Controlling Colloidal Silica Grouts using Microbial Fermentation Activity

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Abstract

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Colloidal silica grouts have long been recognized as an environmentally-conscious ground improvement solution capable of stabilizing weak, problematic soils and achieving large reductions in soil hydraulic conductivities for geotechnical applications including liquefaction mitigation and seepage reduction. In the conventional approach, various chemical accelerants are first added to colloidal silica suspensions, solutions are injected into the intended treatment zone, and gelation occurs over time resulting from both colloidal negative surface charge neutralization and van der Waals attraction between colloids. Although the method holds significant promise for practical ground improvement applications, effectively controlling the gelation time for these grouts and ensuring their stability when subjected to various field-representative subsurface conditions has remained a significant challenge. When high ionic strength brackish/marine subsurface conditions are encountered, silica grouts can set too quickly resulting in a limited extent of improvement with associated hydraulic conductivity reductions that prevent subsequent

treatment attempts. In other cases, unexpected differences in soil mineral and solution chemistries can result in grouts that fail to set long after injections resulting in minimal engineering improvements. In an effort to overcome these limitations, this research investigates the potential of bio-mediated processes to control the rate of colloidal silica gelation thereby forgoing the need for chemical accelerants and improving the probability of treatment success for various subsurface conditions. In this study, the use of microbial fermentation activity was examined which was expected to control gelation through both solution pH reductions and ionic strength changes. A series of batch and soil column experiments were performed to investigate this process using both augmented mixed-acid fermenting bacteria and stimulated microbial communities under varying chemical conditions. Results suggest that both augmented and stimulated bacteria can be used to successively control colloidal silica gelation and that the rate of gelation is dependent on both the magnitude and rate of fermentation-induced pH reductions. Furthermore, soil column experiments, which up-scaled treatment techniques developed in batch, demonstrated the ability of both aqueous solution and geophysical measurements to effectively monitor gelation progression. Although additional characterization of the mechanical behavior of these biologically-improved soils is needed, treated poorly-graded sands in this study exhibited modest tensile strengths and hydraulic conductivity reductions near two orders of magnitude

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Chapter 1: INTRODUCTION

Traditional ground improvement methods oftentimes use either high mechanical energy and/or intensive materials to improve soil engineering properties (Mitchell et al. 2005). Increased awareness of the impacts associated with these processes (i.e., greenhouse gas emissions, aqueous toxicity) has sparked interest in alternative technologies that can obtain comparable engineering improvements with potential reductions in environmental impacts. Over the past two decades, researchers have explored the potential of bio-mediated soil improvement methods, which use microbial metabolic and/or enzymatic activities, to achieve significant enhancements in soil engineering behaviors (Seagren and Aydilek 2010; Burbank et al. 2013; DeJong et al. 2014; Gomez et al. 2016). These processes can leverage natural biogeochemical processes to modify soil geotechnical behaviors through a variety of mechanism including: (1) the precipitation of new mineral phases which bind soil particles, (2) the generation of insoluble gas phases which can alter pore fluid characteristics, and (3) the formation of various biopolymers which can fill pore spaces and act to impart small tensile strengths (DeJong et al. 2006, 2010, 2014; Gomez et al. 2016; and others). Of these bio-mediated soil improvement technologies, perhaps the most widely researched is ureolytic bio-cementation, which has garnered significant interest from both academia and engineering practice and has demonstrated that such technologies may have important practical applications moving forward (DeJong et al. 2014; Gomez et al. 2016 and others). While the success of these existing processes has been encouraging, a much larger suite of bio-mediated processes will be needed to address the wide array of different geotechnical use cases for which ground improvement is needed. For example, few existing bio-mediated processes have been identified which can improve successfully improve soils under low pH conditions. In addition, when large

hydraulic conductivity reductions are desired, current processes such as bio-cementation have remained relatively material-inefficient and ineffective.

This research explores the potential of a new bio-mediated process wherein microbial glucose-fermentation is used to control the gelation of otherwise abiotically-stable colloidal silica suspensions. The process is similar to existing colloidal silica grouting techniques, however, rather than supplying purely chemical accelerants, the process investigated herein utilizes microbial activity to improve the control of gelation timing and afford the ability for improved process monitoring. The bio-mediated colloidal silica grouting process investigated in this research has the potential to address a wide variety of new engineering applications for which few existing bio-mediated technologies currently exist. This includes the improvement of soils under low pH conditions for purposes spanning seepage reduction and liquefaction mitigation, all while eliminating potentially environmentally-compromising by-products.

1.1 Colloidal Silica Grouting

Colloidal silica grouts have long been recognized as an environmentally-conscious ground improvement solution capable of stabilizing weak, problematic soils and achieving large reductions in soil hydraulic conductivities for geotechnical applications including liquefaction mitigation and seepage reduction (Gallagher and Mitchell, 2002; Pamuk et al. 2007; Gallagher et al. 2007 a, b; Diaz-Rodriguez et al. 2008; Spencer et al. 2008; Gallagher and Lin 2009; Conlee 2010; Hamderi and Gallagher 2015; Georgianou et al. 2017; and others). Colloidal silica solutions are composed of amorphous, non-porous, spherical silica nanoparticles (diameters = 5 to 100 nm)

existing in a surrounding liquid phase (Gallagher et al. 2012). When the pH of surrounding solutions is either relatively low (pH < 5) or relatively high (pH > 7 to 8), colloidal silica solutions can exhibit low viscosities near that of water due to strong electrical repulsion between colloids, which acts to stabilize suspensions. These interparticle forces are highly dependent on surface charge of adjacent colloids, with changes in pH values and/or the addition of chemical accelerants (i.e., salts) allowing for the formation of inter-colloidal bonds due surface charge neutralization and van der Waals attraction. The formation of such bonds results in "gelation" with rapid increases in viscosities and the eventual formation of solid resonating gels. In the conventional approach, the length of the time required for gelation (i.e., "induction period" or "gelling period") is carefully manipulated by varying the solution chemistry (i.e., salt, pH, silica concentration, and colloid size) of suspensions prior to injection.

Colloidal silica suspensions are most commonly stabilized at high pH using sodium hydroxide-based solutions, which can increase colloidal repulsion via highly negative colloidal surface charges and thus prevent gelling. Other stabilizing solutions can be used, however, such as highly acidic solutions which can act to induce repulsion via highly positive surface charges. In this sense, the mechanisms responsible for colloidal silica stability and aggregation are quite similar to those responsible for clay mineral aggregation/dispersion (i.e., double layer theory) (Persoff et al. 1999) with changes in both ion concentrations, ion valences, and pH being highly influential towards affecting a given solution's stability over time. This pH and ion sensitivity results from the presence of both silanol ($SiOH^-$) functional groups, which exist on the surface of silica colloids. At high pH and/or low cation concentrations, these groups can exhibit strongly negative surface charges, which can be neutralized through either increases in surrounding cation concentration or

pH decreases (H^+ increases). Conversely at low pH, these same groups can exhibit strongly positive surface charges, which can be neutralized through either increases in surrounding anion concentrations or pH increases (H^+ decreases). As these surface charges become increasingly neutralized, inter-colloidal electrical repulsion is decreased, and silica colloids interact to form siloxane bonds (Si-O-Si) between surface groups as the result of van der Waals attraction (Sogaard et al. 2018). Once sufficient bonds have formed, the viscosity of the suspension increases and continued aggregation results in the formation of stiffer and firmer gels that can act to restrain adjacent soil particles when supplied as a grout. While both the pH and ionic strength of surrounding solutions can alter gelation kinetics, when stable solutions are adjusted to obtain pH values between 5 and 7.5, colloidal aggregation is generally favored (DuPont et al. 1997; Gallagher et al. 2000; Sogaard et al. 2018).

In previous geotechnical studies, colloidal silica grouts have been shown to enable significant enhancements in soil engineering behaviors following gelation for both geotechnical and geoenvironmental engineering applications (Gallagher and Mitchell 2002; Pamuk et al. 2007; Spencer et al. 2008; Gallagher and Lin et al. 2009; Conlee et al. 2010; Hamderi and Gallagher et al. 2015; Georgianou et al. 2017). For example, soil hydraulic conductivity measurements on Monterey 0-30 Sand have shown that reductions of up to 2 order of magnitude are possible when > 5 % by mass colloidal silica grouts are used (Persoff et al. 1999). Loose sands (D_r = 22 %) treated with 5 to 30% by mass colloidal silica grouts have also been shown to achieve unconfined compressive strengths ranging between 30 and 220 kPa (Gallagher et al. 2002). Furthermore, in cyclic triaxial tests, Gallagher et al. (2002) showed that the cyclic resistances of loose sand (D_r = 22%) specimens to liquefaction triggering could be significantly improved when treated with 5%

and 10% by mass colloidal silica grouts. Following liquefaction triggering, strain accumulation during cyclic loading was also shown to be more limited in treated soils versus similar untreated specimens. Such engineering enhancements are afforded by the presence of firm colloidal silica gels, which can act to impart a small tensile strength, restrict soil volumetric tendencies during shearing, and in turn limit excess pore pressure generation (Gallagher et al. 2002, Diaz-Rodriguez et al. 2004; Gallagher et al. 2007, Gallagher et al. 2009, Gallagher et al. 2012; Georgiannou et al. 2017).

1.2 Limitations of Traditional Colloidal Silica Grouting

Although colloidal silica grouts can be advantageous for a variety of different geotechnical use cases, several limitations have continued to limit the adoption of this technology in engineering practice. First, effectively controlling the gelation time of colloidal silica grouts and ensuring their stability when subjected to various field-representative subsurface conditions has remained a significant challenge (Gallagher and Mitchell et al. 2002). For example, when high ionic strength brackish/marine subsurface conditions are encountered, silica grouts can set too quickly resulting in a limited extent of improvement with associated hydraulic conductivity reductions that prevent subsequent treatment attempts. In other cases, unexpected differences in soil mineral and solution chemistries can result in grouts that fail to set long after injections resulting in minimal engineering improvements. Moving forward, the ability to better regulate gelation timing will likely be critical towards achieving successful and more uniform improvement of soils over practical treatment distances. Secondly, when applied to subsurface soils almost no methods exist which can effectively monitor the colloidal silica gelation process in-situ and successfully verify improvement after treatment application. Gallagher et al. (2007) conducted a field trial at a silty

sand site along the Fraser River in B.C., Canada and found that neither CPT sounding measurements (e.g. q_c, f_s) nor shear wave velocity measurements could effectively verify resulting improvement over existing soil heterogeneity. In order for this technology to achieve more widespread adoption, it is clear that these limitations must be addressed.

1.3 Bio-mediated Processes to Enhance the Control of Colloidal Silica Grouts

In an effort to overcome some of the aforementioned limitations of traditional colloidal silica grouting processes, this research aimed to investigate the potential of microbial processes to control the rate of colloidal silica gelation, thereby forgoing the need for chemical accelerants and improving the probability of treatment success for various subsurface conditions. While the use of a biological process to control colloidal silica gelation had not yet been demonstrated, it was hypothesized that microbial fermentation activity could be used to control colloidal silica gelation through both controlled solution pH reductions and ionic strength increases. Fermentation is a ubiquitous metabolic pathway performed by a variety of different microorganisms under anaerobic conditions in order to generate energy. During fermentation, supplied carbohydrates (i.e. saccharides) are converted into a variety different end-products including ethanol, CO_2, H_2, and many different organic acids without utilizing oxygen, thus yielding energy (e.g. ATP). While the use of microbial fermentation pathways has not been extensively investigated for geotechnical applications, the fermentation process was expected to be ideal for subsurface geotechnical applications wherein oxygen is oftentimes limited. In particular, it was suspected that microbial fermentation activity could be used to control colloidal silica gelation by gradually reducing solution pH and increasing the ionic strength of highly alkaline colloidal silica suspensions after

injection. Such solutions would otherwise remain highly stable abiotically and therefore would likely afford increased stability under varying environmental conditions.

While the process was conceptualized, it remained unclear if fermentative microorganisms would be able to tolerate highly alkaline initial pH values (near 9.5) and other constituents present in colloidal silica suspensions (e.g. salts). In all experiments it was suspected that glucose, an abundant simple sugar, could be included in colloidal silica solutions as a fermentative substrate with either augmented cells supplied directly or growth factors provided to enrich native fermentative microorganisms. In order to effectively design these treatment strategies, however, and understanding of potential candidate microorganisms for this process was needed.

Although this research initially considered the use of lactic acid bacteria to facilitate this process, initial experiments were unable to successfully mediate colloidal silica gelation using a single bacterial isolate introduced under sterile experiments. For this reason, such results are not presented herein. Moving forward, the potential of both augmented mixed-acid fermenting bacteria and stimulated fermentative microbial communities to facilitate this process was explored. Mixed-acid fermenting bacteria were of interest due to their abundance in natural systems and their ability to generate organic acids mixtures which largely had pKa values that were consistent with targeted pH ranges (e.g. lactic acid pKa = 3.86; acetic acid pKa = 4.76; succinic acid pKa_1 = 4.21, pKa_2 = 5.64; formic acid pKa = 3.75). Mixed-acid fermenting bacteria include a diverse set of gram-negative anaerobic bacteria, which can ferment supplied carbohydrates (e.g. glucose) to yield a variety of different by-products including lactate, acetate, succinate, formate, CO_2, H_2, with associated pH reductions. Among other genera, mixed-acid fermenting bacteria can include those

from *Escherichia*, *Salmonella*, and *Shigella*, and are frequently used for commercial industrial processes and biotechnology applications (Brock et al. 2003). **Figure 1.1** presents a simplified schematic of the mixed-acid fermentation pathway following Ciani et al. (2013).

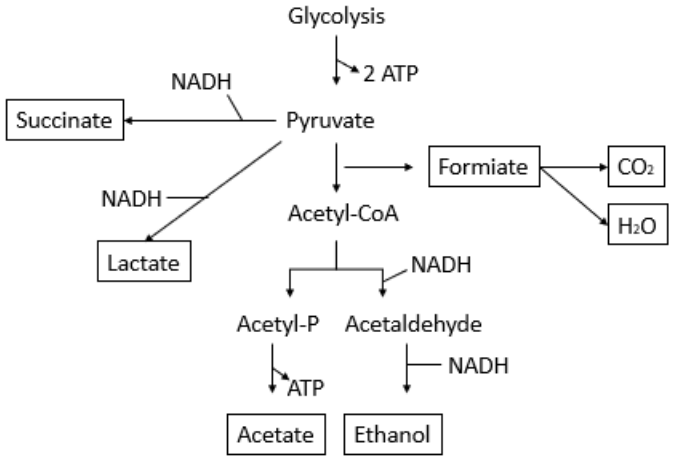

Figure 1.1. Simplified schematic of the mixed-acid fermentation pathway following Ciani et al. (2013)

Although it was hypothesized that mixed-acid fermenting bacteria could be used to facilitate the proposed fermentation process, it remained unclear how process kinetics might be affected as a function of treatment strategy, microbial cell densities, treatment solution chemical conditions, soil types, augmented strain types, and a multitude of other factors. Similar to treatment approaches employed for bio-cementation, two main treatment strategies were considered for achieving sufficient microbial glucose fermentation activity: (1) bio-augmentation wherein bacterial isolates would be supplied directly at sufficient cell densities, and (2) bio-stimulation wherein growth factors and substrates would be supplied to natural soils with the intention of enriching native microbial communities for microorganisms with the ability to successfully ferment glucose and generate increasingly acidic conditions. While it was suspected that augmentation could provide an opportunity to better understand the effect of treatment conditions on fermentation activities by

removing the need for cell growth and enrichment, it was also suspected that such a process might necessitate increases in treatment costs and environmental impacts due to the need for microbial cell culturing, transportation, and injection. In contrast, while stimulation was expected to provide practical advantages with respect to treatment costs, environmental impacts, and cell spatial uniformity, it was also suspected that such experiments would be more challenging to understand given the need for simultaneous enrichment/growth of cells and sufficient fermentation activity after treatment injections. For this reason, both treatment strategies were explored in this work in an effort to better understand the feasibility and efficacy of a biologically-controlled colloidal silica gelation process.

1.4 Thesis Organization

In an effort to overcome the limitations of traditional colloidal silica grouting, this research investigated the potential of bio-mediated processes to control the rate of colloidal silica gelation thereby forgoing the need for chemical accelerants and improving the probability of treatment success for various subsurface conditions. A series of batch and soil column experiments were performed to investigate this process using both augmented mixed-acid fermenting bacteria and stimulated microbial communities under varying chemical conditions. Chapter 2 presents a series of abiotic batch experiments aimed at understanding the effect of solution salinity and pH on the kinetics of colloidal silica gelation without biological activity. Chapters 3 and 4 present batch experiments aimed at improving our understanding of the effect of treatment and environmental factors on the kinetics of glucose fermentation by stimulated and augmented microbial communities. Chapter 5 further explores differences and similarities between the investigated stimulated and augmented biologically-controlled processes including glucose utilization

efficiencies and inhibition by NaCl. Chapter 6 presents a series of soil column and batch experiments, wherein previously developed stimulation treatment techniques were up-scaled to more representative soil volumes to explore a variety of different monitoring methods and assess post-treatment engineering improvements. Finally, Chapter 7 presents conclusions and final thoughts regarding this research work.

Chapter 2: ABIOTIC EXPERIMENTS

2.1 Introduction

Controlling the gelation of colloidal silica requires an understanding of various chemical factors that can influence the process. This includes determining the ideal conditions under which gelation can occur as well as the consideration of processes by which gelation progression can be monitored and assessed. Surrounding solution chemistry as well as silica concentrations and colloidal sizes can all influence the temporal rate of colloidal silica gelation (Gallagher et al. 2000; Sogaard et al. 2018). At high pH and low ion concentrations, silica colloids exhibit high negative surface charge, which acts to keep colloids in a disperse suspension with a viscosity similar to that of water. Colloidal gelation or aggregation can therefore be controlled by either adding charge-neutralizing cations and/or decreasing the surrounding solution pH (increasing H^+ concentrations) which allows for colloidal attraction via van der Waals forces to dominate (Sogaard et al. 2018). In past studies, changes in solution chemistries through pH and/or ion concentration variations have been shown to afford both increases and decreases in gel times depending on the desired effects (Gallagher et al. 2008; Conlee et al. 2012). Overall, these studies have shown expected trends: (1) the addition of ions increases gelation rates through surface charge neutralization, and (2) pH variations can either increase or decrease gelation rates depending on their magnitudes. Gelation rates have been shown to be generally fastest when surrounding fluids have pH values between 5 and 7 with pH values above or below this range corresponding to increases in gel times (i.e. decreases in gelation rates) (Gallagher et al. 2000). Past studies have shown that increasing solution colloidal silica concentrations can also increase gelation rates (Gallagher et al. 2009).

While it was hypothesized that microbial fermentation could be used to induce colloidal silica gelation, it remained unclear what final pH values should be targeted and how such targeted ranges might vary as a function of solution salinity changes. In this study, a series of abiotic gelation experiments were performed to better understand the collective effects of solution pH and ion concentration changes on the gelation rate of alkaline colloidal silica suspensions. From this, it was expected that generated relationships between pH, salt additions, and gelation rates would provide a basis for designing the bio-mediated gelation process.

