (10%) 1.71 (20%)
Ethanol	C_2H_6O		No change ≤ 10% l **Increase ≥ 20%**	1.52 (20%)
Butanol	$C_4H_{10}O$		No change ≤ 20%	None
2,3- Butanediol	$C_4H_{10}O_2$		No change ≤ 20%	None
Formic Acid	CH_2O_2		No change ≤ 10% **Increase ≥ 20%**	1.57 (20%)
Acetic Acid	$C_2H_4O_2$		No change ≤ 10% **Increase ≥ 20%**	2.22 (20%)
Lactic Acid	$C_3H_6O_3$		No change ≤ 10% **Increase ≥ 20%**	1.64 (20%)
Propionic acid	$C_3H_5O_2$		No change ≤ 10% **Increase ≥ 20%**	1.72 (20%)
Butyric Acid	$C_4H_8O_2$		No change ≤ 20%	None
Succinic acid	$C_4H_6O_2$		No change ≤ 20%	None
Valeric Acid (Pentanoic)	$C_5H_{10}O_2$		No change ≤ 20%	None
Caproic Acid (Hexanoic)	$C_6H_{12}O_2$		No change ≤ 20%	None

a Change in concentrations considered statistically significant at p=0.05 using the Student's t-test.
b Solubility increase normalized to average of unamended controls (triplicate run) within same experimental set.
c Percent based on volume per volume (vol/vol) basis
d Cosolvent fraction at which solubility increase observed. The reported percentage value is the fraction of cosolvent initially added.

Field and reactor-fermented samples were used in NAPL partitioning experiments to determine whether TCE solubility was enhanced. The addition of non-fermented molasses (both 10% and 20% by volume) was also evaluated for TCE solubility enhancement. As shown in Figure 2, the solutions with the greatest impact on TCE solubility were the fresh molasses solutions. A 2.3- and 2.7-fold increase in TCE solubility was observed with the fresh 10% and 20% molasses solutions, respectively. The fermented 10% molasses solution (after 234 days of fermentation) did not have an impact on solubility. Similarly, the field samples did not affect TCE solubility (data not shown). The fermented 20% molasses solution (after 239 days of fermentation) did, however, increase TCE solubility, although this varied with fermentation time (Figure 3.2).

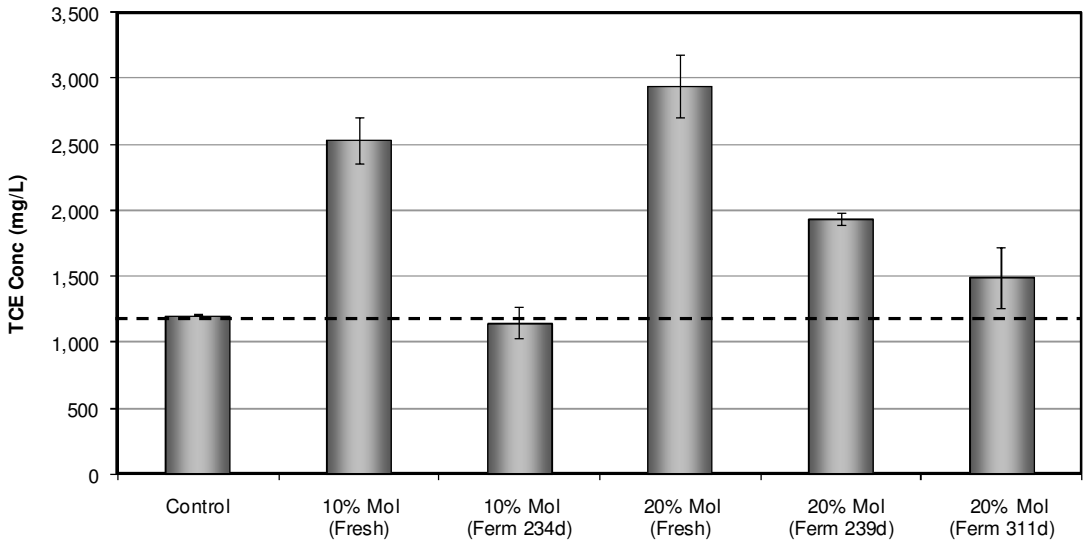


Figure 3.2 Concentrations of soluble TCE in vials containing a NAPL phase and media only (control), media amended with unfermented molasses (Fresh), media amended with molasses that was allowed to ferment (Ferm) for 234 days (10% molasses), 239 days (20% molasses) and 311 days (20% molasses). TCE solubility in water (1.1. g/L) is indicated by the dashed line. Data points represent the average of triplicate runs, with error bars showing the standard deviation.

The solubility enhancement with fresh molasses solutions is similar to that observed by Macbeth et al. (2006), where up to a 6.7-fold increase in TCE solubility was observed when in the presence of a fresh 10% whey solution. Macbeth and coworkers (2006) attributed the solubility enhancement to the protein fraction (10 to 13%) present in the whey powder. Protein can increase solubility through β-sheet structures where a chain of amino acids folds back and forth upon itself, exposing hydrogen atoms. This can undergo extensive hydrogen bonding (Madigan et al. 2006) and thereby alter the properties of water and increase the solubility potential for non-polar compounds such as TCE. The blackstrap molasses used in these experiments contained approximately 3% crude protein, which is believed to be responsible for the solubility enhancement. Accordingly, the observed decrease in TCE solubility over time in the fermented 20% molasses reactor solution is likely a result of the degradation of the protein fraction responsible for the initial solubility increase. The total organic carbon concentration in the 20% molasses reactor decreased by approximately 2,600 mg/L between days 239 and 311 of fermentation indicating continued fermentation and subsequent growth of organisms. The protein component of the organic carbon was not specifically measured, but it is likely that the material responsible for the initial solubility enhancement in the fresh molasses reactors (e.g. protein) was degraded over this period.

To confirm that cosolvency was not the reason for solubility enhancement in the fermented 20% molasses reactor, the liquid in the fermenting reactors and field samples was analyzed for compounds that could potentially serve as cosolvents. Table 3.2 summarizes the observed concentrations of potential cosolvents resulting from molasses

fermentation in the field samples and the liquid from the fermenting reactors. As shown, high levels of volatile fatty acids (VFAs) and ethanol were detected in the reactors undergoing molasses fermentation. In addition to VFAs and ethanol, the fermented 10% molasses reactor contained acetone and low levels of butanol. Acetone and butanol are the primary fermentation products in the later stages of the Acetone-Butanol (AB) fermentation pathway (Jones and Woods 1986). The fermented 20% molasses reactor did not contain detectable amounts of acetone or butanol. These data, along with the higher concentrations of VFAs and lower level of ethanol in the fermented 20% molasses reactor indicate that it was likely not as advanced in the fermentation process as the fermented 10% molasses reactor. The field samples contained lower to non-detectable concentrations of VFAs. The detection of acetone within Field 1 was not considered significant due to the large standard deviation observed in the triplicate samples. Field 2 did not contain significant levels of any cosolvents. Field 1 and Field 2 contained approximately 400 mg/L and < 100 mg/L of organic carbon, respectively. The low levels of these cosolvents in the field samples are attributed to the small amount of carbon present in these samples and the long period of fermentation that they had been subjected to (9 and 14 months for Field 1 and Field 2 samples, respectively). The fermented 10% and 20% molasses reactors contained approximately 27,400 and 52,400 mg/L of organic carbon, respectively.

Table 3.2 Detected concentrations from field samples and 10% and 20% fermented molasses reactors.

Compound	Average Concentration (mg L^{-1})			
	Field 1[a]	Field 2[b]	10% Fermented[c]	20% Fermented[c]
Succinic Acid	ND[d]	ND	1,990 (37)[e]	6,139 (4,019)
Lactic Acid	99 (172)	ND	27,706 (790)	74,235 (9,162)
Formic Acid	95 (13)	4 (6)	1,260 (69)	37,111 (2,163)
Acetic Acid	239 (95)	11 (2)	633 (26)	12,811 (6,325)
Propionic Acid	ND	15 (13)	4,273 (299)	16,744 (4,430)
Butyric Acid	ND	ND	14,420 (366)	12,684 (2,489)
Valeric Acid	ND	ND	ND	ND
Methanol	ND	ND	ND	ND
Ethanol	ND	ND	2,360 (771)	863 (169)
Acetone	69 (102)	ND	370 (74)	ND
Methyl Ethyl Ketone (MEK)	ND	ND	ND	ND
Butanol	ND	ND	800 (268)	ND
Methyl Isobutyl Ketone (MIBK)	ND	ND	ND	ND

Note: Values represent the average of triplicate values.
a Field sample with < 100 mg/L of TOC
b Field sample with 400 mg/L of TOC
c Samples collected after approximately 6 and 10 months of fermentation, from the 10% and 20%molasses reactors, respectively
d No detection (ND)
e Sample standard deviation (n=3)

Individually, the cosolvents listed in Table 3.2 were present at levels below the fractions determined to affect TCE solubility within the NAPL partitioning experiments. The combination of several cosolvents at low concentrations may, however, have an effect on TCE solubility. Accordingly, the highest detected concentration of each cosolvent from Table 3.2 was combined and used in a NAPL partitioning experiment to determine whether cosolvency may have contributed to the solubility enhancement observed in the fermented 20% molasses reactor samples. The combination of cosolvents did not enhance solubility (data not shown), further supporting the premise that the

solubility enhancement observed in the 20% fermented reactor was a result of a degradable solubilizing component within the fresh molasses, not the production of fermentation products.

Although solubility was not substantially increased by the fermentation products present in the fermented 10% molasses reactor, the NAPL phase surface area was visibility altered. As shown in Figure 3.3(A), the NAPL formed a microemulsion with the fermented 10% molasses solution (234 days of fermentation) that continued to be stable >100 days after settling, while the control resumed two distinct phases (NAPL and aqueous) immediately after mixing (Figure 3.3). Fresh molasses (10%) also formed a microemulsion immediately following removal from the rotator; nevertheless, the emulsion was not stable and returned to two distinct phases after 48 hours of settling (Figures 3.3[B1] and 3.3[B2]). Field 1, the field sample that contained some cosolvents (Table 3.2), also formed an emulsion that was slightly less stable than the one observed in the fermented 10% molasses reactor, as some breakdown occurred after 67 days of settling (Figures 3.3[C1] and 3.3[C2]). It should be noted that the fermented 20% molasses reactor liquid (after 311 days of fermentation) behaved similarly to the fresh 10% molasses (data not shown) where the NAPL returned to a distinct phase after 48 hours of settling. This is consistent with our other results that suggest that fermentation had progressed further in the 10% molasses reactor than in the 20% reactor. These data suggest that some type of surfactant was formed after substantial molasses fermentation (i.e., in the Field 1 sample and fermented 10% molasses reactor) and influenced the NAPL surface area upon physical mixing between the NAPL and fermented fluid phases.

Indeed, a unique property of surfactants is the lowering of interfacial tension between immiscible fluids, which can cause the formation of a stable emulsion (if an energy input, such as mixing, occurs). The formation of emulsion during microbial growth has been widely observed and has been attributed to the production of biosurfactants (Desai and Banat 1997; Desai et al 1988; Reisfeld et al 1972).

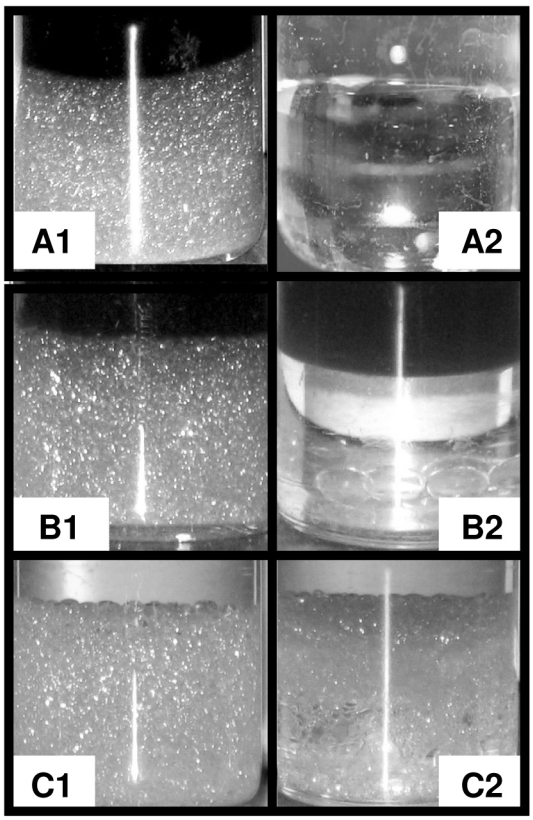


Figure 3.3 Photographs showing changes in NAPL surface area as affected by fermented versus non-fermented substrate and emulsion behavior over time. (A1) TCE emulsion resulting from mixing NAPL-phase TCE with 10% fermented molasses solution, shown 118 days after mixing, (A2) NAPL surface area after mixing with control reactor media. (B1) TCE emulsion immediately following mixing with fresh non-fermented molasses (<24 hrs), (B2) NAPL >48 hours following mixing with fresh molasses where return to solid state indicates initial emulsion was not stable. (C1) TCE emulsion 4 days after mixing with fermented field sample, Field 1, (C2) Emulsion 67 days after mixing with Field 1 where NAPL emulsion is starting to break down. Note: Glass beads are visible on the bottom of vials in (A2) and (B2).

Our results indicate biosurfactants were not produced at a high enough level to increase TCE solubility (Figure 3.2). But, as stated above, the formation of a stable emulsion from both the 10% fermented molasses and the sample from Field 1 (Figure 3.3) indicates that biosurfactants were present at levels sufficient enough to promote the formation of a NAPL emulsion within the aqueous media after substantial fermentation had occurred.

Soil Partitioning Experiments

The effect of fermented molasses on soil partitioning was evaluated by assessing the soil distribution coefficient (K_d) of TCE while in the presence of fermented fluid. Data were used to calculate K_d for each sample by dividing the mass of TCE sorbed to the soil by the equilibrium concentration of TCE in the liquid phase. An averaged value of triplicates was used for each dataset. Fermented 10% molasses (after 234 days fermentation), fresh 10% molasses, Field 1, Field 2, and media from the control reactor were used in the experiments. As shown in Figure 3.4, the fermented molasses sample appeared to result in a decreased K_d value when compared to the control. This decrease is likely attributed to the presence of biosurfactants, which have been shown to enhance desorption of hydrophobic compounds in soil applications (Christofi and Ivshina 2002). The difference in K_d values, however, was not significant at the 95% confidence level ($p = 0.12$) because of the high variability in the dataset. Accordingly, more work is needed to determine whether the K_d value is truly affected by the products generated during the fermentation of organic carbon.

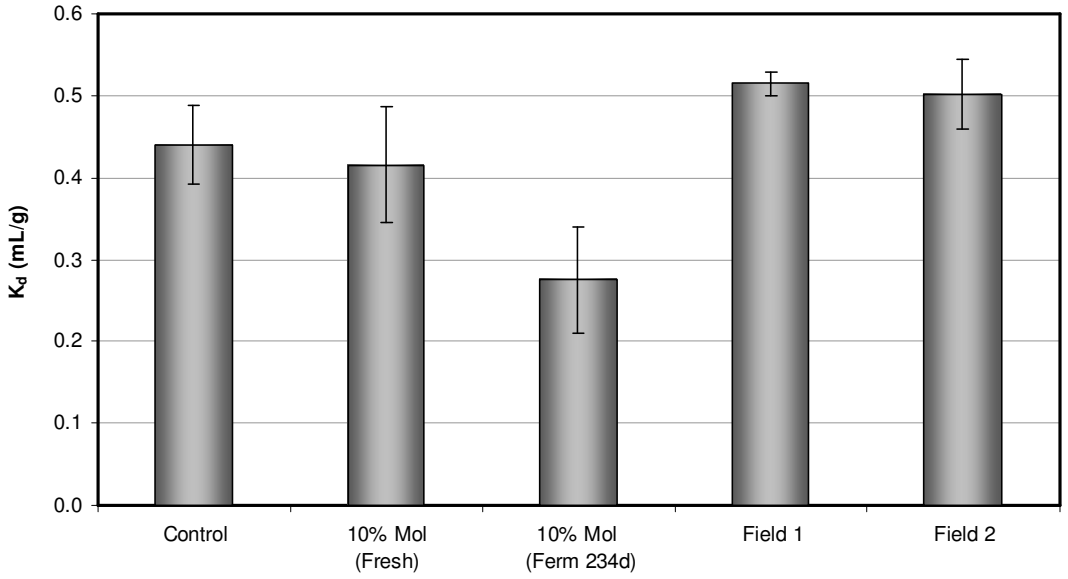


Figure 3.4 K_d values for TCE in soil partitioning experiments with vials containing media only (control), media amended with unfermented 10% molasses (Fresh), media amended with 10% molasses that was allowed to ferment (Ferm) 234 days, and vials containing field fermented samples (Field 1 and Field 2). Data points represent the average of triplicate runs, with error bars showing the standard deviation.

3.4 Conclusions

Our studies indicate that enhanced dissolution of TCE NAPL can occur through the addition and subsequent fermentation of dilute molasses solutions. The mechanism by which this occurs, however, is different depending on whether the solution is fresh or has been allowed to ferment. Enhanced mass transfer occurs through an increased concentration gradient when fresh molasses is present, as the solubility of TCE is increased greater than two-fold. This solubility increase is attributed to the crude protein component present within the molasses itself. Data indicate that cosolvency of TCE does not occur with fermented products; therefore, after the liquid has fermented, solubility

enhancement does not contribute to enhanced mass transfer. Biosurfactants appear to be generated during the fermentation process, however, as evidenced by the stable emulsion formed when the NAPL and fermented aqueous phases were mixed. The resulting increase in surface area could greatly affect the mass transfer of TCE NAPL into the aqueous phase. In addition, fermented carbon may have an effect on the soil partitioning coefficient, indicating that fermented liquid may also enhance the dissolution rate of sorbed mass into the aqueous phase.

In summary, the addition and subsequent fermentation of carbon, such as molasses, appears to affect NAPL mass, and may also affect sorbed mass. These results are important for remediation applications, specifically when bioremediation is used to polish residual source zones. In the case of a NAPL residual source, optimization of remedial strategies can be implemented to promote the mass transfer of NAPL to the aqueous phase, such as pumping to increase mixing in the subsurface thereby promoting the formation of a NAPL emulsion. This topic warrants further research.

3.6 References

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CHAPTER 4 Carbon addition strategies affect *Eubacteria* and *Archaea* community structures, function, and diversity

4.1 Introduction

Organic carbon is commonly added to groundwater to treat plumes contaminated with compounds such as chlorinated solvents or certain metals (Suthersan and Payne, 2005). This process is called biostimulation, and works by promoting the *in situ* biological destruction of contaminants using indigenous organisms that can dechlorinate or reduce the targeted contaminants. Significant research has been conducted on optimizing the growth of these contaminant-reducing species (Fennel et al., 1998; Smidt and de Vos, 2004; Finneran et al. 2002). The function of the entire microbial community that develops in response to carbon inputs, however, has been generally ignored.

Carbon introduction for *in situ* bioremediation technology commonly includes continuously-fed and pulsed-fed systems (Moretti, 2005). The effect that pulsed versus continuous carbon inputs have on microbial communities have been studied in terms of biomass distribution (Peyton, 1996) and bacterial community dynamics (Konopka et al, 2007; Carrero-Colón et al, 2006a; Carrero-Colón et al, 2006b). Substantial differences in bacterial community structure have been shown in continuous versus pulsed systems that received the same overall carbon loading (Carrero-Colón et al, 2006a), and varied frequencies of nutrient addition (Carrero-Colón et al, 2006b). Evidence has also shown that a shift in bacterial community structure can occur from an increase in carbon loading (Temudo et al., 2008) suggesting that bacterial communities are also impacted by continuous inputs of different concentrations of the same type of carbon. In addition, pulsed substrate has been shown to result in less biofouling over time than continuously-fed systems (Khan and Spalding, 2003; Hoelen et al., 2006).

These studies provide evidence that high versus low carbon concentrations can affect bacterial community structure, which will influence the formation of fermentation products. This could have broad impacts that extend beyond *Eubacteria* communities as hydrogen and acetate, two common fermentation products, are used by methanogenic organisms to generate methane. Indeed, bioremediation case study data have documented that high levels of methane can be produced after carbon has been injected into an aquifer (Suthersan and Payne, 2005), indicating that the addition and subsequent fermentation of carbon can greatly affect *Archaea*, the domain to which methanogenic organisms belong.

To date, research on pulsed versus continuously-fed systems has focused on the shifts that occur in bacterial communities. The changes in bacterial community structure stemming from different carbon input methods should also directly translate to structural changes in the archaeal community as they depend on the generation of fermentation products for growth. The design of different carbon input strategies to either enhance or suppress fermentation product and/or methane generation could be a valuable tool for bioremediation systems. This has not been investigated.

The objective of this study was to determine the effect of two different carbon application strategies (low-dose, continuously-fed versus high-dose, pulsed-fed) on fermentative community structure, diversity, density, and the subsequent generation of fermentation products and methane. We hypothesize that the amount and concentration of the fermentation products depend on both the bacterial community structure and density, which are influenced by the method of carbon application. The resulting shifts in the bacterial community will directly affect the structure of the archaeal community and

subsequent production of methane. Once these interactions are understood, systems can potentially be manipulated to enhance or manage the generation of specific fermentation products, control biofouling, and mitigate methane generation.

4.2 Materials and Methods

Experimental Setup

Soil Columns

Three soil columns were continuously operated over a 145-day period to investigate the effect of different carbon input strategies on the indigenous microbial communities and the generation of fermentation products, including methane. All columns were packed with an aquifer material mixture. One column was supplied with a continuous dilute molasses feed (0.44% by volume in a minimal groundwater media), one with a periodic pulsed input of high strength molasses (10% by volume in minimal groundwater media) and the third served as a control (continuous feed of minimal groundwater media only).

Aquifer Material. Aquifer material was obtained from an unimpacted area of site located in Southwestern Minnesota. Soil cuttings included both vadose and saturated zone aquifer material which were homogenized and sieved through a 2-mm mesh to remove large particles prior to use. The native aquifer material contained a high amount of silt and clay; therefore, sand (TCC Materials, Mendota Heights, MN) was added at 1:1 ratio (sand to sieved cuttings) to increase the permeability of the aquifer material. The

final mixture was comprised of 90% sand, 1.8% silt, 8.3% clay, and had an average organic matter fraction of 0.6% (by mass).

Construction. Columns were constructed using 1.5-m lon, 0.1-m inner diameter polyvinyl chloride (PVC) plastic pipes. The column ends were sealed with standard 0.1-m PVC end caps fitted with influent and effluent connections. Six threaded sample ports fitted with Mininert valves (Valco, 3 mm NPT female) were tapped along each column length. Stainless-steel screen (150 mesh) tubes were placed into each sample port to prevent aquifer material from clogging syringe needles during sampling. Columns were operated horizontally to mimic groundwater flow in an aquifer setting. In addition to sample ports, several vertical 6 mm diameter glass tubes were connected to the top of the column via compression fittings (Cole-Parmer Instrument Co., Vernon Hills, IL) to allow for potential off-gassing during column operation. Tubing ends were connected to a headspace trap using flexible PVC tubing (Fisher Scientific, Pittsburg, PA) to inhibit the diffusion of oxygen into the columns during the experiment. A schematic of the columns used in this experiment is shown in Figure 4.1.

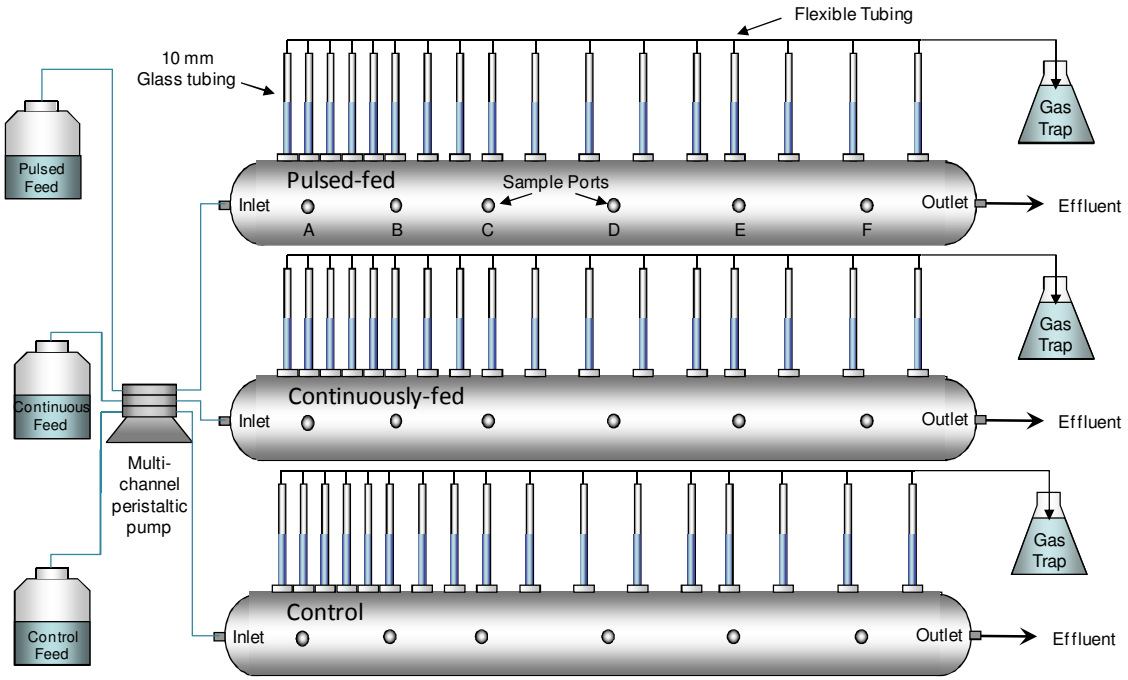


Figure 4.1 Schematic of the column system setup.

Operation and Maintenance. The minimal groundwater media used in column operation was adapted from Wilbur et al. (1995) and contained trace nutrients and mineral components consistent with natural groundwater. The media contained the following (per L of distilled water): 20 mg $NaNO_3$, 20 mg $MgSO_4 \cdot 7H_2O$, 0.7 mg $FeCl_2 \cdot 4H_2O$, 95 mg $K_2HPO_4 \cdot 3H_2O$, 40 mg K_2CO_3, 16.3 mg $MgCl_2 \cdot 6H_2O$, 11.8 mg $CaCl_2 \cdot 2H_2O$, 0.05 mg $CoCl_2 \cdot 6H_2O$, 0.05 mg KI, 0.06 mg $MnCl_2 \cdot 4H_2O$, 0.007 $ZnCl_2$, 0.01 mg $NaMoO_4 \cdot 2H_2O$, 0.007 mg H_3BO_3, 0.01 mg $NiCl_2 \cdot 6H_2O$, 0.008 mg $CuCl_2 \cdot 2H_2O$, 40 mg K_2CO_3, 0.4 mg NH_4HCO_3 and 12 mg $NaHCO_3$. Wholesome Sweeteners™ dark (blackstrap) molasses (unsulphured) was used as the carbon source for the continuously

and pulsed-fed columns. The feed solution was pumped into each column using a Masterflex L/S 07523-70 digital drive pump equipped with a Masterflex 07519-20 cartridge pump head (Cole Parmer, Vernon Hills, IL). Influent flow to the columns was 0.06 mL/min. The pH of the influent was adjusted to 8 throughout the experiment using 10% HCl. The feed to the columns was changed every 3 to 4 days to minimize the degradation of molasses and/or media components. The pulsed addition of molasses occurred approximately every 30 days and consisted of a 3-day step input of 10% molasses (by volume, in minimal groundwater media) followed by 27 days of minimal groundwater media addition. The continuously-fed column consisted of a continuous input of 0.4% molasses, by volume in minimal groundwater media. Column influent lines were disinfected twice weekly using a 10% (by volume) bleach solution to remove biomass growth within the tubing and to prevent clogging of the feed lines. Periodic flushing of the column inlets was also performed, with the frequency based on individual column performance.

Sampling. Aqueous samples were collected weekly from each sampling port for pH, COD, volatile fatty acid, cosolvent and methane analysis. Samples were withdrawn using syringes equipped with 5 cm long 22-gauge needles. All samples were filtered through a 0.2-µm polyethersulfone syringe filter (Nalgene, Rochester, NY) prior to analysis. A total of 8 mL was collected from each port during each sampling event.

Experimental Procedures

Soil collection and DNA extraction

Soil columns were sacrificed after the final liquid sampling event 145 days following introduction of molasses. The columns were disconnected from the feed solutions and drained of excess liquid. The columns were cut at the mid-point between each sampling port using a reciprocating saw. The saw blade was disinfected between cuts by immersion in ethanol followed by drying with autoclaved paper towels to remove ethanol residue. Soil from each end of the cut column section was removed with a metal spatula until approximately 2.5 cm of soil remained in the cross-section on each side of the sampling port location. This remaining soil, assumed to represent the microbial community at each sampling port, was homogenized and immediately frozen at -20^{o}C until further analysis. Additionally, soil nearest to the inlet of each column was sampled and frozen following the same protocol. Genomic DNA was extracted using a PowerSoil DNA Kit (MOBIO Laboratories, Inc., Carlsbad, CA). Triplicate extractions were performed on material collected at each sampling port location.

Automated Ribosomal Intergenic Spacer Analysis (ARISA)

Eubacteria ribosomal intergenic spacer (ITS) regions were amplified using primers ITSF (5'-GTC GTA ACA AGG TAG CCG TA-3') and ITSReub (5'-GCC AAG GCA TCC ACC-3') (Cardinale et al., 2004). *Archaea* ribosomal ITS regions were amplified using primers 1389F (5'-ACG GGC GGT GTG TCG AAG -3') and 71R (5'-TCG GYG CCG

AGC CGA GCC ATC C -3') (Qu et al., 2009). Fungal ribosomal ITS regions were amplified using primers 2234C (5'-GTT TCC GTA GGT GAA CCT GC-3') and 3126T (5'-ATA TGC TTA AGT TCA GCG GGT-3') (Ranjard et al., 2001). Each of the forward were labeled with the phosphoramidite dye 6-carboxy-1,4-dichloro-2',4',5',7'-tetra-chlorofluorescein (HEX).

Conventional polymerase chain reaction (PCR) was performed using a DNA Engine Thermal Cycler (Biorad, Hercules, CA).The PCR final 50 μL reaction mixture for *Eubacteria*, *Archaea* and fungal ARISA contained: 1 X PCR buffer (Promega, Madison, WI), 4 nmol deoxynucleoside triphophates, 25 pmol forward and reverse primers, 1.25 units of *taq* polymerase (Promega) and ~1 ng of template DNA. The PCR protocol for *Eubacteria* ARISA included a 3 min initial denaturation at 94^{o}C followed by 35 cycles of 94^{o}C for 45 s, 55^{o}C for 1 min, and 72^{o}C for 2 min and a final extension for 7 min at 72^{o}C. *Archaea* ARISA PCR protocol included a 5 min initial denaturation at 95^{o}C followed by 35 cycles of 94^{o}C for 1 min, 55^{o}C for 1 min, and 72^{o}C for 2 min and a final extension for 7 min at 72^{o}C.. Fungal ARISA PCR amplification included a 3 min initial denaturation at 94^{o}C followed by 35 cycles of 94^{o}C for 1 min, 55^{o}C for 30 s, and 72^{o}C for 1 min and a final extension for 5 min at 72^{o}C.

ARISA was performed on each of the triplicate samples collected at the inlet and sampling port locations. Amplified products were resolved by capillary electrophoresis using an ABI 3130xl capillary instrument (Applied Biosystems Inc, Foster City, CA) using Map Marker 1000 size standard. Fragment peak areas were analyzed using Gene Profiler software. Resulting data were analyzed using the following protocol: (1)

fragment lengths <122 base pair were removed to eliminate primer dimer fragments, (2) peaks <0.5% of the total peak area were removed to filter out signal noise, (3) manual alignment of the remaining fragment lengths between triplicate samples, (4) manual alignment of fragment lengths between sampling port locations of the same column, and (5) manual alignment of fragment lengths between columns. Each manual alignment considered fragments +/- 1 base pair to be the same.

Quantitative PCR

Methanogen 16S rRNA and *Eubacteria* 16S rRNA communities were quantified in each sample by quantitative real-time PCR (qPCR) using a Realplex^2 Mastercycler (Eppendorf) thermocycler equipped with Eppendorf Mastercycler ep Realplex software. Methanogen qPCR primers Met630F (5'-GGA TTA FAT ACC CSG GTA GT-3') and Met803R (5'-GGT GAR TCC AAT TAA ACC GCA-3') (Hook et al., 2009) were used to enumerate the methanogens, and primers 338F (5'-CCT ACG GGA GGC AGC AG -3') and 518R (5'- ATT ACC GCG GCT GCT GG -3') (Muyzer et al., 1993) were used to enumerate the *Eubacteria* community. Each 25 µL qPCR reaction mixture contained 12.5 µL of 2x Power SYBR Green Master Mix (Applied Biosystems, Foster City, CA), 25 µg of bovine serum albumin, optimized quantities of forward and reverse primers, and 1 ng of template. Methanogens were quantified using a protocol of 95°C for 2 min, followed by 50 cycles of 95°C for 30 s, 60°C for 30 s and 72°C for 1 min. Total *Eubacteria* were quantified using a protocol of 95°C for 10 min, followed by 40 cycles of 95°C for 15 s, 60°C for 1 min and 95°C for 15 s. The quantity of target DNA in samples was calculated

using standard curves generated with known quantities of template DNA. qPCR was performed for each of the triplicate DNA extractions from each sampling port location. Standards for *Eubacteria* qPCR were prepared by PCR amplification of 16S rRNA genes from *E. coli* K12, ligation into pGEM-T Easy vectors (Promega, Madison, WI), and transformation into *E. coli* DH5α. Primers 27F (5'-AGA GTT TGA TCC TGG CTC AG-3') (Edwards et al., 1989) and 907R (5'- CCG TCA ATT CCT TTG AGT TT-3') (Muyzer et al., 1995) were used to amplify *Eubacteria* 16S rRNA genes. Plasmids were purified using alkaline lysis (Sambrook et al., 1989). Methanogen standards were prepared by cloning PCR-amplified 16S rRNA genes of methanogens from this column study into pGEM-T Easy plasmid vectors and transformation into *E. coli* DH5α. Primers 8aF (5'-TCY GGT TGA TCC TGC C-3') (Qu et al., 2009) and Met803R were used to amplify *Archaea* 16S rRNA genes. Plasmid DNA for both *Eubacteria* and methanogen 16S rRNA standards was quantified using Hoeschst 33258 dye and measured on a TD-700 fluorometer (Turner Designs, Sunnyvale, CA) using calf thymus as a DNA standard. Ten-fold serial dilutions of plasmid standard were prepared and run on the thermal cycler to generate standard curves. Detection limits were 3×10^4 gene copies and 6×10^2 gene copies per gram of soil (wet weight) for the *Bacteria* and methanogens, respectively.

Verification of the methanogen standard was performed by PCR amplification of the plasmid insert using M13 forward (5´-GTT TTC CCA GTC ACG AC-3´) and reverse (5´-CAG GAA ACA GCT ATG AC-3´) primers (Promega, Madison, WI), purification using the GeneClean II Kit (MP Biomedicals), and sequenced at the University of Minnesota BioMedical Genomics Center an ABI PRISM 3730xl DNA Analyzer. The

PCR product was sequenced in both directions using M13 primers. The resulting sequences were aligned using the software CodonCode Aligner (Codon Corp, Dedham, MA). The aligned sequence was most closely related to *Methanosarcina* sp. (99% identity, Accession# AY780570.1) based on NCBI's BLAST Database.

Analytical Methods

Ketones (acetone, methyl ethyl ketone, methyl isobutyl ketone), alcohols (methanol, ethanol, butanol) and methane were analyzed using on-column injection with a 7673 automatic liquid sampler fit with a nanoliter adaptor and connected to a Hewlett Packard (HP) 5820 Series II gas chromatograph (Agilent Technologies, Foster City, CA) equipped with a flame ionization detector. A sample volume of 0.2 µL was directly injected onto a 5-m 0.53-mm diameter deactivated fused silica pre-column connected to a 30-m 0.32-mm Rtx-1 (Restek Corp, Bellefonte, PA) capillary column. Peak resolution was performed using an oven temperature of 60°C for 9 min followed by an oven ramp of 10°C/min to a final temperature of 150°C with a 5 min hold. Helium (99.99%) was used as the carrier gas at a flowrate of 17.2 cm/s. The inlet temperature was set to track 3°C above the oven temperature at all times. The detector was maintained at 300°C. Absolute 200 proof ethanol, HPLC grade methanol, and high purity ACS-grade butanol, acetone, methyl ethyl ketone, methyl isobutyl ketone were used to prepare standards. Detection limits were: 11 mg/L for methanol, 2 mg/L for ethanol. 2 mg/L for acetone, 1 mg/L for methyl ethyl ketone, 1 mg/L for butanol, 1 mg/L for methyl isobutyl ketone, and 0.5 mg/L for methane.

Volatile fatty acid analysis was performed using a high performance liquid chromatograph (Agilent Technologies 1200 Series, Foster City, CA) equipped with a diode-array detector (210 nm wavelength) and a 300-mm long Aminex HPX-87H column (Biorad, Hercules, CA). A sample volume of 25 µL was injected with an isocratic mobile phase of 0.005 M H_2SO_4 at a flow rate of 0.6 mL/min. Concentrated acids (85% or higher) were used to prepare standards. Standard curves were adjusted to account for purity. Detection limits were 18 mg/L for lactate, 6 mg/L for formate, 6 mg/L for acetate, 8 mg/L for propionate, 12 mg/L for valerate, and 16 mg/L for butyrate.

Chemical oxygen demand (COD) was analyzed using an Accu-TEST (range 20 to 900 mg/L) Mercury-free Micro-COD system (Bioscience Inc., Bethlehem, PA) following the manufacturer's protocol. COD standards were prepared using a potassium hydrogen phthalate standard (ACROS). A Beckman 32 digital pH meter equipped with a combination electrode was used for pH measurement.

Statistical Analysis

Ordination Analysis

Ordination statistical analyses were performed using R (R Development Core Team, 2009). The package *vegan* was used to analyze ARISA community structure using non-metric multidimensional scaling (NMDS). The function *metaMDS* was used to perform the NMDS analysis with the dissimilarity matrix calculated for both species and sample locations using Bray-Curtis distance measure (function *vegdist*) (Oksanen, 2009). NMDS is recognized as the most generally effective ordination method for ecological community

data (McGune and Grace, 2002). Iterative calculations are performed to find the best solution (minimum stress) for the data set. Stress between 10 and 20 are common for ecological community data, and values in the lower half of this range are considered satisfactory (McGune and Grace, 2002). The species ordination was overlain by environmental factors (COD, volatile fatty acids, ketones, and *Eubacteria* /methanogen gene copies) using the function *envfit* to determine the relationship between these parameters and the ordination of species (Oksanen, 2009; Turich et al., 2007).

Diversity Analysis

Diversity calculations were performed using the Shannon-Wiener index (Eqn. 1).

(1) $$H = -\sum_{i=1}^{S} p_i \ln p_i$$

Where H is the calculated value of the Shannon-Wiener index, p_i is the proportion of peak area of the ith ITS fragment, and S is the total number of ITS fragments within the sample. Evenness (measure of relative abundance of species within a sample, E_H) was calculated using Eqn. 2.