2.2 Materials and Methods

Batch Experiments

A series of batch experiments were performed to investigate effect of pH values and NaCl concentration variations on the gelation rate of colloidal silica solutions. All experiments contained 500 mL of a 6% by mass colloidal silica solution in a glass beaker which was prepared using a NaOH-stabilized colloidal silica stock solution (Ludox SM-30, colloid particle sizes = 7 to 22 nm). Thirty-nine different batch experiments were completed at 13 different pH values (pH = 4 to 10, increments of 0.5) and 3 different NaCl concentrations (0 g/L, 5 g/L and 10 g/L). pH adjustments were completed using either NaOH (Fisher Scientific) or HCl (Fisher Scientific) and NaCl additions were added as dried NaCl masses (Fisher Scientific) and mixed using a Thermo-Scientific magnetic stirrer. During experiments, the viscosity of the solutions was monitored in time using a Brookfield AMETEK Low-Range Viscometer (100 to 240 VAC) with No. 61, No. 62, No. 63, and No. 64 spindles at least once every 2 days for 120 days. The “gel time” was assessed as the time required to reach a viscosity of 2000 cP or greater. Specimen pH values were monitored in time every one to two days for 120 days or until the gel time was reached using a

semi-micro pH electrode and meter system (Orion Versa Star Meter, Thermo Fisher) that was calibrated daily using three buffers (4.01, 7.00, 10.00) and had ± 0.05 pH unit accuracy. Viscosity and pH measurements were completed until solutions achieved viscosities greater than 2000 cP or for up to 120 days after mixing solutions (for solutions which did not gel fully).

2.3 Results and Discussion

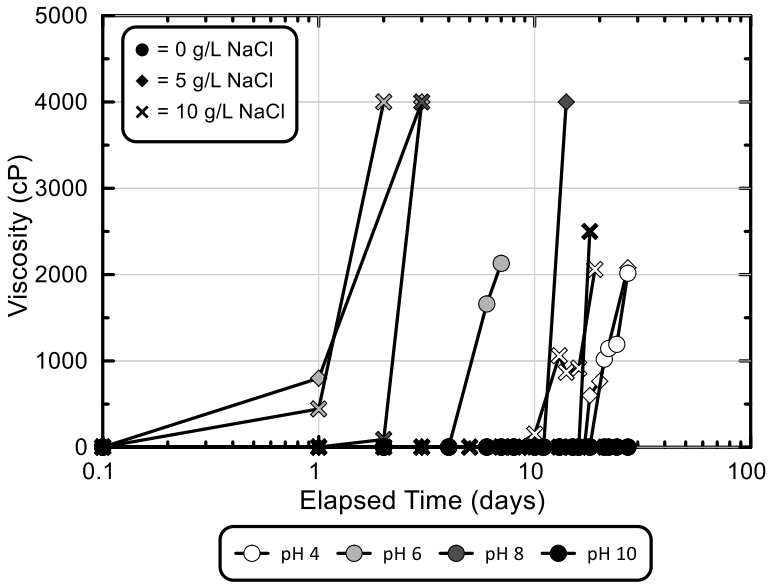

Figure 2.1. Measurements of solution viscosities in time for select batch specimens at pH values of 4, 6, 8, and 10 with NaCl concentrations between 5 g/L and 10 g/L.

Figure 2.1 presents measurements of solution viscosities in time for select specimens at initial pH values of 4, 6, 8, and 10 with NaCl concentrations between 5 g/L and 10 g/L. As shown, dramatic variations in gelation rates with both changes in solution pH and NaCl additions. For solutions with the same pH, decreases in the time required to achieve the gel point (viscosity > 2000 cP) were observed. For similar salt additions, solutions prepared to the lowest (pH = 4) and highest

(pH = 10) pH values considered required the longest time to gel, while solutions with a pH of 6 had the shortest gel times.

Figure 2.2 presents measurements of solution pH values in time for select specimens at initial pH values of 4, 6, 8, and 10 with NaCl concentrations between 5 g/L and 10 g/L. As shown, for almost all experiments, pH values remained relatively constant in time with small variations in values likely due to atmospheric equilibration. Stable pH values were expected, given the lack of reactive substrates, and the presence of purely abiotic gelation.

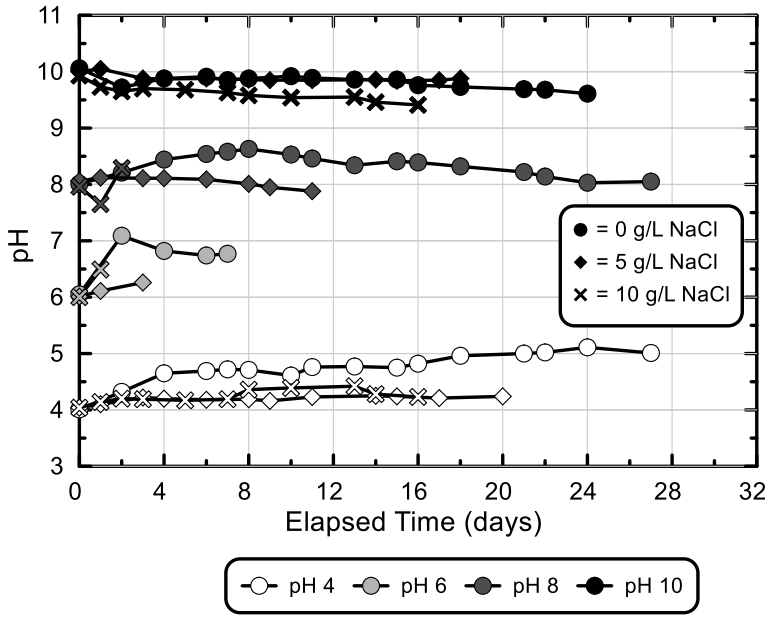

Figure 2.2. Measurements of pH values in time for select batch specimens at pH values of 4, 6, 8, and 10 with NaCl concentrations between 5 g/L and 10 g/L.

Figure 2.3 summarizes the relationship between initial solution pH and NaCl concertation variations and resulting gel times. For all colloidal silica solutions with different NaCl

concentrations, initial pH values for which the gel times were the shortest were referred to as optimal pH ranges. As shown, when NaCl additions were not added (NaCl = 0 g/L), the optimal pH range was between 5 and 6 and most specimens required approximately 6 to 8 days to reach the gel point. Interestingly, at pH values outside of this range, gel times increased significantly. For example, for pH values greater than 7.5 gelation was not observed even after 120 days of monitoring. For specimens with NaCl concentrations of 5 g/L, the optimal pH range expanded and was between 5 and 7 with approximately 2 days required to reach the gel point for most solutions within this range. At pH values below and above this range, most gel times increased to between 5 and 45 days, however, with only one solution at pH = 10 not achieving gelation after 120 days. Lastly, for solutions with NaCl concentrations of 10 g/L, optimal pH ranges further broadened to values between 5 and 8.5 with gel times again near 2 days. Outside of this pH range, 10 g/L NaCl specimens had gel times that were a maximum of only 20 days.

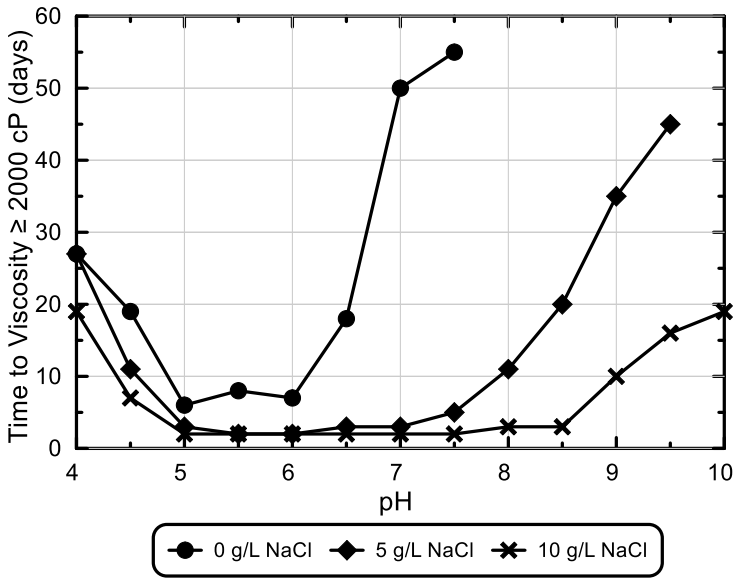

Figure 2.3. Relationships between the time required to achieved solution viscosity increases ≥ 2000 cP (i.e. gel time) with initial solution pH and NaCl concentration variations for all specimens.

2.4 Conclusions

An experiment was performed to examine changes in colloidal silica gel times with variations in initial solution pH and NaCl concentrations. While gelation was abiotically induced in these experiments, results were expected to inform targeted final pH values for the bio-mediated colloidal silica gelation process. Obtained results were consistent with past studies (Persoff et al. 1999) and showed that NaCl concentration increases can dramatically decrease gel times and broaden optimal pH ranges required for gelation. When NaCl was not added to experiments, colloidal silica solutions were extremely stable at high pH values (pH > 7.5) with gel times exceeding 120 days. With increases in NaCl concentrations, however, even specimens at high pH were able to experience abiotic gelation within 60 days. From these results, the use of colloidal silica solutions without added NaCl were hypothesized to offer several distinct advantages for the biologically-controlled process, namely: (1) a narrower optimal pH range, and (2) superior abiotic stability at high pH values. Both characteristics would likely afford the ability of pH changes resulting from microbial fermentation to have greater control of colloidal silica gelation timing while increasing solution insensitivity to changes in surrounding environmental conditions (e.g. site soil and groundwater pH and ionic strength variations). Moving forward, further experiments using the biologically-controlled process would therefore involve colloidal silica suspensions with high initial pH (pH = 9.5) and no NaCl additions; conditions under which suspensions could remain highly stable (minimal gelation) in the absence of microbial fermentation. In order to achieve more rapid gelation, it was expected that the microbial fermentation process should reduce solutions from this initial high pH (pH = 9.5) to values within the optimal pH range for solutions without NaCl (pH = 5 to 6). Successful microbial fermentation under such conditions were expected to pose unique challenges given the large pH changes required.

Chapter 3: STIMULATION BATCH EXPERIMENTS

Bio-stimulation involves the injection of solutions into natural soils, which contain growth factors/substrates and/or impose selective environmental conditions to enrich for native microorganisms with desired metabolic capabilities in-situ (Fujita et al. 2000, 2008; Gomez et al. 2016). While the process has been used extensively for bio-remediation applications (Atlas and Bartha 1973; Gibson and Sewell 1992) as well as ureolytic bio-cementation (Fujita et al. 2000; Tobler et al. 2011; Burbank et al. 2011; Gat et al. 2016; Gomez et al. 2014, 2016; Graddy et al. 2018), it remained unclear if highly alkaline colloidal silica solutions could be supplemented to allow for enrichment of native glucose-fermenting microorganisms present in geotechnically-relevant soils. While stimulation was expected to provide afford practical advantages over augmentation due to expected reductions in treatment costs, environmental impacts, and increases in the spatial uniformity of microbial activity, it was also suspected that the enrichment process would be more challenging to develop and understand given the need for simultaneous enrichment/growth of cells and sufficient fermentation activity after treatment injections (Gomez et al. 2016, 2018; San Pablo et al. 2020). In particular, it remained unclear whether or not sufficient native glucose-fermenting microorganisms would be present in geotechnically-relevant poorly-graded sands and whether or not such microorganisms would be tolerant of large pH changes and conditions imposed by injected colloidal silica solutions. A series of batch experiments were first performed to evaluate the feasibility of using stimulated glucose-fermenting microorganisms to facilitate the colloidal silica gelation process in two different natural poorly-graded sands. In these experiments, small masses of various sand materials were added to sterile colloidal silica solutions which were supplemented with glucose, a fermentable substrate, as well as yeast extract, which

provided trace nutrients and other factors required for cell growth. Following these experiments, additional experimentation was then completed to further explore changes in enrichment and pH reduction behaviors resulting from stimulated fermentation activity as a function of varying substrate concentrations, soil type and masses added, and solution salinity. The results of these experiments provide important insights regarding the potential of stimulated glucose-fermenting microorganisms to facilitate the colloidal silica gelation process.

3.1 Stimulation Feasibility

Although it was hypothesized that glucose-fermenting native microorganisms could be enriched in a variety of different soils, it remained unclear if such microorganisms would be plentiful in poorly-graded sands and whether or not these microorganisms would be tolerant of the high initial pH (9.5) and conditions present within colloidal silica solutions. In order to assess stimulation feasibility, a series of batch experiments were performed wherein glucose and yeast extract were provided in colloidal silica solutions to provide growth factors (i.e. carbon, proteins, trace nutrients) for enrichment of native microorganisms as well as a fermentable substrate (glucose) to enable biologically-controlled pH reductions in time. In order to assess microbial abundances as a function of soil material, varying masses of two different natural sands (Delta Sand, Concrete Sand) were also included in experiments. In all batch experiments, supplied yeast extract and glucose concentrations were identical, however, it was hypothesized that supplied yeast extract concentrations would control enriched microbial cell densities and therefore reaction rates and that supplied glucose concentrations would control final pH magnitudes.

3.1.1 Materials and Methods

Soil Materials

Two different natural sands were included in stimulation feasibility batch experiments. Delta Sand was a poorly-graded marine sand obtained from the San Francisco Bay in CA and had a D_{50} of ≈0.3 mm and a fine contents of ≈1.3%. Concrete Sand was a poorly-graded alluvial sand obtained from Cache Creek in Woodland, CA and had a D_{50} of ≈1.5 mm and a fine contents of ≈1.1%.

Batch Experiments

Eight batch experiments were completed in sterile plastic bottles containing 500 mL volumes of a 6% by mass colloidal silica solution along with 0 mg/L, 0.5 mg/L, 5 mg/L, or 50 mg/L of sand material. Colloidal silica solutions were prepared using a NaOH-stabilized colloidal silica stock solution (Ludox SM-30, colloid particle sizes = 7 to 22 nm) and were supplemented with 5 g/L yeast extract and 10 g/L glucose. All solutions were pH-adjusted using either NaOH (Fisher Scientific) or HCl (Fisher Scientific) to an initial value of 9.5. Solutions were filter-sterilized after all chemical additions using vacuum filtration units with 0.2-micron filters. Following solution sterilization, soil masses were added to experiments and a 5 mm-thick layer of sterile heavy mineral oil was placed at the surface of all bottles to inhibit oxygen transfer and bottles were sealed using sterile caps. In order to minimize the potential for contamination, all exposed surfaces (i.e. bottle mouth, cap) were flame-sterilized using a portable torch (Bernzomatic) whenever bottles were opened. Solution samples (≈2 mL) were obtained from experiments using sterile pipettes at least once every two days to monitor pH changes. pH measurements were completed immediately after collection using a semi-micro pH electrode and meter system (Orion Versa Star Meter, Thermo Fisher) that was calibrated daily using three buffers (4.01, 7.00, 10.01) and had ± 0.05 pH unit accuracy. pH measurements were performed in all Concrete Sand experiments for up to 19 days and all Delta Sand experiments for up to 10 days.

3.1.2 Results and Discussion

Figure 3.1 presents pH measurements in time for experiments containing Concrete Sand. As shown, pH reductions from 9.5 to ≈7 were observed between 4 and 17 days depending on the masses of added soil. pH reduction rates increase as soil concentrations increased likely reflecting increases in initial microbial cell densities with increases in added soil masses. It should be mentioned that pH trends reflected both the time required for microbial growth and enrichment as well as the time required for sufficient glucose fermentation. Interestingly, for all experiments containing 5 mg/L soil or greater, pH trends were similar and minimum pH values reduced to within the targeted range of 5 to 6 identified in earlier abiotic experiments. Lastly, only limited pH changes were observed in the sterile control experiment (0 mg/L C.S.) suggesting that no detectable contamination occurred during experiments.

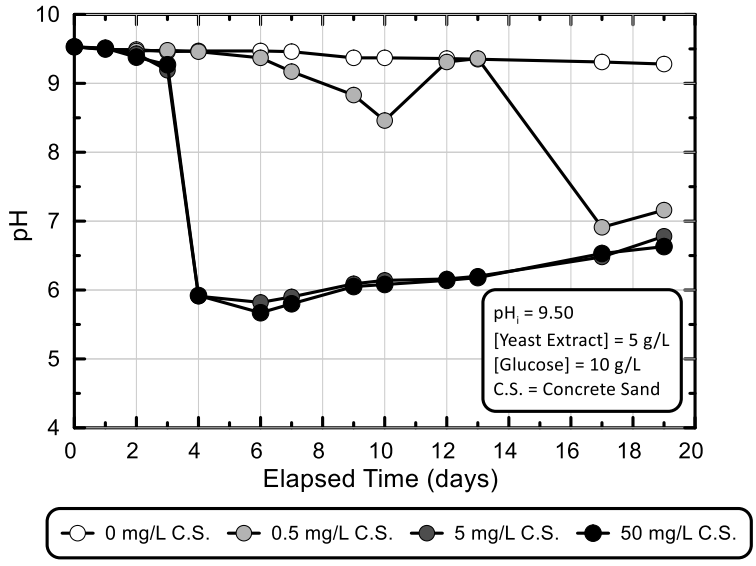

Figure 3.1. Measurements of pH values in time for batch experiments with 0 mg/L, 0.5 mg/L, 5 mg/L and 50 mg/L Concrete Sand and 6% by mass colloidal silica solutions with 5 g/L yeast extract and 10 g/L glucose.

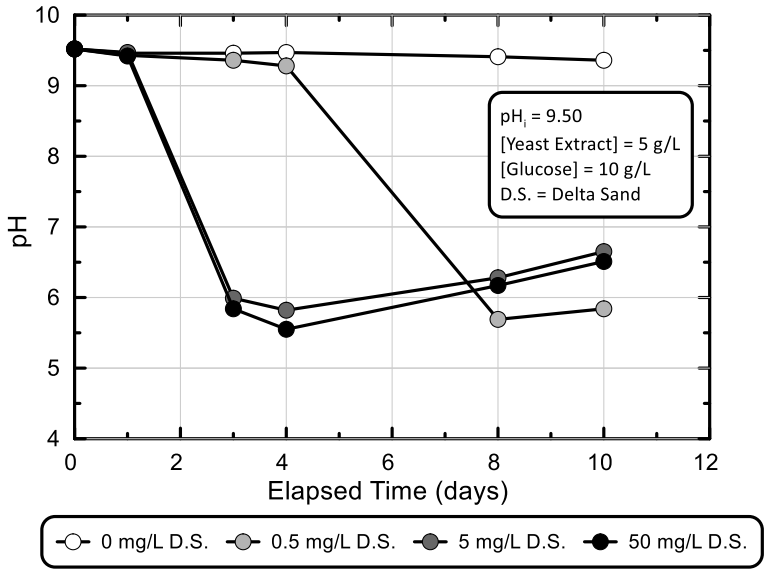

Figure 3.2. Measurements of pH values in time for batch experiments with 0 mg/L, 0.5 mg/L, 5 mg/L and 50 mg/L Concrete Sand and 6% by mass colloidal silica solutions with 5 g/L yeast extract and 10 g/L glucose.

Figure 3.2 presents pH measurements in time for experiments containing Delta Sand. As shown, in all experiments, pH reductions from 9.5 to ≈7 were observed within 2.5 to 7 days with pH reduction rates again dependent on the mass of supplied soil. pH reduction rates were considerably faster than those observed for Concrete Sand (at similar added masses) and may be reflective of either (1) more rapid enrichment of glucose-fermenting microorganisms in Delta Sand, (2) a larger initial inoculum of glucose-fermenting microorganisms (per mass of soil), and/or (3) differences in the native microbial communities present in these two different soils. Faster pH reductions observed in the Delta Sand material may also reflect its marine origin and the presence of microorganisms with likely greater tolerance to high pH and high ionic strength conditions. Similar to the Concrete Sand experiments, pH reduction rates were proportional to added soil masses with

experiments containing at least 5 mg/L soil exhibiting minimum pH values within the target range (pH = 5 to 6). The sterile control experiment also exhibited minimal pH differences.

3.1.3 Conclusions

Stimulation feasibility batch experiments conducted using two natural poorly-graded sands confirmed that glucose fermentation activity could be enriched for when alkaline solutions containing 6% colloidal silica, glucose, and yeast extract were supplied. In all experiments, pH reduction rates were proportional to the amount of soil added suggesting that initial densities of glucose-fermenting microorganisms were a function of supplied soil masses. Experiments containing larger masses of soil (> 5 mg/L) achieved large pH reductions between 3 and 4 days with minimum pH values within the targeted range of 5 to 6. Interestingly, for similar soil masses, fermentation activity was fastest in the Delta Sand experiments reflective of differences initial microbial cell densities, enrichment potential, and/or tolerance to conditions imposed by colloidal silica solutions. From the results of this experiment, Delta Sand was selected as a baseline material for all future experiments examining the effect of various factors on the stimulation process.

3.2 Effect of Substrate Concentrations

Earlier experiments demonstrated that enrichment of glucose-fermenting microorganisms was feasible in poorly-graded sands using nutritive alkaline colloidal silica solutions. It remained unclear, however, if the rate of colloidal silica gelation could be reliably regulated by controlling pH reduction rates and magnitudes resulting from microbial glucose fermentation. It was hypothesized that supplied yeast extract concentrations would govern enriched cell densities and therefore offer the ability to regulate pH reduction rates. Furthermore, it was hypothesized that supplied glucose concentrations would control final pH reduction magnitudes by virtue of controlling the amount of fermentable substrates available. The effect of such variations on the enrichment process and resulting microbial fermentation activity, however, remained unknown. A series of batch experiments were therefore performed to evaluate the effect of yeast extract and glucose variations on stimulated glucose fermentation activity.

3.2.1 Materials and Methods

Batch Experiments

Nine batch experiments were completed using sterile plastic bottles containing 500 mL volumes of a 6% by mass colloidal silica solution along with 50 mg/L of Delta Sand material. Colloidal silica solutions were prepared using a NaOH-stabilized colloidal silica stock solution (Ludox SM-30, colloid particle sizes = 7 to 22 nm) and were supplemented with varying yeast extract (0.1 g/L, 1 g/L, or 10 g/L) and glucose (2.5 g/L, 3.75 g/L, or 5 g/L). Selected yeast extract concentrations were intended to vary growth substrates by up to two order of magnitude between experiments. Selected glucose concentrations focused on a narrower concentration range and were intended to achieve final pH values near the targeted range of 5 to 6. Three additional batch experiments served

as sterile controls and contained similar colloidal silica solutions (5 g/L glucose with either 0.1 g/L, 1 g/L, or 10 g/L yeast extract) without added soil. All solutions were pH-adjusted using either NaOH (Fisher Scientific) or HCl (Fisher Scientific) to an initial value of 9.5. Solutions were filter-sterilized after all chemical additions using vacuum filtration units with 0.2-micron filters. Following solution sterilization, soil masses were added to non-sterile experiments and a 5 mm-thick layer of sterile heavy mineral oil was placed at the surface of all bottles to inhibit oxygen transfer and bottles were sealed using sterile caps. In order to minimize the potential for contamination, all exposed surfaces (i.e. bottle mouth, cap) were flame-sterilized using a portable torch (Bernzomatic) whenever bottles were opened. Solution samples (≈2 mL) were obtained from experiments using sterile pipettes at least once every two days to monitor pH changes. pH measurements were completed immediately after collection using a semi-micro pH electrode and meter system (Orion Versa Star Meter, Thermo Fisher) that was calibrated daily using three buffers (4.01, 7.00, 10.01) and had ± 0.05 pH unit accuracy. pH measurements were performed in all experiments for up to 9 days.

3.2.2 Results and Discussion

Figure 3.3 presents pH measurements in time for experiments with 2.5 g/L glucose and varying yeast extract concentrations. As shown, pH reductions from 9.5 to ≈8 were observed in all experiments within 2 to 5.5 days. Faster pH reductions were consistently observed in experiments with higher yeast extract concentrations likely reflecting higher enriched microbial densities. Similar minimum pH values between 7 and 7.5 were observed in the 1 g/L and 10 g/L yeast extract experiments with the 0.1 g/L yeast extract experiment achieving a lower minimum pH near ≈6. Minimum pH values that were almost always higher than the targeted pH range between 5 and 6,

suggested that higher supplied glucose concentrations were likely needed. Although qualitative observations suggested that viscosities in these specimens were increasing in time, solid gels were not observed over the course of the 9-day monitoring period.

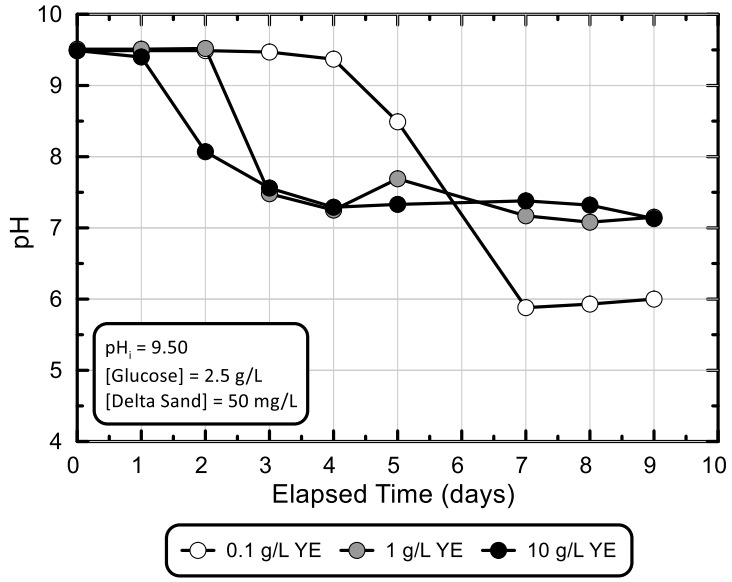

Figure 3.3. Measurements of pH values in time for stimulated batch experiments (50 mg/L Delta Sand) with 2.5 g/L glucose and 0.1 g/L, 1 g/L, or 10 g/L yeast extract in 6% by mass colloidal silica solutions.

Figure 3.4 presents pH measurements in time for experiments with 3.75 g/L glucose and varying yeast extract concentrations. As shown, pH reductions from 9.5 to ≈8 were again observed in all experiments within 2 to 5.5 days with pH reduction rates proportional to supplied yeast extract concentrations. When compared to the previous 2.5 g/L glucose experiments, lower minimum pH values were also observed with values between 5.5 and 6.5, which were similar to the target pH range. Qualitative observations suggested that gelation occurred in all specimens within 2 to 7 days after the minimum pH was measured.

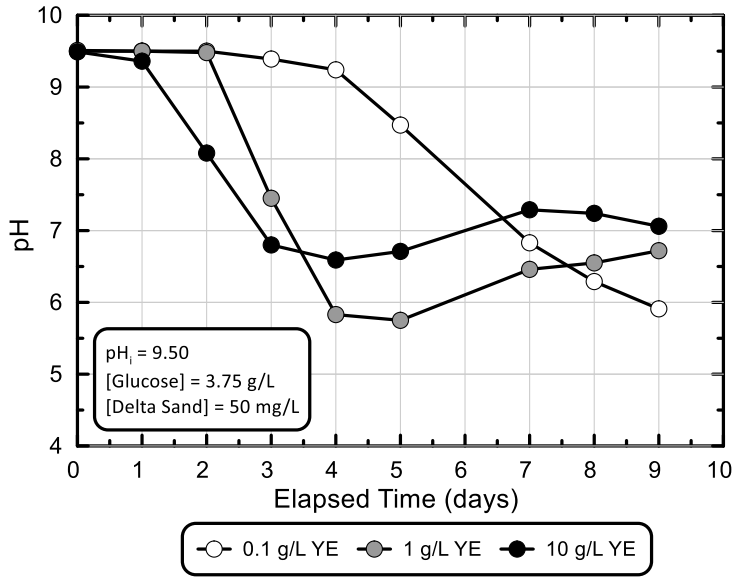

Figure 3.4. Measurements of pH values in time for stimulated batch experiments (50 mg/L Delta Sand) with 3.75 g/L glucose and 0.1 g/L, 1 g/L, or 10 g/L yeast extract in 6% by mass colloidal silica solutions.

Figure 3.5 presents pH measurements in time for experiments with 5 g/L glucose and varying yeast extract concentrations. Similar to other glucose concentrations, pH reductions from 9.5 to ≈8 were similarly observed within 2 to 6 days with pH reduction rates proportional to supplied yeast extract concentrations. Minimum pH values between 5 and 6 were also observed in all flasks within 3 to 9 days suggesting that 5 g/L glucose provided sufficient fermentable substrate to achieve pH values within the targeted range. Qualitative observations suggested that gelation occurred in all specimens within 2 to 5 days after the minimum pH was measured. Interestingly, after achieving the minimal pH values all experiments experienced significant pH increases in time, which may be reflective of the limited ability of the pH probe to successfully assess pH for solutions that

started to gel. All sterile control experiments exhibited minimal pH changes reflective of no added soil materials and continued sterility in time.

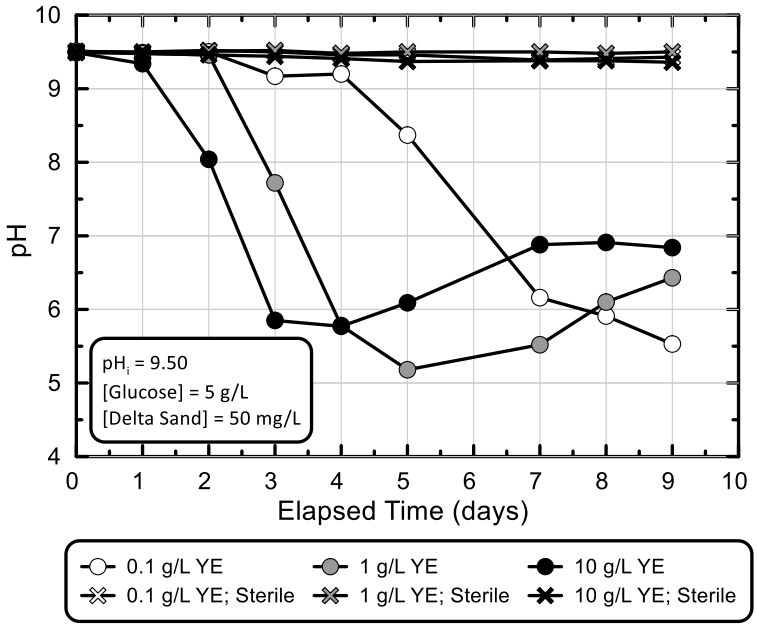

Figure 3.5. Measurements of pH values in time for stimulated batch experiments (50 mg/L Delta Sand) with 5 g/L glucose and 0.1 g/L, 1 g/L, or 10 g/L yeast extract in 6% by mass colloidal silica solutions.

Figure 3.6 presents the time required for solutions to achieve pH values of 8.5 versus supplied yeast extract concentrations for all experiments with varying glucose concentrations. Although the selection of this threshold pH value was arbitrary, changes in the time required for this pH reduction were expected to be reflective of differences in initial fermentation rates. As shown, the time required for solutions to achieve pH values of 8.5 correlated well with changes in supplied yeast extract independent of the concentration of glucose supplied. This suggested that supplied yeast extract was the primary variable influencing pH reduction rates and could likely be used to

control gelation rates. Interestingly, rate differences between the 0.1 g/L and 1 g/L yeast extract experiments were significant, however, as yeast extract further increased to 10 g/L more minimal increases in rates were observed. The non-linear increase in pH reduction rates at higher yeast extract concentrations may suggest that fermentation enrichment and activity became limited by other factors not addressed by simply increasing yeast extract (e.g. cellular waste, trace nutrients).

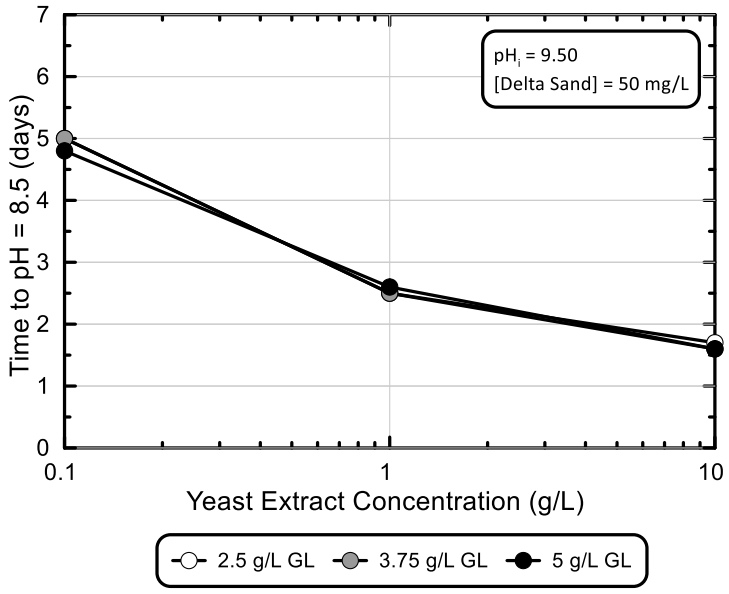

Figure 3.6. Relationships between time required for solutions to achieve pH values of 8.5 (reflective of fermentation rates) versus supplied yeast extract concentrations for all experiments with varying glucose concentrations.

Figure 3.7 similarly presents the time required for solutions to achieve pH values of 7.5 versus supplied yeast extract concentrations for all experiments with varying glucose concentrations. As shown, times required for solutions to achieve pH values of 7.5 were similar between specimens with identical yeast extract concentrations, with some variations resulting from changes in supplied glucose. Although yeast extract again was the primary factor influencing fermentation rates,

variations between specimens with identical yeast extract but different glucose concentrations may reflect small differences in reaction rates as glucose concentrations approached zero near the end of reactions (at low pH values).

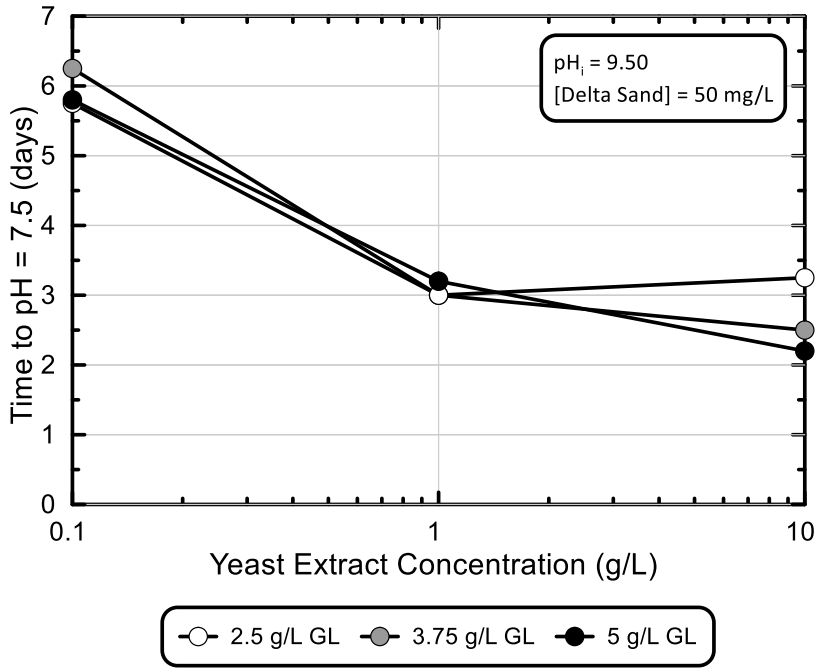

Figure 3.7. Relationships between time required for solutions to achieve pH values of 7.5 (reflective of fermentation rates) versus supplied yeast extract concentrations for all experiments with varying glucose concentrations.

3.2.3 Conclusions

Stimulated batch experiments containing varying chemical concentrations confirmed the role of yeast extract and glucose on resulting pH reduction behaviors. While yeast extract additions appeared to control the rate of fermentation-induced pH reductions via changes in microbial cell densities, glucose additions appeared to control the magnitudes of fermentation-induced final pH values by altering the magnitudes of available fermentable substrates. In all experiments, small

masses of Delta Sand (concentration of 50 mg/L) were sufficient to enable effective enrichment of glucose-fermentation activity, suggesting that such microorganisms are likely plentiful in natural soils. When examining minimum pH values between experiments, 5 g/L glucose was found to reliably result in pH values that were within the target pH range (pH = 5 to 6). For solutions with minimum pH values between 5 and 6.5, gelation was qualitatively observed between 2 and 7 days after the minimum pH was measured, which was consistent with gel times from earlier abiotic experiments. Although changes in yeast extract were shown to reliably alter obtained fermentation rates, the relationship between pH reduction rates and yeast extract concentrations were non-linear suggesting that activity was likely limited by other factors at high concentrations. Although further characterization of the enrichment process is needed, the obtained results were encouraging and suggested that stimulation solutions could be designed to achieve a spectrum of pH reduction behaviors and therefore a spectrum of biologically-controlled gelation rates.

3.3 Effect of Salt Concentrations

While abiotic experiments confirmed that NaCl additions could be used to expedite the colloidal silica gelation process, it remained unclear if microbial fermentation could be used in conjunction with NaCl accelerant additions to enable more rapid gelation rates than could be achieved with a solely biological process. A series of stimulation batch experiments were conducted to provide a preliminary assessment of the effect of NaCl additions on stimulated fermentation activity. Although it was unclear what effect NaCl additions might have, it was suspected that such additions might influence both the enrichment process and fermentative microbial activity through changes in osmotic pressures and potential interferences with cellular transport processes. Beyond its use as a chemical accelerant, higher NaCl concentrations may also be encountered in marine soils, and therefore its investigation was also of interest to assess the impact of environmental ionic strength differences.