(2) $$E_H = \frac{H}{\ln S}$$

The Student's t-test was used to evaluate statistically significant changes in diversity indices and evenness values between the control and carbon-treated columns.

4.3 Results

Fermentation Products

Figure 4.2 summarizes the COD concentrations (representing total carbon) detected in Port A over time in each column. As shown in the continuously-fed column, an initial increase in COD concentration was followed by a steady decline until a low quasi-steady state COD concentration of 751 ± 147 mg/L was reached around Day 90. In contrast, pulsed inputs of 10% molasses resulted in spikes of COD that dropped quickly as the carbon was consumed and/or diluted through the column matrix.

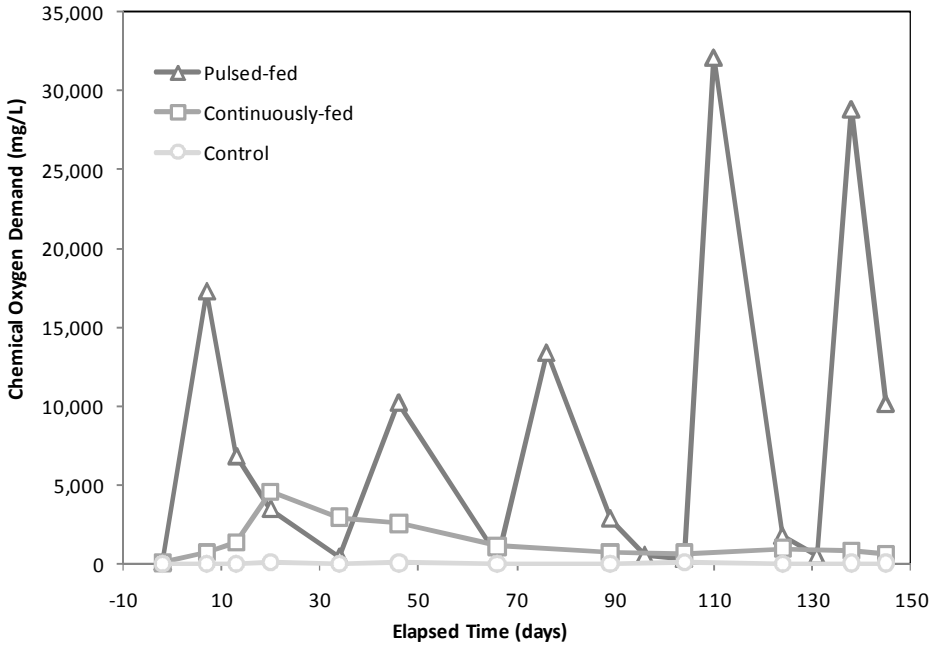


Figure 4.2 Chemical oxygen demand concentrations versus time in Port A of the three columns. Carbon introduction began at Time = 0.

Each pulse of molasses resulted in the generation of several fermentation products (ethanol, butanol, and butyrate, acetate and propionate) along the column length (Figure 4.3[a], [b] and [c]). At the beginning of the study, acetate was the dominant product in all sampling ports of the pulsed-fed column (ranging up to 4,000 mg/L), followed by a shift to butyrate which was produced in excess of 7,000 mg/L in Port A after Day 70. Propionate was also detected in greater quantities towards the end of the study (Day 140) in this column. Butanol and ethanol were also produced as a result of pulsed carbon input, but at much lower levels (<150 mg/L) and only at the beginning of the column. Conversely, butanol and ethanol were never detected in the continuously-fed column, and acetate, butyrate, and propionate were only present through Day 125 at levels < 1,200 mg/L (Figure 4.3[d], [e] and [f]). No fermentation products were detected within the control column. Overall, the microbial community in the continuously-fed column appeared to adapt to the carbon input over a period of 90 to 125 days, meeting the carbon supply with a concomitant demand and consuming most of the fermentation products that were produced. The community in the pulsed-fed column never adapted to the changing input of carbon over the 145-day experiment, resulting in the variable consumption of carbon and production of fermentation products that shifted in predominance with time.

Acetate and hydrogen can be produced during fermentation processes and are the dominant species through which methane is generated via acetoclastic or hydrogenotrophic methanogenesis (Conrad, 1999). Accordingly, methane production was measured throughout this experiment. Figure 4.4 shows the dissolved methane concentrations in Port C of the pulsed-fed and continuously-fed columns throughout the

column study. As shown, the methane concentrations produced in the two columns was similar, suggesting similar methanogen populations were present in both columns. No methane was detected in the control column.

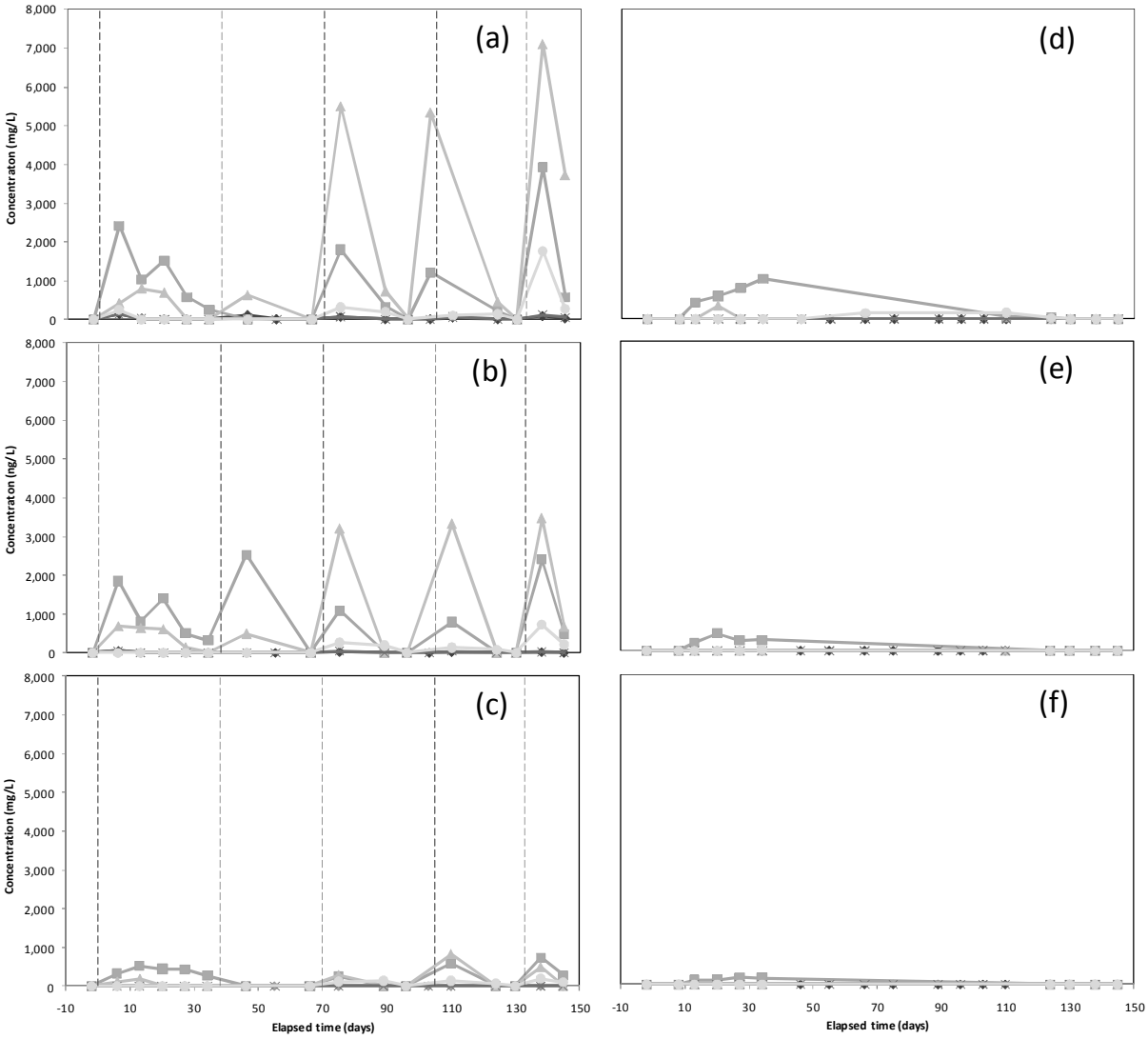


Figure 4.3 Detected fermentation products versus time within the: pulsed-fed molasses column (a) Port A, (b) Port C, (c) Port F, and continuously-fed column (d) Port A, (e), Port C, and (c) Port F. Vertical dashed lines in the pulsed-fed graphs denote the times of pulsed addition of carbon. Symbols are: ethanol (◆), acetate (■), butyrate (▲), butanol (✕), and propionate (●). Time = 0 represents the start of molasses introduction.

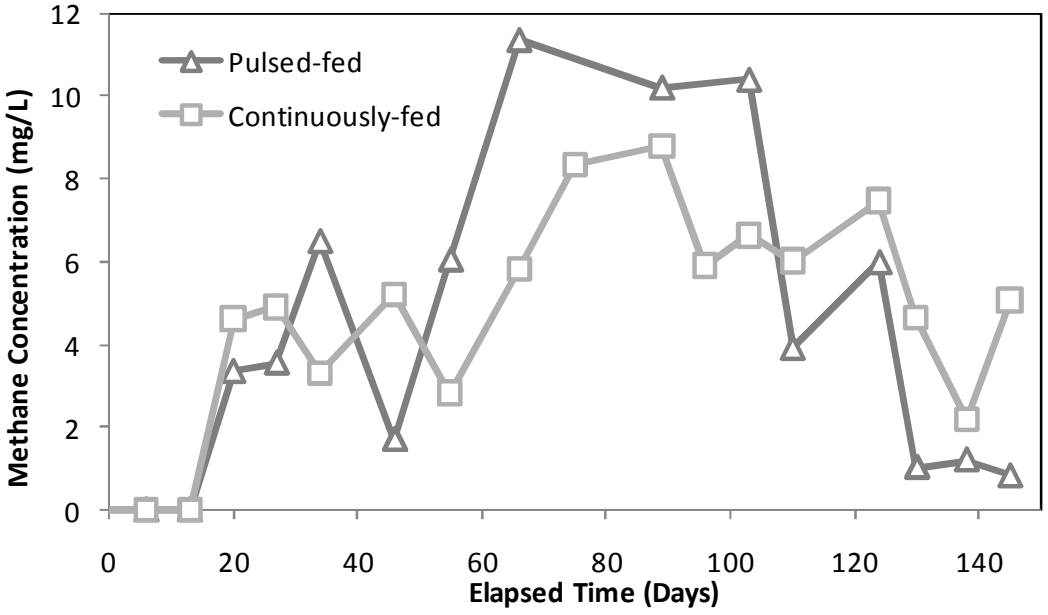


Figure 4.4 Dissolved methane concentrations versus time in Port C of the continuously-fed and pulsed-fed columns. The dashed line represents the calculated dissolved methane (2 mg/L) above which partitioning can occur above methane's lower explosive limit (LEL). Carbon introduction began at Time = 0.

Microbial Communities

qPCR

Figure 4.5 summarizes the results for both *Eubacteria* and methanogen qPCR analysis. The error bars are large, but in general, the pulsed-fed column showed equivalent numbers of bacteria and larger numbers of methanogens than the continuously-fed column. The exception was Port A, located closest to the inlet, where elevated numbers of methanogens were also observed in the continuously-fed column. The number of methanogens along the length of the pulsed-fed column is consistently

high, and much higher than the number of methanogens detected in the continuously-fed column, yet the methane concentrations observed in both columns were similar. This indicates that the number of methanogens in these systems do not necessarily correlate to methane production (r^2=0.2185), and instead perhaps correlate better to available carbon (COD), which drives the reductive poise of an anaerobic system (r^2=0.7779).

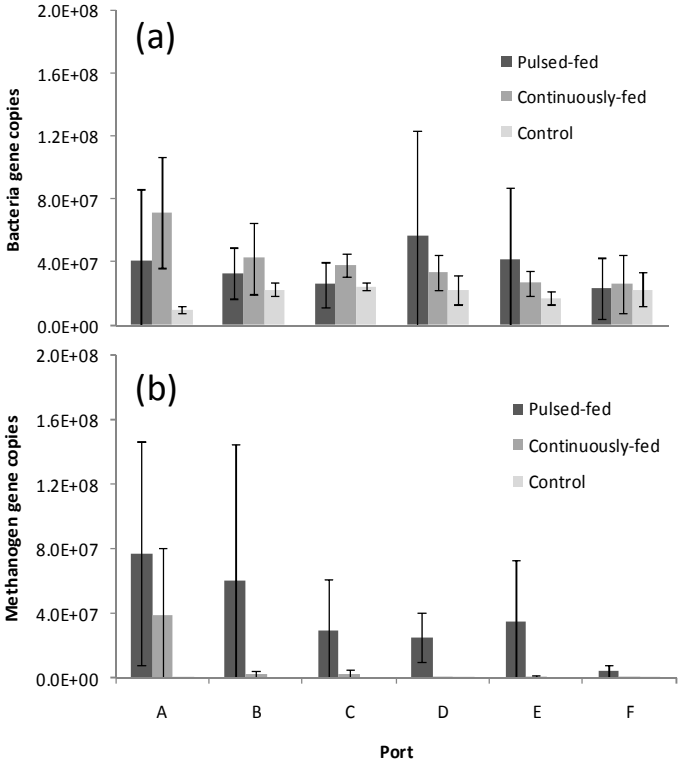
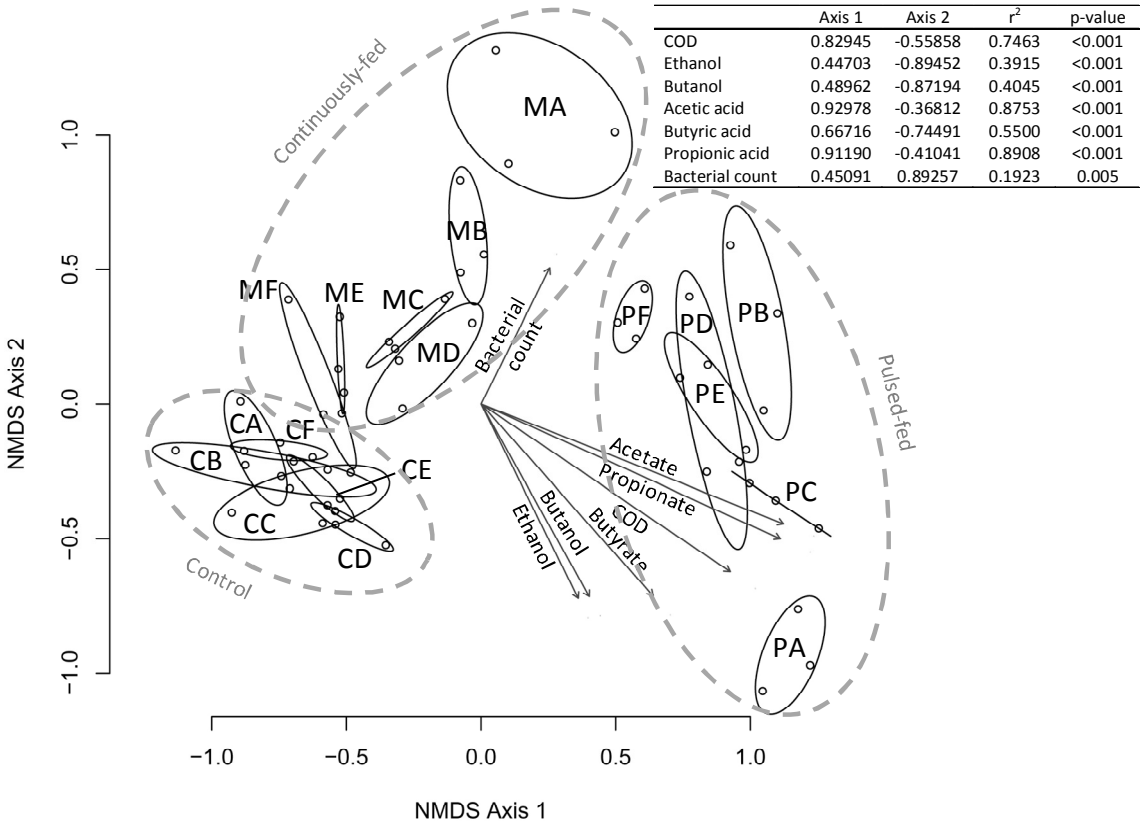


Figure 4.5 Summary of average (a) *Bacteria*, and (b) methanogen gene copies resulting from qPCR analysis. Data represent the average of results from triplicate DNA extractions for each sample. Error bars show the standard deviation of each data set. All values represent gene copies per gram of soil, dry weight.

ARISA

Both *Eubacteria* and *Archaea* community structure were evaluated using ARISA (García-Martínez et al., 1999), which can be used to examine microbial community

structure and diversity (Fisher and Triplett, 1999). Analysis of the ARISA results was performed using NMDS, allowing mapping of the data onto a two dimensional space to visualize similarity (spatial proximity).



	Axis 1	Axis 2	r^2	p-value
COD	0.82945	-0.55858	0.7463	<0.001
Ethanol	0.44703	-0.89452	0.3915	<0.001
Butanol	0.48962	-0.87194	0.4045	<0.001
Acetic acid	0.92978	-0.36812	0.8753	<0.001
Butyric acid	0.66716	-0.74491	0.5500	<0.001
Propionic acid	0.91190	-0.41041	0.8908	<0.001
Bacterial count	0.45091	0.89257	0.1923	0.005

Figure 4.6 Results of non-metric multidimensional scaling analysis of *Eubacteria* communities in column sample ports. Sample port labels are depicted by: column ID (M for continuously-fed molasses, C for control, P for pulsed-fed molasses), and port location (A-F). Vectors show direction of most rapid change in microbial community structure relative to environmental variables. Vector length corresponds to correlation with the ordination axes. Ellipses represent 95% confidence interval of triplicate samples (Bray-Curtis distance measure, Stress 9.78). Dashed outlines group together the sample ports belonging to each column.

Eubacteria Community Structure. Results of the NMDS analysis for *Eubacteria* are shown in Figure 4.6. The ARISA data from the column inlets were not included in the

NMDS analysis as they were highly disturbed because of the disinfection activities necessary to control biomass growth during the experiment. The results from the inlet of the control column (data not shown) indicated that the microbial community was very different in the inlet sample when compared to the rest of the column where the community appeared to be fairly homogenous (Figure 4.6). Accordingly, no inlet samples were used in the NMDS analysis.

Eubacteria ARISA results from the control column (CA through CF) cluster together indicating that *Eubacteria* communities are similar throughout the control column. The *Eubacteria* community from Port A of the pulsed-fed column (PA) showed the greatest dissimilarity with respect to all other column sampling ports, indicating a significantly different community structure is present at this location compared to all other column port locations. Port A of the pulsed-fed column received a larger carbon load than any other port which agrees well with the ordination analysis of *Eubacteria* ARISA results. Furthermore, the ARISA results from the pulsed-fed column show the greatest dissimilarity with respect to the control column, as they are located the furthest distance from the clustered control column results.

Interestingly, Port F of the pulsed-fed column is located closest and therefore is most similar, to Port A of the continuously-fed column, and Port F of the continuously-fed column is most similar to Port A of the control column. These data indicate that the communities shift along a carbon gradient. To further investigate this, Figure 4.7 compares the COD concentrations in Port A of the continuously-fed column to Port F of the pulsed-fed column, and Port A of the control column to Port F of the continuously-fed

column. As shown, the COD values shown in Figure 4.7(a) were of similar magnitude for the first 70 days and there was in excess of 500 mg/L of COD present at all times in Port F of the pulsed-fed columns and Port A of the continuously-fed column, indicating a similar "background" COD level for microbial growth at these two locations in the two different columns. The total mass of COD that was present at these ports was calculated by integrating under the COD versus time curve for each sampling location (Table 4.1). Port A of the continuously-fed column had a total of 35.2 grams of COD present over the column experiment, and Port F of the pulsed-fed column had 40.0 grams of COD. Similarly, the COD concentrations at Port F of the continuously-fed column were <400 mg/L for the entire study (Figure 4.7[b]). Port F of the continuously-fed column contained 4.1 grams of COD over the column experiment compared to 0.8 grams of COD in Port A of the control column. The comparatively low levels of carbon observed at Port F of the continuously-fed column appeared to have a minimal effect on community structure, leading to the close association of *Eubacteria* communities in this port to the communities in the control column.

Table 4.1 Total mass (grams) of COD detected in column sampling ports during column experiment.

Column ID	Port A	Port B	Port C	Port D	Port E	Port F
Control	0.8	0.8	1.0	0.9	0.8	0.7
Continuous	35.2	17.4	14.6	10.1	9.0	4.1
Pulsed	253.3	164.4	166.1	105.2	60.4	40.0

Vectors representing the concentrations of COD and fermentation products on Day 145 were superimposed on the NMDS ordination analysis to evaluate the significance of these compounds with respect to community structure. The vector direction shows the most rapid change in community patterns (gradient), and the lengths are proportional to the correlation between the variable and the ordination (Oksanen 2009). As shown in Figure 4.6, the presence of COD and fermentation products are most strongly correlated with the communities present in the pulsed-fed column. Goodness of fit statistics and p-values for permutation tests (n=1,000) indicate propionate (r^2=0.8892, $p<0.001$), acetate (r^2=0.8737, $p<0.001$), and COD (r^2=0.7463, $p<0.001$) most strongly correlate to NMDS Axis 1, where the pulsed-fed column communities are located. In addition to the chemistry data, the number of *Eubacteria* gene copies was also overlain on the ordination axis. These values did not show a strong correlation with the ARISA data (r^2=0.1912, $p<0.005$).

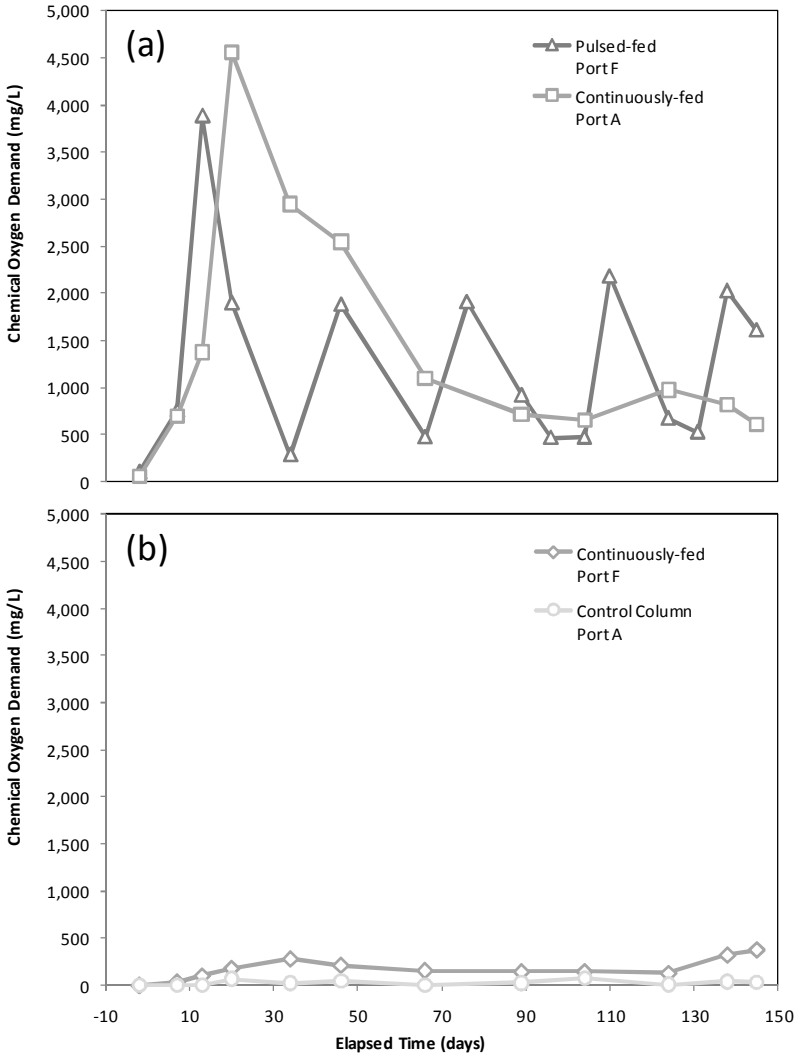


Figure 4.7 Comparison of chemical oxygen demand concentrations in (a) Port A of the continuously-fed and Port F of the pulsed-fed columns, and (b) Port F of the continuously-fed and Port A of the control columns. Carbon introduction began at Time = 0.

Archaea Community Structure. The structure of the *Archaea* communities from the column study are presented in Figure 4.8. Similar to the *Eubacteria* results, the control column communities clustered together, showing similar community structure throughout the control column length. Also similar to the *Eubacteria* results, the community from the

last port (Port F) of the continuously-fed column was most similar to the control results, and ARISA data from Port A of the continuously-fed column were most similar to the pulsed-fed column data. Two noticeable differences between the *Eubacteria* and *Archaea* results is that several locations of the pulsed-fed column clustered together (Ports B, D, E and F), and the spacing between the continuously-fed *Archaea* communities was farther apart than the continuously-fed *Eubacteria* communities. The larger spacing indicates that a greater overall shift in communities occurred in *Archaea* populations along the length of the continuously-fed column. Taken with the pulsed-fed community data, the community appears to significantly shift as a result of carbon input (and subsequent fermentation), reaching a point where the presence of carbon results in less significant changes. These data indicate that the *Archaea* community may reach a point of steady-state more rapidly than the *Eubacteria* communities.

As with the *Eubacteria* ARISA analysis, the COD concentrations on Day 145 correlated most strongly with the *Archaea* pulsed-fed column ARISA data (r^2=0.6372, $p<0.001$), followed by the methanogen gene copy numbers (r^2=0.3528, $p<0.001$). Methane concentrations on Day 145, however, were more strongly correlated to the continuously-fed column (r^2=0.2068, $p=0.003$) further supporting that methane concentrations and methanogen numbers were not correlated in this column study.

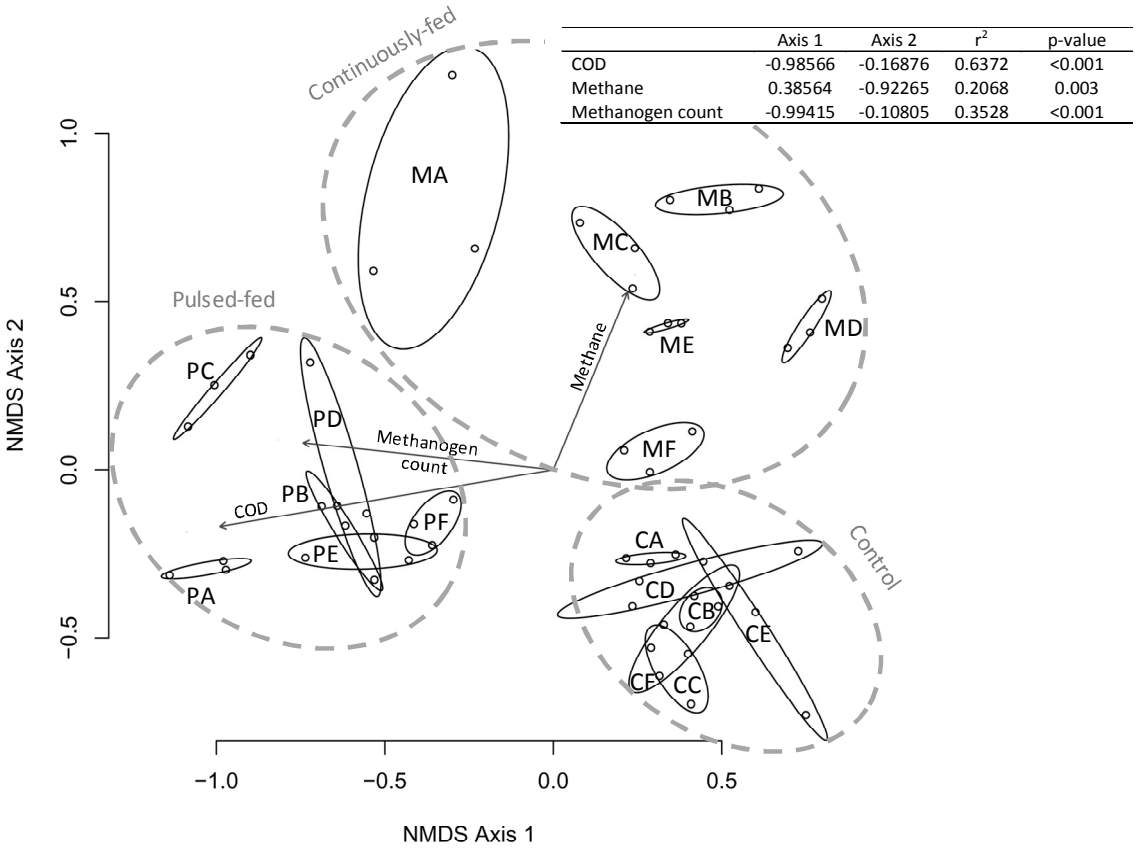


Figure 4.8 Results of non-metric multidimensional scaling analysis of *Archaea* communities in column sampling ports. Sample port labels are depicted by: column ID (M for continuously-fed molasses, C for control, P for pulsed-fed molasses), and port location (A-F). Vectors show direction of most rapid change in microbial community structure relative to environmental variables. Vector length corresponds to correlation with the ordination axes. Ellipses represent 95% confidence interval of triplicate samples (Bray-Curtis distance measure, Stress 15.94). Dashed outlines group together the sample ports belonging to each column.

Diversity Indices. The Shannon-Wiener index (H) is a widely used measure of ecological diversity that relates the total number of species (richness) to relative species abundance (evenness) within the community (Atlas and Bartha, 1997). Application to microbial communities is a recent adaptation of this index and has been shown to be

sufficient for comparing relative diversity between microbial community samples (Shaw et al., 2008). H and evenness values (E_H) were calculated for both *Eubacteria* and *Archaea* using data obtained from ARISA analysis (Tables 4.2 and 4.3). As shown, *Eubacteria* diversity in the continuously-fed column communities did not exhibit statistically significant changes when compared to the control column results. The diversity of *Eubacteria* communities in Ports A and D of the pulsed-fed column, however, did exhibit statistically significant lower values ($p<0.05$) indicating a loss of diversity at these locations. In addition, these same port communities (pulsed-fed column Ports A and D) and two continuously-fed ports (B and E) demonstrated a statistically significant decrease of evenness values ($p\leq0.05$).

Table 4.2 Results of Shannon's Diversity Index calculations within column sampling ports using ARISA data. Data reported as averaged value of triplicate sample calculations.

	Column ID	Port A	Port B	Port C	Port D	Port E	Port F
Eubacteria Community	Control	3.33 (0.08)[a]	3.31 (0.32)	3.48 (0.28)	3.66 (0.08)	3.47 (0.16)	3.42 (0.18)
	Continuous	2.83 (0.85) p=0.44	3.35 (0.18) p=0.90	3.62 (0.11) p=0.33	3.59 (0.20) p=0.68	3.61 (0.09) p=0.29	3.41 (0.16) p=0.97
	Pulsed	2.51 (0.29)** p=0.02	3.03 (0.62) p=0.57	2.96 (0.31) p=0.13	3.06 (0.11)** p=0.03	3.30 (0.28) p=0.55	3.68 (0.17) p=0.31
Archaea Community	Control	2.87 (0.18)	2.47 (0.07)	2.34 (0.06)	2.75 (0.28)	2.30 (1.16)	2.66 (0.15)
	Continuous	2.44 (0.24)** p=0.01	2.86 (0.30) p=0.97	2.78 (0.15) p=0.10	2.59 (0.13) p=0.07	2.86 (0.17) p=0.75	2.85 (0.29) p=0.92
	Pulsed	2.51 (0.08) p=0.10	2.51 (0.03) p=0.08	2.40 (0.11)** p=0.01	2.42 (0.15) p=0.11	2.53 (0.31) p=0.27	2.57 (0.17) p=0.07

a Standard deviation in parentheses

** Change in diversity considered statistically significant at $p \leq 0.05$ when compared to the Control column using the Student's t-test

Table 4.3 Results of Evenness calculations within column sampling ports using ARISA data.
Data reported as average value of triplicate samples.

	Column ID	Port A	Port B	Port C	Port D	Port E	Port F
Eubacteria Community	Control	0.95 (0.02)[a]	0.96 (0.02)	0.93 (0.02)	0.94 (0.02)	0.94 (0.01)	0.94 (0.02)
	Continuous	0.74 (0.18) p=0.17	0.86 (0.03)** p=0.02	0.93 (0.01) p=0.83	0.89 (0.05) p=0.35	0.93 (0.01)** p=0.03	0.95 (0.02) p=0.61
	Pulsed	0.75 (0.04)** p=0.03	0.81 (0.14) p=0.18	0.79 (0.05) p=0.07	0.80 (0.03)** p=0.02	0.85 (0.05) p=0.13	0.92 (0.03) p=0.11
Archaea Community	Control	0.77 (0.04)	0.70 (0.01)	0.68 (0.01)	0.74 (0.05)	0.65 (0.26)	0.71 (0.02)
	Continuous	0.71 (0.05) p=0.06	0.77 (0.05) p=0.68	0.75 (0.02) p=0.11	0.72 (0.03) p=0.10	0.76 (0.02) p=0.59	0.75 (0.05) p=0.66
	Pulsed	0.76 (0.03) p=0.09	0.73 (0.01) p=0.25	0.73 (0.3)** p=0.04	0.69 (0.02) p=0.08	0.72 (0.05) p=0.39	0.72 (0.02)** p=0.04

a Standard deviation in parentheses
** Change in diversity considered statistically significant at $p \leq 0.05$ when compared to the Control column using the Student's t-test

Changes in *Archaea* community diversity and evenness were also determined in all of the columns. Statistically significant differences were observed for both the continuously-fed and pulsed-fed *Archaea* communities with distance in the columns. A less diverse community was present in Port A of the continuously-fed column ($p=0.01$). In contrast, a more diverse community was present in Port C of the pulsed-fed column ($p=0.01$). The relative change in index values, however, was less than that observed in the *Eubacteria* communities. A statistically significant increase in evenness was also observed in Ports C and F ($p \leq 0.05$) of the pulsed-fed column.

4.4 Discussion

The magnitude and breadth of fermentation products generated in the carbon-amended columns appear to be dependent on the method of carbon application. As discussed above, the continuously-fed column reached a quasi-steady state condition on Day 130, at which time no fermentation products were detected and the majority of the COD added to the column was routinely consumed. In contrast, the pulsed-fed column generated high concentrations of volatile fatty acids and alcohols after each pulsed input of molasses. Temporal data suggests this behavior would continue indefinitely. *Eubacteria* ordination and environmental variable (vector) data indicated that elevated carbon (COD) concentrations and the concentration of fermentation products were highly correlated to *Eubacteria* (and *Archaea*, in the case of COD) community structure observed in the pulsed-fed column. The overall number of *Eubacteria* (qPCR data), however, were more strongly correlated to the continuously-fed column communities.

These data suggest that intermittent inputs of high carbon loading do not correspond to an overall greater density of *Eubacteria*, but rather, to a shift in community structure which likely results in higher proportions of fermentative organisms. The presence of COD corresponded to an increased number of methanogens, and therefore methanogen numbers also correlated strongly to the pulsed-fed *Archaea* communities.

The abundance of certain populations within a diverse community can be characterized by use of two different growth strategies: r-strategists (high reproductive rates) and K-strategists (optimal use of substrate) (Atlas and Bartha, 1997). At low initial population densities, as expected in an unamended/unimpacted groundwater aquifer, the r-strategists would dominate after carbon is introduced as a result of their fast reproductive rates. If carbon becomes limited or conditions become overcrowded, the K-strategists would increase in numbers because of their more efficient use of substrate. These types of strategies are evident in the spatial layout of the *Eubacteria* ordination. The control column communities are most similar to those in Port F of the continuously-fed column, which received the lowest carbon load of the carbon-treated columns. Under substrate-limited conditions, the K-strategists would be expected to dominate; therefore, these organisms would be expected to thrive in a low carbon environment. As carbon loading increases, the *Eubacteria* communities begin to shift, likely a result of the growth of r-strategists. The *Eubacteria* community receiving the highest carbon load in the continuously-fed system (Port A) is closest in structure to the communities receiving high-strength periodic doses of carbon (pulsed-fed column). The presence of excess carbon would provide the r-strategists a greater advantage over the K-strategists because

of their more rapid reproduction rates. This scenario corresponds well to both the decreased *Eubacteria* diversity indices and evenness values that were calculated for the pulsed-fed column. A decrease in diversity index values indicates a lower number of species are present, suggesting some species have died off. A decrease in evenness values indicates that certain species are becoming more dominant by representing a larger fraction of the total community. Taken together, the ordination data suggests that certain populations, such as the r-strategists, increase in greater proportion when compared to the *Eubacteria* community as a whole, while other populations, such as K-strategists become less represented.

A significant shift in *Archaea* community structures also occurred as a result of carbon input. Similar to what was observed with the *Eubacteria*, the port receiving the lowest carbon concentrations (continuously-fed Port F) was most similar to *Archaea* control column communities, while the pulsed-fed *Archaea* communities were closest in structure to the members in Port A of the continuously-fed column, where the highest carbon loading occurred. The effect of carbon on *Archaea* diversity and evenness, however, is less obvious. Some *Archaea* communities demonstrated statistically significant increases in diversity and others decreases. No broad inferences can be drawn from these data regarding how *Archaea* communities shift in response to carbon inputs.

The total number of methanogens plus *Eubacteria* was greater in the pulsed-fed column when compared to the continuously-fed column. These findings are similar to the results of Peyton (1996) where higher numbers of colony forming units (CFUs) and protein were observed throughout soil columns in a pulsed-fed system compared to a

continuously-fed system. The overall higher density of biomass in our pulsed-fed column suggests that biofouling should be an issue. This, however, was not observed during the column study. The continuously-fed column exhibited biofouling behavior (i.e. decreased ability to pump fluid into the column) within <30 days of operation, after which significant maintenance of the column was required. The pulsed-fed system did not exhibit biofouling behavior until ~70 days after molasses addition began, and then only required periodic maintenance. Interestingly, the qPCR data from both pulsed- and continuously-fed columns showed similar total numbers of *Eubacteria* plus methanogen gene copies at Port A, located closest to the inlet. These data indicate that the observed biofouling was not simply a result of the quantity of biomass, but rather, from some other factor. Given the similar density of biomass, the reason for biofouling is likely attributed to the types of organisms stimulated via the two different techniques for carbon addition. For example, the production of extracellular polymeric substances (EPS) is considered the most significant factor in membrane fouling (Le-Clech et al., 2006). It is possible that EPS is generated to a greater extent in the continuously-fed system compared to the pulsed-fed system. Therefore it is possible that either the bacteria responsible for the production of EPS had been selected for over time as a result of the continuous supply of substrate, or that the continued presence of relatively constant concentrations of carbon created an environment conducive to production of EPS, such as the formation of biofilms.

To ensure that fungal biomass was not responsible for the observed fouling in the continuously-fed column, fungal ARISA was performed on the sampling port

communities as well. No fungal detections were observed in the sample port locations (data not shown). Although the data from the inlets were not used as they were determined to be excessively disturbed, the inlet samples from the continuously-fed column did contain fungi. Therefore, these organisms may have contributed to the biofouling observed in the continuously-fed column.