3.3.1 Materials and Methods

Batch Experiments

Five batch experiments were completed using sterile plastic bottles containing 500 mL volumes of a 6% by mass colloidal silica solution along with 50 mg/L of Delta Sand material. Colloidal silica solutions were prepared using a NaOH-stabilized colloidal silica stock solution (Ludox SM-30, colloid particle sizes = 7 to 22 nm) and were supplemented with 1 g/L yeast extract, 5 g/L glucose, and varying NaCl (0 g/L, 1 g/L, 2.5 g/L, 5 g/L, or 10 g/L). Selected NaCl concentrations were intended to examine enrichment and fermentation activity in colloidal silica solutions that already contained an abiotic gelation accelerant. All solutions were pH-adjusted using either NaOH (Fisher Scientific) or HCl (Fisher Scientific) to an initial value of 9.5. Solutions were filter-

sterilized after all chemical additions using vacuum filtration units with 0.2-micron filters. Following solution sterilization, soil masses were added to non-sterile experiments and a 5 mm-thick layer of sterile heavy mineral oil was placed at the surface of all bottles to inhibit oxygen transfer and bottles were sealed using sterile caps. In order to minimize the potential for contamination, all exposed surfaces (i.e. bottle mouth, cap) were flame-sterilized using a portable torch (Bernzomatic) whenever bottles were opened. Solution samples (≈2 mL) were obtained from experiments using sterile pipettes at least once every two days to monitor pH changes. pH measurements were completed immediately after collection using a semi-micro pH electrode and meter system (Orion Versa Star Meter, Thermo Fisher) that was calibrated daily using three buffers (4.01, 7.00, 10.01) and had ± 0.05 pH unit accuracy. pH measurements were performed in all experiments for 9 days.

3.3.2 Results and Discussion

Figure 3.8 presents pH measurements in time for stimulated experiments with varying NaCl concentrations. As shown, only minimal differences in pH reduction activities were observed when NaCl concentrations were less than 5 g/L with all experiments achieving pH values between 5 and 6 within 4 days. As NaCl increased beyond 5 g/L, however, more significant inhibition of fermentation activity was observed with the 10 g/L NaCl exhibiting a delay in the onset of pH reductions of about 2 days and a delay in the presence of pH values within the target range of about 3 days. While enriched communities appeared to be relatively insensitive to lower NaCl concentrations, the dramatic inhibition observed at 10 g/L NaCl may be related to inhibition of fermentation activity by ionic strength increases and/or changes in enrichment dynamics and resulting microbial communities.

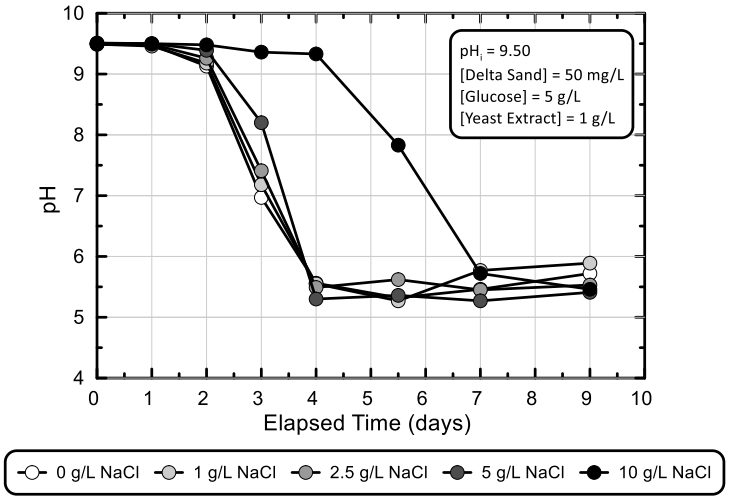

Figure 3.8. Measurements of pH values in time for batch experiments with 0 g/L, 1 g/L, 2.5 g/L, 5 g/L, and 10 g/L NaCl and 6% by mass colloidal silica solutions with 1 g/L yeast extract and 5 g/L glucose.

3.3.3 Conclusions

NaCl accelerant additions were found to have minimal impacts on enriched glucose-fermentation activity when NaCl concentrations were less than 5 g/L. As NaCl increased to 10 g/L, however, significant inhibition was observed and may be related to changes enriched microbial populations and/or inhibition of glucose fermentation activity. While inhibition of stimulation by NaCl may be soil dependent, such results are encouraging as they suggest that the biologically-mediated process can be compatible with small NaCl accelerant additions for applications wherein gelation is desired to occur more rapidly.

3.4 Effect of Glucose Concentration on Minimum pH – All Stimulated Experiments

In order to synthesize boarder trends from stimulation experiments, minimum pH values were plotted versus supplied glucose concentrations for all experiments. **Figure 3.9** presents the relationship between minimum pH values and supplied glucose concentrations for all stimulation experiments conducted at varying yeast extract, glucose, and NaCl concentrations. As shown, supplied glucose concentrations appeared to control minimum pH values, however, some slight variations were observed with changes in yeast extract concentrations likely due to increased solution buffering with increased yeast extract. When glucose concentrations were less than 5 g/L, a near linear relationship between minimum pH and glucose concentration was observed. As glucose concentrations increased above 5 g/L, however, more minimal decreases in minimum pH values were observed. The more minimal reduction in minimum pH values with increases in glucose above 5 g/L may be related to increased solution buffering from produced organic acids (i.e. lactic acid, acetic acid, formic acid) which have pKa values that generally range between 3 and 5 and therefore would not be expected to decrease below this range. When considering the target pH range of 5 to 6 for solutions without supplied NaCl, all experiments with 5 g/L or more glucose achieved minimum pH values within this range. This suggested that despite changes in soil materials, fermentation and enrichment rates, and supplied yeast extract concentration differences, solutions containing 5 g/L glucose generally achieved minimum pH values within the target range and would be expected to accelerate gelation when using stimulated glucose fermentation activity.

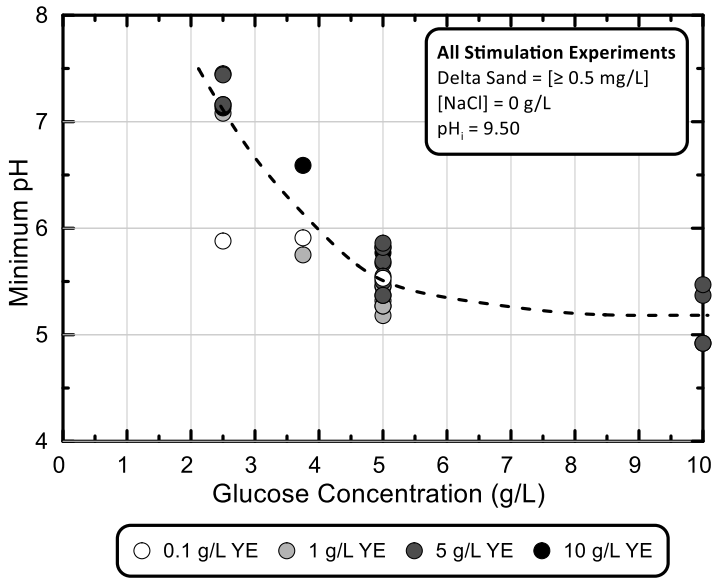

Figure 3.9. Relationship between minimum pH values and supplied glucose concentrations for all stimulation experiments conducted at varying yeast extract, glucose, and NaCl concentrations.

Chapter 4: AUGMENTATION BATCH EXPERIMENTS

Bio-augmentation involves the injection of non-native microorganisms with desired metabolic capabilities into soils in order to complete some biogeochemical process of interest. The approach has been used extensively for bio-remediation purposes and other bio-mediated soil improvement processes such as bio-cementation (DeJong et al. 2006; Martinez et al. 2013; Gomez et al. 2016; and others). It was hypothesized that a bio-augmented approach could be similarly employed for colloidal silica gelation wherein glucose-fermenting microbes would be introduced along with colloidal silica solutions and resulting glucose fermentation activity would be used to decrease pH values overtime and induce gelation. In particular, both lactic acid bacteria (LAB) and mixed acid fermenting bacteria were considered as candidate microorganisms for the augmentation process. Although initial non-sterile experiments involving *Lactobacillus delbrueckii* (an LAB isolate) were successful, several issues were identified in initial experimentation. In particular, *Lactobacillus delbrueckii* was unable to facilitate the gelation process when introduced as a single isolate into sterile experiments and although non-sterile experiments were successful, efforts to isolate gelation facilitating strains from these non-sterile stimulated experiments were unsuccessful. Moving forward, augmentation of experiments with mixed-acid fermenting bacterial isolates were considered instead. In particular, augmentation of colloidal silica solutions with three different mixed-acid fermenting bacteria was explored to assess initial feasibility of these strains to tolerate high pH and high ionic strength conditions present in these solutions and successfully ferment supplied glucose. The three bacterial isolates considered in these experiments included: (1) *Escherichia coli*, a facultative anaerobic mixed acid fermenting gram-negative bacterium, (2) *Paenibacillus Illinoisensis*, a potentially alkali-tolerant facultative anaerobe gram-positive

bacterium, and (3) *Lactococcus lactus*, an aero-tolerant homolactic acid fermenting gram-positive bacterium. Of the three tested, only *Escherichia coli* (*E. coli*) was able to successfully mediate colloidal silica gelation in sterile experiments ($pH_{initial}$ = 9.5, CS = 6% by mass) and was therefore used for all further augmentation experiments. A series of batch experiments were performed to evaluate the impact of supplied *E. coli* cell densities, variations in supplied growth factors and glucose, and changes in surrounding solution salinity on the fermentation activity of *E. coli* in colloidal silica solutions. The results were expected to allow for direct comparisons of activity with similar experiments conducted using stimulated microbial communities.

4.1 Effect of Cell Preparation

The augmentation process requires bacterial cell culturing, rinsing, and pelleting to supply experiments with high cell densities in the absence of growth factors present in initial growth media. While cell pelleting is commonly performed using an isotonic saline solution that contains 9 g/L NaCl, results from the previous stimulated experiments showed that inhibition of cell activity can occur with increases in NaCl. It was therefore hypothesized that NaCl additions during the pelleting process might influence resulting per cell fermentation activities and therefore bulk activities observed in augmented experiments. A series of experiments were performed to evaluate the effect of different cell preparation methods on the fermentation activity of *E. coli* at similar cell densities, with particular focus on the effect of rinse solution compositions.

4.1.1 Materials and Methods

Bacterial Culturing

E. coli cells were grown in glass Erlenmeyer flasks containing a mixed acid fermentation growth media solution (10 g/L yeast extract, 20 g/L glucose), which was inoculated using a -80ºC frozen stock culture that was incubated for 48 hours using a double-orbital shaker at 150 rpm. After this growth period, cells were harvested, centrifuged at 4150 rpm, and pelleted using different rinse solutions and 3 rinse cycles. After pelleting, OD_{600} measurements were completed for all solutions to assess cell densities. During pelleting, several different rinse solutions were used as summarized in **Table 4.1.**

Batch Experiments

Nine batch experiments were completed using sterile plastic bottles containing 500 mL volumes of a 6% by mass colloidal silica solution. Colloidal silica solutions were prepared using a NaOH-

stabilized colloidal silica stock solution (Ludox SM-30, colloid particle sizes = 7 to 22 nm) and were supplemented with 1 g/L yeast extract 5 g/L glucose. Eight batch experiments contained *E. coli* cells at cell densities of either 2.75×10^7 cells/mL or 1.50×10^8 cells/mL, which were prepared using one of the different cell preparation methods outlined in **Table 4.1** (M1 through M4). One batch experiment was not augmented and served as a sterile control. Two cell densities were used in order to examine the effect of cell abundances on the observed glucose fermentation rate. All solutions were pH-adjusted using either NaOH (Fisher Scientific) or HCl (Fisher Scientific) to an initial value of 9.5.

Table 4.1. Summary of Cell Preparation Methods.

Cell Preparation Method	**Rinse Solution Composition**	**Notes about Preparation Methods**
M-1	9 g/L NaCl	Isotonic saline rinse solution. The standard rinse solution used for cell pelleting.
M-2	1.5 g/L NaCl	Rinse solution with $1/6^{th}$ NaCl of standard rinse solution. To examine effect of pelleting solution salinity on resulting cell activity.
M-3	5 g/L yeast extract, 5 g/L glucose	Rinse solution with growth factors. To eliminate presence of saline solutions during pelleting.
M-4	N/A, Grown Growth Media (10 g/L yeast extract, 20 /L glucose, cells)	No rinsing or pelleting. Addition of cells in grown growth media. To control for impacts of rinsing and pelleting.

Six additional batch experiments were performed without the presence of colloidal silica to examine differences in cellular activity in solutions that did not have initially high pH values or high ionic strengths. These experiments were referred to as "activity controls" as they allowed for potential differences in per cell activities to be better assessed. Activity control experiments contained 500 mL volumes of a 5 g/L glucose solution in sterile plastic bottles with the exception of one experiment (M-5), which contained glucose but was also supplemented with 5 g/L yeast extract to examine if continued cell growth would affect fermentative activity. All activity control solutions were pH-adjusted using either NaOH (Fisher Scientific) or HCl (Fisher Scientific) to an initial value of 7.10. Five of the activity control batch experiments were supplied with *E. coli* cells at a cell density of 1.50×10^8 cells/mL which were prepared using one of the four different preparation methods. The fifth experiment (M-5) was the specimen which also contained yeast extract. A sixth batch experiment was not augmented and served as a sterile control.

In all experiments, solutions were filter-sterilized after all chemical additions using vacuum filtration units with 0.2-micron filters. Following solution sterilization, either cell pellets or grown growth media solutions were added to batch experiments, a 5 mm-thick layer of sterile heavy mineral oil was placed at the surface of all bottles to inhibit oxygen transfer, and bottles were sealed using sterile caps. In order to minimize the potential for contamination, all exposed surfaces (i.e., bottle mouth, cap) were flame-sterilized using a portable torch (Bernzomatic) whenever bottles were opened. Solution samples (≈2 mL) were obtained from experiments using sterile pipettes at least once every two days to monitor pH changes. pH measurements were completed immediately after collection using a semi-micro pH electrode and meter system (Orion Versa Star

Meter, Thermo Fisher) that was calibrated daily using three buffers (4.01, 7.00, 10.01) and had ± 0.05 pH unit accuracy. pH measurements were performed in all experiments for up to 3.5 days.

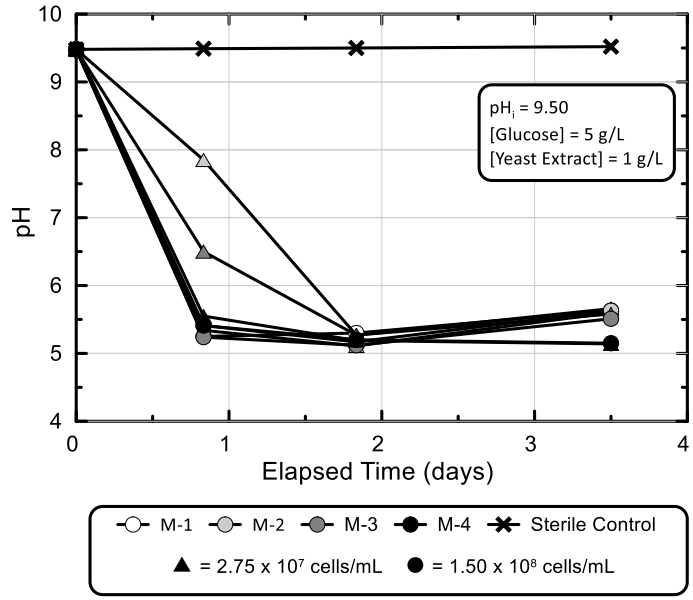

Figure 4.1. Measurements of pH values in time for batch experiments containing *E. coli* cell densities of 2.75 x 10^7 cells/mL and 1.50 x 10^8 cells/mL prepared using 4 different cell preparation methods. Solutions contained 6% by mass colloidal silica solutions with 1 g/L yeast extract and 5 g/L glucose.

4.1.2 Results and Discussion

Figure 4.1 presents pH measurements in time for all colloidal silica batch experiments containing cells prepared using different cell preparation methods outlined in **Table 4.1**. As shown, pH reductions were observed in all experiments from initial values of 9.5 to values within the targeted pH range (pH = 5 to 6) within 1 to 2 days. Similar responses in all experiments containing cells prepared using different methods suggested that cell preparation methods likely had minimal impacts on per cell fermentation activities. Similar pH reductions were also observed between all

high cell density (≈10^8 cells/mL) and low cell density (≈10^7 cells/mL) experiments, with slightly lower pH reduction rates observed in some experiments with low cell densities (M-2 and M-3).

Figure 4.2 presents pH measurements in time for all activity control batch experiments containing cells prepared using different cell preparation methods outlined in **Table 4.1**. As shown, pH reductions were observed in all experiments from initial values of 7.10 to values between ≈4 and 5 within one day. Although some variations in pH trends in time were observed between experiments, similar responses between experiments with different cell preparation procedures, again suggested that such methods likely had minimal impacts on per cell activities. Interestingly, all experiments containing cells prepared with nutritive media obtained higher final pH values, potentially indicative of differences in augmented cell densities. Cells prepared using isotonic saline rinse solutions (Method M-1) behaved similar to all other methods investigated.

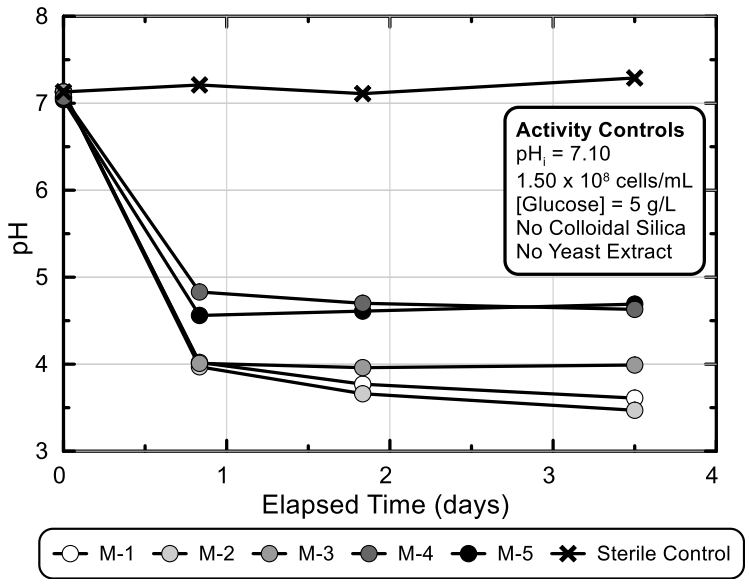

Figure 4.2. Measurements of pH values in time for activity control batch experiments containing *E. coli* cell densities of 1.50 x 10^8 cells/mL prepared using 5 different methods. Solutions contained 5 g/L glucose and no colloidal silica.

4.1.3 Conclusions

Although some small differences in pH trends were observed between experiments augmented with *E. coli* cells prepared using various preparation methods, overall cell pelleting/rinsing techniques appeared to have minimal impacts on observed per cell activities both in colloidal silica experiments and activity control experiments. Moving forward, isotonic saline solutions (9 g/L NaCl) were used to pellet and rinse *E. coli* cells for all further augmentation experiments.

4.2 Effect of Substrate Concentrations

Previously conducted stimulation batch experiments demonstrated that supplied yeast extract concentrations were able to largely control pH reduction rates with supplied glucose concentrations able to largely control pH reduction magnitudes. However, it remained unclear whether similar trends would be observed in experiments augmented with *E. coli* cells. Similar to the stimulated experiments, it was hypothesized that supplied yeast extract concentrations would enable further growth of augmented *E. coli* cell densities and therefore offer the ability to increase pH reduction rates. It was also expected that supplied glucose concentrations would control final pH reduction magnitudes by virtue of controlling the amount of fermentable substrates available. The effect of such variations on the cell growth process and resulting microbial fermentation activity, however, remained unknown. A series of batch experiments were therefore performed to evaluate the effect of yeast extract and glucose variations on the glucose fermentation activity of augmented *E. coli* cells in colloidal silica solutions. These experiments would allow for fermentation rates and substrate utilization efficiency to be compared between augmented and stimulated colloidal silica experiments.

4.2.1 Materials and Methods

Batch Experiments

Eighteen batch experiments were completed using sterile plastic bottles containing 500 mL volumes of a 6% by mass colloidal silica solution. Colloidal silica solutions were prepared using a NaOH-stabilized colloidal silica stock solution (Ludox SM-30, colloid particle sizes = 7 to 22 nm) and were supplemented with varying yeast extract (0 g/L, 0.1 g/L, 1 g/L) and glucose (0 g/L, 1 g/L, 2.5 g/L, 5 g/L, 10 g/L) concentrations as well as varying cell densities (2.75×10^7 cells/mL,

1.50 x 10^8 cells/mL). Selected yeast extract concentrations were intended to vary growth substrates by up to two order of magnitude between experiments. Selected glucose concentrations were intended to investigate the relationship between supplied glucose concentrations and final pH values over a wide range of conditions. All solutions were pH-adjusted using either NaOH (Fisher Scientific) or HCl (Fisher Scientific) to an initial value of 9.5. Nine additional batch experiments served as activity controls and contained solutions with only added glucose (0 g/L, 1 g/L, 2.5 g/L, 5 g/L, 10 g/L) and supplied *E. coli* cells (2.75 x 10^7 cells/mL, 1.50 x 10^8 cells/mL), but no colloidal silica. The activity control solutions were pH-adjusted using either NaOH (Fisher Scientific) or HCl (Fisher Scientific) to initial values of 7.10. Solutions were filter-sterilized after all chemical additions using vacuum filtration units with 0.2-micron filters. Following solution sterilization, *E. coli* cells were added to all experiments (excluding sterile controls) and a 5 mm-thick layer of sterile heavy mineral oil was placed at the surface of all bottles to inhibit oxygen transfer and bottles were sealed using sterile caps. In order to minimize the potential for contamination, all exposed surfaces (i.e. bottle mouth, cap) were flame-sterilized using a portable torch (Bernzomatic) whenever bottles were opened. Solution samples (≈2 mL) were obtained from experiments using sterile pipettes at least once every two days to monitor pH changes. pH measurements were completed immediately after collection using a semi-micro pH electrode and meter system (Orion Versa Star Meter, Thermo Fisher) that was calibrated daily using three buffers (4.01, 7.00, 10.01) and had ± 0.05 pH unit accuracy. pH measurements were performed in all experiments for up to 8 days.

4.2.2 Results and Discussion

Figure 4.3 presents pH measurements in time for experiments with 5 g/L glucose, varying yeast extract concentrations, and varying cell densities. As shown, similar pH reductions from 9.5 down to values within the target pH range (pH = 5 to 6) were observed for all experiments with higher cell densities (10^8 cells/mL) within approximately 2 to 3 days. Variations in yeast extract were found to reliably control the rate of pH reductions as expected due to the presence of additional cell growth beyond augmented cell densities. Solutions with high cell densities and no supplied yeast extract exhibited detectable, but slower fermentation activity. For all experiments containing *E. coli* cells at ≈10^7 cell/mL no pH reductions were observed suggesting that low cell densities may have been inhibited by high initial pH conditions and presence of colloidal silica. Qualitative observations suggested that gelation occurred in all specimens containing high cell densities within 2 to 5 days after the minimum pH was measured.

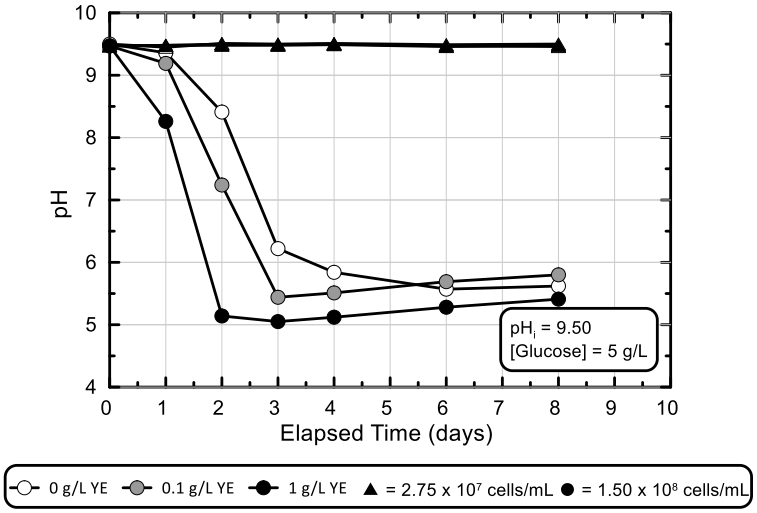

Figure 4.3. Measurements of pH values in time for augmented batch experiments containing *E. coli* cells at two cell densities (2.75 x 10^7 cells/mL and 1.50 x 10^8 cells/mL) with 5 g/L glucose and varying yeast extract (0 g/L, 0.1 g/L, or 1 g/L) in 6% by mass colloidal silica solutions.

Figure 4.4 presents pH measurements in time for experiments with 1 g/L yeast extract and varying glucose concentrations and augmented *E. coli* cell densities. Again, experiments containing low *E. coli* cell densities (10^7 cell/mL) achieved minimal pH reductions suggesting potential inhibition of activity from alkaline colloidal silica solutions. At high cell densities, small differences in pH reduction rates were observed between experiments containing varying glucose despite identical yeast extract concentrations. Small increases in fermentation rates in experiments containing higher glucose concentrations may be related to process enzyme kinetics and expected increases in rates at higher substrate concentrations. Similar to stimulation experiments, final pH reduction magnitudes appeared to be proportional to the supplied glucose concentrations with stable pH values near 7 and 5 observed within 2 days in the 2.5 g/L and 5 g/L glucose experiments, respectively. Qualitative observations suggested that gelation occurred in all specimens within 2 to 6 days after the minimum pH was measured.

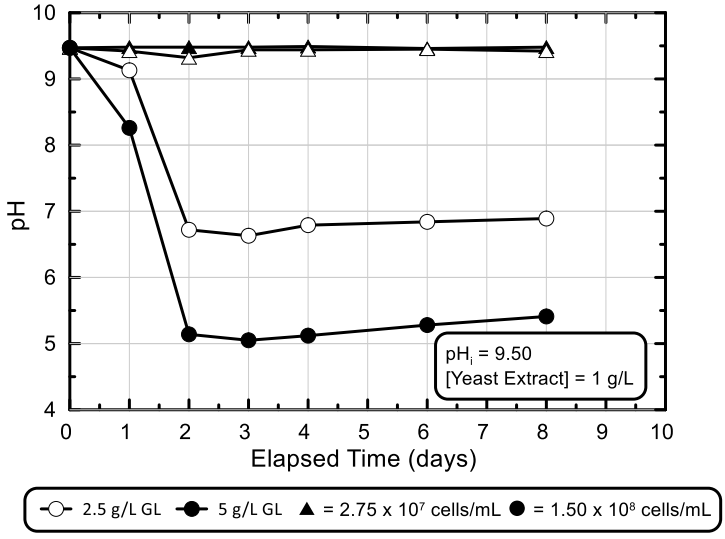

Figure 4.4. Measurements of pH values in time for augmented batch experiments containing *E. coli* cells at two cell densities (2.75 x 10^7 cells/mL and 1.50 x 10^8 cells/mL) with 1 g/L yeast extract and varying glucose concentrations (2.5 g/L or 5 g/L) in 6% by mass colloidal silica solutions.

Figure 4.5 presents pH measurements in time for activity control experiments with varying glucose concentrations and cell densities, but no colloidal silica or yeast extract. Interestingly, all activity control experiments achieved similar pH responses regardless of augmented *E. coli* cell densities. This suggested that cells provided at low cell densities were indeed initially active but were inhibited when supplied to colloidal silica solutions as shown in **Figure 4.3** and **Figure 4.4**. Since these activity control solutions were relatively unbuffered, minimum pH values approached values near ≈3.7, which again may reflect the pKa values of the mixed organic acids produced. Moving forward it was hypothesized that augmenting with high *E. coli* cell densities (≈10^8 cells/mL) may be required to ensure sufficient fermentation activity when cells are supplied in high pH colloidal silica solutions.

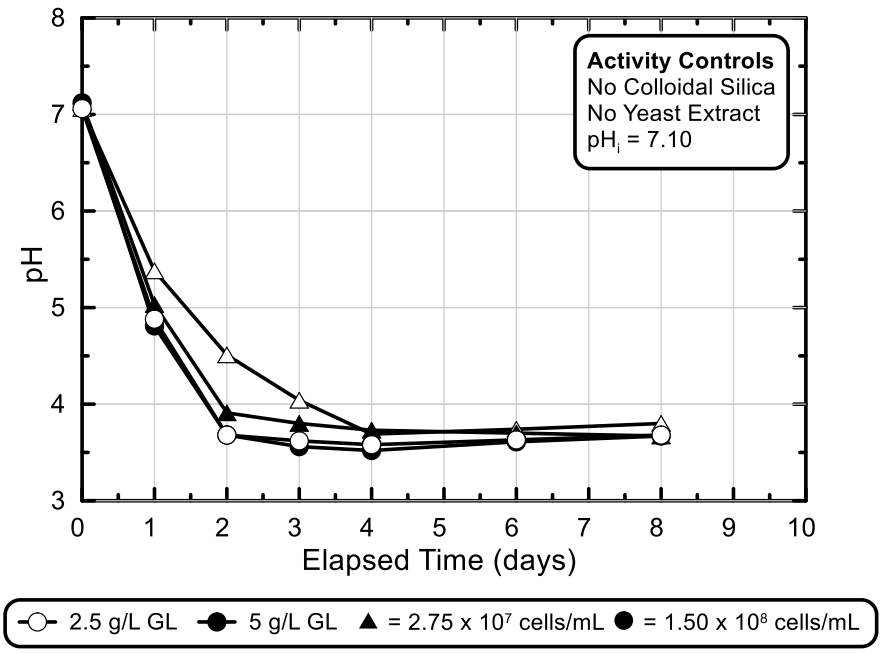

Figure 4.5. Measurements of pH values in time for activity control batch experiments containing *E. coli* cell densities of 2.75 x 10^7 and 1.50 x 10^8 cells/mL. Solutions contained either 2.5 g/L or 5 g/L glucose and no colloidal silica.

Figure 4.6 presents pH measurements in time for colloidal silica experiments containing *E. coli* cell densities of 1.50 x 10^8 cells/mL with 1 g/L yeast extract and varying glucose concentrations. As shown, final pH reduction magnitudes were proportional to supplied glucose concentrations when glucose concentrations were less than 5 g/L. For solutions supplied with 1 g/L, 2.5 g/L and 5 g/L glucose, minimum pH values were near ≈8, 6.5, and 5.5, respectively. Limited reductions in minimum pH values when glucose concentrations increased from 5 g/L to 10 g/L likely reflected increases buffering of solutions by produced organic acids. Again, initial pH reduction rates were similar between experiments containing different glucose concentrations (and identical yeast extract concentrations) but did exhibit some small increases in initial pH reduction rates with increases in supplied glucose, which were likely related to enzyme kinetics and enzyme half-saturation coefficients. As expected, the sterile control experiment exhibited no pH reductions in time.

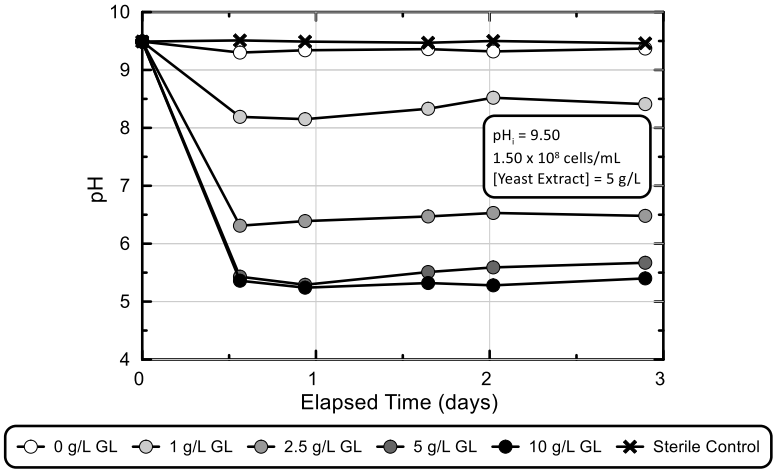

Figure 4.6. Measurements of pH values in time for augmented batch experiments containing *E. coli* cell densities of 1.50 x 10^8 cells/mL in 6% by mass colloidal silica solutions with 1 g/L yeast extract and varying glucose (0 g/L, 1 g/L, 2.5 g/L, 5 g/L or 10 g/L).

Figure 4.7 presents pH measurements in time for activity control experiments with varying glucose concentrations and no colloidal silica or yeast extract. Activity control experiments all achieved similar pH reduction trends in time regardless of the glucose concentrations supplied. Again, minimum pH values approached ≈3.7 most likely due to the organic acids produced, which buffered solutions near their pKa values.

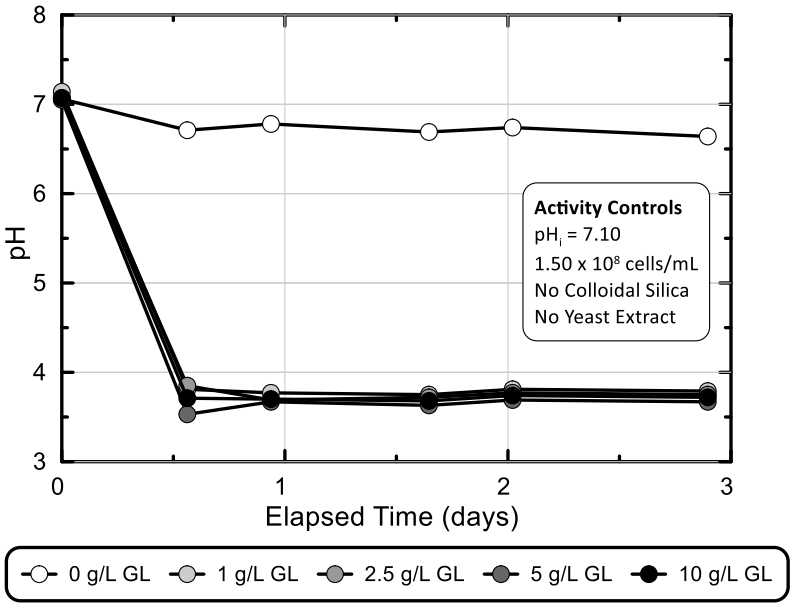

Figure 4.7. Measurements of pH values in time for activity control batch experiments containing *E. coli* cell densities of 1.50 x 10^8 cells/mL and varying glucose (0 g/L, 1 g/L, 2.5 g/L, 5 g/L or 10 g/L) without supplied yeast extract or colloidal silica.

4.2.3 Conclusions

Augmentation batch experiments containing varying chemical concentrations confirmed the role of supplied yeast extract and glucose on pH reduction behaviors. Similar to stimulated experiments, yeast extract additions appeared to control the rate of fermentation-induced pH reductions via changes in microbial cell densities. Moreover, glucose additions appeared to control the

magnitudes of fermentation-induced final pH values by altering the magnitudes of available fermentable substrates. For all experiments conducted at low cell densities (10^7 cells/mL), inhibition of augmented cells by high pH colloidal silica solutions was observed, which prevented sufficient fermentative activity. In contrast, experiments containing higher cell densities of 1.50 x 10^8 cells/mL exhibited significant glucose fermentation activity and moving forward this high cell density was used for all further augmentation experiments. Similar to the stimulation experiments, 5 g/L glucose was found to reliably result in minimum pH values that were within the target pH range (pH = 5 to 6). For solutions with minimum pH values between 5 and 6.5, gelation was qualitatively observed between 2 and 7 days after the minimum pH was measured, which was consistent with gel times from earlier abiotic experiments.

4.3 Effect of Salt Concentrations

Similar to stimulated experiments, the effect of salt additions on augmented *E. coli* glucose fermentation activity remained relatively unknown. Further study of the effect of NaCl additions on the augmentation process was of interest in order to understand both the potential impact of varying salinity from different subsurface environments as well as the effect of NaCl additions when included in colloidal silica solutions as a gelation accelerant. A series of augmented batch colloidal silica experiments were conducted to assess the potential impact of varying NaCl concentrations on augmented *E. coli* fermentation activity. It was suspected that increased inhibition of *E. coli* fermentation activity would be observed with increases in supplied NaCl concentrations, however, the particular magnitudes of inhibitory concentrations and their impacts on fermentation rate magnitudes were unknown. Experiments were identical to the previous stimulation batch experiments, however, rather than supplying experiments with a Delta Sand inoculant, experiments were augmented directly with *E. coli* cells. Experiments were expected to provide a direct comparison of NaCl inhibition between augmented *E. coli* and stimulated microbial communities, while acknowledging that stimulated experiments would likely exhibit slower pH reductions in time due to the need for initial cell growth (i.e. cells are not directly supplied).

4.3.1 Materials and Methods

Batch Experiments

Six batch experiments were completed using sterile plastic bottles containing 500 mL volumes of a 6% by mass colloidal silica solution along with *E. coli* cells at a cell density of 1.5×10^8 cells/mL. Colloidal silica solutions were prepared using a NaOH-stabilized colloidal silica stock solution

(Ludox SM-30, colloid particle sizes = 7 to 22 nm) which was supplemented with 1 g/L yeast extract, 5 g/L glucose, and varying NaCl (0 g/L, 1 g/L, 2.5 g/L, 5 g/L, or 10 g/L). Selected NaCl concentrations were intended to examine augmented fermentation activity in colloidal silica solutions that already contained an abiotic gelation accelerant. A sterile control experiment was also completed, which contained neither supplied NaCl nor *E. coli* cells. All solutions were pH-adjusted using either NaOH (Fisher Scientific) or HCl (Fisher Scientific) to an initial value of 9.5. Solutions were filter-sterilized after all chemical additions using vacuum filtration units with 0.2-micron filters. Following solution sterilization, *E. coli* cells were added to sterile experiments and a 5 mm-thick layer of sterile heavy mineral oil was placed at the surface of all bottles to inhibit oxygen transfer and bottles were sealed using sterile caps. In order to minimize the potential for contamination, all exposed surfaces (i.e., bottle mouth, cap) were flame-sterilized using a portable torch (Bernzomatic) whenever bottles were opened. Solution samples (≈2 mL) were obtained from experiments using sterile pipettes at least once every day to monitor pH changes. pH measurements were completed immediately after collection using a semi-micro pH electrode and meter system (Orion Versa Star Meter, Thermo Fisher) that was calibrated daily using three buffers (4.01, 7.00, 10.01) and had ± 0.05 pH unit accuracy. pH measurements were performed in all experiments for 7 days.