In summary, microbial community structure, function, diversity and density are all affected by different carbon input strategies. A system with an input consisting of a continuous low-dose of carbon, such as molasses, will eventually reach steady-state conditions and essentially yield no detectable fermentation products. Conversely, pulsing in high doses of molasses will yield better biomass distribution, intermittent detection of fermentation products and higher quantities of methanogenic organisms. Dissolved methane, however, does not appear to be generated in higher quantities in one system or the other as methane concentrations did not correlate to methanogen numbers in these columns. This observation can likely be extrapolated to larger systems if off-gassing is allowed to occur. In addition, the community physical structure (i.e. matrix) appeared to be affected by the type of carbon input. A continuous feed of low-concentration of carbon was observed to cause greater biofouling as a result of the *Eubacteria* communities that were selected for over time using this method of carbon input. Fungal organisms may also have played a role in biofouling, and their role in biofouling warrants additional research.

This study shows that the use of high concentration, pulsed-fed systems can encourage the production of fermentation products, but will result in less biofouling than

a continuously-fed system. A continuously-fed, low concentration of carbon will reach a pseudo steady-state with no detectable concentrations of fermentation products, but will require additional maintenance to address the biofouling issues that will undoubtedly occur. Methane generation will occur in both types of systems, with the potential for a pulsed-fed system to generate larger amounts of methane due to the higher numbers of methanogens stemming from this method of carbon addition. The likelihood of greater methane detection in a pulsed-fed system, however, may be dependent on whether off-gassing is able to occur.

These results are directly applicable to remediation systems, giving us clear information on methods to optimize or minimize fermentative communities, biomass distribution, or biofouling during carbon application. Nevertheless, if used with caution they can also tell us a great deal about the response of microbial communities to carbon addition in general. This could have applications to other systems where carbon may be either intermittently or steadily discharged, as in the case of pulsed carbon inputs from freeze-and-thaw cycles in natural environments or accidental spills or leaks of compounds that can serve as substrate, such as liquid manure or ethanol-based fuel.

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CHAPTER 5 Impact of an Ethanol-based Fuel on Native Aquifer Community Structure and Methane Production

5.1 Introduction

The Energy Independence and Security Act of 2007 requires the production of 36 billion gallons of renewable fuel, including ethanol-based fuels, by 2022 (RFA, 2007). This constitutes a 75% increase in renewable fuel production over the next 14 years. As a result, increased availability of ethanol-based fuels at local filling stations will undoubtedly occur over the next decade, leading to an increased likelihood of ethanol-based fuel spills during transportation and storage.

Fuel spills can result in profound side effects. Ethanol, a common component of renewable fuel, can serve as a microbial food source and promote the growth of broad fermentative communities capable of generating alcohols, ketones and volatile fatty acids (Madigan and Martinko, 2006). These compounds can be further broken down to acetate and hydrogen, which are used by methanogenic organisms to generate methane. Methane is explosive when present as 5% of the surrounding atmosphere (NIOSH, 2006). Using an average Henry's law constant for methane (Sander, 1999), a dissolved concentration of less than 2 milligrams per liter (mg/L) of methane indicates an explosion hazard in the gas phase. In previous research on ethanol addition to an aquifer, dissolved methane concentrations reached up to 16 mg/L (Feris et al, 2008). Methane production coincided with increased numbers of *Archaea*, the domain to which methanogens belong (Feris et al., 2008; Cápiro et al., 2008). No research on broader microbial community shifts, particularly with respect to fermentation processes, has been performed.

The fermentation of ethanol can yield multiple products, including acetate, propionate, butyrate, and butanol, with acetate constituting the highest proportion of the

generated fatty acids (Madigan and Martinko, 2006; Schink et al., 1985). Butyrate is produced by clostridia bacteria through the butyryl-CoA pathway, which can also be used to produce butanol and acetone (Madigan and Martinko, 2006). While butanol and acetone do not currently have a federal Maximum Contaminant Level (MCL) assigned as a primary standard, they may be regulated on a state level. For example, the Minnesota Department of Health has set a Health Risk Limit (HRL) of 0.7 mg/L in groundwater for both n-butanol and acetone (MDH, 2009). The effect of ethanol-based fuel addition on the production of these potentially regulated compounds has not been evaluated.

One factor complicating the degradation of ethanol in fuel is that high levels of ethanol (6% to 18%, by volume, [Ulrich, 1999]) are toxic to microorganisms. Accordingly, the core of an ethanol-fuel plume may serve as a long-term source of carbon for the plume fringes, feeding the anaerobic communities (including methanogenic organisms) that develop in response to the carbon addition. To our knowledge, the toxicity effect of ethanol on complex fermentative communities, which drive the breakdown of ethanol and subsequent methane formation, has not been evaluated.

A spill of ethanol-based fuel to an aquifer environment is a complex problem; it is therefore critical to understand how ethanol stimulates and alters both *Eubacteria* and *Archaea* native soil community structures and the subsequent formation of fermentation products created during the ethanol breakdown process. This study was designed to improve our understanding of these complex processes. These results should facilitate the assessment and modeling of ethanol-based fuel spills, enabling one to predict the human

health risk stemming from fermentation product formation and methane generation resulting from microbial growth on ethanol-based fuels.

5.2 Materials and Methods

Experimental Setup

Soil Columns

Two soil columns were operated continuously for 145 days to investigate the effect of a dilute E85 feed on indigenous microbial communities and their subsequent generation of fermentation products and methane. Both columns were packed with aquifer material from southwestern Minnesota. One column was supplied with a continuous dilute E85 feed (0.52% E85 by volume in a minimal groundwater media) and the other served as a control for microbiological measurements (continuous feed of minimal groundwater media only).

Aquifer material was obtained from an unimpacted area of an E95-contaminated site located in Southwestern Minnesota. Soil cuttings included both vadose and saturated zone aquifer material. Soil cuttings were homogenized and sieved through a 2-mm mesh to remove large particles prior to use. The native aquifer material contained a high amount of silt and clay; therefore, sand (TCC Materials, Mendota Heights, MN) was added at 1:1 ratio (sand to sieved cuttings) to increase the permeability of the aquifer material. The final mixture was comprised of 90% sand, 1.8% silt, 8.3% clay, and had an average organic matter fraction of 0.006 (by mass).

Construction. Each column was constructed from a 60-in (1.5 m) long 4-in (0.1 m) inner diameter polyvinyl chloride (PVC) plastic pipe. The ends of the columns were sealed with standard 4-in (0.1 m) PVC end caps that were fitted with influent and effluent connections. Six threaded sample ports were tapped along the column length and fitted with Mininert valves (Valco, 1/8-in [3 mm] NPT female) to allow for sampling along the length of the columns. Stainless-steel screen (150 mesh) tubes were placed into each sample port to prevent aquifer material from clogging syringe needles during sampling. Columns were operated horizontally to mimic groundwater flow in an aquifer setting. In addition to sample ports, several vertical ¼-in (6 mm) diameter glass tubes were connected to the top of the column via compression fittings (Cole-Parmer Instrument Co., Vernon Hills, IL) to allow for off-gassing during column operation. Tubing ends were connected to a headspace trap via flexible PVC tubing (Fisher Scientific, Pittsburg, PA) to inhibit diffusion of oxygen into the columns during the experiment. A schematic of the column setup is shown in Figure 5.1.

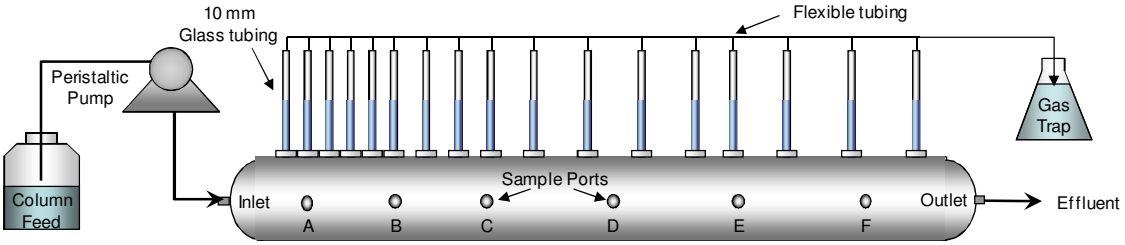

Figure 5.1 Schematic of the column system setup.

The minimal groundwater media used for the influent of both columns contained trace nutrients and minerals consistent with natural groundwater, and is described elsewhere (Nelson and Novak, 2009). Column feed stocks were adjusted to a pH of 8

using either 10 mM NaOH (control column) or 10% HCl (E85-fed column). E85 fuel was obtained from a local filling station (Minneapolis, Minnesota). The content of ethanol within E85 fuel varies with the season; the E85 used in this experiment had an actual denatured ethanol content of 74% (anhydrous ethanol content of 70.3%), as analyzed by the Minnesota Department of Commerce. Adjusting for the above, the anhydrous ethanol percentage in the feed to the E85 column was 0.36%.

Operation and Maintenance. The feed solution was pumped at a flow rate of 0.06 mL/min into each column using a Masterflex L/S 07523-70 digital drive equipped with a Masterflex 07519-20 cartridge pump head containing 07519-85 cartridges (Cole Parmer, Vernon Hills, IL). The feed to the columns was replaced every 3 to 4 days to minimize degradation of E85 and/or media components. The influent lines were disinfected twice weekly with a 10% (by vol) bleach solution followed by a rinse of deionized water and final rinse using the respective feed solutions. This removed biomass growth within the influent tubing and prevented clogging of the feed lines.

Sampling. Aqueous samples were collected weekly from each sampling port for pH, volatile fatty acid, cosolvent, and methane analyses. Samples were withdrawn using syringes equipped with a 2-in (5 cm) long 22-gauge needle. All samples were filtered through a 0.2-µm polyethersulfone syringe filter (Nalgene, Rochester, NY) prior to analysis. A total of 8 mL was collected from each port during each sampling event, equating to <1% of the total column liquid.

Tracer testing. Two bromide tracer tests were performed on the column. The first tracer test was conducted after column construction, but prior to E85 addition; and the second tracer test was performed 123 days after E85 was introduced. A step input of solution containing bromide (0.5 g/L) was added to the columns for three days. Samples were collected from selected sample ports and analyzed for bromide using ion chromatography. The mean residence time of the column and travel time to the various ports were determined by temporal first moment analysis (Das and Kluitenberg, 1996).

Microcosms

Toxicity experiments were conducted in 150-mL bottles fitted with rubber septa sealed with aluminum crimp caps. Each bottle contained 1.5 g of the aquifer mixture (discussed above) and 75 mL of minimal groundwater media containing varying percentages of 200-proof ethanol. Nine treatments were evaluated in triplicate: 0% (control), 0.5%, 1%, 2.5%, 5%, 6%, 8%, 10% and 12% ethanol (by vol). The pH was adjusted to 8 by adding 10% HCl prior to the addition of the aquifer mixture. Microcosms were run for 66 days at which time a liquid sample was collected from each microcosm, filtered through a 0.2-µm polyethersulfone syringe filter and analyzed for cosolvents, volatile fatty acids, and methane. The presence or absence of microbial fermentation products was used to evaluate whether the treatments inhibited microbial activity.

Experimental Procedures

Soil collection and DNA extraction

Soil columns were sacrificed after the final liquid sampling event 145 days after E85 was introduced. The columns were disconnected from the feed solutions and drained of excess liquid. The columns were cut at the mid-point between each sampling port using a reciprocating saw. The saw blade was disinfected between cuts by immersing in ethanol and drying with autoclaved paper towels to remove ethanol residue. Soil from each end of the cut column section was removed with a disinfected metal spatula until approximately 1-inch (2.5 cm) of soil remained on either side of the sampling port. This remaining soil, assumed to represent the microbial community at each sampling port, was homogenized and immediately frozen at -20^{o}C until further analysis. Genomic DNA was extracted using a PowerSoil DNA Kit (MOBIO Laboratories, Inc., Carlsbad, CA). Triplicate genomic DNA extractions were performed on each sampling port location.

Automated Ribosomal Intergenic Spacer Analysis (ARISA)

Eubacteria ribosomal intergenic spacer regions were amplified using primers ITSF and ITSReub (Cardinale et al, 2004). *Archaea* ribosomal intergenic spacer regions were amplified using primers 1389F and 71R (Qu et al, 2009). The primers ITSF and 1389F were labeled with the phosphoramidite dye HEX (6-carboxy-1,4-dichloro-2',4',5',7'-tetra-chlorofluorescein). Polymerase chain reaction (PCR) was performed using a DNA Engine Thermal Cycler (Biorad, Hercules, CA).The PCR final 50 μL reaction mixture for both *Eubacteria* and *Archaea* ARISA contained: 1 X PCR buffer

(Promega, Madison, WI), 4 nmol deoxynucleoside triphophates, 25 pmol forward and reverse primers, 1.25 units of Go Taq DNA polymerase (Promega) and ~1 ng of template DNA. The PCR protocol for *Eubacteria* ARISA included a 3 minute initial denaturation at 94^{o}C followed by 35 cycles of 94^{o}C for 45 seconds, 55^{o}C for 1 minute, and 72^{o}C for 2 minutes and a final extension for 7 minutes at 72^{o}C. *Archaea* ARISA PCR followed the protocol outlined by Qu et al (2009).

ARISA was performed in triplicate using the triplicate genomic DNA extractions from each sampling port location. Amplified products were resolved by capillary electrophoresis using an ABI 3130xl capillary instrument (Applied Biosystems Inc, Foster City, CA). Fragment peak areas were analyzed using Gene Profiler software. Resulting data were analyzed using the following protocol: (1) fragment lengths <122 base pair were removed to eliminate primer dimer fragments, (2) peaks <0.5% of the total peak area were removed to filter out signal noise, (3) manual alignment of the remaining fragment lengths between triplicate samples, (4) manual alignment of fragment lengths between sampling port locations of the same column, and (5) manual alignment of fragment lengths between columns. Each manual alignment considered fragments +/- 1 base pair to be the same.

Quantitative PCR

Methanogen and *Eubacteria* communities were quantified for each sample using quantitative, real-time polymerase chain reaction (qPCR). Methanogen qPCR primers Met630F and Met803R (Hook et al, 2009) were used to enumerate the methanogens, and

primers 338F and 518R (Muyzer et al., 2003) were used to enumerate *Eubacteria*. Each 25 μL qPCR reaction mixture contained 1x Power SYBR Green Master Mix (Applied Biosystems, Foster City, CA), 25 μg of bovine serum albumin, optimized quantities of forward and reverse primers, and 1 ng of template. A Realplex^2 Mastercycler (Eppendorf) thermocycler with Eppendorf Mastercycler ep Realplex software was used for all qPCR protocols. Methanogens were quantified using a protocol of 95°C for 2 minutes, followed by 50 cycles of 95°C for 30 seconds, 60°C for 30 seconds and 72°C for 1 minute. Total *Eubacteria* were quantified using a protocol of 95°C for 10 minutes, followed by 40 cycles of 95°C for 15 seconds, 60°C for 1 minute and 95°C for 15 seconds. A melting curve analysis was performed after each complete run. The quantity of target DNA in samples was calculated using standard curves generated with known quantities of template DNA. qPCR was performed in triplicate for each sampling port location using the triplicate DNA extractions.

Standards for *Eubacteria* qPCR were prepared by PCR amplification of 16S rRNA genes using Primers pA and 907R (Edwards et al, 1989) from *E. coli* K12, ligation into the pGEM-T Easy cloning vector (Promega, Madison, WI), and transformation into *E. coli* DH5α. Methanogen standards were prepared by cloning PCR-amplified 16S rRNA genes of methanogens from this column study into pGEM-T Easy cloning vectors and transformation into *E. coli* DH5α. Primers 8aF and Met803R (Qu et al, 2009) were used to amplify *Archaea* 16S rRNA genes. Verification of the methanogen standard was performed by PCR amplification of the plasmid insert using M13 forward and reverse primers (Promega, Madison, WI), purification using the GeneClean II Kit (MP

Biomedicals), and sequencing at the University of Minnesota BioMedical Genomics Center using a ABI PRISM 3730xl DNA Analyzer. The resulting sequences were aligned using CodonCode Aligner (Codon Corp, Dedham, MA). The aligned sequence was most closely related to *Methanosarcina sp.* (99% identity, Accession# AY780570.1) based on NCBI's BLAST Database. Plasmid DNA for both Eubacteria and methanogen 16S rRNA standards was quantified using Hoeschst 33258 dye and measured on a TD-700 fluorometer (Turner Designs, Sunnyvale, CA) using calf thymus as a DNA standard. Ten-fold serial dilutions of plasmid standard were prepared and run on the thermal cycler to generate standard curves.

Analytical Methods

Analysis for ketones (acetone, methyl ethyl ketone, methyl isobutyl ketone) and alcohols (methanol, ethanol, butanol) was performed using on-column injection with a 7673 automatic liquid sampler fit with a nanoliter adaptor and connected to a Hewlett Packard (HP) 5820 Series II gas chromatograph equipped with a flame ionization detector. A sample volume of 0.2 µL was directly injected onto a 5-m 0.53-mm diameter deactivated fused silica pre-column connected to a 30-meter 0.32 mm Rtx-1 (Restek Corp, Bellefonte, PA) capillary column. Peak resolution was performed using an oven temperature of 60°C for 9 min followed by an oven ramp of 10°C/min to a final temperature of 150°C with a 5 minute hold. Helium (99.99%) was used as the carrier gas at a velocity of 17.2 cm/s. The inlet temperature was set to track 3°C above the oven temperature at all times. The detector was maintained at 300°C. Absolute 200 proof

ethanol, HPLC grade methanol, and high purity ACS-grade butanol, acetone, methyl ethyl ketone, methyl isobutyl ketone were used to prepare standards.

Volatile fatty acid analysis was performed using a high performance liquid chromatograph (Agilent Technologies 1200 Series) equipped with a diode-array detector (210 nm wavelength) and a 300-mm long Aminex HPX-87H column (Biorad, Hercules, CA). A sample volume of 25 µL was injected with an isocratic mobile phase of 0.005 M H_2SO_4 at a flow rate of 0.6 mL/min. Concentrated acids (85% or higher) were used to prepare standards. Standard curves were adjusted to account for purity.

Bromide analysis was performed using a Metrohm 761 Compact Ion Chromatograph (Metrohm US Inc., Riverview, TX) equipped with a Metrosep A Supp5 column. A sample volume of 1.4 mL was injected using an eluent solution consisting of 3.2 mM Na_2CO_3 and 1.0 mM $NaHCO_3$ at a flowrate of 0.7 mL/min (Schnobrich et al 2007). The regenerant solution consisted of 100 mM H_2SO_4. A Beckman 32 digital pH meter equipped with a combination electrode was used for pH measurement.

Statistical Analysis

Ordination statistical analyses were performed using R, a free software environment for data analysis and statistics (R Development Core Team, 2009). The package *vegan* was used to analyze ARISA community structure using non-metric multidimensional scaling (NMDS). The function *metaMDS* was used to perform the NMDS analysis with the dissimilarity matrix calculated for both species and sample locations using Bray-Curtis distance measure (function *vegdist*) (Oksanen, 2009).

5.3 Results and Discussion

Microcosm Experiments

Fermentation products detected at Day 66 in the microcosm experiment included butyrate, acetate and butanol, typical end products of anaerobic ethanol fermentation (Figure 5.2).

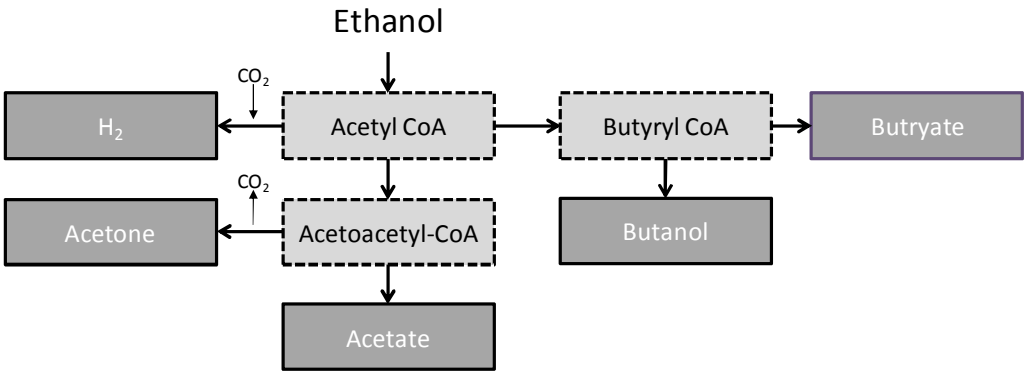

Figure 5.2 Major routes of the anaerobic fermentation of ethanol. Solid outline indicates final product. Note: not all steps are shown in metabolic pathways. Adapted from Madigan and Martinko (2006).

Low concentrations of acetate were detected in the microcosm containing 6% ethanol (Figure 5.3), while two orders of magnitude higher concentrations of acetate were present in the 5% ethanol treatment. Elevated concentrations of butyrate and butanol were also present in the 5% ethanol treatment. These data indicate that the microbial population was strongly inhibited when 6% ethanol was present. No fermentation products were detected in microcosms containing >6% ethanol, indicating complete inhibition of microbial activity and/or toxicity at ethanol concentrations greater than 6%. Conversely,

the microcosms containing 1% ethanol had the overall highest concentration of fermentation products, indicating that microbial activity was stimulated by low levels of ethanol. These data agreed well with previous studies, where ethanol was seen to be inhibitory at levels greater than 6% for enteric bacteria (Ulrich, 1999), and 3.1% (0.67 M) for a pure strain of *E. coli* (Ingram and Vreeland, 1980).

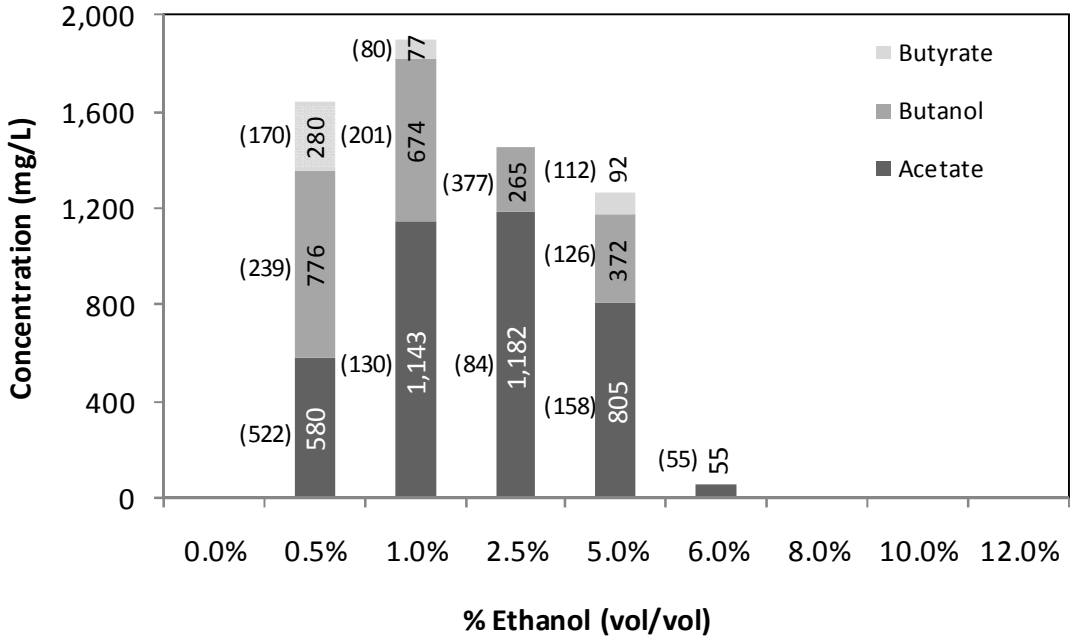

Figure 5.3 Concentrations of ethanol fermentation products on Day 66 in microcosms containing varying percentages of ethanol. Data shown are the average of each triplicate treatment. Standard deviation values are shown in parentheses to the left of the data.

Butanol was detected at concentrations exceeding 600 mg/L in both the 0.5% and 1% ethanol treatments. This concentration is three orders of magnitude above the Minnesota regulatory standard of 0.7 mg/L. Acetone was not detected in the

microcosms, but the presence of butanol indicates the potential for acetone generation via the acetone-butanol fermentation pathway (Jones and Woods, 1986).

Column Study

Fermentation Products

Ethanol and its fermentation products are shown over time for Ports A, C and F in Figure 5.4. Ethanol concentrations decreased over time in Port A, likely as a result of increasing biomass and metabolic activity at that location. The majority of ethanol was consumed prior to reaching Port C (14 days travel time), and was never detected in Port F, located at the end of the column (36 days travel time). These data indicate that ethanol is consumed rapidly when present at levels that are not inhibitory to microbial growth. As was observed in the microcosm study, acetate, butyrate and butanol were the detected products of ethanol fermentation. Port A contained the highest levels of these fermentation products throughout the course of the experiment, indicating that the majority of the biological activity occurred rapidly and in the area of initial exposure to ethanol. The primary fermentation product observed in both Ports A and C was acetate, detected consistently throughout the column experiment. Low levels of butanol (discussed below) and butyrate were detected intermittently throughout the experiment in both Ports A and C. Acetate was present in Port F at the beginning of the study, but was not detected after Day 30. Butyrate and butanol were not detected at Port F during this experiment.

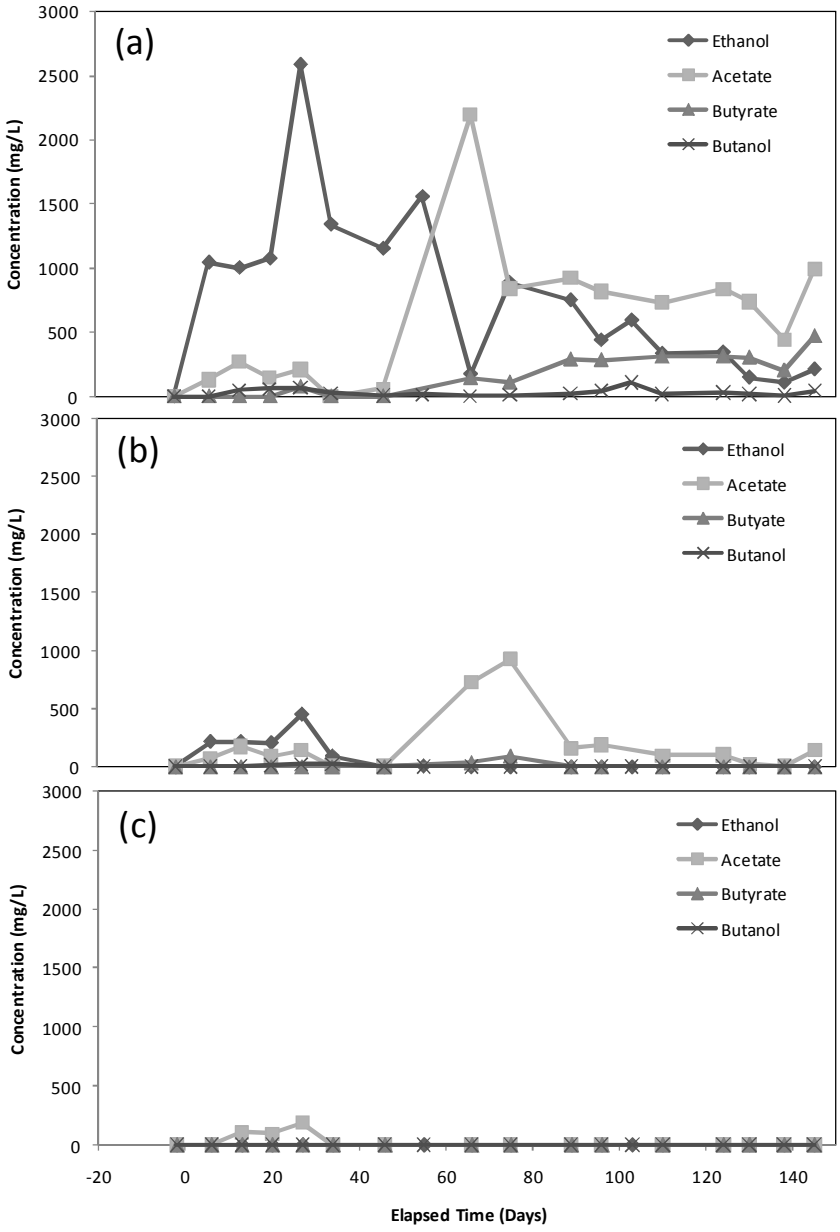

Figure 5.4 Ethanol and fermentation products versus time detected in the E85-fed column in (a) Port A, (b) Port C, and (c) Port F. Time = 0 denotes the start of the E85 feed.

Butyrate and acetate are the predominant products of butyric acid fermentation at a near-neutral pH, and the generation of butanol and acetone is expected when the pH decreases as result of acid generation (Madigan and Martinko, 2006). Figure 5.5 shows butanol concentrations and pH in Ports A, C and F during the experiment. These data shown that butanol was generated when the pH decreased below 7. In the instance of Port A, the pH was < 7 for the majority of the column study, and butanol was consistently detected. Port C showed a decrease in pH at the beginning of the study (first 50 days), during which butanol formation occurred. After the pH increased to >7 (Day 50 to 145), however, butanol was no longer detected. Port F, located at the end of the column, consistently had a pH ≥ 7 throughout the experiment with no detections of butanol.

Fermentation products, such as acetate and hydrogen, can be directly used by methanogenic organisms to produce methane (Schink, 1997). Methane formation was observed in all six sampling ports approximately 30 days following E85 introduction. Figure 6 shows data from Ports A, C and F. Methane was not detected consistently in Port A. Ports C and F, however, contained dissolved methane concentrations above the level at which it could form an explosive gaseous environment (≥2 mg/L). In fact, dissolved methane was detected up to concentrations of 9 mg/L in both Ports C and F throughout the column experiment. No ethanol, fermentation products, or methane were detected in the control column during the experiment.

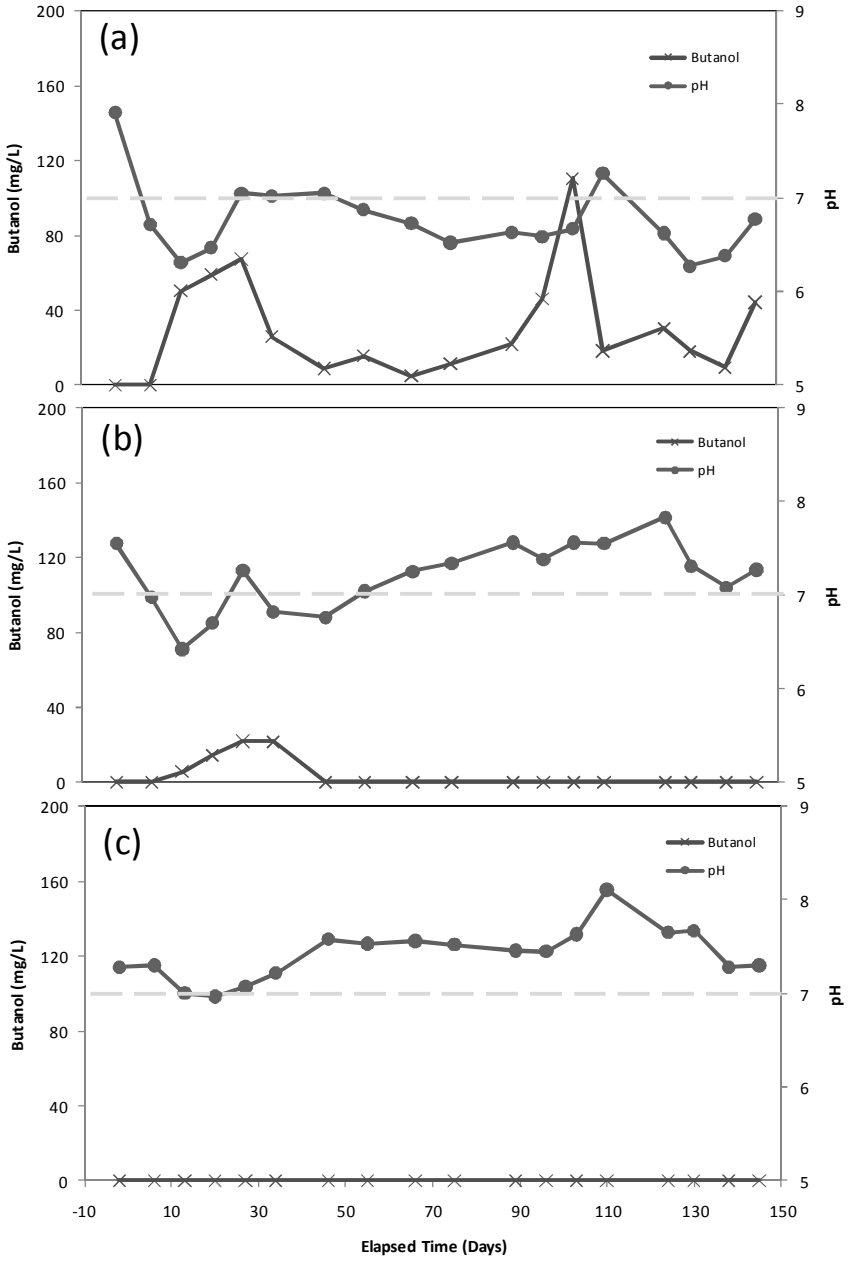

Figure 5.5 Butanol and pH versus time within the E85-fed column in (a) Port A, (b) Port C, and (c) Port F. The dashed line represents pH=7. Introduction of E85 began on day 0.

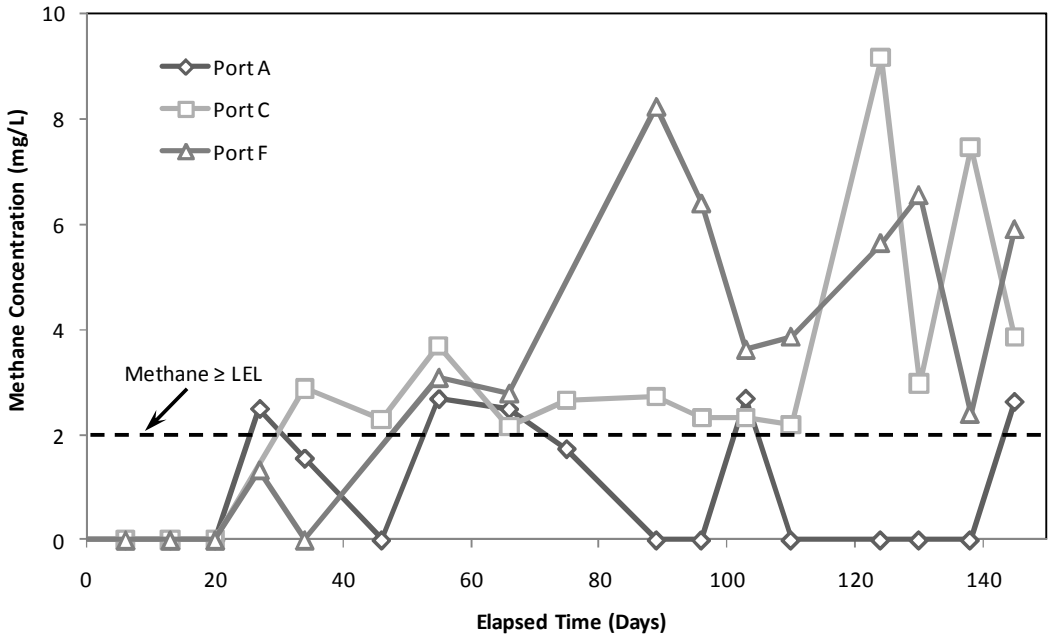

Figure 5.6 Dissolved methane concentrations versus time in Ports A, C and F of the E85-fed column. The dashed line represents the calculated dissolved methane (2 mg/L) above which partitioning can occur above methane's lower explosive limit (LEL). E85 introduction began at Time = 0.

Microbial community structure

Fermentative bacteria, such as *Clostridia* species, and methanogenic organisms belong to separate branches of the tree of life: *Eubacteria* and *Archaea*, respectively. Analysis of both *Eubacteria* and *Archaea* community structures was therefore performed using automated ribosomal intergenic spacer analysis (ARISA). ARISA is a technique that analyzes the length of the spacer region located between the 16S and 23S rRNA gene, which can vary greatly for different microorganisms (García-Martínez et al., 1999). Accordingly, the number of spacer fragments found in a sample can be used to examine microbial community structure (Fisher and Triplett, 1999). Non-metric multidimensional

scaling (NMDS) is a distance-based ordination method that allows species data to be mapped onto two-dimensional space with the distance between points representing dissimilarity (for example, the further two points are located from each other, the greater the dissimilarity). Figures 5.7(a) and 7(b) show the outcome of the NMDS analysis of the ARISA-based *Eubacteria* and *Archaea* community structures, respectively, in both the control and E85-fed columns. As shown, the samples from the control column cluster together. The close spacing between ARISA sample port results in the control column indicates that *Eubacteria* (Figure 5.7[a]) and *Archaea* (Figure 5.7[b]) communities were relatively similar.

The E85-fed column communities are all spaced far from the control column ARISA results, indicating that the addition of E85 substantially changed the community. In Figure 5.7(A), it can be seen that the spacing between the *Eubacteria* communities present in the sample ports of the E85-fed column showed greater dissimilarity along the column length. The community in Port A was the most different of all the sample port locations, as indicated by the large distance between Port A results and the other sampling locations. This difference paralleled the elevated concentrations of fermentation products detected in this port. *Eubacteria* communities from Ports B and C in the E85-fed column appear more similar to one another when compared to Ports D through F. Port B (data not shown) and Port C (Figures 5.4 and 5.5) also contained similar concentrations of fermentation products, which supports the presence of similar fermentative microbial communities in this portion of the column. Ports D through F contained sequentially lower concentrations of fermentation products, with Port F containing no detectable

concentrations of fermentation products after Day 30 of the study. The ARISA results from these ports (D through F) are located sequentially closer to the control column communities, indicating that these portions of the column, particularly the portion closest to Port F, were most similar to the indigenous community as represented by the control column results. These data indicate that a large shift in *Eubacteria* community structures occurred in response to the addition of ethanol, with the magnitude of the shift corresponding to the presence and concentration of fermentation products.

Archaea community ARISA results (Figure 5.7[b]) are similar to those for *Eubacteria* in that the E85-fed column results most similar to those from the control column were from the port locations furthest from the point of E85 introduction (Ports E and F). The data, however, show less of a difference in *Archaea* community structures along the length of the E85-fed column than that observed in *Eubacteria* communities. Increases in *Archaea* in an anaerobic environment have been attributed to methanogenic growth (Feris et al., 2008; Cápiro et al., 2008); accordingly, the shift observed within *Archaea* community structure in the E85-fed column is likely a result of the growth of methanogens. This was further investigated using qPCR.