4.3.2 Results and Discussion

Figure 3.8 presents pH measurements in time for all *E. coli* augmented experiments containing varying concentrations of NaCl. As shown, progressive inhibition of augmented fermentation activity was observed as NaCl concentrations increased. Between experiments, pH values reduced from 9.5 to below 6 between 1 and 7 days with fastest reductions in the 0 g/L NaCl experiment

and slowest reductions in the 10 g/L NaCl experiment. When comparing final pH values between experiments, all experiments had similar final pH magnitudes between 5 and 5.5 suggesting that supplied glucose (which was identical between experiments) likely still controlled final pH magnitudes regardless of the NaCl concentrations present. In contrast to earlier stimulated experiments, detectable slowing of pH reduction rates was observed even for experiments with small NaCl additions (≈1 g/L). While the exact mechanism of NaCl inhibition of *E. coli* fermentation activity remains unknown, NaCl concertation differences may have influenced osmotic pressure differentials and cellular diffusion among other biological processes

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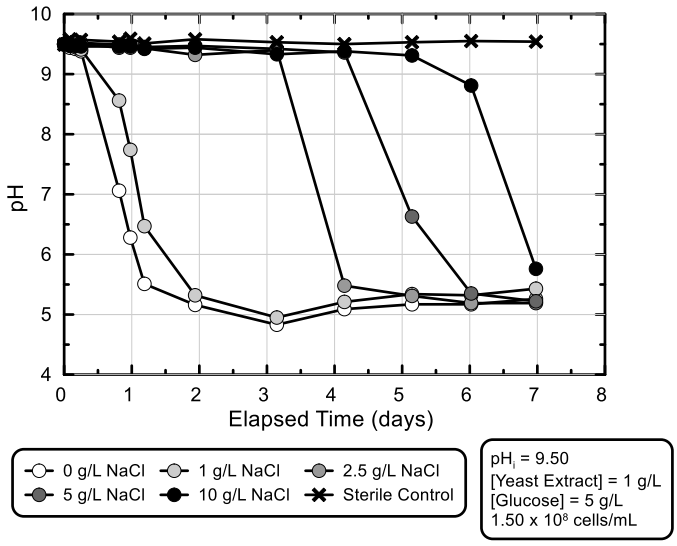

Figure 4.8. Measurements of pH values in time for augmented batch experiments containing *E. coli* cell densities of 1.50 x 10^8 cells/mL in 6% by mass colloidal silica solutions with 1 g/L yeast extract and 5 g/L glucose and varying NaCl concentrations (0 g/L, 1 g/L, 2.5 g/L, 5 g/L, or 10 g/L).

Figure 4.9 presents pH measurement in time for augmented activity control experiments which contained *E. coli* cells at a cell density of 1.5 x 10^8 cells/mL and solutions with 5 g/L glucose and

no colloidal silica or yeast extract. Similar to the previously conducted augmented activity control experiments, this activity control experiment achieved large pH reductions at early time (less than 0.5 days) and final pH values near ≈3.7. This experiment confirmed that augmented *E. coli* cells had similar per cell activities as other augmentation experiments.

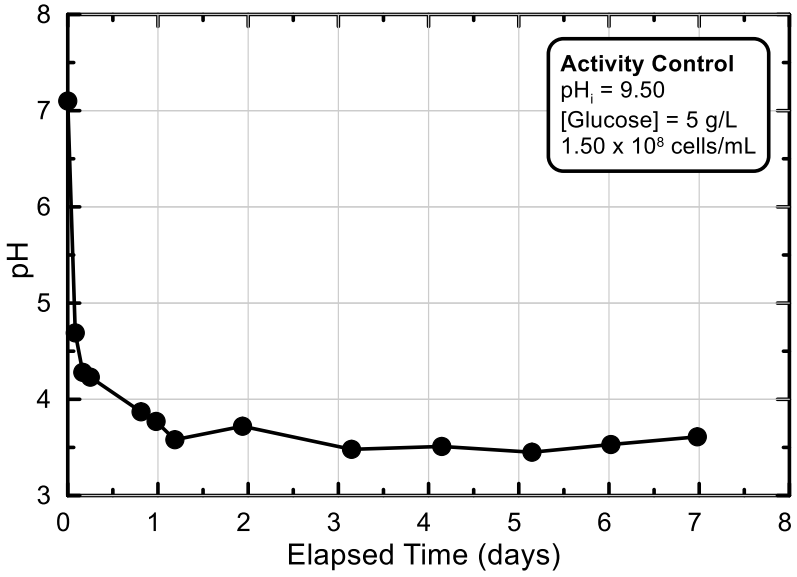

Figure 4.9. Measurements of pH values in time for activity control batch experiments containing *E. coli* cell densities of 1.50 x 10^8 cells/mL and 5 g/L glucose without supplied yeast extract, colloidal silica, or NaCl.

4.3.3 Conclusions

In the above experiments, NaCl additions were shown to significantly inhibit augmented *E. coli* cell fermentation activity. While inhibition of fermentation activity was expected to occur at high NaCl concentrations (>5 g/L), surprisingly, significant inhibition of augmented cells was detected at NaCl concentrations as low as 1 g/L. While it is clear that changes in salinity may impact the kinetics of the augmented colloidal silica gelation process, the ability to slow such rates may be favorable for geotechnical applications wherein injection rates are limited by low hydraulic

conductivity soils and slower gelation may be desired to reach further treatment distances. The greater inhibition of augmented *E. coli* cells by NaCl when compared to simulated communities, may also suggest that stimulated communities may be more tolerant to differences in environmental factors and may offer improved process reliability under more variable conditions expected at field-scale.

Chapter 5: COMPARISON OF AUGMENTATION AND STIMULATION PROCESSES

Batch experiments presented in Chapters 2 through 4 were designed to investigate three main aspects of the biologically-controlled colloidal silica gelation process: (1) process feasibility using stimulated native microorganisms and augmented *E. coli* mixed acid fermenting bacteria, (2) the effect of supplied glucose and yeast extract on microbial fermentation rates and final pH reduction magnitudes, and (3) the effect of NaCl on the fermentation activity of both augmented and stimulated microorganisms in colloidal silica solutions. Although these experiments demonstrated the feasibility of using both stimulated native microorganisms and augmented *E. coli* mixed acid fermenting bacteria to complete this process, it was unclear how important process outcomes (e.g., pH reduction rates, final pH magnitudes) would compare between these two processes. In this chapter, results from experiments using stimulated microorganisms are compared to those completed using augmented *E. coli* cells to compare these two processes and gain insights regarding obtainable reaction rates, pH reduction magnitudes, and the effect of supplied NaCl.

5.1 Comparison of pH Reduction Rates

Figure 5.1 presents pH measurements in time for select stimulated (50 mg/L Delta Sand) and augmented (1.5 x 10^8 cells/mL) experiments completed in 6% by mass colloidal silica solutions with 5 g/L glucose and varying yeast extract concentrations. As shown, a spectrum of reaction rates could be obtained with pH values decreasing from 9.5 to values below 6 between 2 and 8 days. Unsurprisingly, augmented experiments achieved pH reductions to values near 6 between 2 and 5 days faster than stimulated experiments at similar yeast extract concentrations. This was to be expected as augmented experiments contained initially active cells, whereas stimulated

experiments required additional time to achieve sufficient cell growth and activity. Interestingly, when yeast extract concentrations of 10 g/L were supplied to stimulated experiments, pH reductions were only about 1 day slower than augmented experiments containing 1 g/L yeast extract and were nearly identical to the augmented experiment, which did not contain yeast extract (0 g/L). Although desired reaction rates will depend on the particular application, the presence of similar pH reduction rates between stimulated experiments with high yeast extract and augmented experiments without added nutrients was promising and suggested that costs and impacts related to augmentation (i.e. cell culturing and injection) could be eliminated simply by supplying higher concentrations of nutrients without compromising reaction speed. Lastly, nearly identical minimum pH values in both augmented and stimulated experiments suggested that the concentration of supplied glucose, which was identical in all experiments, controlled final pH magnitudes regardless of the microorganisms used.

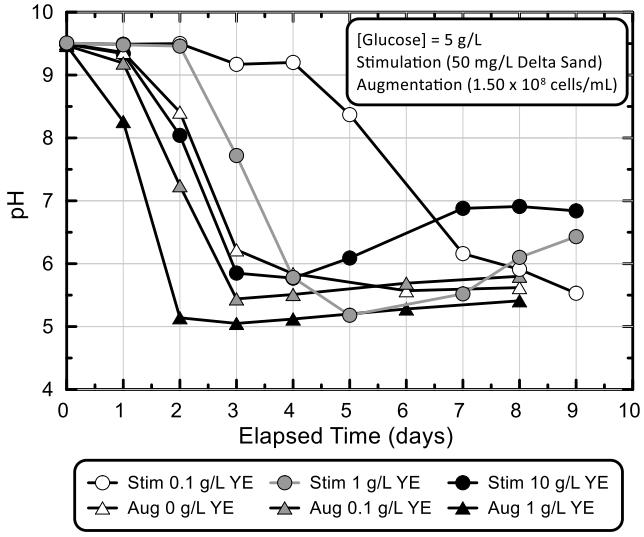

Figure 5.1. Measurements of pH values in time for stimulated (50 mg/L Delta Sand) and augmented (10^8 cells/mL) colloidal silica batch experiments with 5 g/L glucose and varying yeast extract (0 g/L, 0.1 g/L, 1 g/L, or 10 g/L).

Figure 5.2 presents the time required for solutions to achieve pH values of 8.5 versus supplied yeast extract concentrations for the previous stimulated (50 mg/L Delta Sand) and augmented (1.5 x 10^8 cells/mL) experiments. Although the selection of this threshold pH value was arbitrary, changes in the time required for this pH reduction were expected to be reflective of differences in initial fermentation rates. As shown, for the same yeast extract concentration, augmented experiments achieved pH reductions to 8.5 that were between 2 and 3 days faster than similar stimulated experiments. At higher yeast extract concentrations of 10 g/L, however, stimulated experiments achieved pH reductions to 8.5 at similar times as non-nutritive augmented experiments. While augmented experiments with 10 g/L yeast extract were not tested, it is likely that augmented experiments would achieve faster rates than similar 10 g/L yeast extract stimulated experiments. However, such rate increases might necessitate significant additional financial costs and environmental impacts when applied at field-scale.

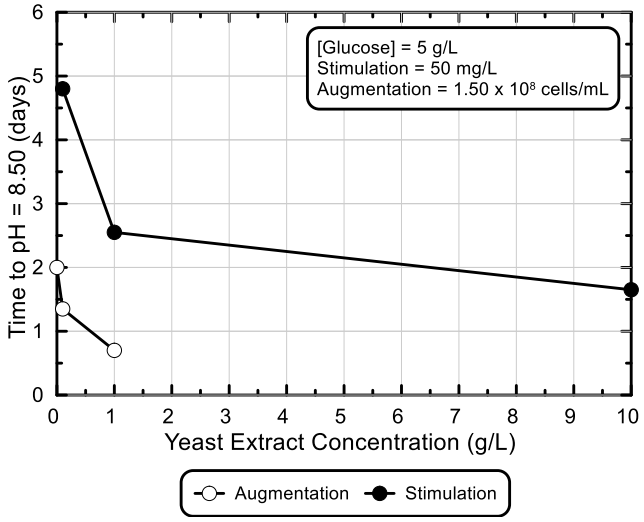

Figure 5.2. Relationships between the time required for solutions to achieve pH values of 8.5 (reflective of glucose fermentation rates) versus supplied yeast extract concentrations for select stimulated (50 mg/L Delta Sand) and augmented (1.5 x 10^8 cells/mL) colloidal silica experiments containing 5 g/L glucose.

Figure 5.3 similarly presents the time required for solutions to achieve pH values of 7.5 versus supplied yeast extract concentrations for select stimulated (50 mg/L Delta Sand) and augmented (1.5×10^8 cells/mL) colloidal experiments containing 5 g/L glucose. As shown, for the same yeast extract concentration, augmented experiments again showed faster fermentation rates. When 10 g/L yeast extract was applied to stimulated experiments, however, pH reduction rates comparable to non-nutritive augmented experiments were achieved.

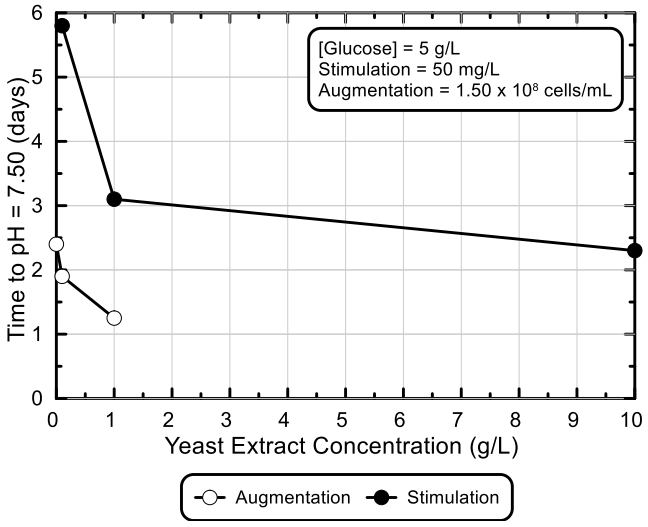

Figure 5.3. Relationships between the time required for solutions to achieve pH values of 7.5 (reflective of glucose fermentation rates) versus supplied yeast extract concentrations for select stimulated (50 mg/L Delta Sand) and augmented (1.5×10^8 cells/mL) colloidal silica experiments containing 5 g/L glucose.

5.2 Comparison of NaCl Inhibition

Figure 5.4 presents the time required for solutions to achieve pH values of 7.5 versus supplied NaCl concentrations for select stimulated (50 mg/L Delta Sand) and augmented (1.5×10^8 cells/mL) colloidal silica experiments containing 5 g/L glucose, 1 g/L yeast extract, and varying NaCl concentrations (0 g/L, 1 g/L, 2.5 g/L, 5 g/L, 10 g/L). As shown, stimulated experiments required

approximately 3 days to achieve pH reductions to values near 7.5 when NaCl concentrations ranged from 0 g/L to 5 g/L, reflective of minimal sensitivity to NaCl additions. At the highest NaCl concentration (10 g/L), however, stimulated experiments did exhibit more significant inhibition, requiring 6 days to achieve this same pH reduction. Augmented experiments, in contrast, exhibited significant rate reductions when NaCl concentrations exceeded 1 g/L with the time to pH reductions near 7.5 increasing from 1 day to 6.5 days. Interestingly, when NaCl concentrations were 2.5 g/L or greater, pH reductions occurred more quickly in stimulated experiments than augmented experiments for similar NaCl concentrations. This was remarkable given that augmented experiments had very high initial cell densities of 10^8 cells/mL, while stimulated experiments required microbial growth to occur during the treatment process. While these results suggested that augmented *E. coli* cells may be more sensitive to changes in solution salinity, stimulated microbial communities may be able to overcome these limitations and tolerate larger variations in NaCl concentrations without resulting in significant changes in reaction rates.

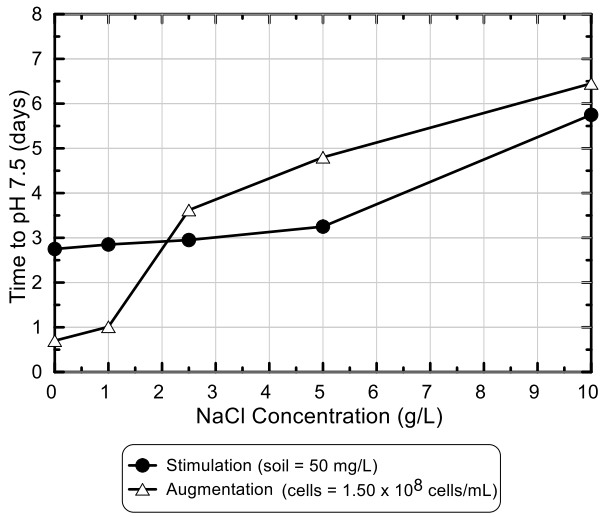

Figure 5.4. Relationships between time required for solutions to achieve pH values of 7.5 (reflective of fermentation rates) versus supplied NaCl concentrations for select stimulated (50 mg/L Delta Sand) and augmented (1.5 x 10^8 cells/mL) colloidal silica experiments containing 5 g/L glucose, 1 g/L yeast extract, and varying NaCl concentrations.

5.3 Comparison of Relationship between Supplied Glucose and Minimum pH

Figure 5.4 presents the relationship between supplied glucose concentrations and minimum pH values for all stimulated and augmented colloidal silica batch experiments regardless of supplied soil concentrations, augmented cell densities, supplied yeast extract concentrations, or NaCl concentrations. As shown, trends between stimulated and augmented experiments were similar, with supplied glucose concentrations governing achieved minimum pH values. Minimal differences between augmented and stimulated experiments suggested that both microbial communities were similarly efficient in utilizing supplied glucose for fermentation and acid generation. As glucose concentrations increased from 2.5 to 5 g/L, near linear decreases in minimum pH values were observed with increases in supplied glucose. When glucose concentrations were near 5 g/L, almost all experiments achieved minimum pH values within the targeted range of 5 to 6, regardless of using stimulation or augmentation. As glucose concentrations exceeded 5 g/L, however, minimal changes in minimum pH values were observed suggesting that glucose concentrations exceeding 5 g/L may be excessive. The lack of further reductions in minimum pH values for glucose concentrations exceeding 5 g/L again may largely reflect the increased buffering of solutions by produced organic acids and/or incomplete utilization of supplied glucose.

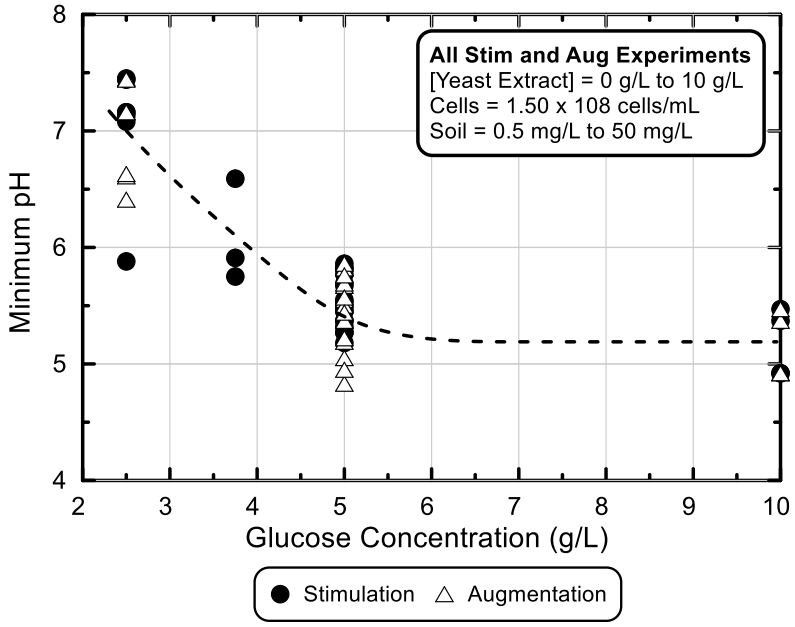

Figure 5.5 Relationship between minimum pH values and supplied glucose concentrations for all stimulation and augmentation colloidal silica batch experiments with varying augmented cell densities and soil additions as well as yeast extract, glucose, and NaCl concentrations.