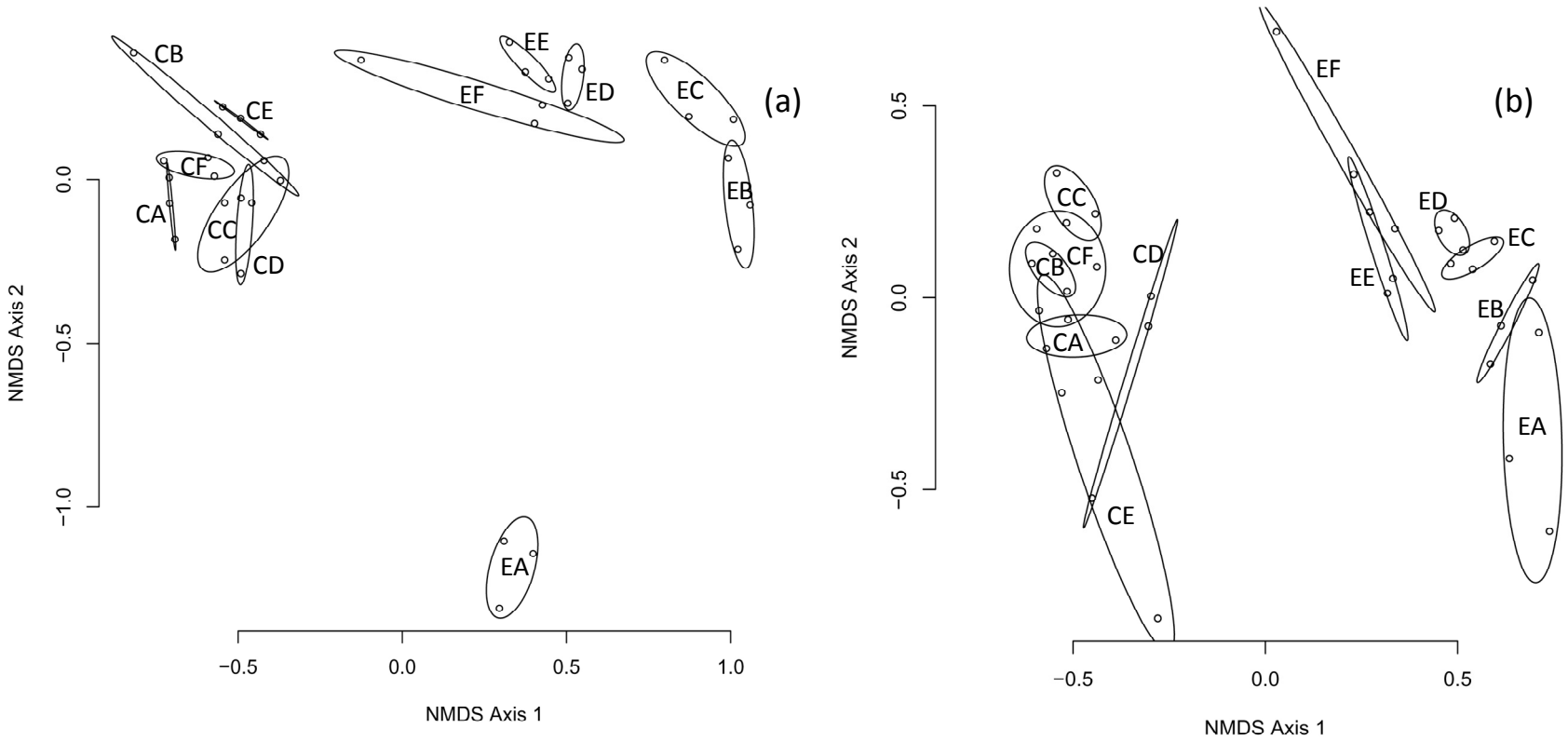

Figure 5.7 Results of non-metric multidimensional scaling analysis for (a) *Bacteria* community structure (Stress 9.46), and (b) *Archaea* community structure (Stress 11.63) using Bray-Curtis distance measure. Sample port labels are depicted by: column ID (C for control, E for E85-fed), and port location (A-F). Ellipses represent 95% confidence interval for triplicate samples.

Figure 5.8 shows the number of methanogen gene copies detected in the soil at each sample port at the end of the experiment. The qPCR data were analyzed using the student's t-test to determine whether differences of methanogen gene copies within the E85-fed and control column were statistically significant. Results indicated that Ports A, B, D, E and F showed a statistically significant increase in methanogens at $p<0.05$. The number of methanogens present along the length of the column strongly correlated (Figure 5.9, $r^2=0.9951$) to acetate concentration, indicating that the degradation of ethanol to acetate stimulated methanogenesis. The number of methanogen gene copies detected in Port A in this study are within the same order of magnitude as those detected in other anaerobic systems containing high concentrations of carbon (Table 5.1) such as wetland soils (Kim et al., 2008), or cow rumen (Hook et al, 2009). Comparison to a similar system in which ethanol was added to soil (Cápiro et al, 2008) shows, however, that the methanogen numbers in this study were two orders of magnitude higher. The difference is likely a result of the style of ethanol application, as Cápiro et al. (2008) added one-time pulse of 100% neat ethanol, likely causing some toxicity, whereas a continuous low dose of E85 was delivered over 145 days in this study.

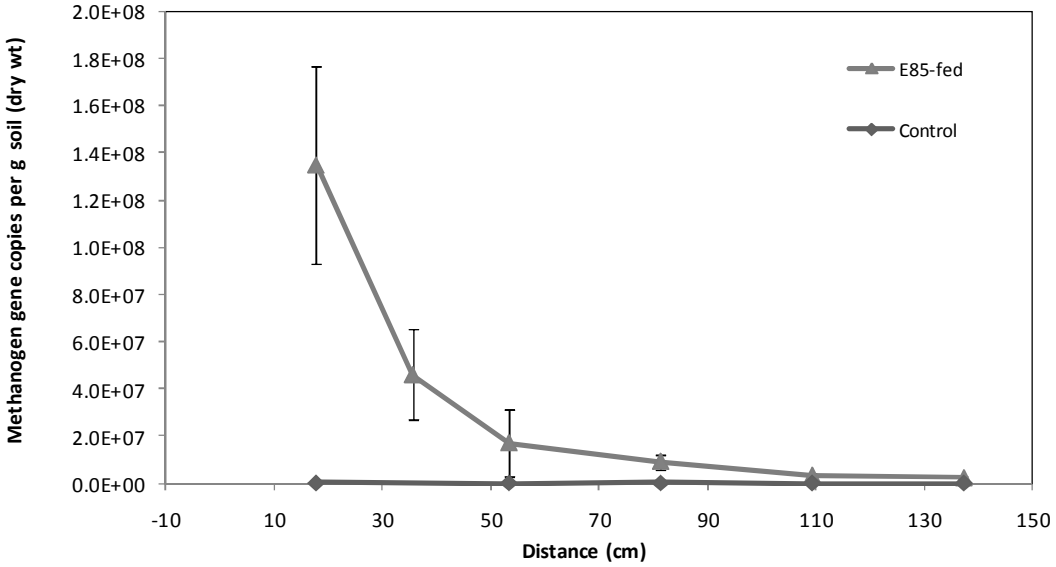

Figure 5.8 Number of methanogen gene copies per gram of soil (dry weight) along E85-fed and Control column lengths on Day 145 of experiment. Error bars represent the standard deviation of triplicate samples.

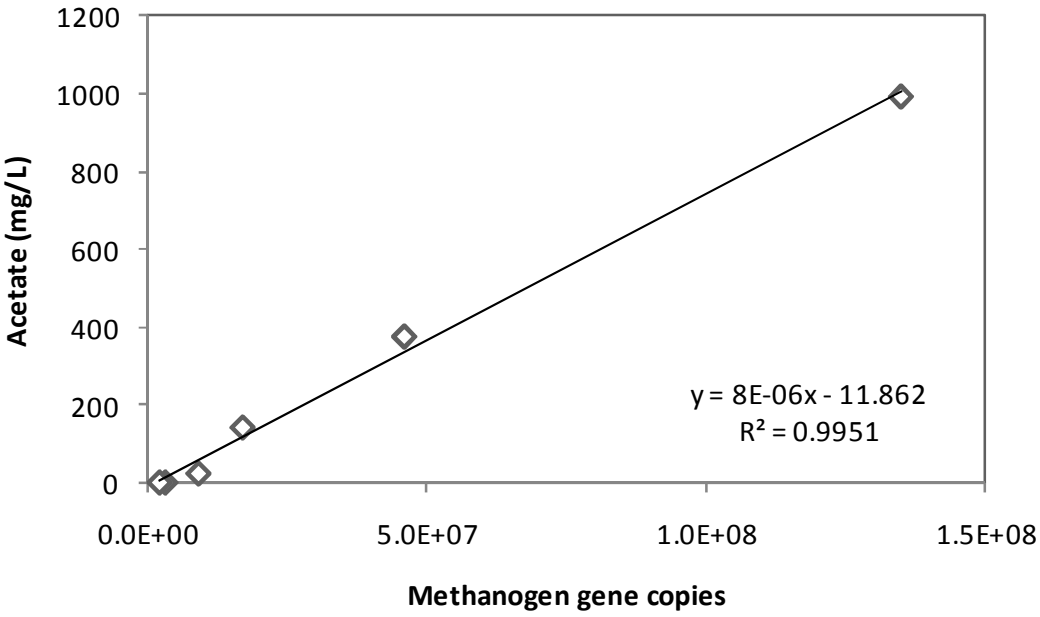

Figure 5.9 Correlation between acetate concentrations and methanogen gene copies along the E85-fed column length.

Table 5.1. Comparison of methanogen quantitative PCR results with other studies.

Number of organisms	Application	Organism/ Target	Reference
1.35×10^8 (copies/g dry wt)	Soil column with continuous E85 Feed (Port A, Average)	Methanogen/ 16S rRNA	This Study
3.3×10^8 (copies/g dry wt)	Wetland (Fen, Control average)	Methanogen/ *mcrA*	Kim et al. (2008)
2.85×10^8 (copies/g wet wt)	Cow Rumen (Day 20, Control average)	Methanogen/ 16S rRNA	Hook et al. (2009)
1×10^6 (copies/g dry wt)	Pilot-scale aquifer tank with one pulsed input of neat ethanol	*Archaea*/ 16S rRNA	Cápiro et al. (2008)

5.4 Conclusions

Results from this study indicate that spills of fuel containing high percentages of ethanol can cause a significant microbial community shift within the subsurface environment and become a lingering source of methane generation. At the core of the spill, the high concentrations of ethanol (>6% by vol) will inhibit microbial growth and subsequent fermentation. At the plume fringes, microbial growth will be stimulated by the presence of ethanol causing a community shift in both *Eubacteria* and *Archaea* populations. Changes in *Eubacteria* communities will generate fermentation products such as acetate, butyrate, butanol, and possibly acetone within areas containing <6% ethanol. This shift in *Eubacteria* community structure will also result in increased

methanogen numbers as they respond to the presence of acetate. The end result is the production of methane at levels considered explosive, if allowed to partition and accumulate within the vadose zone.

The results presented in this study can be used by both practitioners and regulators to assess potential risks posed by the fermentation of ethanol. These data can be used to more effectively model the fate and transport of an E85 spill by including a "switch" to incorporate ethanol toxicity effects. Fermentation processes can be included to assess the potential generation of regulated compounds, such as butanol and acetone. Additionally, the generation of acetate can be correlated to predict the production of methane.

5.6 References

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CHAPTER 6 Conclusions and Recommendations

6.1 Conclusions

The results presented in this dissertation range from the mechanisms by which molasses can enhance the dissolution of trichloroethene to how carbon addition affects the microbial community and structure in an aquifer. The general and specific conclusions drawn as a result of this research are given below.

- Fresh 10% molasses increased the solubility of trichloroethene twofold via the hydrogen bonding of the protein component with water. An increase in solubility directly relates to an increased mass flux of NAPL into aqueous phase.

- Fermentation of molasses resulted in the production of biosurfactants that disrupted the surface area of TCE NAPL. Increased surface area corresponds to an increased rate of mass exchange between the NAPL and fluid, which in turn enhances the dissolution of the NAPL.

- Fermented molasses appeared to affect the TCE soil partitioning coefficient. A lower coefficient was observed ($p=0.12$) for TCE indicating increased dissolution of sorbed TCE mass into aqueous phase can occur in the presence of fermented molasses.

- The method of carbon application affected the quantities and types of fermentation products. Continuously-fed, low concentrations (0.5%) of molasses led to a pseudo steady-state condition after which fermentation products were generated at low to non-detectable quantities. Pulsed-fed, high concentrations (10%) of molasses generated high levels of fermentation products after each pulse of substrate. Engineered systems can use these different methods to either minimize or enhance fermentation products, depending on the objectives of the design.

- Pulsed, high concentrations of molasses resulted in an overall higher distribution of biomass within the column when compared to the low, continuously-fed column. The continuously-fed column, however, exhibited greater biofouling behavior suggesting that the density of organisms was not responsible for the biofouling behavior.

- Both pulsed-fed and continuously-fed molasses columns generated methane at similar concentrations. Methanogens, however, were present in higher numbers along the pulsed-fed column length indicating a larger potential for methane generation.

- Fermentation processes were inhibited in the presence of >6% (by vol) of ethanol. The fermentation of ethanol at levels below this resulted in the production of acetate, butyrate, and butanol, with acetate as the predominant product.

- A dilute feed of E85 (0.36% by vol) resulted in the generation of methane at levels that could be explosive if allowed to accumulate in the atmosphere. The number of methanogens along the E85-fed column strongly correlated to the presence of acetate (r^2=0.9951).

- Butanol was generated in the E85-fed column when the pH dropped below 7. The presence of butanol indicated the acetone-butanol fermentation pathway was active, suggesting the potential for acetone formation. Both butanol and acetone are regulated by Minnesota.

6.2 Recommendations

The application of bioremediation to source areas holds promise as a method to address residual contaminated mass. Optimizing the dissolution of both NAPL and sorbed mass is critical to the success of this technology. Strong evidence was presented in this dissertation regarding the mechanisms that can increase the dissolution of TCE NAPL through addition and fermentation of molasses. The effect on sorbed mass, however, was not found to be statistically significant at a 95% confidence because of the high variability in the experimental dataset. Future research is therefore needed to verify this result, as the success of bioremediation within a source area is dependent on the ability to enhance the removal of the residual sorbed mass. In addition, expanding these results to include other NAPL compounds is a crucial next step in moving this technology forward.

The method of carbon input was shown to affect the fermentative community structure which influences the generation of fermentation products that lead to enhanced dissolution of contaminants. Biofouling, however, was an issue during the application of carbon, and it was proposed that biofilm formation was responsible for the greater biofouling behavior observed in the continuously-fed molasses column. Further research aimed at understanding the physical structure of the biomass resulting from carbon addition is needed. Additionally, the role of fungi in biofouling is not currently understood, and requires additional study.

Finally, dilute E85 significantly impacted the microbial community structure resulting in the production of compounds such as acetate, butanol and methane. E85 was selected for this study because it contained a large fraction of ethanol. This type of fuel, however, can only be used by specially designed flex fuel vehicles. E10 is more widely distributed ethanol-based fuel as it can be used in any vehicle. Future research is needed to understand the potential for methane generation using lower fraction ethanol-based fuels. Greater understanding of these microbial processes would allow for engineering of safeguards to address hazards associated with the buildup of explosive gas.

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Appendix A. Additional information for Chapter 3

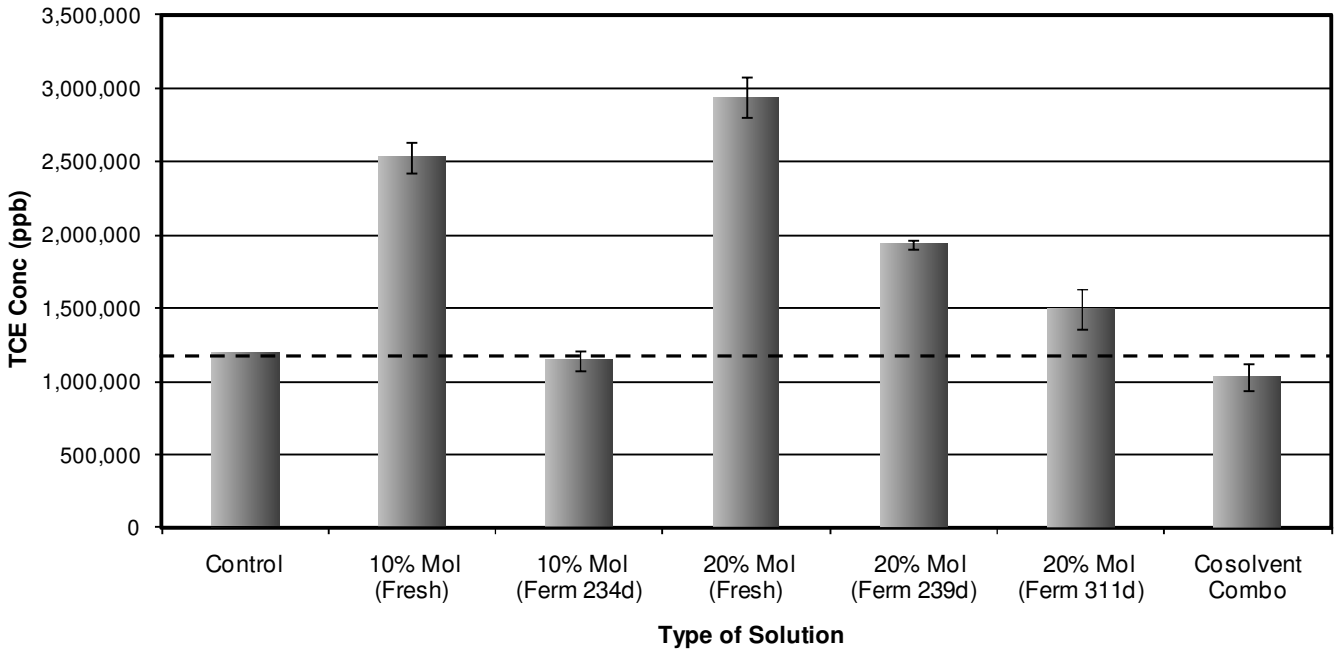

Figure A.1 Results of the NAPL partitioning experiment including the evaluation of a combination of low levels of cosolvents. Error bars represent standard deviation of triplicate runs. The dashed line represents TCE solubility (1.1 g/L).

Appendix B. Additional information for Chapter 4

Aligned sequence for methanogen qPCR standard. Sequence is most closely related to *Methanosarcina sp.* (99% identity, Accession# AY780570.1) based on NCBI's BLAST Database.

TGATCCTGCCAGAGGTTACTGCTATCGGTGTTCGCTAAGCCATGCGAGTCATATGTTCTTCGTG
AACATGGCGTACTGCTCAGTAACACGTGGATAACCTGCCCTTTGGGTCCGGCATAACCCCGGG
AAACTGGGGATAATTCCGGATAACGCATATATGCTGGAATGCTTTATGCGTAAAATGGATTCG
TCTGCCCAAGGATGGGTCTGCAGCCTATCAGGTAGTAGTGGGTGTAATGTACCTACTAGCCAA
CAACGGGTACGGGTTGTGAGAGCAAGAGCCCGGAGATGGATTCTGAGACATGAATCCAGGCC
CTACGGGGCGCAGCAGGCGCGAAAACTTTACAATGCGGGAAACCGTGATGAGGGGACACCGA
GTGCCAGCATCATATGCTGGCTGTCCGGGTGTGTAAAATACACCCGTTAGCAAGGGCCGGGCA
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CGGGGGATACTGCCAGGCTTGGAACCGGGAGAGGTAAGAGGTACTACAGGGGTAGGAGTGA
AATCTTGTAATCCCTGTGGGACCACCTGTGGCGAAGGCGTCTTACCAGAACGGGTTCGACGGT
GAGGGACGAAAGCTGGGGGCGCGAACCGGATTAGATACCCGGGTAGTCCCAGCCGTAAACGA
TGCTCGCTAGGTGTCAGGCATGGCGCGACCGTGTCTGGTGCCGCAGGGAAGCCGTGAAGCGA
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ACGGGTGGAGCCTGCGGTTTAATTGGA

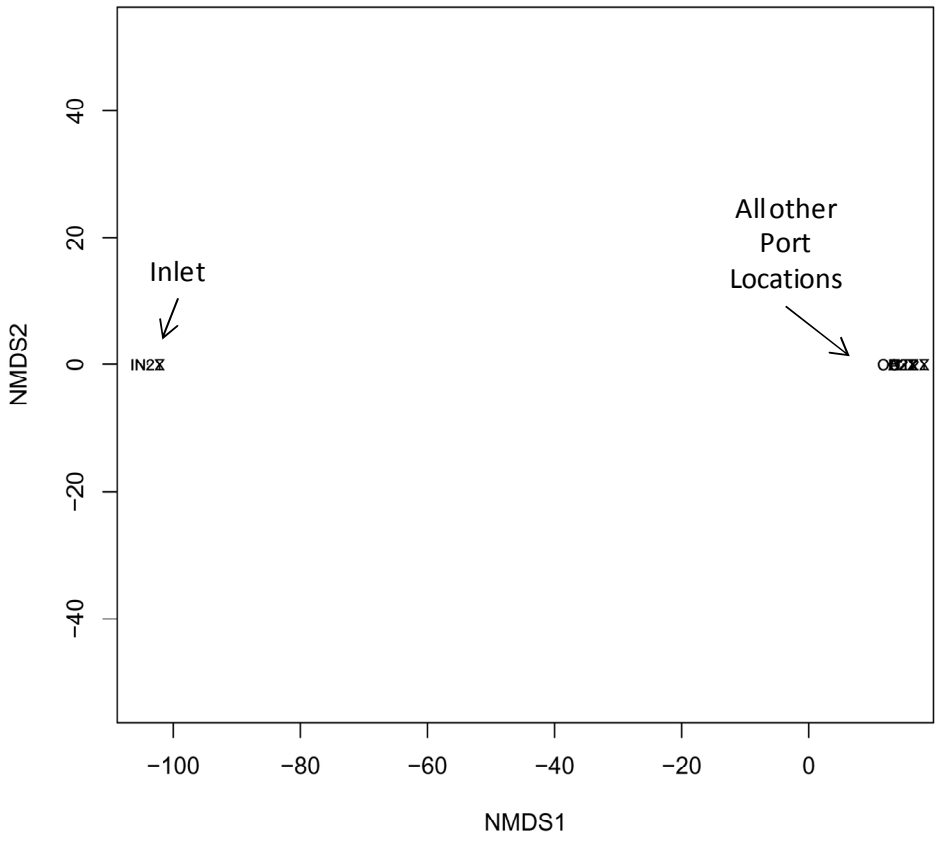


Figure B.1. Results of non-metric multidimensional scaling analysis for *Eubacteria* community structure (Stress = 0.008) in the control column using Bray-Curtis distance measure. Inlet ARISA data used within analysis. Note scale and position of triplicate inlet communities (denoted by IN2X, IN2Y, or IN2Z) with respect to remaining column locations.

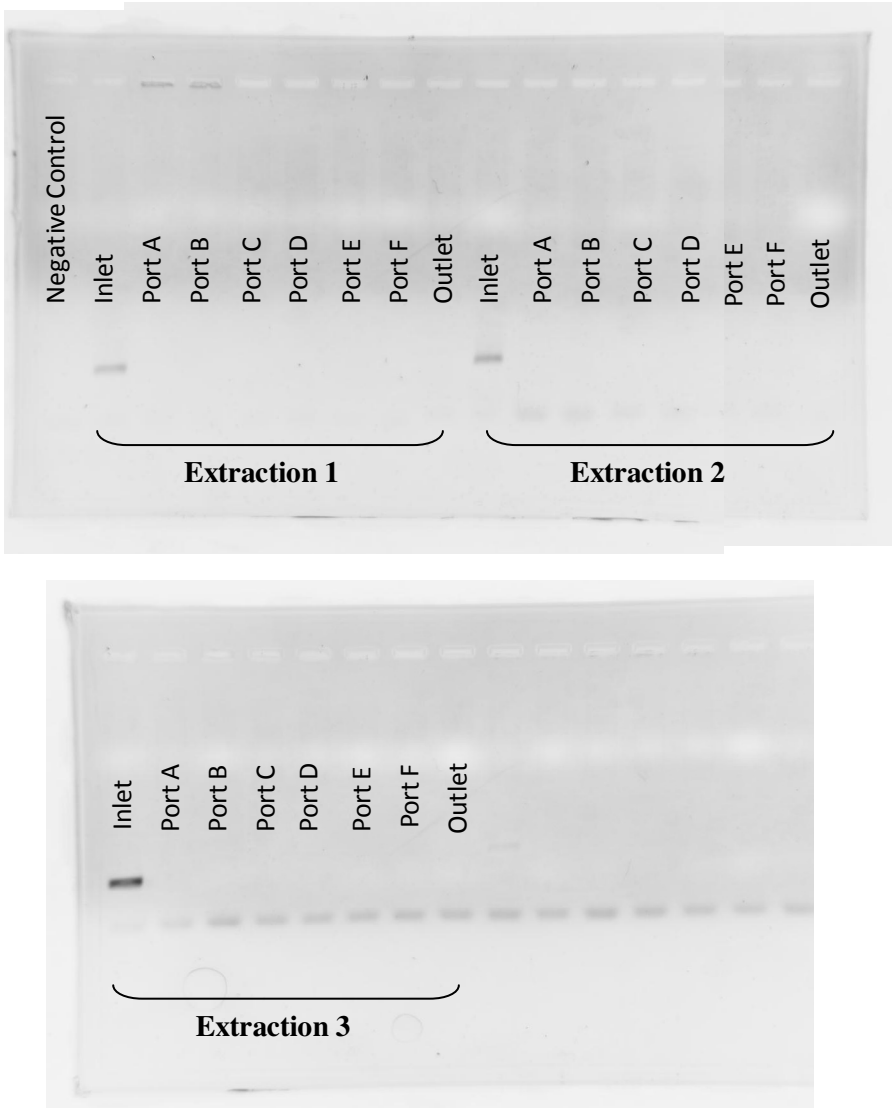


Figure B.2 Results of fungal ARISA from the continuously-fed molasses column (resolved on 1% agarose gel).

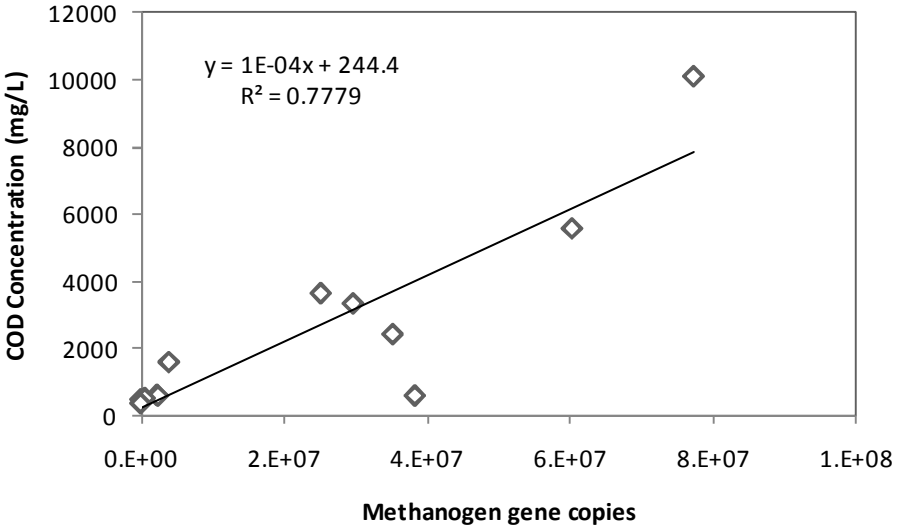


Figure B.3 Correlation between methanogen gene copies and COD concentrations in molasses fed columns.

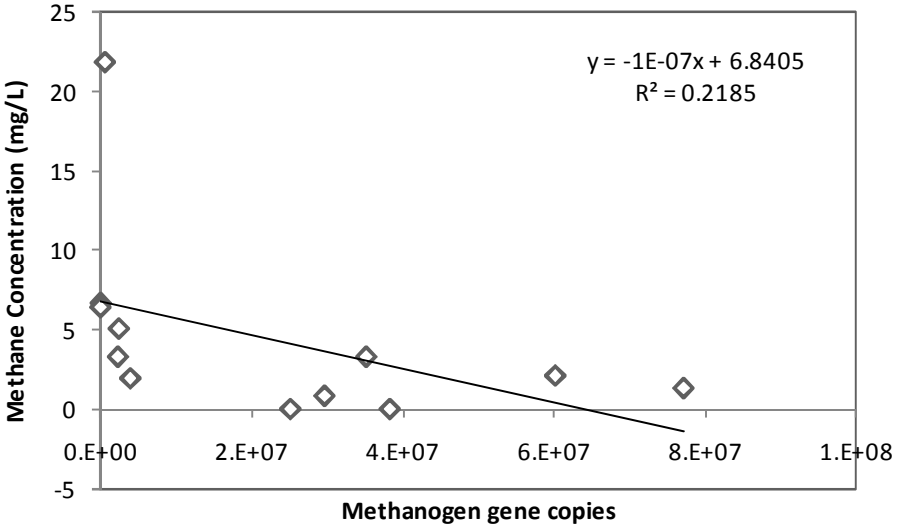


Figure B.4 Correlation between methanogen gene copies and methane concentrations in molasses fed columns. As shown, no correlation existed likely as a result of allowing off-gassing to occur during column operation.

Two bromide tracer studies were performed on the columns. The first tracer test was conducted after column construction, but prior to carbon addition, and the second tracer test was performed 123 days after carbon was introduced. The tracer test consisted of a step input of bromide (0.5 to 1.0 g/L) solution to the columns over three days. Samples were collected from selected sample ports and analyzed by ion chromatography. Mean residence time for the bromide tracer was determined using temporal first moment analysis (Das and Kluitenberg, 1996). Linear velocity was calculated by dividing the distance to the sample port by the mean residence time.

Bromide analysis was performed using a Metrohm 761 Compact Ion Chromatograph (Metrohm US Inc., Riverview, TX) equipped with a Metrosep A Supp5 column. A sample volume of 1.4 mL was injected using an eluent solution consisting of 3.2 mM Na_2CO_3, and 1.0 mM $NaHCO_3$ at a flowrate of 0.7 mL/min (Schnobrich et al. 2007). The regenerant solution consisted of 100 mM H_2SO_4.

Bromide data collected from Port A, located closest to the influent, was used for moment analysis to determine the retention time of the center of mass from the tracer breakthrough curves (Figures B.5 through B.7). Results are summarized in Table B.1. The calculated residence times for the carbon-fed columns were normalized to that of the control column, with the change in normalized residence time reported as the relative percent difference from the initial tracer study. As shown, a 20% decrease in the continuously-fed and 39% decrease in the pulsed-fed column residence times were observed. The observed residence time decrease corresponded to 25% and 65% increases in linear velocity for the continuously-fed and pulsed-fed columns, respectively.

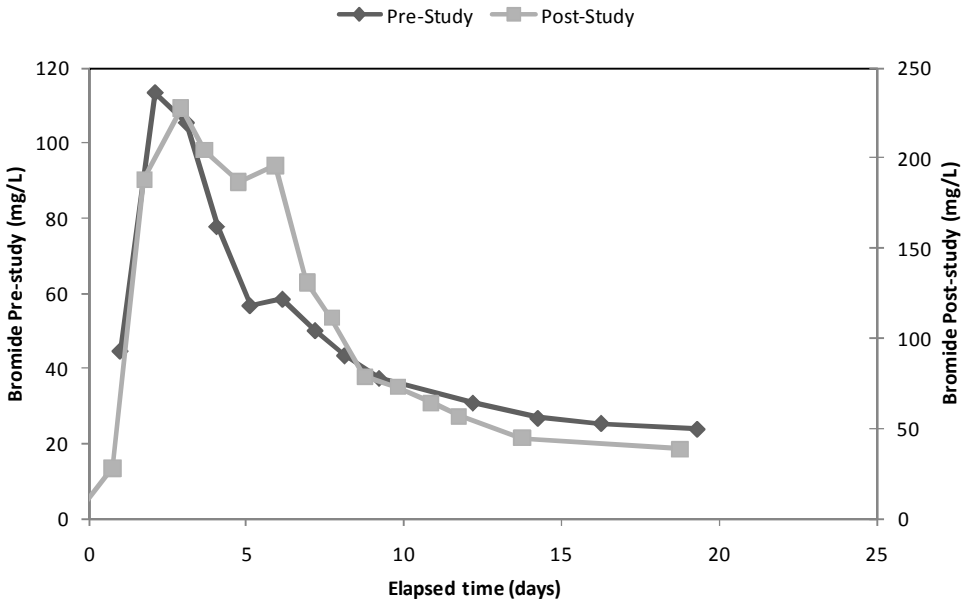


Figure B.5 Breakthrough curves for bromide in Port A of the control column during the pre- and post- carbon addition tracer studies.

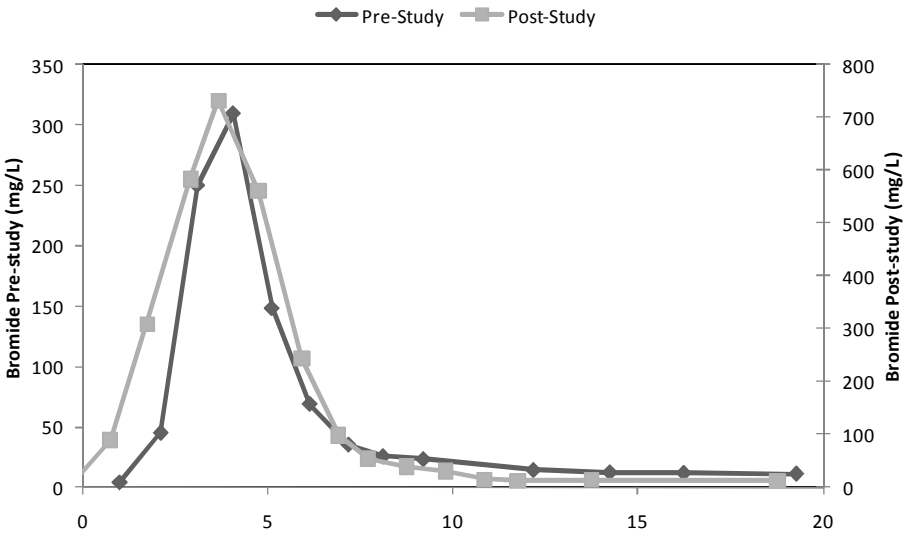


Figure B.6 Breakthrough curves for bromide in Port A of the continuously-fed column during the pre- and post- carbon addition tracer studies.

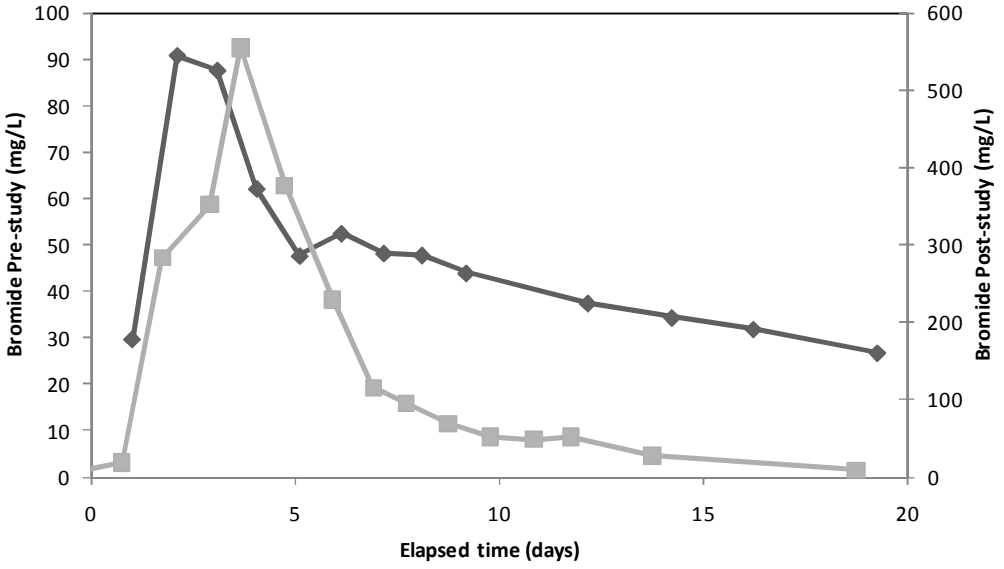


Figure B.7 Breakthrough curves for bromide in Port A of the pulsed-fed column during the pre- and post- carbon addition tracer studies.

Table B.1. Results of temporal first moment analysis for Port A of pre- and post-carbon addition tracer studies.

		Control Column	Continuously-fed Column	Pulsed Column	Normalized Continuous	Normalized Pulsed
Pre-carbon Tracer	t* (d)[a]	6.14	4.12	7.04	0.67	1.15
	v* (in/d)[b]	1.14	1.70	0.99	1.49	0.87
Post-carbon Tracer	t* (d)	5.39	2.90	3.76	0.54	0.70
	v* (in/d)	1.30	2.41	1.86	1.86	1.43
	Δt* (d)	---	---		0.37	0.45
Relative difference	%	---	---		20%	39%
	Δv* (in/d)	---	---		0.37	0.56
Relative difference	%	---	---		25%	65%

a Center of mass mean residence time, in days
b Linear velocity calculated from mean residence time, in inches per day

Appendix C. Additional information for Chapter 5

Bromide data collected from Port A, located closest to the influent, was used for moment analysis to determine the retention time of the center of mass from the tracer breakthrough curves (Figure C.1). Results are summarized in Table C.1. The calculated residence times for the E85-fed column was normalized to that of the control column, with the change in normalized residence time reported as the relative percent difference from the initial tracer study. As shown, a 28% decrease in the E85-fed column residence time was observed. The observed residence time decrease corresponded to a 38% increase in linear velocity for the E85-fed column.

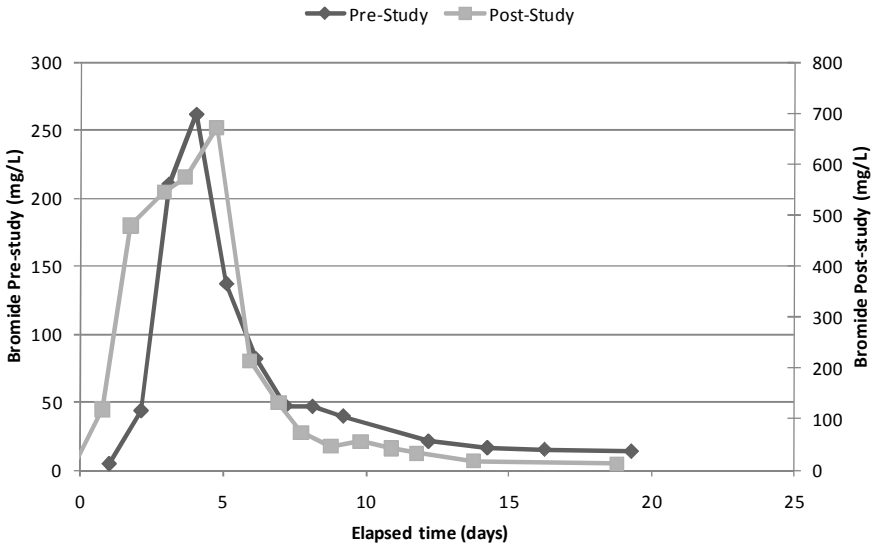


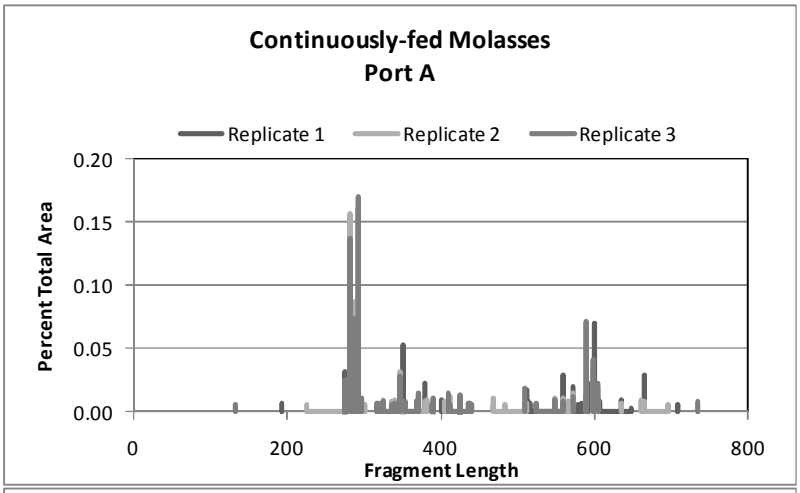
Figure C.1 Breakthrough curves for bromide in Port A of the E85-fed column during the pre- and post- E85 tracer studies.