5.4 Conclusions

The results the performed batch experiments demonstrated that both stimulation and augmentation could be used to reliably ferment glucose supplied in colloidal silica solutions to generate large pH reductions and induce colloidal silica gelation. Although augmentation can generally achieve faster pH reduction rates, the process requires high cell densities, which will necessitate both cell culturing and injection. In addition to significant additional costs and environmental impacts, the augmentation process may also suffer from limited uniformity of supplied cells when applied to actual soils due to colloidal filtration limitations. The stimulation process, however, offers the ability to forgo cell culturing and injection by simply supplying growth factors to enrich native glucose fermenting microorganisms in natural soils. In the performed stimulated experiments

containing high yeast extract concentrations, pH reduction rates were comparable to non-nutritive augmented experiments despite supplying only growth factors and requiring significant cell growth during the treatment period. When considering differences in glucose utilization between stimulation and augmentation processes, both stimulated and augmented experiments achieved nearly identical minimum pH values for similar glucose concentrations suggestive of similar glucose utilization efficiencies. When examining the effect of supplied yeast extract and glucose concentrations, similar effects were observed in both stimulated and augmented experiments, with pH reduction magnitudes controlled by glucose concentrations and pH reduction rates controlled by yeast extract concentrations and either augmented cell densities (augmentation) or supplied soil concentrations (stimulation). When comparing inhibition of microbial activity by supplied NaCl concentrations between these processes, augmentation exhibited significant inhibition at low NaCl concentrations with stimulation exhibiting much less sensitivity to NaCl. While further work is needed to optimize and understand the kinetics of these stimulation and augmentation processes, the presented batch experiments confirmed that biologically-mediated glucose fermentation can be used to control gelation of otherwise abiotically-stable colloidal silica solutions (colloidal silica solutions with 0 g/L NaCl and $pH_{initial}$ = 9.5 required more than 120 days to achieve gelation abiotically). When compared to traditional colloidal silica grouting techniques, the bio-mediated process may afford substantially improved control of grout gelation (i.e., gel times) and new opportunities for in-situ monitoring of colloidal silica grouts for a variety of different geotechnical applications.

Chapter 6: UP-SCALING THE BIO-MEDIATED PROCESS USING SOIL COLUMN EXPERIMENTS

Previous batch experiments investigated the impact of various treatment factors on the activity and pH reduction behavior of stimulated and augmented glucose-fermenting microorganisms in alkaline colloidal silica solutions. Although mediation of glucose fermentation with augmented *E. coli* cells was shown to be successful in the previous batch experiments, the augmentation process required very high initial cell densities ($\approx 10^8$ cells/mL), was more sensitive to changes in NaCl concentrations than stimulated communities and was expected to result in both higher implementation costs and environmental impacts when deployed at field-scale. In contrast, the use of native glucose-fermenting microorganisms was shown to be feasible in stimulated batch experiments and may afford important benefits with respect to reducing treatment costs and environmental impacts. In particular, stimulation can: (1) eliminate potential permitting issues related to the introduction of non-native microorganisms, (2) remove the need for cell culturing and injection and related impacts and costs, and (3) avoid cell transportation issues related to the colloidal filtration of injection cells in porous media. While results from stimulated batch experiments were encouraging, it remained unclear how this enrichment process would proceed under conditions more representative of subsurface soils as well as how gelation progression and fermentation activity could be effectively monitored in-situ.

A series of soil column experiments were performed to investigate the ability of treatment techniques previously developed in batch experiments to be up-scaled to more representative soil

volumes. More specifically, these tests allowed for: (1) investigation of process reaction kinetics under more representative porosities and substrate-to-cell ratios, (2) examination of the potential of various chemical and geophysical monitoring techniques to track glucose fermentation activity and colloidal silica gelation progression, and (3) assessment of the spatial uniformity of stimulated microbial activity in natural soils. In the performed study, six columns received various nutritive treatments intended to evaluate process reaction kinetics and geophysical changes during stimulated bio-mediated gelation and two additional columns served as abiotic controls and received similar solutions that did not contain added nutrients in order to establish expected behaviors without fermentation activity or colloidal silica gelation. In addition to column experiments, a series of corresponding batch experiments were also performed, which contained treatment solutions identical to those supplied to columns but included variations in supplied soil masses. These experiments were performed to allow for direct comparison of results between batch and column experiments. In particular, comparisons were expected to yield important insights regarding: (1) the effect of varying soil-to-fluid ratios (i.e. initial microbial cell densities), (2) the effect of varying substrate concentrations (i.e. glucose, yeast extract) and (3) the effect of varying salinity on resulting pH reduction behaviors and colloidal silica gel times. Batch experiments contained either 0.05 g/L or 0.1 g/L of Delta Sand, however, soil columns contained ≈4.5 g/L of Delta Sand, which was expected to result in significant increases in reaction rates.

6.1 Materials and Methods

Treatment Solutions

Eight centimeter-scale columns containing Delta Sand (≈4.5 g/L) were prepared to an initial relative density of ≈40% and had pore volumes of ≈250 mL. Columns were subjected to a total stress of ≈100 kPa from a reaction frame that applied a vertical force to column top caps. All columns were saturated with deionized water prior to treatments and received a single 8 pore volume injection of colloidal silica treatment solutions at a flow rate of 20 mL/min, intended to fully replace residing pore fluids. Colloidal silica solutions were prepared using a NaOH-stabilized colloidal silica stock solution (Ludox SM-30, colloid particle sizes = 7 to 22 nm) and were pH-adjusted using either NaOH (Fisher Scientific) or HCl (Fisher Scientific) to an initial value of 9.5. Treatments solutions between biotic columns (Column T3 through T8) examined the effect of variations in supplied yeast extract, glucose, and NaCl concentrations. Two abiotic solutions (Column T1 and T2) received similar solutions as biotic columns, but without supplied yeast extract or glucose to establish reference behaviors. **Table 6.1** provides a summary of all column and batch experiment treatment solutions.

Table 6.1. Summary of All Column and Batch Experiment Treatment Solutions

Treatment Solution	Yeast Extract (g/L)	Glucose (g/L)	NaCl (g/L)	Description
T1	0	0	0	Abiotic control solution without NaCl.
T2	0	0	1	Abiotic control solution with NaCl.
T3	0.2	5	0	Biotic solution with a low yeast extract concentration. Evaluates the effect of yeast extract on the pH reduction rate. Compared to T4, T5 and T6.
T4	1	5	0	Biotic solution with a medium yeast extract concentration. Evaluates the effect of yeast extract on the pH reduction rate. Compared to T3, T5 and T6.

T5	1	5	0	Biotic solution with a medium yeast extract concentration. Identical to T4 and evaluates reproducibility and the effect of yeast extract on the pH reduction rate. Compared to T3, T4 and T6.
T6	5	5	0	Biotic solution with a high yeast extract concentration. Evaluates the effect of yeast extract on the pH reduction rate. Compared to T3, T4 and T5.
T7	1	10	0	Biotic solution with medium yeast extract and high glucose concentration. Evaluates the effect of yeast extract and glucose on the pH reduction rate and magnitude. Compared to T4 and T5.
T8	1	5	1	Biotic solution with a medium yeast extract concentration and added NaCl. Evaluates the effect of NaCl on the pH reduction rate. Compared to T4 and T5.

Soil Columns

Soil column specimens were prepared in 15.2 cm high, 7.6 cm inner diameter hollow acrylic cylinders, which contained PFTE caps on top and bottom for solution exchange and various fittings for bender element sensors, solution sampling ports, and electrical conductivity probes. Columns were treated from the bottom upwards to ensure saturation and minimize heterogeneity in transport resulting from solution density differences. Three sampling ports existed along column heights at various distances from the injection location (bottom – 5.08 cm, middle – 10.16 cm, top – 15.24 cm) and were used for timed solution sampling. Soils within columns were confined by caps and porous plastic discs (Porex Inc.) on top and bottom to prevent soil erosion during injections. Columns were treated using constant flow rate peristaltic pumps and solution pressures during injections were monitored using pore pressure transducers at the influent and effluent locations (Omega Inc., pressure range = 0 to 30 psi). 1900 mL solution reservoirs were connected to column effluent tubes, were filled during the colloidal silica treatment injection, and were connected to

columns to enable replacement of columns fluids during sampling events to prevent desaturation. **Figure 6.1** presents a simplified schematic of the configuration of a single soil column.

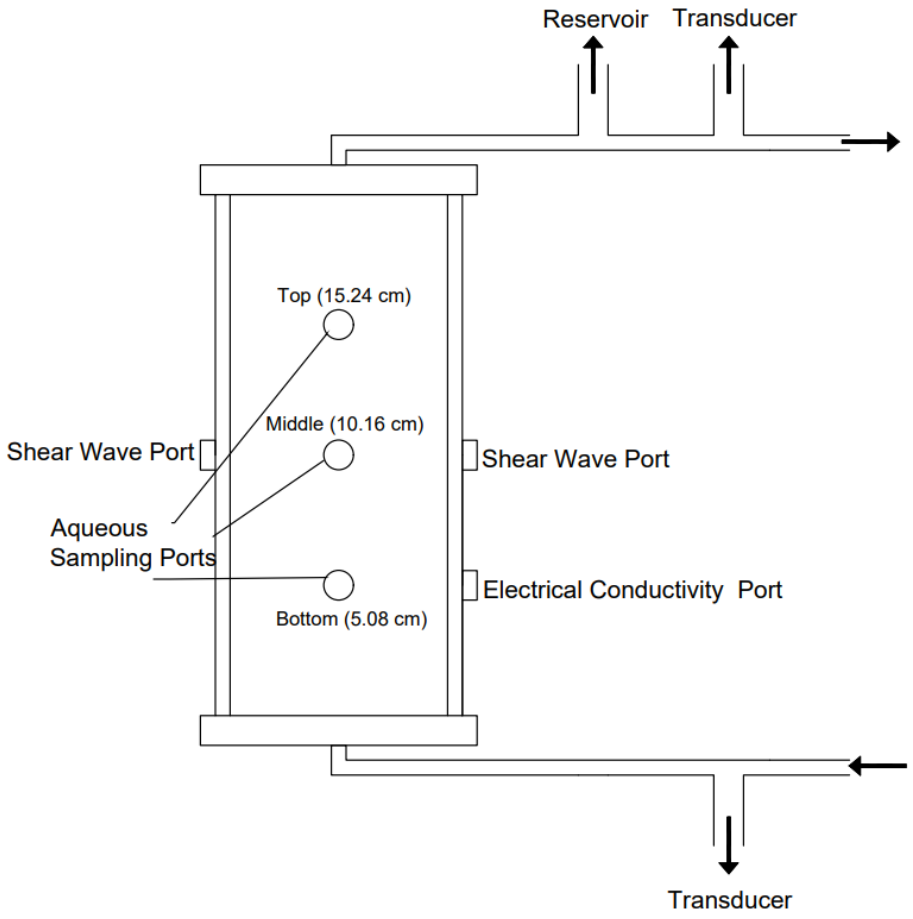

Figure 6.1 Simplified schematic of a single soil column which included three aqueous sampling ports, two bender element (shear wave velocity) ports, one electrical conductivity port (select columns), as well as influent tubing and effluent tubing.

Biogeochemical Monitoring

Aqueous samples were obtained from all three column sampling ports at various times over a 6-day monitoring period to examine changes in solution pH and glucose concentrations. All samples were obtained using sterile needles (Fisher Scientific) and were near 2 mL in volume. The pH of

collected solution samples was measured immediately upon collection using a semi-micro pH electrode and meter system (Orion Versa Star Meter, Thermo Fisher) that was calibrated daily using three buffers (4.01, 7.00, 10.01) and had ± 0.05 pH unit accuracy. Samples for glucose measurements were stabilized in 2M NaOH and frozen immediately after sampling. For stabilization, 0.125 mL of solution was added to 0.585 mL of 2M NaOH to prevent further biological activity and dissolve potentially gelled colloidal silica. A detailed protocol for glucose assay method can be found in **Appendix 1**.

Geophysical Measurements

Electrical conductivity and shear wave velocity (V_s) measurements were performed during soil column experiments to examine the ability of non-destructive geophysical measurements to detect changes in bio-mediated colloidal silica gelation and glucose fermentation activity. Electrical conductivity measurements were only completed for four columns (Column T1, T2, T5, and T8) using calibrated 4-cell conductivity probes (range = 1µS/cm to 200 mS/cm) that were embedded near the bottom of soil columns and measured using a multiuse meter (Orion Versa Star Meter, Thermo Fisher) at various times. Shear wave velocity measurements were completed using two bender element sensors (y-poled, Piezo Inc.), which were placed at the center of columns at known sensor spacings and were measured at least once daily. Initial shear wave velocities in columns were generally between 90 m/s and 110 m/s.

Hydraulic Conductivity Measurements

The initial and final hydraulic conductivity (k) of columns was characterized using pore pressure and flow rate measurements during injections. Initial hydraulic conductivity estimates were obtained during the colloidal silica injection wherein injection pressures were near 0.8 psi and flow rates were near 20 mL/min. Final hydraulic conductivity estimates were obtained 14 days after colloidal silica injections by injecting de-ionized water. During these injections, injection pressures varied between 8 psi and 20 psi and flow rates varied between 20 mL/min and 100 mL/min due to silica gelation. Pressure measurements were recorded at both the influent and effluent of columns during measurements as shown in **Figure 6.1** after steady state flow conditions were achieved.

Unconfined Compressive Strength Measurements

Treated soils were extruded from acrylic cylinders after treatment using a hydraulic jacking device. After extrusion, soil columns were subjected to unconfined compressive strength (UCS) tests. UCS tests were performed using a GDS load frame system in accordance with ASTM D216 using a constant rate of axial strain of 1% per minute.

Batch Experiments

Sixteen batch experiments containing identical treatment solutions as columns (see **Table 6.1**) but with lower soil-to-fluid ratios (0.05 g/L or 0.1 g/L Delta Sand) were completed in sterile plastic bottles containing 250 mL volumes of a 6% by mass colloidal silica solution. Colloidal silica solutions were prepared using a NaOH-stabilized colloidal silica stock solution (Ludox SM-30,

colloid particle sizes = 7 to 22 nm) and were pH-adjusted using either NaOH (Fisher Scientific) or HCl (Fisher Scientific) to an initial value of 9.5 and then filter-sterilized. In order to minimize the potential for contamination, all exposed surfaces (i.e. bottle mouth, cap) were flame-sterilized using a portable torch (Bernzomatic) whenever bottles were opened. Solution samples (≈2 mL) were obtained from experiments using sterile pipettes at least twice a day to monitor pH and glucose concentration changes. pH and glucose measurements were completed using methods similar to the aforementioned soil columns. Post-treatment viscosity changes were assessed for all batch experiments using a Brookfield AMETEK Low-Range Viscometer (100 to 240 VAC) with No. 63 and No. 64 spindles to assess the impact of biological processes on solution gelation.

6.2 Results and Discussion

6.2.1 Solution pH Changes in Columns

Figure 6.2 presents pH measurements in time for both abiotic columns (T1 and T2) at all three sampling locations. As shown, some pH reductions in time were observed in both columns at all the three sampling ports from initial values near 9.5 to values between 7.5 and 8 within approximately 1 day. Although these pH reductions were unexpected given the lack of fermentable substrates and growth factors, the observed pH reductions may have resulted from the equilibration of supplied colloidal silica solutions with existing soil minerals. Although trends were similar along columns, slightly higher pH values were recorded at the port closest to the injection source (5.08 cm).

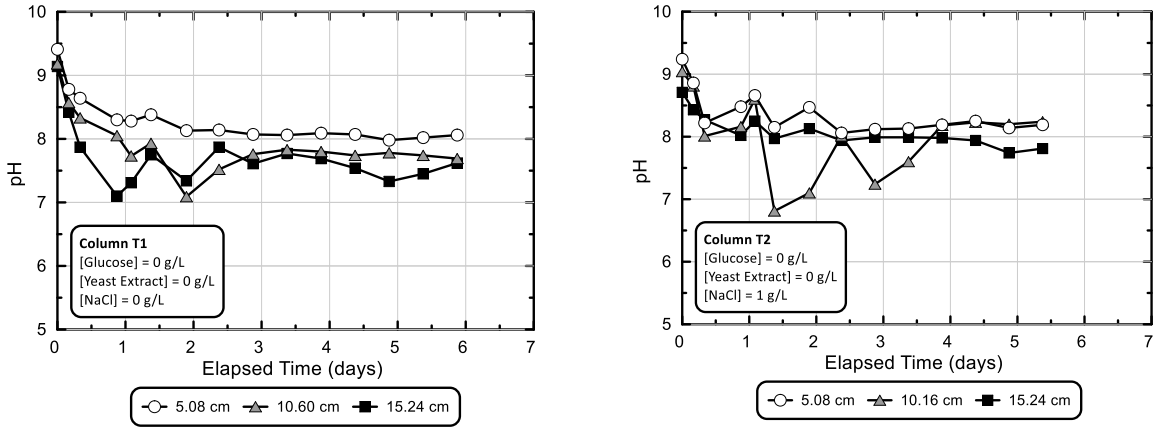

Figure 6.2. Measurements of solution pH in time for the two abiotic columns (T1, T2) at all three sampling ports.

Figure 6.3 presents pH measurements in time for all biotic columns (T3 through T8) for all three sampling locations. For all respective columns, pH reductions in time were similar between sampling ports suggesting that columns had minimal spatial variations in enriched microbial activity. In most columns, pH values reduced from initial values near 9.5 to values below 7 within 2 days. Near the end of experiments, all columns achieved minimum pH values between ≈5 and 7. Although differences between treatment formulations were more difficult to interpret from **Figure 6.3**, the achieved results did suggest that minimal differences existed spatially within columns with mid-port measurements being representative of most column behaviors.