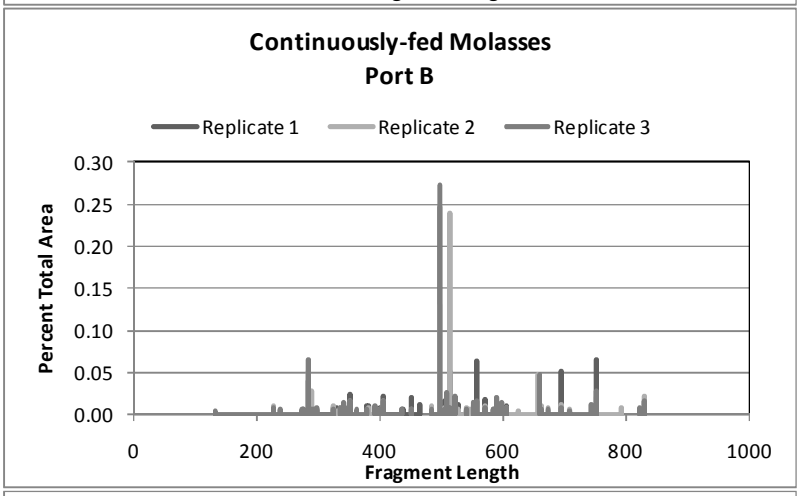
Table C.1 Results of temporal first moment analysis for Port A of pre- and post-E85 addition tracer studies.

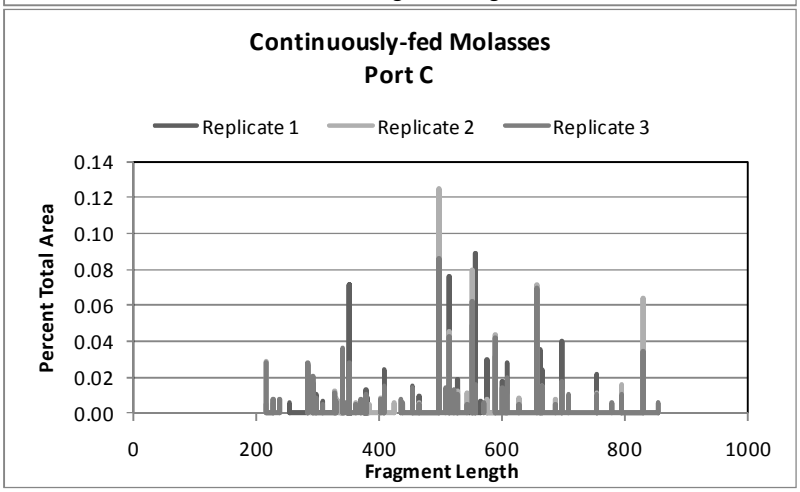
		Control Column	E85-fed Column	Normalized E85 Results
Pre-carbon Tracer	t* (d)[a]	6.14	4.76	0.77
	v* (in/d)[b]	1.14	1.47	1.29
Post-carbon Tracer	t* (d)	5.39	3.02	0.56
	v* (in/d)	1.30	2.32	1.78
	Δt* (d)	---	---	0.21
Relative difference	%	---	---	28%
	Δv* (in/d)	---	---	0.49
Relative difference	%	---	---	38%

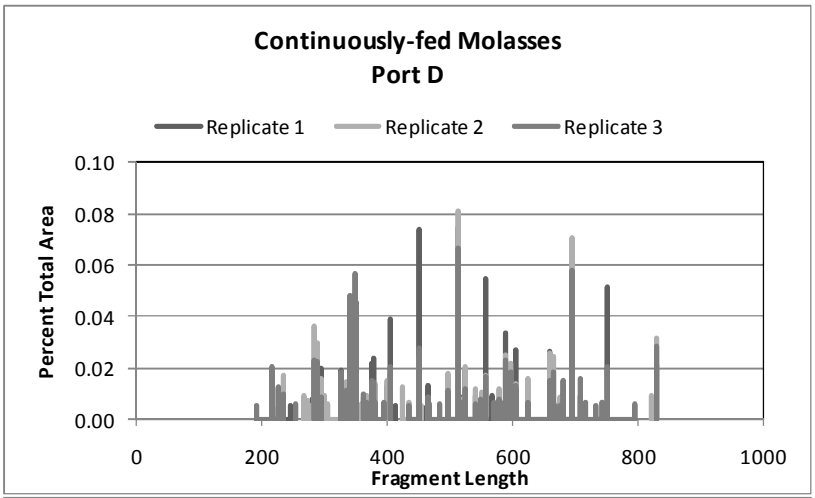
a Center of mass mean residence time, in days
b Linear velocity calculated from mean residence time, in inches per day

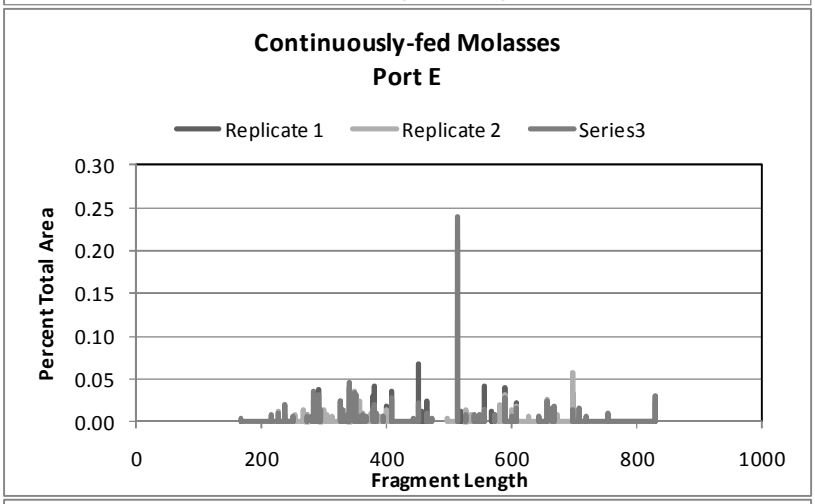
Appendix D. ARISA Data: Graphed (Continuously-fed Molasses Column) and Raw Aligned (All Columns)








Continuously-fed Molasses
Port D
Replicate 1
Replicate 2
Replicate 3
Percent Total Area
0.10
0.08
0.06
0.04
0.02
0.00
0
200
400
600
800
1000
Fragment Length


Continuously-fed Molasses
Port E
Replicate 1
Replicate 2
Series3
Percent Total Area
0.30
0.25
0.20
0.15
0.10
0.05
0.00
0
200
400
600
800
1000
Fragment Length

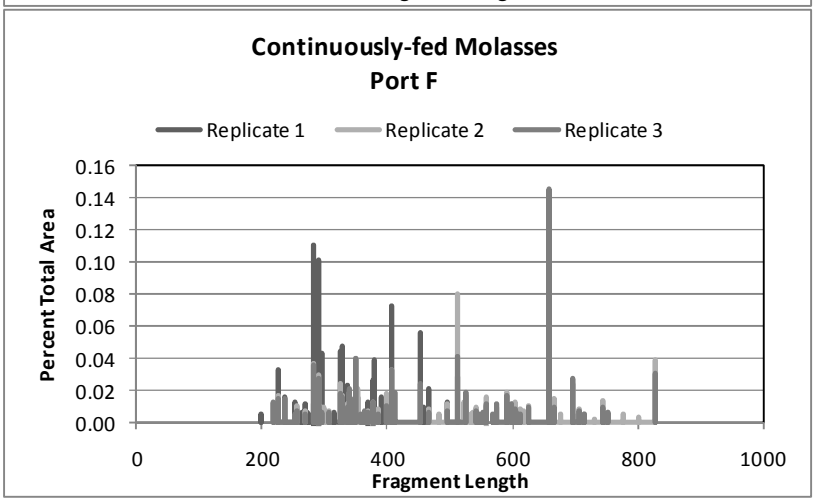

Continuously-fed Molasses
Port F
Replicate 1
Replicate 2
Replicate 3
Percent Total Area
0.16
0.14
0.12
0.10
0.08
0.06
0.04
0.02
0.00
0
200
400
600
800
1000
Fragment Length

Table D.1 ***Eubacteria*** **Automated Ribosomal Intergenic Spacer Analysis**
Aligned E85-fed Column Data

Fragment Length	A-1	A-2	A-3	B-1	B-2	B-3	C-1	C-2
132	0.00%	0.00%	0.58%	0.00%	0.00%	0.50%	0.00%	0.00%
136	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
167	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
194	0.66%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
200	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
217	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.54%	2.91%
222	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
225	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
227	0.00%	0.59%	0.00%	0.54%	0.98%	0.92%	0.57%	0.80%
237	0.00%	0.00%	0.00%	0.00%	0.00%	0.69%	0.00%	0.00%
246	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
254	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.62%	0.00%
256	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
265	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
266	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
267	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
269	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
273	0.00%	0.00%	0.00%	0.00%	0.00%	0.59%	0.00%	0.00%
275	3.26%	1.65%	2.53%	0.71%	0.00%	0.55%	0.00%	0.00%
278	0.00%	0.00%	0.89%	0.00%	0.00%	0.00%	0.00%	0.00%
283	11.19%	15.66%	13.75%	4.08%	5.16%	6.59%	2.68%	2.20%
285	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
290	6.19%	8.73%	7.39%	0.00%	2.78%	0.55%	0.00%	1.63%
292	0.00%	0.00%	0.00%	0.69%	0.00%	0.55%	1.74%	0.55%
295	16.03%	12.26%	16.98%	0.00%	0.00%	0.00%	0.00%	0.00%
296	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	1.07%	0.75%
298	0.69%	0.00%	1.12%	0.61%	0.00%	0.81%	0.00%	0.00%
299	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
302	0.00%	0.53%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
304	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
308	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.65%	0.00%
316	0.00%	0.00%	0.63%	0.00%	0.00%	0.00%	0.00%	0.00%
319	0.52%	0.00%	0.75%	0.00%	0.00%	0.00%	0.00%	0.00%
326	0.00%	0.98%	0.79%	0.85%	1.07%	0.68%	0.79%	1.26%
329	0.00%	0.00%	0.00%	0.77%	0.51%	0.00%	0.82%	0.84%
337	0.00%	0.77%	0.58%	0.54%	0.87%	0.77%	0.00%	0.67%
340	0.00%	0.00%	0.00%	0.65%	0.54%	1.31%	1.90%	1.28%
341	0.00%	0.96%	0.73%	1.00%	0.65%	0.94%	0.00%	1.04%
346	0.00%	0.00%	0.00%	0.53%	0.00%	0.67%	0.00%	0.00%
348	2.73%	3.21%	2.77%	0.00%	0.00%	0.00%	0.00%	0.00%
351	2.15%	0.86%	0.00%	0.94%	1.53%	1.47%	7.18%	1.80%
352	5.30%	0.64%	0.00%	2.45%	0.65%	1.65%	7.10%	1.40%
354	0.00%	0.67%	0.80%	0.00%	0.00%	0.00%	0.00%	0.00%
358	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
363	0.00%	0.00%	0.00%	0.00%	0.00%	0.54%	0.00%	0.60%
368	0.85%	0.83%	0.80%	0.00%	0.00%	0.00%	0.54%	0.00%
370	1.33%	1.15%	1.48%	0.00%	0.00%	0.00%	0.00%	0.00%
373	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
377	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.59%
379	2.23%	0.82%	0.00%	0.98%	0.00%	0.72%	1.35%	0.00%
381	0.00%	0.96%	0.00%	1.02%	0.90%	0.00%	0.88%	0.00%
384	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.50%
389	0.00%	0.00%	0.59%	0.00%	0.00%	0.00%	0.00%	0.00%
390	0.75%	0.64%	1.04%	0.00%	0.00%	0.00%	0.00%	0.00%
392	0.00%	0.73%	0.00%	0.00%	0.71%	1.11%	0.00%	0.00%
395	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
400	0.00%	0.00%	0.00%	0.73%	0.69%	0.54%	0.00%	0.00%
403	0.93%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.83%
407	0.00%	0.79%	0.00%	2.27%	1.55%	1.52%	0.00%	0.00%
409	1.15%	0.00%	1.53%	0.00%	0.00%	0.00%	2.42%	0.84%
411	0.00%	1.19%	0.60%	0.00%	0.00%	0.00%	0.00%	0.00%
415	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
424	0.66%	0.64%	0.58%	0.00%	0.00%	0.00%	0.00%	0.00%
425	1.14%	0.00%	1.38%	0.00%	0.00%	0.00%	0.00%	0.64%
436	0.00%	0.00%	0.65%	0.59%	0.00%	0.61%	0.00%	0.00%
438	0.61%	0.66%	0.70%	0.60%	0.00%	0.00%	0.00%	0.53%
443	0.00%	0.00%	0.58%	0.00%	0.00%	0.00%	0.00%	0.00%
453	0.00%	0.00%	0.00%	2.10%	0.71%	0.67%	1.47%	0.88%
454	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
458	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
461	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%

Table D.1 ***Eubacteria*** **Automated Ribosomal Intergenic Spacer Analysis**
Aligned E85-fed Column Data

Fragment Length	C-3	D-1	D-2	D-3	E-1	E-2	E-3	F-1	F-2	F-3
132	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
136	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
167	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.51%	0.00%	0.00%	0.00%
194	0.00%	0.00%	0.00%	0.52%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
200	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.55%	0.00%	0.00%
217	2.80%	1.58%	2.06%	2.03%	0.00%	0.00%	0.76%	0.00%	0.00%	1.27%
222	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
225	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
227	0.78%	0.50%	1.21%	1.26%	0.00%	1.16%	0.97%	3.27%	1.75%	1.52%
237	0.80%	0.88%	1.70%	0.98%	0.75%	2.05%	1.94%	1.62%	1.32%	1.53%
246	0.00%	0.53%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
254	0.00%	0.00%	0.00%	0.00%	0.00%	0.61%	0.55%	1.29%	0.00%	0.53%
256	0.00%	0.00%	0.00%	0.61%	0.00%	0.88%	0.00%	0.88%	1.07%	0.76%
265	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.68%	0.00%	0.00%
266	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.70%	0.00%	0.00%
267	0.00%	0.00%	0.00%	0.00%	0.00%	1.41%	0.00%	0.00%	0.00%	0.00%
269	0.00%	0.00%	0.91%	0.00%	0.00%	0.00%	0.00%	1.22%	0.78%	0.54%
273	0.00%	0.55%	0.72%	0.00%	0.81%	0.90%	0.69%	0.70%	0.00%	0.00%
275	0.00%	0.50%	0.76%	0.00%	0.00%	0.51%	0.00%	0.58%	0.00%	0.00%
278	0.00%	0.00%	0.50%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
283	2.78%	0.83%	0.00%	0.00%	0.68%	0.00%	0.53%	1.14%	0.00%	0.00%
285	0.00%	2.10%	3.61%	2.29%	2.55%	3.46%	3.66%	11.11%	3.66%	3.59%
290	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.80%	2.99%	2.74%
292	2.10%	2.25%	2.99%	2.22%	3.72%	2.49%	3.24%	10.13%	0.78%	1.06%
295	0.00%	0.00%	0.00%	0.86%	0.91%	0.79%	1.46%	4.35%	0.96%	0.62%
296	0.89%	1.96%	1.59%	0.85%	0.69%	0.79%	0.00%	0.00%	0.00%	0.00%
298	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.90%	1.01%	0.00%
299	0.00%	0.00%	0.00%	0.00%	0.80%	1.40%	0.00%	0.00%	0.00%	0.00%
302	0.00%	0.00%	0.91%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
304	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.65%	0.00%
308	0.51%	0.00%	0.60%	0.00%	0.00%	0.83%	0.00%	0.61%	0.71%	0.68%
316	0.00%	0.00%	0.00%	0.00%	0.00%	0.57%	0.00%	0.66%	0.00%	0.00%
319	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
326	1.12%	1.92%	1.59%	1.87%	2.43%	2.07%	2.39%	4.50%	2.42%	1.83%
329	0.69%	1.50%	0.66%	1.03%	1.41%	1.00%	1.42%	4.72%	1.68%	1.57%
337	0.00%	0.73%	1.47%	1.13%	0.83%	1.50%	0.90%	2.37%	1.20%	0.99%
340	3.64%	2.49%	2.29%	4.84%	3.90%	1.94%	4.57%	1.88%	1.96%	2.17%
341	0.00%	0.00%	0.00%	0.00%	0.00%	1.61%	0.00%	0.00%	1.40%	1.46%
346	0.57%	1.22%	0.93%	1.16%	0.92%	1.02%	0.00%	0.00%	0.68%	0.67%
348	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	1.24%	0.00%	0.00%
351	2.10%	1.92%	4.60%	5.69%	2.74%	3.64%	3.46%	0.78%	4.01%	4.03%
352	2.79%	2.81%	2.87%	4.55%	3.14%	2.38%	3.24%	1.63%	2.11%	0.00%
354	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
358	0.00%	0.00%	0.58%	0.00%	0.69%	2.49%	0.56%	0.00%	0.00%	0.60%
363	0.54%	0.52%	0.88%	1.00%	0.00%	1.00%	0.88%	0.50%	0.66%	0.78%
368	0.76%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	1.25%	0.73%	0.70%
370	0.00%	0.51%	0.95%	0.66%	0.00%	0.00%	0.61%	0.51%	0.00%	0.00%
373	0.00%	0.00%	0.00%	0.00%	0.00%	0.62%	0.00%	0.00%	0.00%	0.00%
377	0.72%	2.21%	1.18%	1.52%	0.00%	1.08%	0.00%	0.00%	1.32%	1.03%
379	1.08%	2.37%	0.77%	1.47%	2.97%	1.00%	1.59%	2.68%	1.16%	1.33%
381	0.00%	0.00%	0.00%	0.00%	4.16%	0.00%	2.10%	3.91%	0.00%	0.00%
384	0.00%	0.00%	1.38%	0.67%	0.59%	0.00%	1.11%	0.77%	0.81%	0.59%
389	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
390	0.00%	0.00%	0.00%	0.00%	0.00%	0.67%	0.00%	0.00%	0.00%	0.00%
392	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	1.58%	0.00%	0.00%
395	0.00%	0.57%	0.00%	0.65%	0.59%	0.00%	0.60%	0.00%	0.00%	0.00%
400	0.00%	1.30%	1.50%	0.00%	1.91%	1.34%	0.00%	1.26%	1.80%	1.11%
403	0.78%	0.00%	0.81%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
407	0.00%	3.90%	1.79%	2.05%	3.52%	2.35%	2.73%	7.26%	3.33%	3.32%
409	1.54%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
411	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	1.89%
415	0.00%	0.54%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
424	0.00%	0.00%	1.24%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
425	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
436	0.78%	0.00%	0.67%	0.57%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
438	0.62%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
443	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.51%	0.00%	0.00%	0.00%
453	1.38%	7.35%	2.43%	2.79%	6.77%	1.94%	2.16%	5.61%	1.93%	2.45%
454	0.00%	0.00%	0.55%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
458	0.00%	0.49%	0.00%	0.00%	1.28%	0.00%	0.00%	0.99%	0.00%	0.00%
461	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%

Table D.1 ***Eubacteria*** **Automated Ribosomal Intergenic Spacer Analysis**
Aligned E85-fed Column Data

Fragment Length	A-1	A-2	A-3	B-1	B-2	B-3	C-1	C-2
465	0.00%	0.00%	0.00%	1.19%	0.00%	0.00%	1.01%	0.62%
468	0.53%	1.12%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
474	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
480	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
484	0.00%	0.53%	0.00%	0.00%	1.01%	0.66%	0.00%	0.00%
486	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
498	0.00%	0.00%	0.00%	24.76%	13.01%	27.21%	3.70%	12.51%
505	0.00%	0.00%	0.00%	0.00%	0.68%	0.64%	0.00%	0.00%
507	0.00%	0.00%	0.00%	1.64%	0.79%	0.00%	0.00%	0.00%
509	0.00%	0.73%	1.90%	0.00%	1.54%	2.67%	0.00%	0.00%
513	1.79%	0.69%	0.00%	5.20%	23.88%	0.74%	7.64%	4.59%
514	0.81%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
515	0.70%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
522	0.00%	0.00%	0.00%	0.00%	0.00%	2.23%	0.00%	0.87%
526	0.57%	0.00%	0.73%	0.00%	0.00%	0.00%	0.00%	0.00%
527	0.00%	0.00%	0.00%	1.21%	0.67%	0.00%	1.89%	1.21%
533	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
536	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
541	0.00%	0.00%	0.00%	0.51%	0.76%	0.69%	0.00%	1.14%
548	0.82%	1.03%	0.99%	0.00%	0.69%	0.00%	0.77%	0.00%
553	0.00%	0.00%	0.00%	0.93%	0.58%	1.39%	4.92%	7.97%
557	0.00%	0.00%	0.00%	6.29%	0.74%	1.66%	8.92%	0.90%
558	2.97%	1.11%	0.88%	0.00%	0.00%	0.00%	0.00%	0.00%
565	0.85%	0.86%	0.00%	0.00%	0.00%	0.00%	0.67%	0.00%
569	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
571	1.97%	1.48%	1.24%	1.81%	1.11%	0.88%	0.00%	0.00%
575	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	2.96%	0.80%
579	0.59%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
582	0.71%	0.00%	0.00%	0.63%	0.00%	0.66%	0.00%	0.00%
591	1.36%	1.01%	7.10%	1.44%	1.65%	1.92%	3.21%	4.35%
592	2.26%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
599	0.00%	3.52%	4.08%	0.63%	0.83%	1.51%	1.80%	1.21%
601	6.98%	1.71%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
605	1.27%	1.93%	2.26%	0.00%	0.78%	0.66%	1.09%	0.70%
607	0.85%	0.00%	0.00%	0.95%	0.56%	0.83%	2.78%	1.08%
611	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
618	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
625	0.00%	0.00%	0.00%	0.00%	0.51%	0.00%	0.00%	0.85%
630	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
637	0.93%	0.72%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
642	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
647	0.25%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
656	0.00%	0.00%	0.00%	0.00%	4.72%	0.00%	0.00%	7.11%
659	0.00%	0.97%	0.00%	3.03%	0.50%	4.73%	0.00%	0.00%
662	0.00%	0.00%	0.00%	0.65%	1.05%	0.59%	3.55%	0.84%
665	2.87%	0.79%	0.00%	0.00%	0.00%	0.00%	2.42%	1.48%
669	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
674	0.00%	0.00%	0.00%	0.00%	0.80%	0.53%	0.00%	0.00%
678	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
684	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.79%
696	0.00%	0.59%	0.00%	5.08%	1.16%	0.82%	3.97%	1.32%
703	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
708	0.61%	0.00%	0.00%	0.00%	0.57%	0.52%	0.00%	0.00%
717	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
733	0.00%	0.65%	0.80%	0.00%	0.00%	0.00%	0.00%	0.00%
738	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
745	0.00%	0.00%	0.00%	0.00%	0.00%	1.15%	0.00%	0.00%
749	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
753	0.00%	0.00%	0.00%	6.60%	1.92%	2.72%	2.16%	1.19%
778	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
793	0.00%	0.00%	0.00%	0.00%	0.77%	0.00%	0.67%	1.63%
800	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
823	0.00%	0.00%	0.00%	0.00%	0.00%	0.88%	0.00%	0.00%
829	0.00%	0.00%	0.00%	1.70%	2.30%	1.70%	3.27%	6.40%
855	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.55%

Table D.1 ***Eubacteria*** **Automated Ribosomal Intergenic Spacer Analysis**
Aligned E85-fed Column Data

Fragment Length	C-3	D-1	D-2	D-3	E-1	E-2	E-3	F-1	F-2	F-3
465	0.51%	1.30%	0.84%	0.90%	2.38%	0.86%	1.03%	2.10%	0.87%	0.68%
468	0.00%	0.00%	0.00%	0.57%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
474	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.50%	0.00%	0.00%	0.00%
480	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
484	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.53%	0.00%
486	0.00%	0.00%	0.00%	0.58%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
498	8.57%	1.46%	1.79%	1.14%	0.00%	0.50%	0.00%	1.26%	1.19%	0.71%
505	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
507	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
509	1.44%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
513	4.24%	7.45%	8.08%	6.66%	21.27%	11.81%	23.98%	2.91%	8.00%	4.12%
514	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
515	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
522	1.36%	0.85%	0.82%	0.68%	1.17%	0.00%	0.00%	0.55%	1.32%	0.00%
526	0.00%	1.95%	2.06%	1.19%	1.15%	1.37%	0.00%	0.71%	1.67%	1.96%
527	1.05%	0.00%	0.00%	0.00%	0.00%	1.18%	0.83%	0.00%	0.00%	0.00%
533	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.50%	0.00%
536	0.00%	0.00%	0.00%	0.00%	0.00%	0.74%	0.00%	0.00%	0.62%	0.00%
541	0.52%	0.92%	1.21%	0.62%	0.72%	0.80%	0.89%	0.00%	1.01%	0.77%
548	0.00%	0.00%	0.00%	0.82%	0.00%	0.00%	0.89%	0.00%	0.00%	0.50%
553	6.21%	0.74%	1.09%	0.70%	0.00%	0.83%	0.63%	0.00%	0.60%	0.60%
557	1.62%	5.47%	1.75%	1.63%	4.15%	0.67%	1.07%	1.05%	0.89%	1.14%
558	0.00%	0.00%	0.00%	0.00%	0.00%	1.38%	1.39%	0.00%	1.59%	0.00%
565	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
569	0.00%	0.92%	0.00%	0.00%	1.29%	0.00%	0.00%	0.00%	0.00%	0.00%
571	0.59%	0.00%	0.00%	0.64%	0.00%	0.00%	0.00%	0.00%	0.00%	0.52%
575	0.00%	0.60%	0.00%	0.00%	0.79%	0.71%	0.78%	0.00%	0.93%	1.21%
579	0.00%	0.51%	1.19%	0.77%	0.00%	0.57%	0.00%	0.00%	0.00%	0.00%
582	0.00%	0.64%	0.00%	0.66%	0.00%	1.94%	0.00%	0.00%	0.00%	0.00%
591	4.21%	3.34%	2.54%	2.32%	4.01%	3.18%	2.82%	0.00%	1.87%	1.72%
592	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
599	1.38%	1.38%	2.16%	1.83%	1.13%	1.52%	0.72%	0.55%	1.18%	1.23%
601	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
605	1.08%	0.90%	0.00%	1.17%	0.74%	0.00%	0.71%	0.00%	1.26%	0.89%
607	2.00%	2.72%	1.41%	1.34%	2.29%	1.89%	1.63%	0.00%	0.59%	0.65%
611	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.84%	0.50%
618	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.77%	0.00%
625	0.51%	0.00%	1.60%	0.69%	0.00%	0.68%	0.00%	0.00%	1.06%	0.98%
630	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
637	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
642	0.00%	0.00%	0.00%	0.00%	0.00%	0.61%	0.64%	0.00%	0.00%	0.00%
647	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
656	6.99%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
659	0.00%	2.66%	2.57%	1.53%	2.36%	2.63%	2.39%	1.16%	3.37%	14.54%
662	1.11%	1.19%	0.93%	0.83%	0.00%	0.77%	0.00%	0.00%	0.77%	0.52%
665	1.49%	0.66%	2.41%	1.85%	1.23%	1.11%	1.61%	0.00%	1.45%	0.99%
669	0.00%	0.00%	0.00%	0.55%	0.00%	1.18%	1.73%	0.00%	0.00%	0.00%
674	0.00%	0.62%	0.85%	0.51%	0.00%	0.80%	0.00%	0.00%	0.00%	0.00%
678	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.52%	0.00%
684	0.53%	1.02%	0.79%	1.53%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
696	1.79%	4.65%	7.05%	5.81%	3.40%	5.77%	1.40%	0.58%	2.47%	2.75%
703	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
708	1.05%	0.92%	0.87%	1.61%	0.00%	0.76%	1.72%	0.00%	0.82%	0.81%
717	0.00%	0.00%	0.00%	0.66%	0.00%	0.00%	0.71%	0.00%	0.60%	0.50%
733	0.00%	0.00%	0.00%	0.52%	0.00%	0.00%	0.00%	0.00%	0.22%	0.00%
738	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
745	0.00%	0.00%	0.69%	0.69%	0.00%	0.00%	0.00%	0.00%	1.41%	0.96%
749	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
753	1.01%	5.17%	1.59%	2.03%	0.00%	0.80%	1.10%	0.00%	0.67%	0.67%
778	0.55%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.51%	0.00%
793	1.01%	0.00%	0.00%	0.60%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
800	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.29%	0.00%
823	0.00%	0.00%	0.93%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
829	3.45%	2.09%	3.20%	2.81%	2.25%	3.02%	3.09%	0.00%	3.95%	3.10%
855	0.63%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%

Table D.2 ***Eubacteria*** **Automated Ribosomal Intergenic Spacer Analysis Aligned Control Column Data**

Fragment Length	A-1	A-2	A-3	B-1	B-2	B-3	C-1	C-2	C-3
137	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
154	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
165	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
178	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
192	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
217	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
228	0.00%	2.75%	0.00%	2.63%	4.11%	1.29%	3.27%	2.97%	2.10%
238	1.91%	4.24%	1.42%	2.88%	0.00%	0.00%	3.60%	1.74%	2.90%
246	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
254	0.00%	0.00%	0.00%	0.87%	0.00%	0.99%	0.00%	0.79%	0.92%
256	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	2.73%	0.90%	0.00%
270	2.66%	0.00%	0.00%	0.00%	3.43%	0.00%	0.00%	1.02%	0.87%
272	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
276	2.00%	0.00%	0.75%	1.20%	0.00%	0.00%	0.00%	0.84%	0.53%
282	3.65%	2.15%	2.09%	0.00%	0.00%	1.18%	9.38%	1.30%	1.05%
284	1.79%	4.14%	3.16%	5.04%	7.10%	3.44%	0.00%	6.91%	6.02%
290	0.00%	3.50%	0.00%	0.00%	0.00%	0.00%	0.00%	1.22%	0.79%
291	2.73%	4.15%	3.38%	2.43%	7.54%	3.24%	0.00%	0.00%	0.00%
292	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	5.16%	4.83%	4.16%
295	1.43%	2.24%	1.28%	1.76%	0.00%	1.70%	4.02%	3.20%	4.24%
297	1.53%	1.65%	2.38%	0.00%	0.00%	1.44%	1.70%	0.00%	0.00%
299	0.00%	1.70%	1.17%	0.00%	0.00%	2.93%	0.00%	0.61%	0.53%
301	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
304	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.57%	0.84%
308	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
316	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.58%	0.93%
320	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
327	2.44%	2.70%	4.01%	2.13%	4.34%	2.85%	2.64%	2.63%	2.59%
329	1.25%	2.32%	2.13%	1.64%	3.46%	2.31%	2.80%	3.07%	2.63%
331	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
334	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
337	1.83%	0.00%	1.03%	1.85%	0.00%	1.83%	2.58%	1.10%	1.39%
339	0.00%	0.00%	0.00%	0.00%	0.00%	0.91%	0.00%	0.00%	0.00%
340	1.36%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.86%	0.62%
341	2.22%	3.54%	1.78%	4.90%	3.17%	1.89%	0.00%	0.81%	0.80%
346	0.00%	0.00%	2.28%	0.00%	0.00%	1.79%	0.00%	0.67%	1.69%
347	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.61%	0.00%
349	6.66%	0.00%	2.53%	0.00%	0.00%	0.00%	6.57%	1.86%	4.54%
351	0.00%	0.00%	0.00%	4.41%	4.68%	3.08%	0.00%	0.00%	0.00%
352	11.59%	7.77%	4.63%	5.22%	4.38%	6.33%	4.07%	3.25%	0.00%
359	0.00%	0.00%	1.87%	0.00%	0.00%	0.00%	0.00%	2.20%	1.51%
363	1.15%	0.00%	0.00%	1.39%	3.99%	1.77%	0.00%	0.00%	0.00%
365	1.18%	2.76%	1.45%	0.00%	0.00%	0.00%	1.33%	1.14%	2.10%
368	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
369	0.00%	0.00%	0.00%	1.22%	0.00%	0.00%	0.00%	0.75%	1.15%
374	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	1.35%
377	1.34%	4.90%	4.57%	1.95%	4.76%	4.40%	1.78%	2.81%	4.08%
378	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	2.36%	2.78%	3.63%
380	4.29%	2.74%	4.14%	2.31%	3.50%	3.83%	0.00%	0.00%	0.00%
382	0.00%	0.00%	0.00%	2.42%	5.90%	1.41%	1.43%	0.74%	0.90%
385	2.32%	1.95%	1.42%	0.00%	0.00%	1.83%	2.22%	1.16%	1.30%
390	0.00%	0.00%	0.95%	1.22%	0.00%	0.00%	0.00%	0.00%	0.59%
391	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
392	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	1.56%	1.35%
400	2.37%	3.06%	2.40%	2.75%	0.00%	2.55%	4.62%	1.17%	2.29%
401	0.00%	0.00%	1.87%	0.00%	0.00%	1.57%	0.00%	1.21%	4.46%
404	0.00%	0.00%	0.00%	0.00%	0.00%	0.62%	0.00%	0.00%	0.00%
406	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
408	6.48%	6.69%	12.83%	7.31%	7.92%	10.71%	10.81%	7.20%	13.40%
421	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
444	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
447	0.00%	0.00%	1.09%	0.00%	0.00%	0.00%	0.00%	0.56%	0.95%
448	4.51%	0.00%	1.58%	0.00%	0.00%	1.69%	0.00%	0.60%	0.00%
453	3.95%	5.99%	7.77%	4.74%	7.10%	4.79%	1.89%	3.86%	2.98%
455	0.00%	0.00%	0.00%	1.47%	0.00%	0.00%	0.00%	0.00%	0.00%

Table D.2 ***Eubacteria*** **Automated Ribosomal Intergenic Spacer Analysis**
Aligned Control Column Data

Fragment Length	D-1	D-2	D-3	E-1	E-2	E-3	F-1	F-2	F-3
137	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.53%	0.00%	0.00%
154	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
165	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
178	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
192	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
217	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
228	2.23%	2.62%	1.78%	3.07%	3.69%	1.97%	2.96%	2.97%	1.85%
238	1.89%	3.03%	2.02%	2.46%	3.00%	2.37%	2.56%	3.16%	3.21%
246	0.65%	0.99%	0.00%	0.93%	0.00%	0.00%	1.06%	1.43%	0.00%
254	1.02%	0.90%	0.94%	1.43%	0.85%	1.20%	0.70%	0.00%	0.00%
256	1.12%	1.18%	0.00%	1.41%	1.29%	0.00%	0.89%	0.00%	0.00%
270	1.23%	1.42%	0.98%	1.06%	0.00%	0.00%	0.00%	0.00%	0.00%
272	0.00%	0.00%	0.45%	0.94%	1.10%	0.00%	0.00%	0.00%	0.00%
276	0.96%	1.12%	0.00%	1.76%	1.41%	0.00%	0.00%	0.00%	0.00%
282	1.04%	1.30%	1.14%	0.64%	1.17%	1.38%	1.16%	1.81%	1.02%
284	4.98%	6.35%	5.52%	5.78%	8.55%	6.30%	6.04%	7.58%	5.69%
290	4.71%	1.36%	1.40%	1.08%	1.45%	1.11%	0.47%	0.00%	0.00%
291	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	6.02%	4.42%	2.00%
292	0.00%	5.68%	4.82%	5.27%	5.87%	4.89%	0.00%	0.00%	3.48%
295	3.21%	2.80%	3.79%	0.00%	0.00%	0.00%	2.26%	2.15%	3.68%
297	0.00%	0.00%	0.00%	3.66%	4.22%	3.47%	0.65%	0.00%	0.00%
299	0.70%	0.00%	0.43%	0.00%	0.00%	0.00%	0.81%	0.00%	0.00%
301	0.00%	0.00%	0.50%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
304	0.00%	0.00%	0.00%	0.00%	0.87%	0.00%	1.22%	0.00%	0.00%
308	0.00%	0.00%	0.88%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
316	0.00%	0.00%	0.65%	0.00%	0.00%	0.00%	0.76%	0.00%	0.85%
320	0.00%	0.00%	0.00%	0.92%	0.00%	0.00%	0.00%	0.00%	0.00%
327	2.20%	3.56%	3.39%	4.90%	4.32%	4.42%	2.83%	3.72%	3.77%
329	1.89%	3.44%	2.80%	3.29%	3.64%	3.92%	3.84%	2.03%	3.33%
331	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
334	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
337	1.65%	1.66%	1.66%	1.50%	1.34%	1.16%	1.57%	2.41%	1.74%
339	0.00%	0.00%	0.00%	0.82%	0.00%	0.00%	0.00%	0.00%	0.00%
340	0.00%	1.25%	0.39%	0.84%	0.00%	0.00%	2.25%	1.52%	0.86%
341	1.96%	1.50%	2.77%	1.67%	1.37%	0.00%	0.00%	1.49%	1.08%
346	1.05%	0.97%	0.86%	1.07%	2.08%	0.88%	0.00%	0.00%	0.00%
347	0.00%	0.88%	0.84%	0.00%	0.00%	1.16%	1.64%	0.00%	1.79%
349	6.71%	3.17%	2.13%	0.00%	0.00%	0.00%	2.71%	3.55%	3.29%
351	0.00%	0.00%	0.00%	3.42%	1.75%	2.33%	0.00%	0.00%	0.00%
352	4.54%	4.95%	3.94%	3.88%	3.08%	4.57%	4.67%	7.02%	4.58%
359	0.00%	1.19%	1.03%	1.87%	1.38%	1.39%	1.29%	1.17%	2.51%
363	0.00%	0.00%	0.00%	1.67%	1.85%	1.98%	0.00%	0.00%	0.00%
365	1.67%	1.37%	2.18%	0.00%	0.00%	0.00%	1.39%	2.66%	1.79%
368	0.00%	0.93%	1.10%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
369	0.00%	0.93%	1.84%	0.00%	1.75%	1.54%	0.91%	0.00%	2.45%
374	0.00%	1.11%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	1.67%
377	1.73%	0.00%	0.00%	0.00%	0.00%	0.00%	2.01%	3.21%	0.00%
378	2.62%	3.51%	3.77%	3.43%	3.38%	4.34%	2.37%	0.00%	4.34%
380	0.00%	3.27%	4.01%	3.56%	2.62%	4.79%	0.00%	2.72%	3.65%
382	0.90%	0.00%	0.57%	2.83%	0.79%	1.83%	0.92%	2.41%	0.00%
385	1.31%	1.26%	0.57%	1.36%	0.00%	0.00%	1.19%	0.00%	1.20%
390	0.76%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	1.31%	0.00%
391	1.09%	0.00%	0.74%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
392	0.00%	1.31%	1.08%	1.29%	1.34%	1.67%	1.04%	1.20%	1.94%
400	3.65%	0.94%	3.79%	3.42%	3.08%	5.47%	2.18%	3.53%	1.86%
401	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
404	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.67%	0.00%	5.24%
406	6.42%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
408	0.00%	7.15%	12.30%	7.39%	8.60%	14.27%	10.08%	6.61%	12.55%
421	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
444	0.00%	0.00%	0.00%	1.20%	0.00%	0.00%	0.00%	0.00%	0.00%
447	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
448	0.59%	0.00%	0.77%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
453	1.50%	0.00%	0.00%	2.59%	2.13%	2.29%	3.68%	2.74%	3.77%
455	1.27%	3.52%	2.81%	0.00%	0.00%	1.63%	0.00%	0.00%	0.00%

Table D.2 ***Eubacteria*** **Automated Ribosomal Intergenic Spacer Analysis**
Aligned Control Column Data

Fragment Length	A-1	A-2	A-3	B-1	B-2	B-3	C-1	C-2	C-3
458	1.98%	2.46%	2.71%	2.55%	2.99%	2.28%	0.00%	0.64%	0.88%
466	0.00%	0.00%	3.83%	1.98%	0.00%	2.31%	1.97%	2.42%	2.73%
467	0.00%	0.00%	0.00%	1.10%	0.00%	1.73%	0.00%	0.00%	1.16%
474	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.64%	0.00%
475	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.68%	0.00%
480	0.00%	0.00%	0.93%	0.00%	0.00%	0.00%	0.00%	2.23%	0.00%
490	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
495	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
496	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
511	0.00%	0.00%	0.00%	1.68%	0.00%	0.00%	0.00%	0.78%	0.00%
520	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.58%	0.00%
537	2.17%	0.00%	1.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
548	0.00%	0.00%	1.06%	1.89%	0.00%	1.44%	1.96%	1.18%	0.65%
553	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
559	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
568	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	1.09%	0.00%
574	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.81%	0.00%
587	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
590	0.00%	0.00%	0.00%	0.00%	0.00%	1.30%	0.00%	0.00%	0.00%
591	3.27%	2.98%	1.11%	3.16%	0.00%	1.76%	5.87%	5.63%	3.13%
593	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
598	0.00%	0.00%	0.00%	0.00%	3.27%	6.29%	0.00%	0.00%	0.00%
602	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
605	2.14%	4.24%	4.11%	5.64%	7.31%	5.21%	2.01%	2.04%	2.19%
606	1.43%	0.00%	1.19%	1.87%	0.00%	1.57%	0.00%	0.00%	0.00%
611	1.48%	2.71%	1.28%	1.40%	6.28%	1.83%	2.07%	1.44%	1.12%
614	1.48%	1.70%	0.78%	0.00%	0.00%	0.00%	0.00%	0.00%	0.80%
622	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
627	0.00%	0.00%	0.00%	1.77%	0.00%	0.00%	2.25%	1.26%	0.00%
629	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
637	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
638	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
641	0.00%	0.00%	0.00%	1.27%	0.00%	0.00%	0.00%	0.00%	0.00%
649	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
652	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
659	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
662	1.89%	0.00%	0.00%	1.92%	0.00%	0.00%	0.00%	1.25%	0.00%
668	4.10%	6.68%	4.28%	0.00%	0.00%	0.00%	2.51%	1.42%	1.41%
671	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
675	0.00%	0.00%	0.00%	2.17%	0.00%	0.00%	0.00%	1.09%	0.00%
686	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
691	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	1.56%	1.32%
693	0.00%	4.91%	0.00%	2.00%	0.00%	0.00%	3.13%	0.59%	0.00%
694	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
706	4.57%	3.40%	1.74%	3.07%	4.77%	1.91%	3.28%	2.58%	1.56%
708	0.00%	0.00%	0.00%	1.21%	0.00%	0.00%	0.00%	0.00%	0.00%
718	0.00%	0.00%	0.00%	1.55%	0.00%	0.00%	0.00%	0.00%	0.00%
726	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
732	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
755	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
759	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
773	2.39%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
829	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
846	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%

Table D.2 ***Eubacteria*** **Automated Ribosomal Intergenic Spacer Analysis**
Aligned Control Column Data

Fragment Length	D-1	D-2	D-3	E-1	E-2	E-3	F-1	F-2	F-3
458	0.00%	0.00%	0.59%	3.23%	0.99%	0.97%	0.00%	0.00%	1.76%
466	1.45%	2.62%	4.03%	2.22%	3.28%	5.04%	3.25%	2.13%	0.00%
467	1.02%	1.55%	0.00%	1.14%	0.00%	0.00%	0.00%	2.04%	4.06%
474	0.82%	0.00%	0.88%	0.00%	0.00%	1.17%	0.00%	0.00%	0.00%
475	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
480	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
490	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
495	0.78%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
496	0.56%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
511	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.94%	0.00%	0.00%
520	0.00%	0.00%	0.00%	0.00%	1.43%	1.79%	0.00%	0.00%	0.00%
537	1.41%	0.00%	0.00%	0.00%	1.61%	0.00%	0.00%	0.00%	0.00%
548	1.18%	1.23%	1.08%	0.00%	1.49%	0.00%	1.17%	0.00%	0.00%
553	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
559	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
568	1.45%	0.00%	1.40%	0.00%	0.00%	0.00%	0.94%	0.00%	0.00%
574	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
587	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
590	3.67%	2.03%	0.95%	2.48%	1.39%	1.25%	4.33%	3.90%	3.08%
591	0.00%	0.36%	1.46%	0.00%	1.73%	0.00%	0.00%	0.00%	0.00%
593	0.00%	1.70%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
598	0.00%	1.19%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
602	0.00%	0.72%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
605	3.71%	2.79%	1.76%	1.19%	1.98%	2.33%	1.62%	6.58%	1.66%
606	0.83%	0.90%	0.00%	0.00%	0.00%	2.07%	1.18%	0.00%	0.00%
611	1.29%	0.00%	1.23%	1.02%	0.00%	0.88%	1.13%	1.61%	0.00%
614	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
622	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
627	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.78%	1.57%	0.00%
629	1.16%	1.92%	1.04%	1.89%	1.72%	0.00%	0.82%	0.00%	0.00%
637	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
638	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
641	0.84%	0.00%	0.00%	0.00%	0.00%	0.00%	0.81%	0.00%	0.00%
649	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
652	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
659	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
662	1.40%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
668	2.32%	2.23%	1.66%	1.55%	2.57%	2.19%	3.08%	6.08%	4.25%
671	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
675	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
686	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
691	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.89%	3.26%	0.00%
693	0.91%	2.27%	1.89%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
694	0.76%	0.00%	0.00%	0.90%	1.46%	0.00%	0.00%	0.00%	0.00%
706	3.22%	0.00%	0.00%	1.99%	0.92%	0.00%	1.71%	0.00%	0.00%
708	1.14%	1.81%	1.40%	0.00%	0.00%	0.00%	0.66%	0.00%	0.00%
718	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
726	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
732	0.00%	0.00%	0.00%	0.00%	1.42%	0.00%	0.00%	0.00%	0.00%
755	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
759	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
773	1.46%	0.00%	0.00%	0.00%	0.00%	0.00%	1.36%	0.00%	0.00%
829	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
846	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%

Table D.3 ***Eubacteria*** **Automated Ribosomal Intergenic Spacer Analysis Aligned Pulsed-fed Molasses Column Data**

Fragment Length	A-1	A-2	A-3	B-1	B-2	B-3	C-1	C-2	C-3
124	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
130	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	1.00%	0.61%	0.78%
131	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.75%	0.00%	0.56%
132	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.87%	0.55%	0.72%
146	0.00%	0.00%	0.00%	0.63%	0.00%	0.00%	0.00%	0.00%	0.00%
147	0.00%	0.00%	0.00%	0.52%	0.00%	0.50%	0.00%	0.00%	0.00%
154	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
158	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
194	0.00%	0.00%	0.00%	0.00%	0.00%	0.52%	0.00%	0.00%	0.00%
217	0.00%	0.00%	0.00%	0.53%	0.51%	0.00%	0.00%	0.00%	0.00%
221	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
227	0.00%	0.00%	0.78%	0.00%	0.00%	0.73%	0.00%	0.00%	0.51%
237	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.55%	0.00%
253	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
259	0.00%	0.00%	0.84%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
268	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
272	0.00%	0.00%	0.00%	0.00%	0.00%	0.63%	0.00%	0.00%	0.00%
275	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
281	0.00%	0.00%	0.00%	0.56%	0.00%	0.00%	0.00%	0.00%	0.00%
284	3.36%	2.44%	3.53%	1.59%	3.14%	2.21%	2.64%	2.90%	3.30%
288	11.17%	17.91%	11.83%	0.00%	0.00%	0.00%	0.60%	0.00%	0.00%
290	0.00%	0.00%	17.57%	5.06%	19.31%	7.46%	9.25%	12.52%	10.84%
291	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
292	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
296	0.00%	0.00%	0.00%	1.94%	1.30%	0.85%	0.96%	0.89%	1.37%
297	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
301	0.62%	0.00%	0.92%	0.72%	0.72%	0.53%	0.67%	0.84%	1.25%
306	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
307	1.97%	3.70%	2.52%	0.00%	0.59%	0.00%	0.66%	0.69%	0.00%
309	1.69%	2.87%	3.46%	0.56%	4.82%	1.43%	4.58%	5.42%	5.52%
310	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
316	0.83%	1.09%	1.74%	0.00%	0.00%	0.00%	0.82%	0.69%	1.27%
318	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.60%
323	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
327	0.54%	0.00%	0.00%	0.78%	0.59%	0.52%	0.00%	0.62%	1.18%
329	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.51%	0.00%
333	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
337	0.52%	0.00%	0.73%	1.25%	0.81%	0.60%	0.00%	0.00%	0.61%
340	0.00%	0.00%	0.00%	0.78%	1.20%	1.45%	0.00%	0.60%	1.14%
341	0.00%	0.00%	0.00%	0.00%	0.65%	0.00%	0.00%	0.00%	0.00%
342	0.00%	0.00%	0.00%	0.00%	0.53%	0.65%	0.00%	0.00%	0.62%
345	1.76%	0.51%	1.03%	0.00%	0.00%	0.00%	0.84%	0.00%	0.00%
346	0.92%	1.28%	1.39%	0.00%	0.95%	0.98%	0.86%	1.10%	1.32%
351	0.00%	0.00%	0.00%	0.77%	0.00%	0.00%	0.00%	0.63%	0.97%
353	0.00%	0.57%	0.00%	2.19%	0.00%	0.00%	0.00%	1.21%	0.59%
354	0.70%	0.95%	1.57%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
359	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
364	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
367	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
369	0.89%	2.06%	1.42%	0.74%	2.14%	1.45%	15.06%	8.50%	6.74%
371	1.38%	0.00%	0.71%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
374	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
377	0.00%	0.00%	0.00%	0.66%	0.00%	0.56%	0.00%	0.00%	0.00%
379	0.00%	0.00%	0.00%	0.90%	0.81%	0.64%	0.00%	0.63%	0.77%
380	0.52%	0.00%	0.51%	0.00%	0.65%	0.00%	0.00%	0.72%	0.59%
382	2.53%	0.77%	0.78%	0.00%	0.00%	0.00%	0.50%	0.00%	0.91%
383	0.00%	0.00%	1.60%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
385	1.71%	2.62%	0.96%	0.00%	0.80%	0.00%	0.56%	0.00%	0.00%
388	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
392	0.00%	0.00%	0.00%	0.87%	0.62%	0.00%	0.50%	0.00%	0.00%
399	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.64%
401	14.39%	4.63%	3.55%	0.54%	0.73%	0.00%	4.27%	1.34%	0.88%
404	0.00%	0.00%	0.00%	0.00%	1.05%	0.00%	0.53%	1.12%	1.48%
407	0.00%	0.74%	0.00%	2.54%	3.11%	1.27%	1.99%	1.77%	0.92%
412	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
419	0.00%	0.00%	0.00%	0.89%	0.00%	0.00%	0.00%	0.00%	0.00%
424	0.00%	0.00%	0.00%	1.83%	1.09%	0.59%	0.99%	0.73%	1.32%
431	0.77%	0.00%	0.65%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
437	0.00%	0.00%	0.00%	1.02%	0.00%	0.00%	0.00%	0.00%	0.00%
438	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
453	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
455	1.53%	3.14%	1.86%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
459	0.55%	0.59%	0.00%	0.00%	0.00%	0.00%	0.69%	0.99%	1.21%

Table D.3 ***Eubacteria*** **Automated Ribosomal Intergenic Spacer Analysis**
Aligned Pulsed-fed Molasses Column Data

Fragment Length	D-1	D-2	D-3	E-1	E-2	E-3	F-1	F-2	F-3
124	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
130	0.00%	0.00%	0.58%	0.55%	0.00%	0.62%	0.00%	0.55%	0.61%
131	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
132	0.00%	0.56%	0.56%	0.56%	0.00%	0.51%	0.00%	0.60%	0.51%
146	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
147	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
154	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.55%	0.00%	0.00%
158	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
194	0.00%	0.00%	0.00%	0.00%	0.00%	0.67%	0.00%	0.00%	0.00%
217	0.00%	0.86%	0.67%	0.00%	0.00%	0.74%	0.95%	1.26%	1.16%
221	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
227	0.51%	0.69%	0.98%	0.53%	0.89%	0.89%	0.68%	0.00%	0.98%
237	0.86%	0.54%	0.67%	0.58%	0.85%	0.00%	0.55%	1.34%	1.39%
253	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.59%	0.00%	0.54%
259	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
268	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.59%
272	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.89%	0.00%	0.66%
275	0.00%	0.00%	0.00%	0.00%	0.55%	0.60%	0.00%	0.00%	0.00%
281	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
284	2.05%	2.36%	3.09%	2.00%	2.48%	2.69%	2.06%	1.70%	2.49%
288	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
290	3.67%	13.28%	12.88%	9.86%	7.47%	7.04%	1.13%	0.68%	1.32%
291	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	1.28%	2.01%	1.70%
292	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
296	0.00%	0.00%	0.00%	0.66%	0.67%	1.45%	1.27%	1.57%	2.07%
297	0.63%	1.06%	1.14%	0.00%	0.74%	0.00%	0.00%	0.00%	0.00%
301	0.80%	2.05%	1.99%	1.68%	1.00%	1.25%	0.87%	0.88%	1.25%
306	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	1.12%
307	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
309	0.00%	1.35%	2.18%	1.60%	0.57%	0.00%	0.00%	0.00%	0.00%
310	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
316	0.00%	0.64%	1.35%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
318	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
323	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.99%	1.37%	0.93%
327	0.76%	1.34%	0.65%	0.59%	0.96%	0.62%	1.00%	1.11%	0.86%
329	0.77%	1.27%	0.72%	0.69%	1.08%	0.56%	0.00%	0.00%	0.00%
333	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
337	0.56%	0.00%	0.58%	0.59%	0.00%	1.14%	0.66%	0.96%	1.01%
340	0.00%	0.00%	0.00%	0.00%	1.39%	0.90%	0.62%	2.52%	1.47%
341	0.00%	1.08%	0.76%	0.00%	0.00%	0.00%	0.65%	0.00%	1.22%
342	0.52%	1.73%	1.33%	1.00%	0.67%	0.94%	0.00%	0.78%	0.67%
345	0.72%	1.28%	0.90%	0.00%	0.00%	0.00%	0.61%	0.00%	0.00%
346	0.00%	0.00%	0.00%	0.79%	1.21%	1.36%	0.00%	0.00%	0.00%
351	0.78%	2.76%	0.67%	0.00%	0.00%	0.00%	1.73%	1.64%	2.30%
353	0.00%	0.00%	0.00%	1.24%	0.83%	0.00%	1.34%	2.99%	1.31%
354	0.00%	0.00%	0.78%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
359	0.00%	0.00%	0.00%	0.00%	0.56%	0.00%	0.00%	0.70%	0.00%
364	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	1.05%	0.00%	0.61%
367	0.66%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
369	1.74%	3.22%	3.25%	4.08%	2.10%	2.66%	0.83%	1.90%	1.48%
371	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
374	0.00%	0.00%	0.00%	0.00%	0.00%	0.66%	0.00%	0.00%	0.79%
377	0.84%	0.00%	0.84%	0.80%	0.00%	0.76%	1.48%	1.95%	1.00%
379	0.98%	0.89%	0.71%	0.81%	0.74%	0.69%	1.53%	1.31%	1.09%
380	0.00%	0.61%	0.00%	0.00%	1.17%	0.00%	0.00%	0.00%	0.00%
382	0.56%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.74%
383	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
385	0.00%	0.00%	0.00%	0.00%	0.82%	0.00%	0.00%	0.73%	0.51%
388	0.00%	0.00%	0.00%	0.00%	0.00%	0.88%	0.00%	0.00%	0.00%
392	0.00%	0.81%	0.00%	0.00%	0.69%	0.63%	0.00%	0.00%	0.00%
399	0.00%	0.00%	0.00%	0.51%	0.00%	0.00%	0.00%	0.00%	0.00%
401	0.82%	1.43%	0.73%	1.57%	1.41%	0.75%	0.81%	0.88%	2.20%
404	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
407	3.97%	4.28%	1.41%	2.60%	5.20%	2.31%	4.18%	4.80%	1.55%
412	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
419	0.00%	0.00%	0.63%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
424	1.07%	0.61%	1.35%	1.33%	0.68%	1.07%	1.38%	1.57%	1.87%
431	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
437	0.00%	0.00%	0.00%	0.00%	0.65%	0.00%	0.00%	0.87%	0.94%
438	0.00%	0.00%	0.73%	0.00%	0.00%	0.00%	0.00%	1.24%	0.91%
453	0.74%	0.00%	0.00%	0.00%	0.68%	0.53%	1.05%	2.34%	0.79%
455	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
459	0.00%	0.54%	0.67%	1.34%	0.73%	0.00%	0.00%	0.00%	0.54%

Table D.3 ***Eubacteria*** **Automated Ribosomal Intergenic Spacer Analysis**
Aligned Pulsed-fed Molasses Column Data

Fragment Length	A-1	A-2	A-3	B-1	B-2	B-3	C-1	C-2	C-3
466	0.00%	0.00%	0.00%	0.86%	0.00%	0.00%	0.64%	0.00%	0.00%
467	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
480	0.69%	0.00%	0.53%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
486	0.00%	0.00%	0.00%	0.92%	0.65%	0.00%	0.73%	0.67%	1.10%
490	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
494	0.53%	0.00%	0.00%	0.00%	0.71%	0.00%	0.00%	0.74%	0.69%
498	0.00%	0.00%	0.00%	0.00%	0.86%	0.00%	0.00%	0.00%	0.00%
502	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
505	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
509	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
513	0.00%	0.00%	0.00%	6.85%	4.55%	33.11%	0.85%	1.41%	0.98%
518	0.00%	0.00%	1.03%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
521	0.00%	0.00%	0.00%	1.28%	1.86%	0.51%	1.47%	1.04%	1.14%
523	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
526	0.72%	1.30%	2.01%	2.96%	1.50%	0.69%	1.05%	0.90%	1.08%
529	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
537	0.00%	0.00%	0.00%	0.52%	0.00%	0.00%	0.00%	0.00%	0.00%
538	0.00%	0.00%	0.00%	1.48%	0.00%	0.00%	0.00%	0.00%	0.00%
541	10.17%	3.70%	3.73%	0.00%	0.72%	0.00%	6.17%	2.86%	3.06%
543	0.00%	0.00%	0.00%	0.00%	0.68%	0.00%	0.00%	0.00%	0.94%
546	0.00%	1.18%	2.18%	0.00%	0.72%	0.00%	0.00%	0.00%	0.00%
548	0.00%	0.00%	0.00%	0.00%	2.36%	0.77%	0.71%	1.47%	2.07%
549	0.00%	0.00%	0.00%	2.14%	0.00%	0.00%	0.00%	0.00%	0.00%
553	0.00%	0.00%	0.00%	2.11%	0.99%	0.00%	0.00%	0.59%	0.00%
558	0.00%	0.00%	0.00%	21.37%	2.31%	0.50%	1.70%	0.81%	1.03%
565	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
570	0.00%	0.00%	0.76%	0.00%	0.78%	0.52%	0.00%	0.80%	0.88%
573	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
578	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
581	1.14%	1.08%	0.76%	9.08%	6.32%	1.62%	0.63%	1.30%	1.13%
589	0.00%	0.00%	0.00%	0.00%	0.00%	0.57%	0.00%	0.00%	0.90%
591	0.00%	0.00%	0.00%	3.21%	0.73%	0.81%	0.87%	1.13%	0.72%
600	0.75%	1.14%	1.49%	8.89%	6.34%	4.06%	1.74%	3.16%	4.58%
606	9.26%	17.40%	12.07%	1.72%	6.78%	3.32%	16.79%	19.63%	16.87%
608	13.20%	14.99%	6.52%	2.67%	4.69%	2.44%	4.64%	6.73%	3.90%
612	0.00%	0.00%	0.00%	0.66%	0.00%	0.00%	0.00%	0.00%	0.00%
617	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
624	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
632	0.54%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
635	0.00%	0.00%	0.00%	1.31%	0.00%	0.00%	0.00%	0.00%	0.00%
637	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
640	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
645	0.00%	0.00%	0.54%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
658	0.00%	0.00%	0.00%	0.96%	0.00%	4.23%	0.00%	0.00%	0.00%
662	0.00%	0.00%	0.00%	2.89%	0.71%	1.71%	0.00%	0.00%	1.01%
665	0.00%	0.00%	1.53%	0.00%	1.85%	0.00%	0.00%	1.46%	0.00%
667	0.00%	0.00%	0.00%	0.75%	0.00%	0.00%	0.00%	0.00%	0.88%
668	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
674	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.64%
679	0.00%	0.00%	0.63%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
683	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
692	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
697	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
698	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
709	0.00%	0.00%	0.00%	0.00%	0.00%	0.75%	0.00%	0.00%	1.47%
712	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
727	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.83%	0.00%	0.00%
730	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.52%	0.00%
733	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
746	0.00%	0.00%	0.00%	0.00%	1.18%	0.00%	0.00%	0.00%	0.00%
754	0.00%	0.00%	0.00%	2.04%	0.00%	0.77%	0.00%	0.00%	0.00%
771	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
793	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
816	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.88%
822	0.00%	0.00%	0.00%	0.86%	0.00%	0.00%	0.00%	0.00%	0.80%
829	0.00%	0.00%	0.00%	16.80%	4.09%	5.24%	0.97%	1.19%	1.66%

Table D.3 ***Eubacteria*** **Automated Ribosomal Intergenic Spacer Analysis**
Aligned Pulsed-fed Molasses Column Data

Fragment Length	D-1	D-2	D-3	E-1	E-2	E-3	F-1	F-2	F-3
466	0.73%	1.29%	0.61%	0.85%	0.92%	0.00%	1.25%	1.39%	0.76%
467	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.99%	0.51%
480	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
486	0.00%	0.73%	0.64%	0.83%	0.72%	0.00%	0.00%	0.00%	0.00%
490	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
494	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
498	17.90%	0.97%	1.19%	1.35%	7.19%	4.49%	6.91%	2.65%	2.44%
502	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
505	1.69%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
509	0.86%	0.00%	0.00%	0.63%	0.00%	1.00%	3.89%	2.87%	3.83%
513	5.82%	0.54%	1.10%	0.00%	7.61%	3.63%	8.57%	4.24%	3.26%
518	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
521	0.74%	2.72%	1.41%	1.93%	1.13%	0.95%	0.74%	0.94%	1.04%
523	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	2.04%	4.50%	1.35%
526	1.26%	0.79%	0.82%	0.94%	0.51%	0.00%	1.88%	0.66%	1.43%
529	1.88%	0.00%	0.00%	0.00%	3.05%	2.66%	0.00%	0.00%	0.00%
537	0.70%	0.00%	0.65%	0.00%	0.00%	0.69%	0.00%	0.73%	0.00%
538	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
541	0.00%	6.63%	5.00%	12.36%	1.34%	2.23%	0.00%	0.00%	0.81%
543	0.56%	0.00%	0.00%	0.00%	0.55%	1.14%	0.00%	0.00%	0.00%
546	0.00%	0.00%	0.50%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
548	0.00%	1.50%	1.14%	0.87%	1.12%	1.47%	0.00%	0.00%	0.00%
549	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	1.40%	1.12%
553	0.00%	0.00%	1.01%	0.00%	1.57%	0.86%	1.11%	1.37%	1.02%
558	2.80%	4.75%	2.09%	3.02%	1.68%	1.91%	2.96%	3.73%	1.58%
565	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
570	0.00%	0.75%	0.76%	0.00%	0.62%	0.72%	0.83%	0.00%	0.00%
573	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
578	0.53%	0.72%	0.00%	0.69%	0.87%	0.51%	0.96%	0.81%	0.75%
581	1.14%	0.00%	2.60%	0.51%	0.76%	0.93%	1.77%	2.18%	1.21%
589	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
591	0.94%	2.87%	1.01%	1.00%	1.72%	1.78%	3.55%	2.42%	2.91%
600	3.27%	3.63%	3.73%	2.85%	4.85%	4.54%	4.80%	6.13%	6.64%
606	3.61%	9.24%	12.78%	8.87%	5.85%	9.15%	1.70%	3.37%	2.79%
608	2.86%	6.42%	3.34%	4.76%	2.68%	4.40%	3.06%	3.27%	3.11%
612	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	1.01%	0.00%
617	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
624	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	1.41%	3.06%	2.54%
632	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
635	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
637	0.00%	0.00%	0.00%	0.00%	0.87%	0.00%	0.00%	0.00%	0.00%
640	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
645	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
658	2.88%	0.00%	0.00%	0.00%	0.00%	6.01%	7.41%	1.23%	1.81%
662	1.77%	0.79%	0.79%	4.66%	2.60%	1.32%	2.07%	3.76%	1.84%
665	0.00%	0.00%	0.00%	2.47%	1.77%	0.74%	0.52%	0.00%	0.54%
667	0.00%	0.00%	0.00%	0.00%	0.74%	0.00%	0.00%	0.00%	0.00%
668	0.00%	1.33%	0.00%	0.85%	0.00%	0.00%	0.00%	0.00%	0.00%
674	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
679	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
683	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.72%
692	0.00%	1.24%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
697	1.47%	0.00%	0.00%	0.00%	0.00%	1.95%	2.42%	2.42%	2.56%
698	0.00%	0.00%	0.00%	0.00%	1.37%	0.00%	0.00%	0.00%	0.00%
709	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	1.10%
712	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
727	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
730	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
733	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
746	1.18%	0.00%	2.88%	0.00%	0.00%	2.48%	0.84%	1.41%	0.87%
754	0.98%	0.00%	0.00%	0.00%	0.96%	1.45%	0.78%	0.00%	1.11%
771	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
793	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
816	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
822	0.00%	0.00%	0.00%	0.91%	0.00%	0.00%	0.00%	0.00%	0.00%
829	7.27%	1.30%	1.12%	1.30%	4.75%	7.13%	3.49%	2.80%	3.92%

Table D.4 ***Eubacteria*** **Automated Ribosomal Intergenic Spacer Analysis**
Aligned Continuously-fed Molasses Column Data

Fragment Length	A-1	A-2	A-3	B-1	B-2	B-3	C-1	C-2	C-3
146	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
153	0.0%	0.0%	0.0%	0.60%	0.0%	0.55%	0.0%	0.0%	0.0%
154	0.0%	0.0%	0.0%	0.59%	0.54%	0.0%	0.0%	0.51%	0.0%
166	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.81%
196	1.69%	0.0%	1.62%	0.0%	0.63%	0.73%	0.0%	0.0%	0.0%
199	0.57%	0.0%	0.0%	0.63%	0.0%	0.83%	0.0%	0.0%	0.0%
217	0.81%	0.67%	0.0%	0.0%	0.0%	0.0%	1.28%	0.72%	0.87%
222	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
225	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
228	0.62%	1.02%	0.69%	1.16%	1.43%	1.26%	1.98%	1.46%	1.57%
231	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
238	0.0%	1.02%	0.0%	0.75%	0.92%	0.51%	1.36%	1.22%	1.02%
247	0.64%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.97%	0.0%
255	0.78%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.66%	0.55%
256	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.52%	0.0%
261	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
265	1.08%	0.0%	0.0%	0.0%	0.0%	0.65%	0.0%	0.0%	0.0%
267	0.0%	0.0%	0.0%	0.0%	0.0%	0.57%	0.0%	0.0%	0.0%
269	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.89%
270	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
273	0.86%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.67%	0.0%
275	0.84%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
277	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
281	0.0%	0.70%	0.0%	0.79%	0.76%	1.12%	0.92%	1.24%	1.23%
284	1.34%	2.38%	1.11%	1.28%	1.74%	2.61%	2.50%	2.59%	2.38%
287	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
289	4.75%	1.75%	1.65%	1.62%	0.96%	0.89%	0.0%	0.0%	0.0%
291	0.59%	0.85%	0.71%	0.0%	1.43%	1.94%	0.74%	1.04%	0.76%
292	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	2.65%	2.45%	3.50%
294	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
297	1.23%	0.84%	0.79%	0.79%	1.22%	0.93%	0.82%	0.87%	1.62%
298	0.0%	0.0%	0.0%	0.0%	0.50%	0.53%	0.97%	1.30%	0.86%
301	0.0%	0.51%	0.0%	0.0%	0.50%	0.0%	0.0%	0.0%	0.0%
307	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
308	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
310	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
314	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
316	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.51%
321	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
328	0.0%	0.73%	0.0%	1.21%	1.19%	1.35%	2.20%	2.11%	2.48%
330	0.66%	0.99%	0.0%	0.62%	1.25%	1.05%	1.68%	1.81%	2.08%
333	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
337	0.79%	0.0%	0.0%	1.00%	0.79%	0.0%	0.96%	0.63%	0.93%
340	0.65%	1.59%	0.0%	2.48%	1.69%	2.90%	0.73%	1.16%	1.26%
341	0.0%	0.0%	0.0%	0.0%	1.20%	0.0%	0.67%	0.97%	0.0%
346	0.0%	0.0%	0.0%	0.0%	1.12%	0.99%	0.0%	0.0%	0.0%
347	0.0%	0.76%	0.63%	0.0%	0.0%	0.0%	1.26%	1.38%	0.0%
350	0.0%	0.0%	0.0%	1.88%	0.0%	0.0%	0.0%	0.0%	0.0%
351	1.23%	1.37%	0.0%	0.67%	0.0%	0.51%	4.36%	4.03%	4.98%
352	0.0%	1.21%	0.90%	0.74%	2.19%	0.96%	4.56%	3.75%	4.97%
355	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
357	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
359	0.0%	0.50%	1.18%	0.0%	2.28%	1.81%	0.0%	0.0%	1.15%
365	0.0%	0.0%	0.0%	0.67%	0.72%	0.0%	0.0%	0.63%	0.75%
366	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
368	0.0%	0.69%	0.0%	0.0%	0.0%	0.53%	0.0%	0.72%	0.0%
370	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.50%	0.0%
375	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
377	0.0%	0.0%	0.0%	1.13%	0.0%	0.0%	1.70%	0.0%	0.0%
379	0.62%	1.11%	0.65%	1.17%	1.67%	1.94%	1.71%	2.57%	2.58%
381	0.0%	1.07%	0.78%	0.0%	1.90%	1.70%	0.0%	2.38%	2.19%
385	0.77%	0.0%	0.0%	0.0%	0.57%	0.0%	0.0%	0.87%	0.65%
392	0.54%	0.0%	0.61%	0.0%	1.27%	1.13%	0.0%	1.47%	1.75%
400	0.78%	0.90%	0.85%	1.54%	1.60%	1.07%	0.0%	1.63%	2.03%
403	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
406	0.0%	0.65%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
408	1.81%	3.71%	2.70%	1.95%	4.14%	4.78%	3.27%	6.51%	6.38%
416	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
427	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
431	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
437	0.0%	0.0%	0.64%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
442	0.62%	0.0%	0.0%	0.82%	0.73%	0.73%	0.0%	0.0%	0.0%
448	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
449	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
453	0.0%	0.54%	0.58%	7.15%	4.04%	5.38%	5.33%	5.91%	7.77%

Table D.4 ***Eubacteria*** **Automated Ribosomal Intergenic Spacer Analysis**
Aligned Continuously-fed Molasses Column Data

Fragment Length	D-1	D-2	D-3	E-1	E-2	E-3	F-1	F-2	F-3
146	0.0%	0.0%	0.0%	0.62%	0.0%	0.0%	0.0%	0.0%	0.0%
153	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
154	0.0%	0.0%	0.0%	0.0%	0.73%	0.0%	0.0%	0.0%	0.0%
166	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
196	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.56%	0.0%
199	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
217	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
222	0.0%	0.0%	0.0%	0.0%	0.80%	0.0%	0.0%	0.0%	0.0%
225	0.0%	0.0%	0.0%	0.0%	0.81%	0.0%	0.0%	0.0%	0.0%
228	0.89%	1.17%	1.23%	1.78%	1.48%	1.89%	3.31%	2.09%	1.88%
231	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
238	1.47%	1.76%	1.47%	1.58%	2.36%	1.55%	3.55%	2.99%	1.97%
247	0.59%	0.0%	0.54%	0.0%	1.04%	0.0%	0.0%	0.0%	0.0%
255	0.82%	0.54%	0.0%	0.0%	0.54%	0.0%	3.08%	0.0%	0.98%
256	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
261	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
265	0.0%	0.56%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
267	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.79%
269	0.0%	0.61%	0.0%	0.0%	1.03%	0.0%	0.0%	0.0%	0.0%
270	0.64%	0.70%	0.82%	0.0%	0.63%	1.19%	0.0%	0.0%	0.0%
273	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.53%
275	0.0%	0.55%	0.69%	0.0%	0.0%	0.58%	0.0%	0.0%	0.0%
277	0.0%	0.57%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
281	2.01%	1.68%	1.51%	1.48%	2.23%	1.68%	4.85%	2.12%	1.11%
284	2.34%	2.94%	2.51%	3.75%	3.47%	3.11%	0.0%	5.37%	4.68%
287	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
289	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
291	1.74%	0.79%	0.72%	3.33%	1.32%	1.89%	0.0%	0.0%	0.98%
292	0.0%	3.10%	3.62%	1.23%	2.96%	4.13%	2.96%	3.23%	2.83%
294	0.96%	0.0%	0.0%	0.0%	0.0%	0.0%	3.08%	3.08%	2.47%
297	0.66%	2.27%	1.94%	1.03%	2.79%	2.08%	0.0%	0.0%	0.0%
298	0.0%	0.0%	0.70%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
301	0.0%	0.0%	0.0%	0.0%	0.0%	0.61%	0.0%	0.0%	0.0%
307	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
308	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
310	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
314	0.0%	0.0%	0.0%	0.0%	0.0%	0.71%	0.0%	0.0%	0.0%
316	0.0%	0.61%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
321	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
328	1.38%	1.92%	1.89%	2.39%	2.05%	2.99%	1.92%	2.69%	2.80%
330	1.02%	2.12%	1.54%	2.14%	2.18%	2.43%	3.10%	2.93%	2.81%
333	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
337	0.99%	0.69%	0.61%	0.98%	0.92%	1.02%	1.91%	1.91%	1.01%
340	0.54%	1.42%	2.91%	0.92%	1.11%	0.0%	1.91%	1.02%	1.68%
341	0.80%	2.55%	0.0%	2.44%	2.76%	4.02%	1.75%	2.03%	2.98%
346	0.54%	0.65%	0.0%	0.0%	0.0%	0.0%	0.0%	1.09%	0.0%
347	0.0%	0.56%	0.51%	0.0%	0.0%	0.0%	0.0%	1.51%	1.94%
350	4.59%	3.03%	4.16%	7.89%	0.0%	0.0%	6.09%	3.14%	3.60%
351	0.0%	0.0%	0.0%	7.09%	3.39%	8.70%	0.0%	0.0%	0.0%
352	3.07%	3.62%	4.36%	0.0%	5.49%	0.0%	2.04%	5.72%	4.08%
355	0.0%	0.0%	0.0%	0.0%	0.0%	0.40%	0.0%	0.0%	0.0%
357	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
359	0.0%	2.18%	0.85%	0.0%	0.98%	0.83%	0.0%	0.0%	0.82%
365	1.18%	1.85%	0.95%	1.32%	2.03%	0.61%	0.0%	2.01%	1.35%
366	0.0%	0.0%	0.0%	0.0%	0.0%	0.70%	0.0%	0.0%	0.0%
368	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.82%
370	0.60%	2.52%	0.77%	0.0%	0.94%	1.65%	0.0%	1.32%	1.83%
375	0.0%	0.54%	0.0%	0.0%	0.83%	0.65%	0.0%	0.0%	0.0%
377	0.94%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
379	0.84%	2.96%	3.09%	1.89%	2.19%	3.31%	0.0%	4.21%	4.14%
381	0.0%	3.07%	2.18%	1.96%	2.35%	2.67%	0.0%	3.58%	3.01%
385	1.14%	1.10%	1.06%	1.00%	0.81%	0.0%	0.0%	1.70%	1.44%
392	0.0%	0.0%	0.59%	0.0%	0.65%	1.00%	0.0%	1.90%	1.06%
400	1.06%	1.82%	1.21%	1.53%	1.66%	1.31%	2.46%	1.34%	1.77%
403	0.0%	0.64%	0.65%	0.0%	1.66%	0.53%	0.0%	0.0%	1.95%
406	3.42%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
408	0.0%	0.0%	5.60%	4.75%	9.20%	9.28%	6.95%	10.88%	10.64%
416	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
427	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
431	0.0%	0.40%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
437	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
442	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
448	0.0%	1.40%	1.01%	0.0%	0.50%	0.0%	0.0%	0.0%	0.77%
449	0.0%	0.0%	0.0%	0.0%	1.81%	1.91%	0.0%	2.98%	1.45%
453	5.13%	7.73%	6.28%	3.89%	7.16%	5.39%	3.79%	5.82%	5.03%

Table D.4 ***Eubacteria*** **Automated Ribosomal Intergenic Spacer Analysis**
Aligned Continuously-fed Molasses Column Data

Fragment Length	A-1	A-2	A-3	B-1	B-2	B-3	C-1	C-2	C-3
458	0.0%	0.0%	0.0%	0.0%	0.79%	0.55%	0.0%	0.53%	1.72%
459	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
466	0.0%	0.0%	0.0%	0.0%	1.77%	1.44%	0.99%	2.71%	2.57%
467	0.0%	1.48%	0.93%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
474	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
477	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
482	1.02%	0.62%	0.0%	0.0%	0.0%	0.50%	0.0%	0.0%	0.0%
485	0.53%	0.68%	0.63%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
488	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
498	0.65%	0.0%	0.0%	11.61%	10.43%	13.91%	5.19%	4.19%	4.38%
509	0.0%	0.0%	0.0%	0.90%	1.63%	0.0%	1.74%	2.44%	3.45%
513	0.79%	1.52%	3.37%	3.19%	2.54%	3.97%	6.27%	2.63%	4.11%
514	4.27%	10.29%	4.36%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
520	0.72%	1.26%	0.66%	0.0%	0.94%	0.68%	0.0%	0.0%	0.0%
523	0.0%	0.0%	0.0%	0.0%	0.0%	0.52%	0.0%	1.17%	0.0%
528	2.11%	0.99%	0.0%	1.60%	2.13%	1.37%	2.41%	2.26%	2.22%
536	0.0%	0.0%	0.0%	0.0%	0.60%	1.18%	0.73%	0.87%	1.12%
538	0.0%	0.0%	0.0%	0.0%	0.96%	0.0%	0.0%	0.0%	0.0%
542	1.08%	0.0%	0.0%	0.96%	1.07%	1.23%	0.0%	0.0%	0.0%
545	0.86%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
548	1.29%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
549	1.01%	1.53%	1.12%	2.42%	3.19%	2.85%	0.0%	0.79%	1.24%
553	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	1.51%	0.57%	0.0%
558	8.64%	8.53%	0.84%	2.14%	2.21%	1.26%	3.77%	1.18%	1.14%
568	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
569	0.0%	0.0%	0.0%	0.0%	1.16%	0.0%	1.88%	1.33%	1.40%
571	1.66%	1.40%	1.85%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
574	1.08%	0.62%	0.0%	0.90%	1.01%	0.0%	1.22%	0.64%	0.0%
581	5.95%	2.03%	0.0%	0.0%	0.0%	0.0%	1.65%	1.78%	1.63%
583	3.38%	0.0%	0.0%	0.0%	0.0%	0.92%	0.0%	0.0%	0.0%
589	4.49%	2.86%	1.11%	3.66%	4.19%	3.49%	0.0%	0.0%	0.0%
590	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	5.38%	3.15%	3.87%
591	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
592	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
598	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
600	11.34%	4.11%	1.93%	1.26%	1.71%	3.49%	4.04%	2.04%	2.56%
605	0.0%	0.0%	0.0%	1.10%	0.85%	1.02%	1.61%	0.96%	0.73%
608	0.70%	0.0%	0.0%	0.86%	0.76%	0.68%	2.08%	1.30%	0.81%
612	0.0%	0.52%	0.0%	0.0%	0.0%	0.0%	0.0%	0.55%	0.61%
617	0.0%	0.0%	1.64%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
624	0.77%	0.64%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
627	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
636	0.0%	0.0%	0.0%	0.60%	0.0%	0.0%	0.0%	0.0%	0.0%
641	1.35%	0.82%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
658	5.88%	2.12%	0.0%	16.69%	7.70%	5.68%	3.79%	1.91%	0.87%
660	0.0%	0.93%	0.50%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
663	3.05%	5.10%	12.29%	0.88%	1.29%	2.63%	1.69%	1.71%	1.32%
665	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
669	0.0%	0.62%	0.0%	1.94%	0.55%	0.60%	0.81%	5.10%	0.0%
674	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
690	0.0%	0.0%	0.72%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
694	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
696	0.95%	1.98%	0.65%	0.64%	0.93%	0.58%	3.17%	1.27%	1.59%
702	1.17%	2.00%	0.0%	0.89%	0.0%	0.65%	0.0%	0.0%	0.0%
706	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
708	1.50%	0.86%	0.0%	0.0%	0.0%	0.0%	0.86%	0.0%	0.0%
714	0.0%	0.0%	0.0%	0.87%	0.57%	0.0%	0.0%	0.0%	0.0%
746	0.78%	0.60%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
754	1.83%	1.96%	0.0%	0.0%	0.73%	0.0%	1.34%	0.0%	0.0%
760	0.0%	0.0%	0.0%	1.93%	0.64%	0.0%	0.0%	0.0%	0.0%
773	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
778	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
823	0.0%	0.0%	0.70%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
829	3.54%	0.0%	0.0%	9.75%	5.95%	5.78%	0.0%	0.0%	0.0%
830	0.0%	6.24%	0.63%	0.0%	0.0%	0.0%	5.97%	2.07%	2.12%
856	0.0%	0.0%	0.0%	0.92%	0.0%	0.0%	0.0%	0.0%	0.0%

Table D.4 ***Eubacteria*** **Automated Ribosomal Intergenic Spacer Analysis**
Aligned Continuously-fed Molasses Column Data

Fragment Length	D-1	D-2	D-3	E-1	E-2	E-3	F-1	F-2	F-3
458	1.46%	1.57%	1.65%	1.18%	3.52%	2.40%	1.97%	2.42%	2.33%
459	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
466	0.50%	3.54%	0.0%	1.60%	2.67%	0.0%	0.0%	0.0%	0.0%
467	0.79%	0.0%	2.97%	1.39%	0.0%	3.77%	0.0%	4.12%	3.46%
474	0.0%	0.57%	0.83%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
477	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
482	0.0%	0.65%	0.0%	0.0%	1.06%	0.0%	0.0%	0.0%	0.99%
485	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
488	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
498	1.68%	1.07%	1.94%	1.10%	0.0%	0.0%	4.57%	2.17%	1.19%
509	0.88%	0.79%	1.79%	2.00%	1.33%	0.99%	0.0%	0.0%	0.0%
513	0.67%	1.14%	0.74%	0.0%	0.0%	1.69%	0.0%	2.28%	2.40%
514	0.74%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
520	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
523	0.0%	0.0%	0.86%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
528	2.82%	0.67%	2.10%	0.0%	0.0%	1.63%	0.0%	0.0%	0.0%
536	0.0%	0.0%	0.92%	3.39%	0.0%	0.0%	0.0%	0.0%	0.0%
538	1.01%	0.0%	1.15%	0.74%	0.74%	0.80%	3.23%	0.0%	0.0%
542	1.08%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
545	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
548	0.0%	0.60%	0.80%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
549	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
553	0.86%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
558	7.85%	1.59%	2.24%	3.56%	1.13%	0.84%	4.54%	1.50%	1.25%
568	0.60%	0.97%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
569	0.50%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
571	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
574	2.11%	0.83%	2.26%	1.46%	0.84%	1.27%	0.0%	1.24%	0.0%
581	2.10%	0.0%	1.80%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
583	0.0%	0.0%	0.0%	0.0%	0.0%	0.56%	0.0%	0.0%	0.0%
589	0.0%	0.0%	0.0%	1.64%	0.0%	0.0%	1.61%	0.0%	0.0%
590	5.45%	2.40%	4.56%	0.00%	0.71%	0.0%	5.57%	4.00%	3.97%
591	0.0%	0.0%	0.0%	1.90%	0.58%	1.59%	0.0%	0.0%	0.0%
592	0.0%	0.0%	0.0%	0.0%	1.14%	2.47%	0.0%	0.0%	0.0%
598	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	1.93%	0.0%	0.0%
600	2.34%	1.56%	1.35%	0.75%	0.89%	0.70%	0.0%	0.0%	0.0%
605	1.16%	1.43%	1.93%	2.37%	2.25%	2.05%	5.96%	2.37%	2.94%
608	1.59%	1.22%	2.13%	0.0%	0.96%	0.64%	0.0%	0.0%	1.17%
612	0.76%	0.66%	0.79%	1.99%	0.92%	1.37%	0.0%	0.0%	1.06%
617	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
624	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
627	0.89%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
636	0.66%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
641	0.73%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
658	3.07%	0.0%	1.31%	2.84%	1.67%	0.91%	2.73%	0.0%	0.68%
660	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.78%
663	1.41%	0.89%	2.48%	2.03%	0.76%	0.0%	2.69%	0.0%	0.0%
665	0.0%	0.0%	0.0%	0.0%	0.90%	1.71%	0.0%	0.0%	0.0%
669	0.0%	0.54%	0.85%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
674	0.0%	0.0%	0.0%	1.07%	0.0%	0.0%	0.0%	0.0%	0.0%
690	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
694	0.0%	0.0%	0.65%	0.0%	0.0%	0.88%	0.0%	0.0%	0.0%
696	4.03%	1.34%	1.73%	3.88%	1.17%	1.32%	2.55%	0.0%	0.0%
702	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
706	0.85%	0.0%	0.0%	1.92%	0.0%	0.0%	2.84%	0.0%	0.0%
708	0.0%	0.0%	0.0%	1.00%	0.89%	0.0%	0.0%	0.0%	0.0%
714	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
746	0.60%	0.0%	0.88%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
754	0.86%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
760	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
773	0.51%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
778	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
823	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
829	4.29%	1.60%	2.59%	0.0%	0.0%	0.0%	7.06%	2.38%	2.60%
830	0.0%	0.0%	0.0%	7.05%	2.60%	2.12%	0.0%	0.0%	0.0%
856	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%

Table D.5 ***Archaea*** **Automated Ribosomal Intergenic Spacer Analysis**
Aligned E85-fed Column Data

Fragment Length	A-1	A-2	A-3	B-1	B-2	B-3	C-1	C-2	C-3
123	0.00%	2.57%	0.00%	3.25%	0.67%	2.81%	0.69%	0.57%	0.00%
124	0.00%	0.00%	0.00%	3.80%	0.00%	3.41%	1.04%	4.78%	5.54%
125	0.78%	0.94%	1.18%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
126	0.53%	0.00%	0.00%	0.00%	0.00%	0.00%	0.76%	0.54%	0.00%
127	0.00%	0.00%	0.00%	0.00%	2.30%	0.58%	1.53%	0.00%	0.00%
129	1.97%	4.21%	2.54%	0.55%	1.28%	0.00%	0.00%	0.00%	0.00%
130	2.37%	0.00%	2.24%	1.09%	0.62%	2.10%	0.00%	1.08%	1.00%
131	0.00%	2.41%	1.43%	0.96%	1.81%	2.21%	0.00%	0.00%	0.00%
133	0.00%	0.00%	0.00%	0.76%	0.00%	0.00%	1.32%	1.99%	0.00%
134	1.04%	0.00%	0.00%	1.52%	0.59%	1.57%	3.32%	2.59%	0.92%
135	0.00%	0.00%	0.00%	1.37%	3.58%	1.92%	0.00%	0.00%	0.00%
136	4.16%	2.82%	5.28%	0.00%	0.00%	0.00%	2.85%	3.32%	2.31%
137	0.55%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	1.00%	0.53%
138	3.92%	4.66%	3.00%	3.92%	0.66%	0.00%	0.00%	0.00%	0.00%
139	0.00%	0.00%	0.00%	0.89%	0.00%	0.00%	0.00%	0.00%	0.00%
140	0.00%	0.80%	0.00%	0.00%	0.00%	4.98%	0.00%	0.00%	0.00%
141	27.76%	10.88%	7.73%	30.31%	24.22%	14.96%	35.80%	23.75%	19.65%
143	0.00%	1.67%	0.00%	0.00%	7.07%	0.00%	0.00%	0.00%	0.00%
145	0.00%	1.38%	0.68%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
146	0.00%	0.00%	0.00%	0.94%	0.00%	1.15%	0.00%	0.00%	0.00%
148	0.00%	0.00%	0.00%	0.00%	0.00%	0.90%	1.38%	0.00%	1.30%
150	13.39%	8.54%	12.74%	6.48%	7.69%	5.75%	5.56%	6.80%	4.95%
153	0.00%	0.60%	1.07%	0.00%	1.97%	1.08%	1.04%	0.66%	0.00%
156	1.06%	0.00%	0.54%	1.12%	0.00%	0.66%	0.00%	0.00%	0.00%
158	0.51%	0.61%	0.68%	4.97%	0.00%	3.36%	2.21%	0.51%	0.00%
160	1.91%	1.90%	0.53%	0.00%	1.94%	0.00%	0.00%	0.00%	0.00%
162	0.69%	0.83%	0.58%	0.00%	0.59%	0.52%	0.81%	0.66%	0.93%
166	0.00%	0.59%	0.00%	0.00%	1.41%	0.00%	0.00%	0.00%	0.51%
167	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.80%	0.82%	0.00%
170	0.00%	0.92%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
172	0.73%	2.21%	0.00%	2.34%	0.00%	0.56%	0.00%	1.86%	2.15%
174	0.00%	0.00%	0.00%	0.57%	2.01%	0.00%	0.00%	0.78%	0.00%
175	0.00%	0.93%	1.21%	0.00%	0.00%	0.00%	1.02%	1.65%	0.00%
178	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
179	0.64%	0.60%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	1.18%
181	0.74%	0.75%	1.08%	0.00%	0.87%	0.56%	0.00%	0.56%	1.28%
183	0.00%	0.90%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
186	0.00%	0.00%	0.00%	0.00%	1.18%	0.51%	0.56%	0.00%	2.46%
191	0.98%	0.00%	0.77%	0.00%	1.09%	0.00%	0.00%	0.00%	2.17%
193	0.57%	0.00%	0.00%	1.07%	0.88%	2.53%	0.00%	0.00%	0.00%
195	2.61%	2.41%	3.72%	0.00%	1.41%	0.00%	2.10%	7.48%	3.42%
196	0.00%	2.31%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
200	0.00%	0.00%	0.00%	0.00%	0.58%	0.00%	0.00%	0.00%	0.00%
203	0.00%	0.00%	0.66%	0.00%	0.97%	0.76%	1.29%	1.10%	1.39%
206	0.00%	1.69%	0.60%	0.00%	0.72%	0.00%	0.00%	0.00%	0.00%
209	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.86%	0.72%
212	2.86%	3.33%	9.30%	0.60%	0.58%	1.86%	0.00%	0.57%	0.87%
215	0.00%	0.64%	0.00%	0.60%	0.00%	0.88%	4.29%	0.66%	0.00%
220	0.00%	0.88%	0.61%	0.00%	1.10%	1.05%	0.00%	1.40%	2.35%
222	0.00%	0.57%	0.00%	0.00%	0.00%	0.56%	0.00%	0.00%	0.00%
227	0.00%	0.00%	0.00%	0.00%	0.59%	0.00%	0.00%	0.00%	0.00%
231	0.00%	0.00%	0.58%	0.00%	0.00%	0.83%	0.00%	0.00%	0.00%
235	0.00%	3.10%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
238	0.00%	1.49%	0.81%	7.15%	0.52%	0.00%	0.54%	0.58%	0.00%
244	0.00%	0.00%	0.00%	0.54%	0.00%	0.00%	0.00%	0.00%	0.00%
246	0.79%	0.57%	0.00%	0.54%	0.98%	0.70%	0.92%	0.63%	1.77%

Table D.5 ***Archaea*** **Automated Ribosomal Intergenic Spacer Analysis Aligned E85-fed Column Data**

Fragment Length	D-1	D-2	D-3	E-1	E-2	E-3	F-1	F-2	F-3
123	0.00%	0.00%	0.00%	2.33%	0.94%	0.00%	0.70%	0.74%	0.00%
124	2.89%	3.23%	15.14%	0.00%	0.00%	3.63%	4.53%	3.10%	6.08%
125	0.00%	0.00%	0.00%	2.32%	2.28%	7.50%	0.00%	0.00%	0.00%
126	7.23%	0.67%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
127	0.00%	0.00%	0.00%	1.26%	0.60%	0.00%	0.00%	0.00%	0.00%
129	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
130	0.00%	0.00%	0.00%	0.00%	0.61%	0.87%	0.00%	1.88%	0.61%
131	0.84%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.80%	0.00%
133	0.00%	0.00%	0.00%	1.32%	0.83%	1.17%	0.00%	0.00%	0.00%
134	0.00%	0.99%	1.87%	0.00%	0.00%	0.00%	2.95%	1.73%	1.75%
135	3.14%	2.53%	2.79%	1.52%	1.84%	2.36%	0.00%	0.00%	0.00%
136	0.00%	0.00%	0.00%	1.10%	3.70%	3.03%	4.10%	6.12%	2.36%
137	1.69%	1.55%	0.74%	1.89%	0.69%	0.91%	1.09%	0.89%	1.05%
138	0.00%	0.00%	0.00%	1.03%	0.59%	0.00%	0.00%	0.67%	0.54%
139	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
140	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	35.02%	9.44%	6.73%
141	33.94%	35.94%	19.81%	27.16%	32.56%	8.27%	0.00%	24.50%	15.62%
143	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
145	0.00%	0.00%	0.00%	0.55%	0.92%	1.11%	0.80%	0.00%	0.88%
146	0.00%	0.00%	0.00%	0.00%	0.00%	1.42%	0.00%	0.00%	0.50%
148	0.73%	0.78%	0.89%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
150	7.96%	3.41%	4.49%	4.74%	6.24%	3.11%	2.91%	5.73%	3.33%
153	1.98%	0.00%	0.57%	1.71%	0.00%	0.52%	0.00%	0.00%	0.00%
156	0.72%	1.39%	0.59%	2.38%	0.00%	0.00%	1.08%	1.54%	1.04%
158	0.00%	0.00%	0.00%	1.93%	1.13%	12.75%	0.85%	0.00%	0.00%
160	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
162	0.00%	0.00%	1.09%	1.32%	0.53%	0.00%	0.00%	0.00%	1.00%
166	0.00%	0.00%	0.67%	0.00%	0.00%	0.00%	0.80%	0.00%	0.90%
167	0.00%	0.00%	0.75%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
170	0.00%	0.00%	0.00%	0.00%	0.55%	1.65%	0.00%	0.00%	0.00%
172	1.10%	3.22%	1.54%	0.00%	1.27%	0.00%	1.68%	0.77%	0.62%
174	1.08%	0.91%	1.37%	0.00%	0.00%	0.00%	0.59%	0.85%	0.00%
175	0.00%	0.00%	0.00%	0.63%	2.44%	0.65%	0.00%	0.00%	0.00%
178	0.00%	0.00%	0.00%	0.61%	0.55%	0.54%	0.00%	0.00%	5.47%
179	0.94%	0.93%	0.00%	0.68%	0.81%	0.00%	0.71%	1.14%	2.20%
181	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.84%	0.00%	0.00%
183	0.00%	0.53%	0.00%	0.52%	0.70%	1.00%	0.00%	0.00%	0.72%
186	0.70%	0.00%	0.00%	1.61%	0.00%	0.00%	0.00%	0.00%	0.00%
191	0.00%	0.00%	0.00%	0.00%	0.00%	1.02%	0.00%	0.00%	0.00%
193	0.00%	1.32%	1.77%	0.00%	0.56%	0.65%	1.55%	0.00%	0.76%
195	3.68%	4.54%	4.39%	2.26%	4.31%	4.48%	1.91%	5.95%	2.03%
196	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
200	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.84%	0.00%
203	0.00%	0.00%	0.95%	0.82%	0.00%	0.56%	0.00%	0.00%	0.00%
206	0.00%	0.00%	0.00%	0.66%	0.00%	0.00%	0.00%	0.00%	0.00%
209	0.00%	0.69%	1.46%	0.00%	0.00%	0.54%	0.00%	0.50%	0.58%
212	1.16%	0.00%	0.00%	0.00%	0.00%	0.66%	0.00%	0.00%	0.00%
215	0.00%	0.00%	0.00%	0.00%	0.00%	0.60%	0.93%	0.00%	0.00%
220	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
222	0.95%	0.00%	0.53%	0.00%	0.50%	0.00%	0.00%	0.99%	0.00%
227	0.00%	0.00%	0.00%	0.00%	0.00%	0.68%	0.00%	0.00%	0.00%
231	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
235	0.00%	0.00%	0.51%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
238	0.67%	1.01%	0.53%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
244	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
246	0.82%	0.00%	1.35%	1.03%	1.15%	1.71%	1.06%	1.06%	0.85%

Table D.5 ***Archaea*** **Automated Ribosomal Intergenic Spacer Analysis**
Aligned E85-fed Column Data

Fragment Length	A-1	A-2	A-3	B-1	B-2	B-3	C-1	C-2	C-3
250	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	1.37%	0.55%	0.61%
252	0.00%	0.58%	0.00%	0.00%	0.50%	0.00%	0.00%	0.00%	0.00%
254	0.00%	0.59%	0.66%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
259	0.00%	0.00%	0.00%	1.19%	1.98%	0.50%	0.91%	0.91%	0.00%
260	0.91%	1.19%	0.00%	0.00%	0.00%	0.61%	0.00%	0.00%	0.00%
268	0.00%	0.81%	0.00%	0.00%	0.64%	0.00%	0.00%	0.00%	0.00%
270	0.76%	0.00%	0.80%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
274	1.56%	0.69%	2.46%	0.79%	0.00%	2.73%	0.00%	0.00%	1.18%
276	1.74%	1.46%	3.24%	1.41%	1.60%	4.68%	0.00%	0.50%	1.83%
285	0.00%	0.00%	0.00%	0.00%	1.55%	0.00%	0.00%	0.00%	0.00%
295	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
299	0.00%	0.00%	0.50%	0.00%	0.00%	0.00%	0.00%	0.00%	0.59%
308	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.50%
314	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	1.51%
322	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
323	11.61%	10.06%	7.27%	10.76%	9.24%	11.55%	15.66%	14.97%	15.21%
326	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
339	0.00%	0.00%	1.86%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
351	0.00%	0.00%	0.00%	0.00%	1.65%	0.00%	0.00%	2.84%	0.93%
356	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
365	0.00%	0.00%	0.00%	2.58%	1.59%	1.46%	1.46%	0.77%	1.17%
375	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
385	0.00%	0.00%	0.80%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
402	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
409	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
430	0.00%	0.63%	0.00%	0.50%	0.55%	0.64%	0.00%	0.00%	0.00%
439	0.00%	0.00%	0.00%	0.59%	0.00%	0.82%	0.00%	0.00%	0.52%
446	3.29%	2.68%	3.05%	0.00%	0.88%	0.51%	0.00%	0.00%	0.00%
449	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	1.92%	0.50%
452	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
498	0.00%	0.00%	0.00%	0.00%	0.00%	0.79%	0.00%	0.00%	0.87%
508	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
525	1.92%	2.52%	5.14%	0.00%	0.00%	0.85%	0.00%	0.00%	0.00%
538	0.00%	0.62%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
544	0.00%	2.80%	0.00%	0.00%	0.00%	0.00%	0.00%	0.88%	0.00%
557	0.00%	0.00%	0.00%	0.00%	0.00%	0.66%	1.65%	0.00%	0.00%
770	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
796	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
833	0.00%	0.00%	0.55%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
886	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.58%

Table D.5 ***Archaea*** **Automated Ribosomal Intergenic Spacer Analysis Aligned E85-fed Column Data**

Fragment Length	D-1	D-2	D-3	E-1	E-2	E-3	F-1	F-2	F-3
250	1.08%	1.40%	1.40%	1.64%	0.99%	3.14%	0.75%	2.53%	3.95%
252	0.00%	0.00%	0.00%	0.50%	0.00%	0.00%	0.00%	0.00%	0.00%
254	0.00%	0.73%	0.68%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
259	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.74%	1.21%
260	0.56%	0.92%	0.58%	0.94%	0.62%	0.62%	0.00%	0.00%	0.00%
268	0.00%	0.99%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	1.07%
270	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
274	0.00%	0.00%	0.73%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
276	0.79%	0.51%	1.02%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
285	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
295	0.84%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
299	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
308	0.73%	0.00%	0.00%	0.73%	0.00%	0.00%	0.00%	0.70%	0.00%
314	0.00%	0.00%	0.83%	0.00%	0.00%	1.39%	0.74%	0.00%	0.66%
322	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
323	18.15%	16.81%	13.39%	19.70%	20.26%	14.98%	21.98%	24.93%	19.10%
326	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
339	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
351	1.56%	1.33%	0.00%	0.75%	0.57%	0.79%	0.00%	0.00%	0.00%
356	1.18%	0.00%	0.00%	1.04%	0.00%	0.00%	0.51%	0.00%	0.00%
365	0.57%	0.00%	0.00%	0.50%	0.00%	0.00%	0.00%	0.00%	0.00%
375	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
385	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
402	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
409	0.00%	0.00%	0.00%	0.00%	0.00%	0.54%	0.00%	0.00%	0.00%
430	0.00%	0.00%	0.00%	0.00%	0.00%	0.83%	0.00%	0.00%	0.83%
439	0.00%	0.62%	0.82%	0.00%	0.82%	0.70%	0.58%	0.00%	1.35%
446	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
449	1.53%	2.82%	0.58%	3.90%	2.79%	0.99%	1.45%	1.96%	0.95%
452	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
498	0.00%	0.00%	0.51%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
508	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
525	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
538	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
544	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
557	0.79%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.55%	0.00%
770	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
796	0.00%	0.00%	0.70%	0.00%	0.00%	0.00%	0.00%	0.50%	0.00%
833	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
886	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.51%

Table D.6 ***Archaea*** **Automated Ribosomal Intergenic Spacer Analysis Aligned Control Column Data**

Fragment Length	A-1	A-2	A-3	B-1	B-2	B-3	C-1	C-2	C-3
122	2.32%	2.92%	0.55%	1.86%	0.00%	0.59%	0.00%	0.00%	0.00%
124	0.00%	0.00%	1.29%	0.55%	0.00%	0.00%	0.00%	0.00%	0.00%
125	2.43%	2.07%	2.40%	2.82%	2.40%	7.54%	0.80%	0.75%	3.16%
126	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.69%	1.13%
128	0.00%	0.00%	1.19%	0.00%	0.57%	0.00%	0.00%	0.00%	0.00%
130	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
131	0.00%	0.00%	0.00%	0.00%	0.00%	0.52%	0.00%	0.62%	0.00%
132	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
134	3.56%	1.17%	3.78%	2.78%	3.23%	3.34%	2.09%	1.43%	1.63%
135	2.20%	4.45%	3.28%	3.55%	3.64%	4.73%	1.73%	3.23%	2.58%
136	0.00%	0.81%	0.70%	0.00%	0.00%	0.00%	1.57%	0.00%	0.64%
138	0.00%	0.00%	0.00%	0.00%	0.00%	1.05%	0.00%	0.00%	0.00%
139	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
141	0.00%	2.39%	2.02%	0.76%	0.79%	1.15%	2.64%	6.57%	2.20%
142	6.66%	1.46%	4.19%	2.51%	0.61%	1.67%	0.00%	0.00%	2.25%
144	0.69%	0.54%	1.29%	0.00%	0.00%	1.10%	0.72%	0.00%	0.50%
146	1.83%	1.72%	0.70%	0.86%	2.18%	0.62%	0.00%	1.86%	1.16%
148	0.00%	0.72%	0.00%	0.00%	0.00%	0.00%	0.00%	0.63%	0.93%
150	2.99%	1.33%	0.00%	1.09%	0.00%	0.67%	0.00%	0.00%	0.00%
151	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	1.82%	1.91%	1.31%
152	0.00%	0.00%	0.00%	0.00%	0.59%	1.05%	0.00%	0.00%	0.00%
154	0.76%	1.08%	1.29%	0.85%	0.64%	0.00%	0.00%	1.95%	0.00%
156	0.00%	0.00%	0.00%	0.00%	1.04%	0.00%	0.00%	0.00%	0.00%
158	0.00%	1.50%	0.55%	0.00%	0.00%	0.51%	0.62%	0.95%	1.11%
159	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.90%	0.00%	0.00%
161	0.91%	1.40%	0.50%	0.00%	0.75%	1.01%	0.00%	0.00%	0.00%
162	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	1.39%	0.57%	0.60%
163	0.00%	1.33%	0.50%	3.05%	1.84%	1.40%	0.00%	0.00%	0.00%
164	0.00%	0.87%	0.00%	0.00%	0.00%	0.00%	0.86%	0.00%	0.62%
167	0.00%	0.91%	0.60%	0.00%	0.00%	0.00%	1.67%	0.00%	0.00%
169	0.00%	0.00%	0.58%	0.57%	0.00%	0.66%	0.00%	0.00%	0.00%
171	3.64%	0.75%	0.00%	0.00%	1.20%	0.00%	1.34%	4.66%	0.61%
172	0.68%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	1.14%	0.00%
174	1.24%	3.04%	3.43%	2.67%	4.55%	5.63%	3.56%	2.33%	4.49%
175	0.00%	2.40%	1.43%	1.48%	0.00%	1.86%	0.00%	0.92%	0.99%
178	0.00%	2.63%	2.52%	0.00%	0.66%	0.00%	0.00%	0.00%	0.00%
179	0.00%	0.00%	0.00%	1.75%	1.08%	2.75%	1.56%	2.16%	2.09%
180	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
182	0.63%	0.90%	0.00%	0.00%	0.53%	0.00%	0.00%	0.55%	0.82%
184	0.00%	1.70%	1.58%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
187	0.78%	0.00%	1.61%	0.00%	1.71%	0.00%	0.00%	0.80%	0.00%
189	0.00%	0.82%	0.74%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
191	1.44%	3.78%	1.73%	1.07%	4.66%	2.47%	0.93%	1.48%	1.32%
192	0.66%	0.00%	0.00%	0.72%	0.81%	0.00%	0.00%	0.00%	0.00%
194	0.70%	2.47%	2.91%	1.36%	7.57%	5.21%	1.07%	4.58%	3.11%
196	1.24%	0.00%	0.00%	1.18%	0.00%	0.00%	0.69%	0.00%	0.55%
200	0.76%	0.00%	0.70%	0.00%	1.71%	0.00%	1.79%	2.51%	0.00%
203	0.00%	0.00%	0.60%	0.00%	0.00%	0.00%	0.00%	0.66%	0.00%
206	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
208	0.00%	0.00%	1.10%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
211	0.00%	0.00%	0.00%	0.91%	0.66%	0.00%	0.00%	0.00%	0.00%
213	0.55%	1.94%	0.00%	0.00%	0.00%	0.00%	0.00%	0.63%	2.77%
216	1.25%	0.00%	0.69%	0.56%	0.00%	0.00%	0.00%	0.00%	0.74%
218	0.00%	1.17%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
221	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.60%
224	0.00%	0.00%	0.54%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
227	0.00%	0.71%	0.51%	0.86%	0.60%	0.00%	0.00%	0.00%	0.00%
232	0.54%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
235	2.26%	0.00%	0.58%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
238	0.00%	0.00%	0.00%	0.60%	0.00%	0.00%	0.00%	0.74%	0.51%
241	0.00%	0.62%	0.00%	2.26%	2.89%	0.00%	0.00%	0.00%	0.00%
244	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	4.69%	0.00%	2.07%

Table D.6 ***Archaea*** **Automated Ribosomal Intergenic Spacer Analysis Aligned Control Column Data**

Fragment Length	D-1	D-2	D-3	E-1	E-2	E-3	F-1	F-2	F-3
122	0.00%	1.24%	0.00%	0.00%	0.54%	0.55%	0.00%	0.00%	0.00%
124	1.49%	0.70%	0.00%	0.00%	0.00%	0.00%	0.00%	0.78%	0.00%
125	0.58%	0.59%	0.58%	0.00%	0.00%	1.50%	1.16%	0.67%	3.36%
126	0.00%	0.00%	0.00%	0.00%	1.44%	0.00%	0.00%	0.00%	0.00%
128	0.00%	1.52%	0.53%	0.00%	1.36%	2.36%	0.90%	0.00%	0.00%
130	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	2.77%	0.00%
131	0.00%	0.00%	1.14%	0.00%	0.00%	0.00%	0.00%	0.99%	0.00%
132	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.75%	0.59%
134	0.61%	4.09%	3.58%	0.81%	4.69%	1.52%	1.52%	2.16%	1.26%
135	0.00%	5.21%	3.06%	2.01%	5.33%	2.95%	1.37%	2.88%	2.06%
136	0.97%	0.75%	1.31%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
138	0.00%	0.00%	0.55%	0.00%	0.87%	1.59%	0.00%	0.00%	0.93%
139	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
141	2.78%	5.28%	6.34%	1.28%	1.32%	2.55%	0.00%	0.00%	3.91%
142	1.10%	4.37%	3.64%	0.00%	0.00%	0.00%	1.10%	1.67%	1.15%
144	1.61%	0.00%	0.97%	0.00%	0.99%	4.39%	0.55%	0.00%	1.23%
146	0.51%	1.31%	0.70%	0.00%	1.23%	0.79%	1.39%	0.00%	1.25%
148	0.00%	0.00%	0.77%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
150	0.00%	1.06%	0.73%	0.00%	0.00%	1.22%	0.59%	1.28%	1.56%
151	0.00%	1.12%	1.00%	0.00%	0.00%	0.00%	0.00%	0.99%	0.67%
152	1.36%	1.40%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
154	0.00%	1.64%	0.99%	0.66%	1.40%	0.00%	0.00%	1.61%	2.06%
156	1.67%	0.99%	0.00%	0.00%	0.55%	0.00%	0.00%	0.00%	1.08%
158	0.00%	0.00%	0.00%	0.00%	3.19%	4.38%	0.00%	0.00%	0.00%
159	0.67%	0.71%	0.59%	0.00%	0.00%	0.51%	0.00%	2.07%	1.14%
161	0.00%	0.00%	0.00%	0.00%	0.00%	1.43%	0.00%	0.76%	0.00%
162	0.00%	0.55%	1.92%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
163	0.00%	0.00%	0.55%	0.00%	6.17%	0.80%	1.27%	0.89%	0.54%
164	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
167	0.00%	0.00%	1.04%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
169	0.00%	0.00%	0.72%	0.00%	0.00%	0.69%	0.00%	0.00%	0.00%
171	8.11%	3.98%	0.00%	0.99%	0.00%	4.63%	0.00%	0.00%	2.02%
172	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.72%	0.00%	0.53%
174	1.54%	4.16%	3.98%	0.98%	0.00%	4.89%	3.91%	2.25%	2.14%
175	0.00%	0.00%	0.85%	0.00%	2.45%	1.75%	1.21%	0.00%	2.09%
178	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
179	0.00%	2.53%	0.53%	0.00%	2.17%	1.76%	2.48%	1.61%	1.12%
180	0.00%	0.00%	0.78%	0.00%	0.00%	0.95%	0.00%	0.00%	0.00%
182	0.97%	0.77%	0.53%	0.75%	0.54%	0.84%	0.89%	0.00%	0.00%
184	0.00%	1.20%	0.00%	0.00%	0.00%	0.00%	0.72%	1.34%	0.00%
187	0.53%	0.00%	0.50%	0.00%	0.00%	0.00%	0.53%	0.00%	0.81%
189	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.57%
191	0.00%	1.56%	3.81%	0.00%	0.00%	0.00%	2.71%	2.74%	2.75%
192	0.00%	0.00%	0.00%	0.00%	0.75%	0.51%	0.00%	0.00%	0.00%
194	0.00%	0.00%	0.00%	0.00%	0.00%	2.01%	1.25%	2.35%	1.08%
196	4.30%	4.98%	3.05%	0.79%	3.40%	6.67%	0.00%	0.00%	0.00%
200	0.00%	0.51%	1.48%	0.00%	0.00%	0.00%	0.00%	0.56%	0.84%
203	0.00%	0.00%	0.00%	0.00%	3.30%	0.00%	0.67%	0.00%	0.79%
206	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.58%	0.00%	0.00%
208	0.00%	0.00%	0.00%	0.00%	0.00%	1.52%	1.23%	0.00%	0.00%
211	0.00%	0.00%	0.90%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
213	0.81%	0.00%	0.66%	0.00%	0.00%	0.81%	0.00%	0.00%	0.67%
216	0.00%	0.00%	0.00%	0.00%	0.00%	1.55%	0.00%	0.00%	0.00%
218	0.00%	0.00%	0.00%	0.00%	0.00%	0.54%	0.00%	0.56%	0.00%
221	0.00%	0.68%	0.55%	0.00%	0.00%	1.27%	0.00%	0.00%	0.00%
224	0.00%	0.00%	0.00%	0.00%	0.00%	0.77%	0.00%	0.00%	0.00%
227	0.50%	0.00%	1.82%	0.00%	0.00%	0.54%	0.00%	0.00%	1.09%
232	0.00%	0.00%	0.53%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
235	0.57%	0.00%	0.00%	0.00%	0.00%	0.72%	1.31%	0.00%	0.00%
238	0.89%	0.00%	1.53%	0.55%	0.00%	0.00%	1.36%	1.73%	0.00%
241	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	2.25%	0.00%	1.44%
244	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%

Table D.6 ***Archaea*** **Automated Ribosomal Intergenic Spacer Analysis Aligned Control Column Data**

Fragment Length	A-1	A-2	A-3	B-1	B-2	B-3	C-1	C-2	C-3
246	2.39%	2.97%	1.40%	3.33%	0.72%	1.69%	0.00%	0.00%	0.00%
250	2.87%	0.00%	0.00%	0.00%	0.00%	0.00%	1.24%	0.00%	0.00%
253	1.17%	0.52%	0.00%	0.00%	0.00%	0.00%	1.04%	0.00%	0.00%
255	0.83%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
259	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.83%	0.00%	1.42%
261	0.00%	0.65%	0.00%	0.67%	0.00%	0.58%	0.00%	0.00%	0.00%
265	0.00%	0.00%	1.22%	1.07%	0.00%	0.00%	0.00%	0.00%	0.00%
268	0.70%	0.79%	0.00%	0.00%	1.00%	0.00%	0.00%	0.00%	0.00%
270	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
273	0.00%	1.43%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
277	0.00%	0.00%	0.00%	0.00%	0.00%	0.89%	0.00%	0.00%	0.00%
285	0.00%	0.00%	0.00%	0.00%	0.87%	0.71%	0.00%	0.00%	0.00%
290	0.00%	0.00%	0.00%	0.88%	0.00%	0.00%	0.00%	0.00%	0.00%
299	0.00%	0.00%	0.00%	0.50%	0.00%	0.66%	0.00%	0.00%	0.00%
304	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
306	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.58%	0.52%	0.00%
308	0.00%	0.00%	0.00%	0.00%	0.00%	0.55%	0.00%	0.00%	0.00%
313	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
314	3.22%	1.41%	0.92%	1.33%	2.02%	1.74%	1.21%	0.54%	1.06%
317	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
322	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
323	25.75%	18.79%	24.93%	30.13%	30.86%	33.76%	28.92%	36.62%	34.46%
326	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
339	0.00%	0.00%	0.00%	0.69%	0.00%	0.00%	0.00%	0.00%	0.00%
347	0.84%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
352	0.00%	0.64%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	1.00%
357	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
364	0.00%	1.02%	0.00%	0.53%	0.00%	0.00%	0.00%	0.00%	0.00%
373	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
383	0.00%	0.00%	0.69%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
387	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	1.39%	0.00%	0.00%
391	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
396	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
397	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
402	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
409	0.67%	0.77%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
422	0.58%	0.62%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
430	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.76%
439	1.03%	1.76%	0.00%	0.93%	1.02%	0.58%	1.41%	0.66%	1.11%
444	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	16.33%	5.57%	4.78%
449	17.19%	4.95%	10.74%	8.05%	2.70%	1.55%	0.00%	0.00%	0.00%
465	0.63%	1.09%	0.87%	0.00%	0.00%	0.00%	0.96%	0.00%	0.00%
485	0.85%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
489	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
507	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
516	0.56%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
522	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
526	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
542	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
591	0.00%	0.00%	0.51%	0.00%	0.78%	0.57%	0.00%	0.00%	0.00%
668	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
739	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
785	0.00%	0.00%	0.00%	1.25%	0.00%	0.00%	0.00%	0.00%	0.00%
803	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
830	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
885	0.00%	0.55%	0.00%	0.58%	0.00%	0.57%	0.94%	0.00%	0.00%

Table D.6 ***Archaea*** **Automated Ribosomal Intergenic Spacer Analysis**
Aligned Control Column Data

Fragment Length	D-1	D-2	D-3	E-1	E-2	E-3	F-1	F-2	F-3
246	1.16%	1.45%	2.95%	0.92%	3.69%	1.79%	4.72%	7.25%	1.40%
250	0.50%	0.00%	0.56%	0.00%	0.00%	0.00%	0.70%	0.87%	0.00%
253	0.71%	0.00%	0.00%	1.10%	0.65%	0.97%	0.71%	0.00%	0.74%
255	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.82%	0.00%
259	0.00%	0.64%	0.62%	0.00%	0.00%	0.00%	0.00%	1.86%	0.00%
261	0.00%	0.79%	0.00%	0.00%	0.00%	0.00%	0.81%	0.00%	0.00%
265	0.67%	0.00%	0.00%	0.00%	0.83%	0.00%	0.00%	0.00%	0.00%
268	0.00%	0.00%	0.92%	0.00%	0.00%	0.00%	1.75%	0.53%	1.51%
270	0.00%	0.68%	0.50%	0.60%	0.00%	0.96%	0.00%	0.00%	0.00%
273	0.00%	0.00%	0.00%	0.00%	0.00%	0.46%	0.00%	0.00%	0.00%
277	0.00%	0.00%	0.00%	0.00%	0.00%	0.88%	0.91%	0.00%	0.00%
285	0.67%	0.76%	1.22%	0.00%	1.54%	0.53%	0.97%	1.13%	2.12%
290	0.61%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
299	0.00%	0.00%	0.00%	0.00%	0.00%	0.50%	1.36%	0.00%	0.00%
304	0.00%	0.00%	0.00%	0.00%	0.61%	0.00%	0.00%	0.00%	0.00%
306	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.61%	0.00%	0.00%
308	0.96%	0.00%	0.50%	0.00%	0.00%	0.00%	0.81%	0.00%	0.00%
313	0.87%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
314	0.63%	1.06%	0.94%	0.56%	1.43%	2.61%	1.80%	1.23%	0.00%
317	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.59%	0.00%
322	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
323	17.11%	21.41%	23.99%	10.27%	22.10%	18.90%	26.27%	33.25%	34.44%
326	0.00%	0.00%	0.00%	0.00%	0.00%	1.08%	0.00%	0.00%	0.00%
339	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
347	0.61%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.62%	0.00%
352	0.56%	0.00%	0.00%	0.00%	2.64%	0.00%	0.00%	0.00%	0.00%
357	0.00%	0.00%	0.00%	0.00%	0.68%	0.00%	0.68%	0.00%	0.00%
364	1.93%	0.00%	0.00%	0.00%	0.00%	0.00%	0.59%	0.50%	0.00%
373	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.78%	0.00%
383	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
387	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
391	0.00%	0.00%	0.00%	0.00%	0.72%	0.00%	0.00%	0.00%	0.00%
396	0.00%	0.00%	0.00%	0.00%	0.63%	0.00%	0.00%	0.00%	0.00%
397	0.00%	0.00%	0.00%	0.00%	0.50%	0.00%	0.51%	0.00%	1.16%
402	0.00%	0.00%	0.00%	0.86%	0.00%	0.00%	0.00%	1.30%	0.00%
409	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
422	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
430	0.00%	0.70%	0.53%	0.00%	0.00%	0.53%	0.53%	0.81%	0.73%
439	1.13%	1.13%	1.81%	0.00%	1.12%	1.80%	0.83%	1.05%	1.88%
444	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
449	26.42%	3.38%	2.03%	3.39%	10.68%	1.51%	10.60%	0.00%	1.89%
465	0.74%	1.12%	1.18%	0.69%	1.55%	1.26%	0.00%	1.28%	1.05%
485	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
489	0.00%	0.00%	0.59%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
507	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
516	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.51%	0.00%	0.00%
522	0.75%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
526	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
542	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.69%	0.00%	0.00%
591	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.55%	0.00%	0.00%
668	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
739	0.00%	0.00%	0.00%	0.00%	0.57%	0.00%	0.00%	0.50%	0.00%
785	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.50%	0.00%	0.00%
803	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
830	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
885	0.54%	0.51%	0.50%	0.00%	0.00%	0.00%	0.62%	0.51%	0.00%

Table D.7 ***Archaea*** **Automated Ribosomal Intergenic Spacer Analysis Aligned Pulsed-fed Molasses Column Data**

Fragment Length	A-1	A-2	A-3	B-1	B-2	B-3	C-1	C-2	C-3
122	2.65%	1.60%	1.04%	0.00%	1.07%	0.50%	1.35%	0.51%	0.00%
124	0.00%	0.00%	0.00%	5.11%	0.00%	4.93%	0.00%	0.00%	0.83%
125	0.00%	0.59%	0.79%	0.00%	0.00%	0.00%	1.03%	3.40%	1.63%
127	0.00%	0.00%	0.51%	1.53%	0.55%	0.00%	0.00%	0.00%	0.00%
129	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	1.12%	0.00%
130	0.00%	0.00%	0.00%	3.71%	3.27%	1.77%	0.00%	0.00%	0.00%
132	0.62%	4.06%	1.15%	0.00%	0.00%	0.00%	1.05%	0.00%	0.00%
133	2.47%	2.10%	1.08%	0.81%	0.90%	0.59%	0.92%	0.00%	0.57%
134	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
136	1.91%	0.00%	0.97%	0.50%	1.68%	0.90%	1.27%	1.49%	0.82%
138	0.86%	1.22%	1.04%	0.00%	0.00%	0.00%	0.00%	0.00%	0.98%
140	0.00%	0.00%	0.00%	4.60%	6.04%	4.85%	4.99%	7.83%	9.63%
141	5.95%	5.44%	6.34%	0.00%	0.00%	0.00%	0.00%	8.37%	0.00%
142	15.17%	13.91%	14.96%	16.62%	20.31%	18.39%	19.96%	11.95%	22.08%
144	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
147	1.17%	0.00%	0.55%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
148	1.24%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.94%
150	0.00%	0.00%	0.00%	0.95%	0.99%	5.01%	0.00%	2.61%	3.14%
152	0.00%	0.00%	0.00%	0.00%	0.00%	0.56%	0.00%	0.59%	0.00%
154	0.00%	0.00%	0.58%	0.00%	0.00%	0.53%	0.00%	0.00%	0.00%
158	3.96%	8.50%	5.66%	1.88%	2.15%	2.56%	1.12%	0.78%	1.88%
161	0.83%	0.00%	0.00%	0.00%	0.99%	0.96%	0.00%	0.00%	0.00%
163	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	1.44%	0.00%
164	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
168	0.00%	0.51%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
171	0.00%	0.00%	0.00%	0.86%	1.12%	0.00%	0.00%	0.00%	0.00%
173	0.00%	0.00%	0.91%	3.43%	0.00%	5.18%	3.55%	0.00%	0.84%
175	0.73%	0.00%	0.76%	0.00%	0.00%	0.55%	0.62%	0.51%	1.16%
178	1.88%	3.82%	1.16%	0.98%	3.64%	2.29%	10.15%	9.58%	8.69%
180	1.92%	0.00%	1.10%	0.90%	0.00%	0.00%	0.00%	0.00%	0.00%
181	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.59%
184	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.67%	0.00%
186	0.00%	0.00%	0.60%	0.00%	0.00%	0.55%	0.00%	0.00%	0.00%
190	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
191	0.54%	0.00%	1.25%	0.00%	0.78%	1.31%	1.71%	0.00%	1.29%
192	0.00%	0.00%	0.00%	0.00%	0.64%	0.00%	0.00%	0.00%	0.00%
194	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
195	3.54%	1.32%	2.65%	3.90%	2.55%	5.03%	4.89%	3.44%	4.33%
196	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
200	0.55%	0.95%	0.54%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
202	0.00%	0.00%	0.66%	0.00%	0.86%	0.00%	0.00%	0.00%	0.00%
205	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.92%	0.59%	0.00%
206	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
207	0.00%	0.00%	0.60%	0.88%	0.00%	0.00%	0.52%	0.00%	1.35%
209	0.00%	0.00%	0.00%	0.84%	0.00%	0.00%	0.00%	0.00%	0.00%
213	0.00%	1.65%	1.39%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
216	0.00%	0.00%	0.00%	1.22%	0.53%	0.00%	0.00%	0.00%	0.00%
220	0.00%	0.00%	0.00%	0.00%	0.54%	0.00%	0.00%	0.00%	0.74%
223	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
227	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.72%
234	0.78%	0.00%	0.00%	0.00%	0.00%	0.00%	0.54%	0.00%	0.00%
238	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.52%	0.00%	0.00%
242	0.00%	2.23%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
245	0.00%	0.00%	0.00%	0.71%	0.91%	1.19%	0.94%	1.11%	1.02%
249	0.00%	0.00%	0.00%	0.94%	0.00%	0.00%	1.15%	0.00%	0.00%
252	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
253	0.00%	0.00%	0.00%	0.00%	0.00%	0.50%	0.00%	0.00%	0.00%

Table D.7 ***Archaea*** **Automated Ribosomal Intergenic Spacer Analysis Aligned Pulsed-fed Molasses Column Data**

Fragment Length	D-1	D-2	D-3	E-1	E-2	E-3	F-1	F-2	F-3
122	0.00%	0.00%	0.74%	1.57%	1.38%	0.00%	0.00%	1.31%	0.00%
124	9.86%	0.00%	1.45%	2.28%	4.20%	2.43%	3.39%	7.08%	7.34%
125	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	2.97%	0.00%	0.00%
127	0.00%	0.53%	1.38%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
129	0.00%	0.00%	0.00%	0.58%	0.00%	1.75%	0.00%	0.00%	0.00%
130	1.76%	0.00%	0.58%	0.00%	0.00%	0.00%	1.21%	0.66%	0.00%
132	0.00%	0.75%	0.83%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
133	0.83%	0.00%	1.07%	0.75%	0.00%	0.96%	0.00%	0.00%	0.00%
134	1.33%	0.57%	0.83%	1.30%	1.40%	0.65%	3.65%	2.76%	1.12%
136	1.84%	0.64%	0.70%	1.78%	1.56%	1.08%	2.53%	3.47%	1.51%
138	0.00%	0.00%	0.00%	0.00%	0.00%	0.73%	0.00%	0.67%	0.00%
140	5.57%	17.69%	5.09%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
141	0.00%	0.00%	0.00%	0.00%	6.39%	7.97%	7.17%	6.32%	7.03%
142	19.82%	0.00%	18.04%	23.09%	14.02%	18.46%	17.81%	17.94%	17.09%
144	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.75%	0.00%	0.00%
147	0.50%	0.00%	0.00%	0.00%	0.68%	0.00%	0.94%	0.00%	0.00%
148	0.66%	0.00%	0.71%	0.51%	1.19%	0.00%	0.52%	0.00%	0.00%
150	1.73%	1.90%	1.67%	0.92%	1.37%	2.29%	3.02%	2.79%	4.96%
152	0.00%	0.53%	0.61%	0.00%	0.55%	0.00%	0.00%	0.00%	0.62%
154	0.00%	0.52%	0.00%	0.65%	0.00%	0.00%	0.90%	0.00%	0.00%
158	1.31%	1.94%	1.21%	3.30%	1.54%	0.80%	0.00%	0.00%	0.00%
161	0.92%	0.00%	0.00%	0.00%	0.00%	0.00%	0.59%	1.58%	0.00%
163	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.58%	0.81%	0.53%
164	0.00%	0.00%	0.00%	0.00%	1.24%	0.57%	0.00%	0.71%	0.00%
168	0.00%	0.00%	0.00%	0.00%	0.70%	0.72%	1.30%	0.00%	1.51%
171	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	1.00%	0.77%	0.00%
173	1.71%	1.04%	0.00%	0.73%	2.63%	1.81%	0.87%	0.00%	1.00%
175	0.00%	0.37%	1.08%	0.00%	0.00%	0.00%	0.50%	0.00%	1.56%
178	1.66%	9.11%	8.08%	9.02%	4.28%	4.76%	0.50%	1.96%	0.58%
180	0.83%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
181	0.68%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
184	0.53%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
186	0.56%	0.87%	0.00%	0.00%	0.00%	0.00%	0.62%	0.51%	0.57%
190	0.59%	1.00%	1.13%	0.00%	0.00%	0.00%	0.00%	0.95%	0.00%
191	0.89%	0.00%	0.00%	0.00%	0.95%	0.00%	0.00%	0.00%	0.00%
192	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.67%	1.12%	1.00%
194	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
195	1.07%	4.41%	1.51%	3.75%	2.41%	3.94%	4.54%	6.67%	5.92%
196	0.83%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
200	0.00%	0.00%	0.00%	0.00%	0.00%	0.84%	0.00%	1.13%	0.00%
202	0.00%	0.60%	0.78%	0.00%	0.57%	1.31%	0.00%	0.00%	0.00%
205	0.00%	0.59%	0.64%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
206	0.00%	0.00%	0.00%	1.33%	0.00%	0.51%	0.00%	0.00%	0.00%
207	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.56%	0.52%
209	0.00%	0.60%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
213	0.00%	0.00%	0.00%	0.00%	0.00%	0.89%	0.60%	0.00%	0.00%
216	0.00%	0.60%	1.02%	0.00%	0.95%	1.40%	0.68%	0.00%	0.00%
220	0.00%	0.00%	0.00%	0.56%	0.00%	0.00%	0.91%	0.00%	0.60%
223	0.00%	0.00%	0.00%	0.00%	0.94%	0.00%	0.00%	0.00%	0.00%
227	0.59%	0.00%	0.94%	0.00%	0.56%	0.00%	0.00%	0.00%	0.00%
234	0.00%	0.00%	0.00%	0.00%	0.00%	1.09%	0.00%	0.00%	0.00%
238	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.59%
242	0.00%	0.00%	0.00%	0.00%	0.00%	0.75%	0.97%	0.00%	0.73%
245	1.34%	1.53%	0.86%	0.62%	0.66%	0.56%	0.62%	0.53%	0.72%
249	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
252	0.00%	0.00%	0.74%	0.00%	0.00%	0.00%	0.00%	0.00%	1.01%
253	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.75%	0.56%	0.56%

Table D.7 ***Archaea*** **Automated Ribosomal Intergenic Spacer Analysis Aligned Pulsed-fed Molasses Column Data**

Fragment Length	A-1	A-2	A-3	B-1	B-2	B-3	C-1	C-2	C-3
259	0.00%	0.00%	0.00%	0.00%	0.96%	0.00%	0.00%	0.58%	0.00%
265	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
267	0.77%	0.97%	0.93%	0.00%	0.00%	0.57%	0.66%	0.00%	0.00%
269	4.36%	6.58%	4.70%	0.51%	0.54%	0.00%	0.00%	0.00%	0.00%
274	0.00%	0.00%	0.00%	2.87%	4.13%	3.05%	1.32%	1.17%	0.00%
276	0.00%	0.00%	0.00%	5.01%	6.30%	3.98%	0.99%	0.95%	0.00%
279	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
278	0.55%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
286	0.00%	0.78%	0.88%	0.00%	0.00%	0.00%	0.94%	0.00%	0.00%
288	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
290	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
294	1.30%	2.32%	1.31%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
297	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
302	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
308	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
314	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.72%	0.00%
316	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
322	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	11.82%	14.37%	11.75%
324	5.30%	2.96%	4.44%	19.20%	13.20%	14.28%	0.00%	0.00%	0.00%
334	0.00%	0.00%	0.00%	0.69%	0.00%	0.00%	0.00%	0.00%	0.00%
347	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
351	0.00%	0.52%	0.00%	0.75%	1.04%	0.64%	1.06%	0.75%	0.00%
369	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
386	0.51%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
396	0.00%	0.00%	0.00%	0.69%	0.00%	0.00%	0.00%	0.00%	0.00%
401	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
405	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.64%	0.00%
410	0.97%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
430	0.00%	0.00%	0.00%	0.52%	0.57%	0.66%	0.86%	0.00%	0.65%
439	0.00%	0.00%	0.00%	0.62%	0.82%	0.67%	0.73%	0.00%	0.55%
446	0.92%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
449	0.00%	0.00%	0.00%	0.71%	0.00%	0.00%	0.00%	0.00%	0.00%
452	9.09%	7.59%	8.87%	0.55%	3.27%	2.40%	10.84%	14.14%	6.33%
454	15.32%	13.62%	15.63%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
498	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
503	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.89%
506	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
525	0.00%	0.00%	0.00%	0.53%	0.53%	0.00%	0.00%	0.00%	0.00%
530	0.00%	0.00%	0.53%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
544	0.00%	0.00%	0.00%	1.68%	2.56%	1.02%	0.00%	0.54%	0.00%
664	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
795	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
831	0.00%	0.00%	0.00%	1.67%	0.00%	0.52%	0.00%	0.00%	0.00%
834	2.21%	1.83%	1.06%	0.00%	0.66%	0.00%	1.17%	0.80%	0.79%
848	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%

Table D.7 ***Archaea*** **Automated Ribosomal Intergenic Spacer Analysis Aligned Pulsed-fed Molasses Column Data**

Fragment Length	D-1	D-2	D-3	E-1	E-2	E-3	F-1	F-2	F-3
259	0.59%	0.00%	0.00%	0.00%	0.70%	0.79%	0.75%	0.74%	0.00%
265	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.81%	0.00%
267	0.72%	0.00%	0.00%	0.00%	1.11%	0.81%	0.00%	0.86%	0.93%
269	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
274	2.22%	0.00%	0.92%	0.00%	3.32%	2.72%	1.46%	1.20%	1.23%
276	2.35%	0.00%	0.93%	0.50%	3.86%	3.68%	1.82%	1.78%	1.86%
279	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
278	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
286	0.00%	0.00%	0.00%	0.00%	0.61%	0.00%	0.00%	0.98%	0.00%
288	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.56%	0.00%	0.00%
290	0.00%	0.00%	0.50%	0.00%	0.00%	0.00%	0.55%	0.00%	0.00%
294	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
297	0.00%	1.02%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
302	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
308	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.61%	0.00%	0.00%
314	0.00%	0.00%	0.96%	0.00%	0.00%	0.00%	0.42%	1.40%	0.00%
316	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
322	0.00%	6.89%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
324	14.13%	13.99%	17.26%	15.93%	18.20%	15.88%	13.16%	13.10%	16.90%
334	0.73%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
347	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
351	0.00%	0.00%	0.00%	0.75%	0.00%	0.00%	0.00%	0.00%	0.53%
369	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
386	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
396	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.70%	0.00%	0.00%
401	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
405	0.00%	0.73%	0.00%	0.66%	0.00%	0.00%	0.00%	0.55%	0.00%
410	0.00%	0.00%	0.00%	0.00%	0.00%	0.53%	0.54%	0.00%	0.00%
430	0.77%	0.65%	0.00%	0.58%	0.50%	1.39%	0.00%	0.00%	0.67%
439	0.87%	0.00%	0.51%	0.00%	0.80%	0.92%	1.10%	0.51%	1.75%
446	0.67%	0.45%	0.00%	0.57%	0.80%	0.72%	0.00%	0.00%	0.00%
449	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	2.74%	1.56%	1.86%
452	1.19%	15.71%	12.08%	12.92%	2.39%	2.21%	0.00%	0.00%	0.52%
454	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
498	0.73%	0.00%	0.00%	0.00%	0.76%	0.64%	0.00%	0.00%	0.00%
503	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
506	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
525	0.00%	0.00%	0.00%	0.00%	0.00%	1.04%	0.00%	0.00%	0.00%
530	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
544	1.95%	0.73%	0.89%	0.52%	1.13%	0.93%	1.03%	0.55%	0.00%
664	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
795	0.00%	0.00%	0.00%	0.00%	0.00%	0.50%	0.00%	0.54%	0.00%
831	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
834	0.81%	0.00%	0.66%	0.93%	1.18%	1.59%	0.00%	0.00%	0.00%
848	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%

Table D.8 ***Archaea*** **Automated Ribosomal Intergenic Spacer Analysis**
Aligned Continuously-fed Molasses Column Data

Fragment Length	A-1	A-2	A-3	B-1	B-2	B-3	C-1	C-2	C-3
123	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	1.18%	0.00%	0.88%
124	0.57%	2.51%	1.42%	0.00%	1.48%	0.00%	0.00%	2.11%	1.66%
125	3.41%	1.92%	0.00%	3.17%	2.26%	6.10%	5.17%	1.71%	1.60%
126	0.00%	0.00%	0.00%	0.62%	2.07%	1.72%	0.00%	1.19%	0.00%
129	2.16%	0.66%	1.52%	0.00%	1.35%	0.58%	0.55%	1.14%	1.83%
131	0.00%	0.00%	0.00%	1.61%	2.70%	0.00%	0.00%	0.00%	0.00%
132	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
133	0.86%	1.96%	2.74%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
134	0.93%	1.49%	1.12%	0.00%	1.84%	0.00%	2.72%	4.84%	1.85%
135	0.00%	0.00%	0.00%	1.71%	1.04%	1.28%	1.86%	2.04%	1.95%
136	0.50%	3.19%	2.95%	0.00%	0.00%	0.00%	0.58%	0.59%	2.20%
137	0.00%	0.00%	0.00%	1.73%	2.80%	4.28%	0.00%	0.00%	0.00%
140	11.44%	11.67%	22.24%	0.00%	0.00%	0.00%	21.79%	18.71%	26.19%
141	0.00%	16.68%	0.00%	3.22%	14.10%	28.22%	4.86%	5.10%	5.99%
142	32.19%	9.25%	0.00%	2.54%	3.21%	0.00%	0.00%	0.00%	0.00%
144	2.27%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.65%
145	1.21%	0.71%	1.42%	0.00%	0.00%	0.00%	0.00%	0.00%	0.99%
147	1.06%	0.00%	0.00%	0.50%	1.24%	0.00%	0.00%	0.00%	1.63%
149	3.66%	2.39%	1.00%	3.86%	2.28%	3.26%	2.14%	0.91%	5.11%
151	0.80%	1.38%	0.75%	0.00%	0.90%	0.00%	0.00%	0.00%	0.00%
153	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	1.45%	0.57%	0.00%
154	0.00%	0.00%	0.00%	1.78%	0.73%	0.00%	0.00%	0.00%	0.00%
156	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	1.41%	0.82%	0.00%
157	1.21%	0.00%	1.63%	2.97%	1.53%	1.12%	0.00%	0.00%	0.00%
159	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	1.93%	0.86%
161	0.85%	2.85%	0.00%	0.65%	2.36%	0.00%	0.00%	0.00%	0.00%
163	0.00%	0.94%	0.00%	0.65%	0.00%	0.00%	1.05%	1.28%	0.58%
164	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
166	0.00%	0.00%	0.00%	0.54%	1.00%	0.90%	0.54%	0.57%	0.00%
168	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
170	2.91%	3.76%	2.30%	1.32%	0.00%	0.00%	0.00%	0.00%	0.00%
171	0.00%	0.00%	0.00%	2.71%	3.50%	2.79%	2.81%	4.94%	2.81%
172	1.25%	0.00%	0.73%	0.51%	0.80%	0.82%	0.50%	1.13%	0.50%
174	0.00%	0.00%	0.00%	0.00%	0.72%	0.87%	0.00%	0.00%	0.00%
177	0.72%	1.42%	2.05%	0.00%	0.00%	0.65%	2.09%	0.66%	0.56%
178	0.00%	0.00%	0.00%	0.00%	0.00%	1.15%	0.00%	0.00%	0.00%
179	0.00%	0.00%	0.00%	2.16%	1.07%	0.94%	0.51%	0.93%	0.00%
181	0.56%	0.00%	0.00%	1.79%	0.61%	0.00%	0.66%	0.00%	0.00%
184	0.00%	0.00%	0.00%	0.93%	0.79%	0.00%	0.00%	0.00%	0.00%
186	0.00%	0.00%	0.00%	0.60%	0.00%	0.68%	0.00%	0.00%	0.00%
187	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.75%	0.00%	0.00%
189	0.00%	0.00%	0.83%	0.00%	0.00%	0.00%	1.39%	1.79%	0.00%
190	1.72%	0.68%	0.92%	1.32%	0.00%	1.88%	0.99%	0.61%	1.05%
192	0.00%	2.96%	10.40%	0.00%	0.00%	0.00%	0.00%	0.50%	0.00%
194	5.77%	4.48%	26.65%	8.89%	6.60%	6.18%	4.33%	9.48%	4.26%
195	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	2.93%
199	0.61%	0.00%	0.00%	0.96%	0.55%	0.00%	0.00%	0.00%	0.00%
202	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	1.02%	0.00%	0.00%
204	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	1.18%	0.57%	1.66%
207	0.00%	0.00%	0.00%	0.92%	1.08%	0.00%	0.00%	0.00%	0.00%
209	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
211	1.39%	0.00%	0.00%	1.14%	0.00%	0.00%	0.00%	0.62%	0.00%
213	0.00%	0.00%	0.00%	0.67%	0.00%	0.00%	0.00%	0.00%	0.00%
214	0.63%	0.54%	0.00%	0.84%	1.07%	0.00%	0.51%	0.00%	0.00%
221	0.83%	0.00%	0.00%	4.40%	0.89%	0.82%	1.32%	0.00%	0.73%
225	0.00%	0.00%	0.00%	0.41%	0.00%	0.00%	0.00%	0.00%	0.00%
227	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.63%	0.00%	0.00%
234	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
237	0.00%	0.00%	0.00%	2.05%	1.47%	1.46%	0.72%	0.75%	0.00%
239	0.82%	0.00%	1.43%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
241	0.00%	0.00%	0.68%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
244	0.57%	1.35%	0.00%	0.91%	1.19%	0.88%	0.87%	0.76%	0.90%
246	0.00%	0.00%	0.00%	0.83%	0.00%	1.00%	0.00%	0.00%	0.00%
251	0.65%	0.00%	0.00%	0.00%	0.52%	0.53%	0.00%	0.00%	0.00%

Table D.8 ***Archaea*** **Automated Ribosomal Intergenic Spacer Analysis**
Aligned Continuously-fed Molasses Column Data

Fragment Length	D-1	D-2	D-3	E-1	E-2	E-3	F-1	F-2	F-3
123	1.81%	0.61%	0.65%	0.57%	0.60%	0.72%	1.08%	0.00%	0.00%
124	0.00%	0.00%	0.65%	0.00%	0.00%	0.98%	3.53%	1.62%	0.79%
125	0.55%	1.59%	1.41%	1.25%	0.00%	2.14%	0.85%	2.75%	3.13%
126	0.00%	0.57%	0.00%	1.10%	0.00%	0.71%	0.90%	0.00%	0.00%
129	0.00%	0.97%	0.00%	0.00%	0.51%	0.00%	0.00%	0.57%	0.00%
131	0.00%	0.00%	0.00%	1.13%	0.79%	0.51%	0.78%	0.87%	0.00%
132	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
133	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
134	0.82%	2.16%	2.23%	1.87%	3.27%	2.51%	0.00%	1.37%	1.16%
135	0.82%	3.52%	2.28%	1.58%	2.19%	2.62%	2.13%	1.87%	0.81%
136	0.00%	0.00%	0.00%	0.75%	2.08%	1.50%	0.00%	1.86%	0.00%
137	1.34%	0.00%	2.24%	0.00%	0.00%	0.00%	0.00%	1.32%	0.51%
140	0.00%	0.00%	0.00%	21.99%	13.39%	15.83%	8.17%	0.00%	5.90%
141	26.64%	14.93%	26.89%	0.00%	0.00%	0.00%	2.29%	15.48%	1.58%
142	0.00%	0.00%	0.00%	0.00%	3.20%	1.11%	0.00%	0.00%	0.00%
144	0.00%	1.50%	1.87%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
145	0.00%	0.90%	0.94%	0.45%	1.31%	0.00%	0.00%	0.00%	0.00%
147	0.00%	0.00%	0.00%	2.70%	0.00%	0.00%	0.00%	0.00%	1.49%
149	1.22%	3.50%	1.25%	1.43%	0.66%	0.75%	2.07%	1.57%	2.20%
151	0.00%	0.93%	1.24%	0.00%	0.52%	0.53%	0.00%	2.88%	0.00%
153	0.00%	0.00%	0.00%	2.19%	0.50%	0.64%	0.00%	0.00%	0.00%
154	0.00%	0.00%	1.31%	0.00%	2.20%	0.00%	0.00%	0.00%	0.00%
156	0.00%	0.00%	0.00%	0.00%	0.62%	0.00%	0.93%	3.85%	0.00%
157	0.00%	0.00%	0.00%	0.00%	1.91%	3.52%	0.00%	0.00%	0.00%
159	1.21%	0.76%	1.76%	0.00%	0.00%	0.00%	0.63%	0.88%	0.00%
161	0.84%	1.01%	0.65%	0.00%	0.00%	0.00%	0.59%	0.59%	0.99%
163	0.00%	0.00%	0.00%	0.71%	0.00%	0.00%	0.66%	0.00%	0.67%
164	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
166	1.08%	1.04%	0.00%	0.64%	1.16%	0.00%	0.00%	0.00%	0.00%
168	0.00%	0.00%	0.00%	0.00%	0.50%	0.00%	0.70%	1.09%	0.00%
170	0.00%	0.00%	0.00%	1.22%	0.00%	3.64%	3.06%	1.03%	1.64%
171	7.62%	7.52%	5.61%	0.00%	1.74%	0.64%	0.00%	0.00%	0.00%
172	0.00%	0.00%	1.01%	0.00%	0.66%	0.00%	0.00%	0.00%	0.68%
174	0.00%	1.52%	2.32%	3.69%	1.65%	2.79%	0.67%	0.83%	1.68%
177	0.91%	0.85%	0.00%	0.00%	0.00%	0.00%	1.33%	0.00%	0.00%
178	0.00%	0.00%	0.00%	0.69%	1.41%	1.81%	0.00%	0.00%	0.00%
179	0.00%	0.76%	1.41%	0.00%	1.34%	0.85%	1.07%	2.17%	1.22%
181	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	1.07%	1.39%	0.72%
184	0.00%	0.00%	0.00%	0.67%	0.00%	0.00%	0.00%	0.00%	0.00%
186	0.00%	0.59%	0.00%	0.00%	0.00%	0.00%	0.50%	0.60%	0.00%
187	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.51%	0.00%	0.00%
189	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
190	0.00%	0.00%	0.00%	0.62%	0.54%	0.00%	0.00%	0.00%	0.00%
192	1.95%	1.42%	0.73%	0.63%	1.15%	1.39%	0.00%	1.20%	0.51%
194	4.19%	8.59%	4.62%	4.55%	1.88%	4.50%	0.00%	0.84%	0.58%
195	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	2.61%	3.56%	4.74%
199	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.93%	0.00%	0.00%
202	0.00%	0.00%	0.59%	0.56%	0.91%	0.59%	0.63%	0.65%	0.00%
204	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.52%	0.00%
207	0.00%	0.63%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.57%
209	0.00%	0.00%	0.00%	0.67%	0.67%	0.00%	0.00%	0.00%	1.08%
211	0.00%	0.00%	0.00%	0.00%	0.52%	0.00%	0.50%	0.00%	0.00%
213	0.00%	0.58%	0.00%	0.00%	0.00%	0.00%	0.73%	0.00%	0.00%
214	0.00%	0.79%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
221	0.00%	0.00%	0.75%	0.50%	0.00%	0.00%	0.62%	0.00%	0.71%
225	0.98%	0.00%	0.83%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
227	0.78%	0.00%	0.00%	0.52%	0.00%	0.00%	0.91%	0.00%	0.93%
234	0.00%	0.99%	0.00%	0.84%	0.00%	0.51%	0.00%	0.00%	0.80%
237	2.14%	0.74%	0.64%	0.00%	0.00%	0.00%	0.00%	0.00%	0.90%
239	0.00%	0.00%	0.00%	0.90%	0.71%	0.00%	1.13%	0.57%	0.00%
241	1.28%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.85%	0.58%
244	2.56%	0.00%	1.50%	0.98%	4.29%	2.38%	3.34%	4.63%	7.79%
246	0.66%	1.19%	0.00%	0.00%	1.10%	0.00%	0.61%	0.00%	0.00%
251	0.98%	3.07%	0.00%	1.85%	0.58%	0.00%	0.00%	0.60%	4.44%

Table D.8 ***Archaea*** **Automated Ribosomal Intergenic Spacer Analysis**
Aligned Continuously-fed Molasses Column Data

Fragment Length	A-1	A-2	A-3	B-1	B-2	B-3	C-1	C-2	C-3
253	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	2.13%	1.48%	0.95%
254	0.00%	0.00%	0.00%	1.71%	0.00%	0.53%	0.00%	0.00%	0.54%
258	0.00%	0.92%	0.00%	0.56%	0.00%	0.00%	0.70%	0.81%	1.24%
259	0.00%	0.61%	0.00%	1.80%	1.69%	1.94%	0.52%	1.26%	0.66%
261	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
267	0.00%	0.00%	0.00%	0.59%	0.82%	0.00%	0.00%	0.00%	0.00%
269	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
273	0.81%	1.34%	1.40%	0.54%	0.00%	0.00%	1.00%	0.67%	0.77%
275	1.18%	2.03%	1.49%	0.74%	1.36%	1.14%	0.00%	0.00%	0.00%
277	0.70%	0.00%	0.00%	0.00%	0.00%	0.53%	0.00%	0.00%	0.00%
281	0.00%	0.00%	0.00%	0.96%	0.00%	0.74%	0.00%	0.00%	0.00%
283	0.00%	0.00%	0.00%	0.00%	0.00%	0.66%	0.00%	0.00%	0.00%
286	0.00%	1.54%	0.53%	0.00%	0.00%	0.00%	0.00%	0.62%	0.00%
289	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
295	0.00%	0.00%	0.00%	0.61%	0.00%	0.00%	0.00%	0.00%	0.00%
298	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
308	0.00%	0.00%	0.00%	1.84%	0.00%	0.00%	0.00%	0.00%	0.00%
312	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
314	0.54%	2.19%	0.00%	5.46%	1.78%	3.13%	1.34%	1.96%	1.81%
316	0.22%	0.00%	0.00%	0.00%	0.00%	0.00%	0.57%	0.00%	0.00%
322	0.00%	0.00%	0.00%	0.80%	5.60%	2.93%	0.00%	0.00%	1.24%
323	1.53%	1.39%	1.74%	0.00%	0.00%	0.00%	4.96%	3.03%	1.11%
326	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.75%	0.00%
335	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
345	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.73%
352	0.94%	0.00%	0.00%	0.00%	0.93%	1.19%	0.76%	0.00%	0.00%
355	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
365	0.00%	0.00%	0.00%	0.56%	0.00%	0.60%	0.00%	0.00%	0.00%
376	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
380	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
396	0.00%	0.00%	0.00%	0.52%	0.79%	0.00%	0.56%	0.60%	0.00%
410	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.57%	0.00%
416	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
423	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
431	0.88%	0.63%	0.51%	0.00%	0.69%	0.91%	0.00%	1.93%	0.72%
440	0.58%	0.00%	0.00%	0.00%	0.00%	0.46%	0.00%	0.00%	0.00%
450	0.00%	0.00%	0.00%	4.48%	2.20%	2.78%	6.25%	3.52%	4.68%
465	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.82%	0.50%	0.00%
479	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
488	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
516	0.00%	0.00%	0.00%	0.61%	0.00%	0.00%	0.00%	0.00%	0.00%
522	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
526	0.00%	0.73%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
533	0.00%	0.00%	0.00%	0.00%	1.47%	0.00%	0.00%	0.00%	0.00%
542	0.00%	0.00%	0.59%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
545	1.78%	0.63%	0.00%	3.26%	0.78%	0.00%	1.73%	1.32%	0.00%
557	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.57%	0.63%
590	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
739	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
794	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.50%
830	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
832	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
834	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
884	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.52%	0.52%	0.00%
925	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
962	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.75%	0.00%

Table D.8 ***Archaea*** **Automated Ribosomal Intergenic Spacer Analysis**
Aligned Continuously-fed Molasses Column Data

Fragment Length	D-1	D-2	D-3	E-1	E-2	E-3	F-1	F-2	F-3
253	0.00%	1.00%	0.68%	0.00%	1.21%	1.29%	0.00%	0.00%	0.00%
254	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
258	0.93%	0.00%	0.00%	1.18%	1.44%	0.51%	1.20%	0.00%	0.00%
259	0.99%	1.64%	0.52%	0.00%	0.00%	0.60%	0.57%	0.80%	0.00%
261	0.00%	0.00%	0.67%	0.00%	0.00%	0.00%	0.66%	0.00%	0.00%
267	0.73%	0.00%	0.92%	0.91%	0.00%	1.24%	1.09%	1.50%	0.00%
269	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.50%	0.00%	0.00%
273	0.00%	0.00%	0.61%	0.50%	0.00%	0.00%	0.00%	0.00%	0.00%
275	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
277	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
281	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.61%	0.00%	0.00%
283	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
286	0.56%	1.02%	0.00%	0.00%	0.66%	0.59%	0.00%	1.04%	1.23%
289	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
295	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.54%	0.00%	0.00%
298	0.52%	0.00%	0.00%	1.25%	0.00%	0.00%	0.88%	0.97%	0.00%
308	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.50%	0.00%
312	1.37%	0.80%	0.79%	0.61%	0.00%	0.99%	1.18%	1.29%	0.00%
314	2.87%	3.14%	1.65%	5.02%	5.41%	3.44%	0.96%	1.69%	3.13%
316	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.26%	0.00%	0.00%
322	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
323	3.10%	2.21%	1.78%	7.95%	3.75%	4.48%	15.36%	12.47%	17.61%
326	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
335	0.00%	0.00%	0.00%	0.00%	0.60%	0.00%	0.00%	0.00%	0.00%
345	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	1.04%	0.00%	0.00%
352	0.00%	0.00%	0.78%	0.68%	0.00%	0.00%	0.64%	0.00%	0.00%
355	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.52%	0.00%	0.00%
365	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	1.08%
376	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	3.03%	0.00%	0.51%
380	0.00%	0.00%	0.00%	0.70%	0.00%	0.00%	0.00%	0.00%	0.00%
396	0.00%	0.55%	0.00%	0.00%	0.00%	0.73%	0.00%	0.00%	0.00%
410	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.68%	0.00%	0.00%
416	0.00%	0.00%	0.00%	0.00%	2.65%	0.00%	0.00%	0.00%	0.00%
423	0.00%	0.00%	0.00%	0.00%	0.50%	0.00%	0.00%	0.00%	0.00%
431	0.00%	0.00%	0.00%	0.52%	0.89%	0.00%	0.00%	0.00%	0.00%
440	0.75%	0.59%	0.00%	0.74%	0.83%	0.91%	1.09%	1.03%	1.76%
450	12.76%	11.24%	9.83%	10.89%	11.83%	15.46%	12.64%	7.75%	8.38%
465	0.00%	0.00%	0.00%	0.00%	0.49%	0.00%	1.21%	0.74%	0.74%
479	0.60%	0.00%	0.51%	0.67%	0.88%	0.00%	0.00%	0.00%	1.84%
488	0.00%	0.00%	0.00%	0.00%	0.58%	0.00%	0.00%	0.00%	0.00%
516	0.00%	0.00%	0.00%	0.00%	0.62%	0.00%	0.00%	0.00%	0.00%
522	0.51%	0.00%	0.00%	0.00%	0.00%	0.00%	0.63%	0.00%	0.00%
526	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
533	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
542	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
545	0.75%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
557	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
590	0.00%	0.00%	0.60%	0.00%	0.00%	0.67%	0.61%	0.00%	0.81%
739	0.00%	0.00%	0.00%	0.00%	0.00%	0.61%	0.00%	0.00%	0.00%
794	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
830	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
832	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
834	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
884	0.00%	0.00%	0.54%	0.97%	0.97%	0.92%	0.64%	0.83%	0.00%
925	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
962	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%