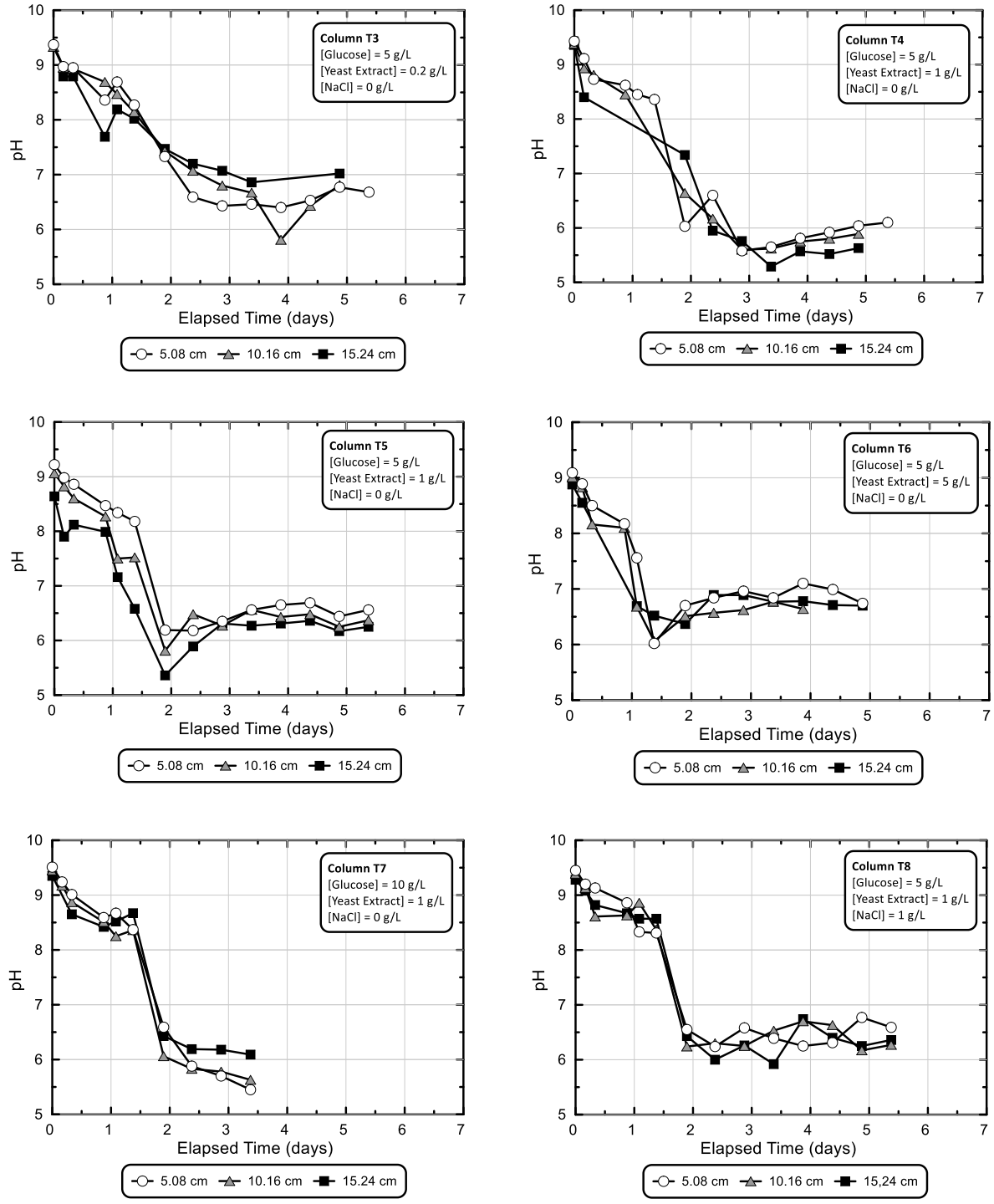

Figure 6.3. Measurements of solution pH in time for the six biotic columns (T3 through T8) at all three sampling ports.

6.2.2 Comparison of Solution pH Changes Between Column and Batch Experiments

Figure 6.4 presents pH measurements in time for all mid-port locations in abiotic columns (T1 and T2) as well as corresponding batch experiments, which received identical treatment solutions. As shown, significant pH reductions were observed in both columns, however, no detectable pH changes were observed in batch experiments. Again, the reduction in pH values observed in columns over time were attributed to the equilibration of colloidal silica solution with soil minerals, while batch experiments may have experienced more minimal pH changes in time due to the presence of much larger solution-to-soil ratios.

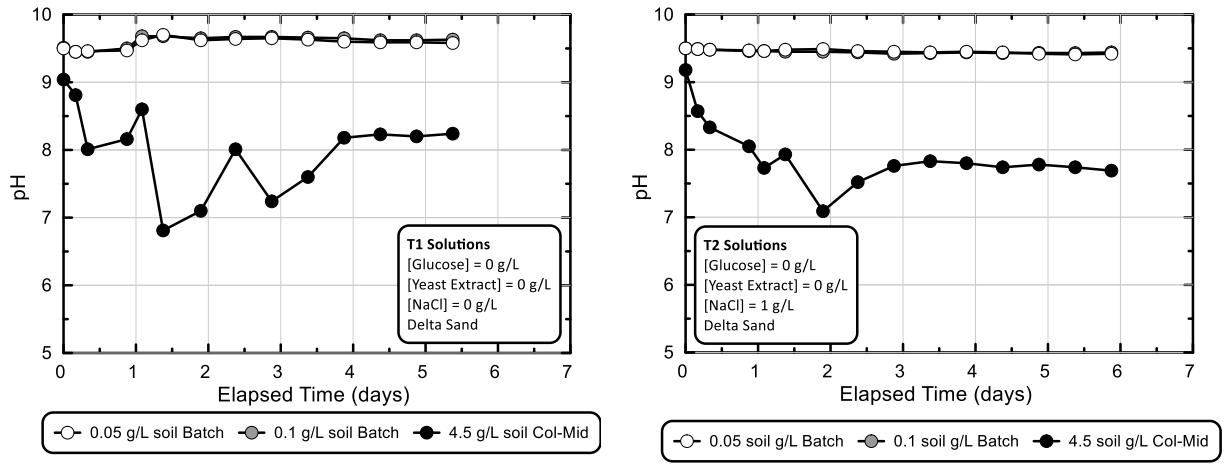

Figure 6.4. Comparison of solution pH measurements in time for abiotic columns (T1, T2) which contained ≈4.5 g/L Delta Sand and corresponding batch experiments which contained either 0.05 g/L or 0.1 g/L Delta Sand.

Figure 6.5 presents pH measurements in time for all mid-port locations in biotic columns (T3 through T8) as well as corresponding batch experiments, which received identical treatment solutions. All biotic experiments contained growth factors and fermentative substrates (i.e. yeast extract, glucose) intended to stimulate native glucose fermenting microorganisms and permit pH reductions in time and colloidal silica gelation. As expected, when comparing pH reductions in

time from soil columns with similar batch experiments, significant faster pH reductions were observed in soil columns reflective of the higher soil-to-fluid ratio in these columns, which likely resulted in much larger initial microbial densities. In all batch experiments, minimum pH values within the targeted pH range of 5 to 6 were achieved between 2.5 and 5.5 days, which was consistent with similar previous batch experiments. In batch experiments containing 0.1 g/L Delta Sand, faster pH reductions were also achieved when compared to 0.5 g/L Delta Sand experiments, however, differences between pH trends appeared to be no more than a 1-day offset. Although pH reduction rates were much faster initially in soil columns when compared to batch experiments, interestingly, minimum pH values were significantly higher and ranged between 5.5 and 6.5. Again, these higher pH values may have been reflective of increased solution buffering in columns via equilibration with larger added soil masses, but may also have been reflective of potential differences in enriched microbial communities and their respective fermentation activities.

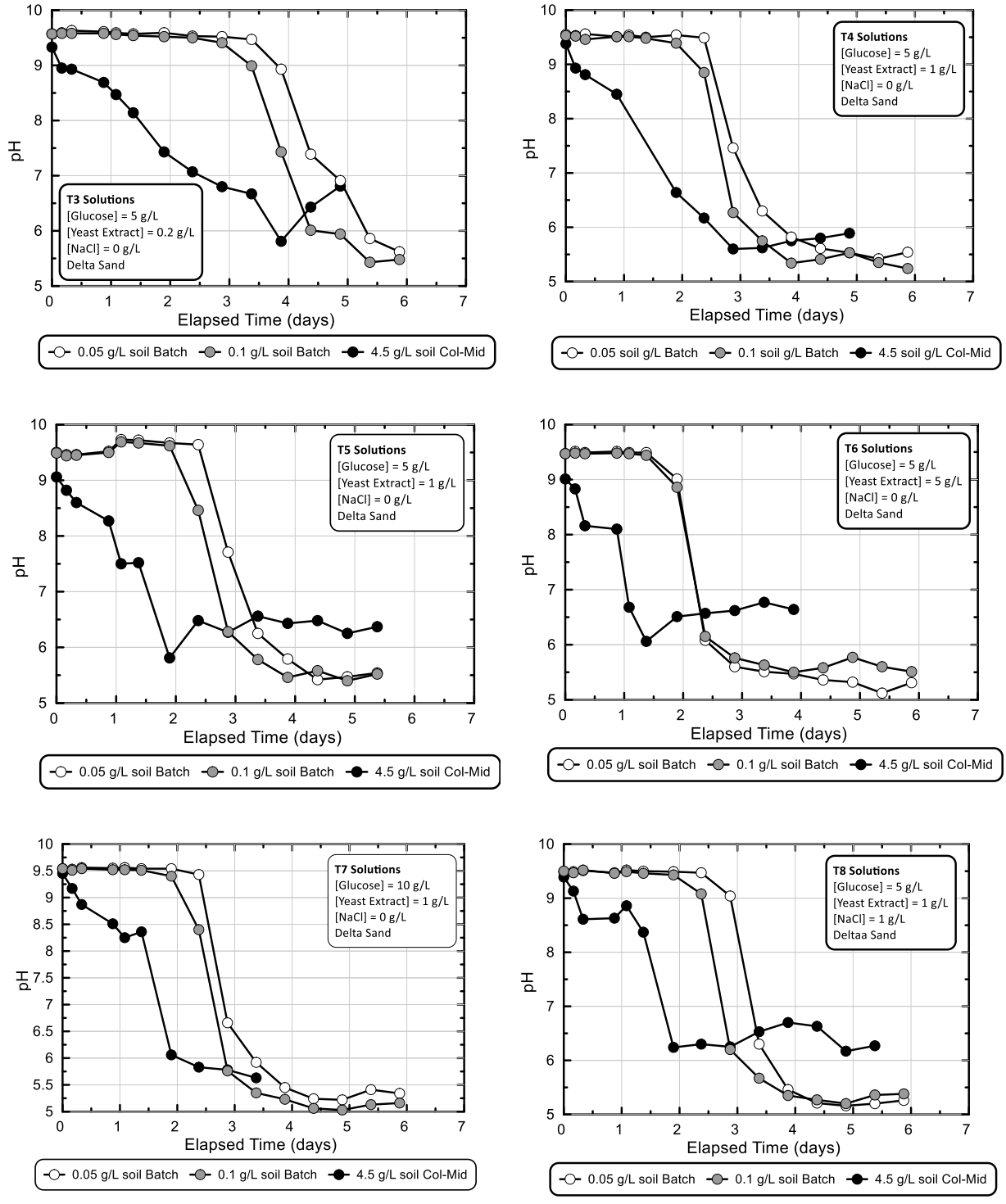

Figure 6.5. Comparison of solution pH measurements in time for biotic columns (T3 through T8) which contained ≈4.5 g/L Delta Sand and corresponding batch experiments which contained either 0.05 g/L or 0.1 g/L Delta Sand.

6.2.3 Comparison of Glucose Concentration Changes Between Column and Batch Experiments

Figure 6.6 presents glucose concentration measurements in time for all mid-port locations in biotic columns (T3 through T8) as well as corresponding batch experiments, which received identical treatment solutions. Although solution samples were obtained at consistent times for all columns, glucose measurements were only completed for select samples after reviewing corresponded pH reductions in time. As shown, columns and corresponding batch experiments T3 through T6 and T8 had initial glucose concentrations near 5 g/L, which corresponded with the injected solution concentration. Although batch experiments had minimal variations in initial glucose concentrations, small differences in initial glucose concentrations were observed between columns, most likely due to solution mixing. After 2 to 4 days after injections, this 5 g/L concentration was nearly fully degraded in all columns due to microbial fermentation activity. In the 10 g/L glucose column (column T7), 5 g/L glucose was degraded within approximately 2.5 days, however, full degradation was not observed due to the gelation of columns solutions, which prevented further sampling after ≈3.5 days. Similar gelation was also observed in T7 batch experiments, prior to full degradation of the supplied glucose concentrations. When comparing batch experiments to similarly treated columns, again similar trends were observed as pH measurements with: (1) glucose degradation occurring faster in columns due to a larger soil inoculants, (2) increases in glucose degradation rates observed in 0.1 g/L Delta Sand experiments in comparison to the 0.05 g/L Delta Sand experiments with the exception of the high yeast extract solution (T6), and (3) batch experiments experiencing an initial lag in glucose degradation between 1.5 and 4 days, with glucose degradation proceeding at a similar rate as soil columns after that. Although glucose measurements could have been performed for abiotic columns, such measurements were not

completed due to the lack of supplied glucose in these columns. Measurements were completed on previous batch experiments not containing glucose, however, and confirmed that the glucose assay measurement did not detect any measurable glucose and/or inferences from colloidal silica or soil minerals (data not shown).

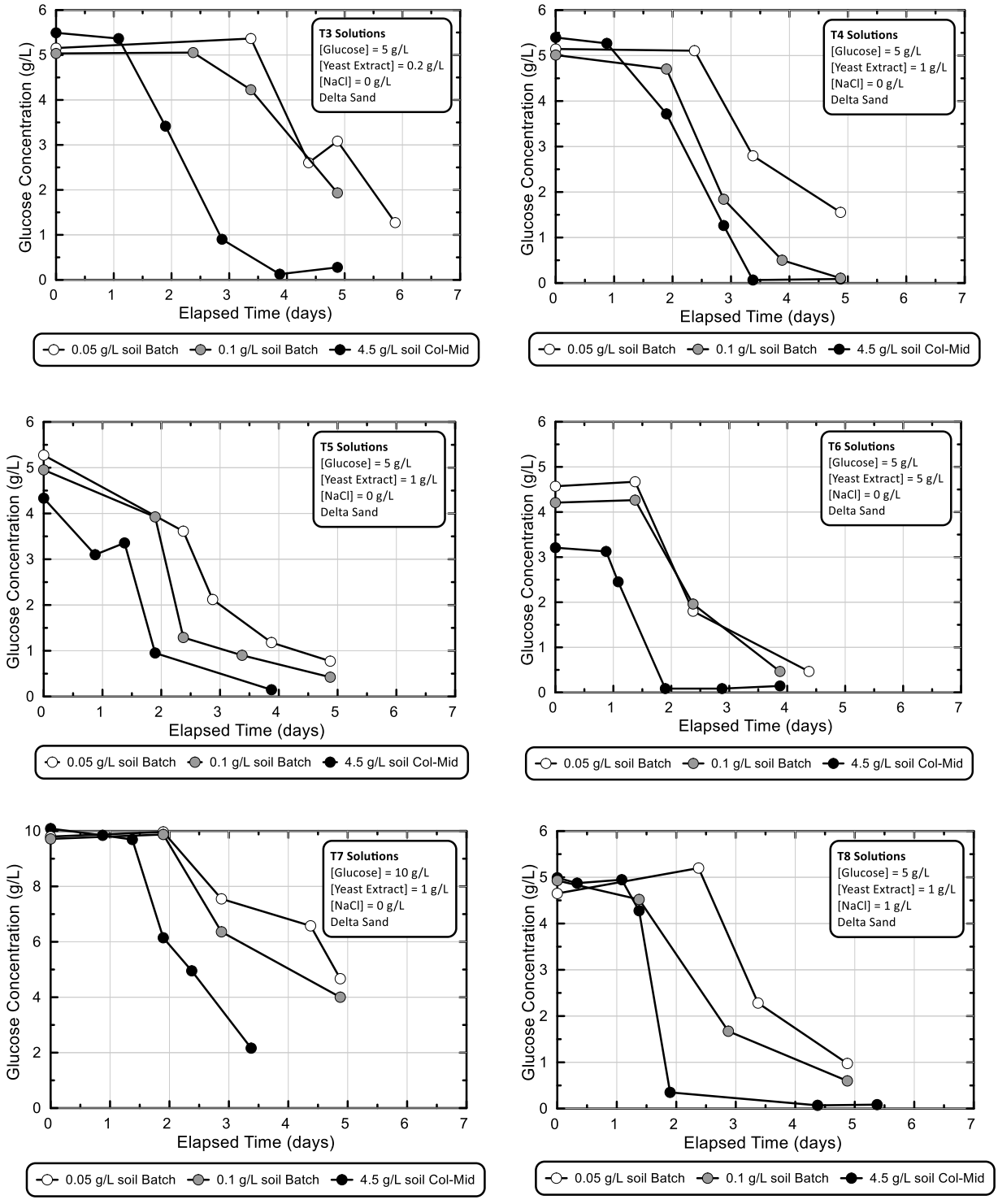

Figure 6.6. Comparison of glucose measurements in time for biotic columns (T3 through T8) which contained ≈4.5 g/L Delta Sand and corresponding batch experiments, which contained either 0.05 g/L or 0.1 g/L Delta Sand.

6.2.4 Comparison of pH Changes in Time Due to Treatment Solution Variations

Figure 6.7 presents pH measurements in time from mid-port locations in all columns (red trendlines = abiotic columns, blue trendlines = biotic columns). When comparing trends between abiotic and biotic columns, clear differences can be observed with abiotic columns achieving initial pH reductions from 9.5 to values between 7 and 8 after about 1 day with final values near 8 approached after about 4 days. In contrast, biotic columns achieved similar initial pH reductions to values between 6 and 8 after 1.5 days, however, pH reductions continued in time with final values between 5.5 and 7 achieved in most columns after 3 days. Given that glucose concentrations were 5 g/L or greater in all biotic columns, minimum pH values were larger than expected in soil columns based upon the response of previous batch experiments. Again, these higher final pH values may be reflective of differences in enriched microbial communities in columns versus batch experiments and/or potential differences in acid generation efficiency per mass of supplied glucose.

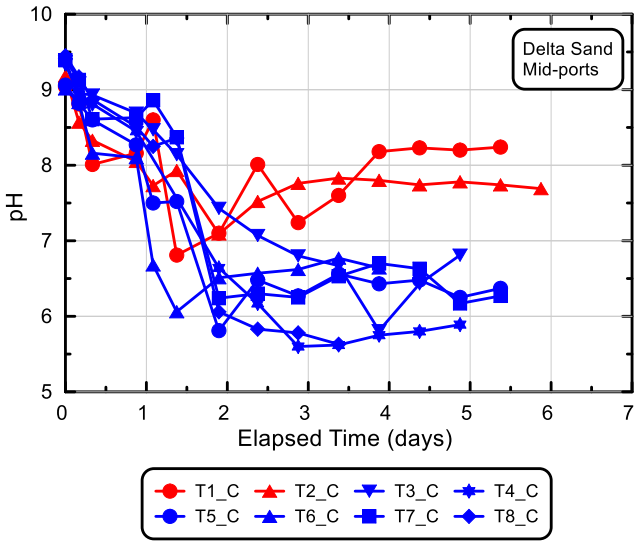

Figure 6.7. Comparison of pH measurements in time from all column mid-port locations. Red trendlines are reflective of abiotic columns which contained no added glucose or yeast extract. Blue trendlines are reflective of biotic columns with supplied glucose and yeast extract concentrations.

Figure 6.8 presents pH measurements in time from mid-port locations in all columns which received solutions with 1 g/L yeast extract and varying glucose concentrations (T4, T5, and T7). It should be noted that two of the columns (T4 and T5) received identical solutions and were expected to behave similarly. When comparing pH trends between columns, all columns achieved nearly identical pH reductions in time regardless of supplied glucose concentrations. This result was consistent with previous batch experiments, which indicated that when glucose concentrations exceeded 5 g/L, minimal differences in final pH magnitudes were observed. Although it was hypothesized that higher glucose concentrations might slightly increase pH reduction rates in time, surprisingly, the 10 g/L glucose column (T7) had a pH reduction rate that was slightly slower than the other 5 g/L glucose columns (T4, T5). Despite these similarities, however, the 10 g/L glucose column (T7) did achieve gelation approximately 2 days faster than the 5 g/L glucose columns (T4, T5).

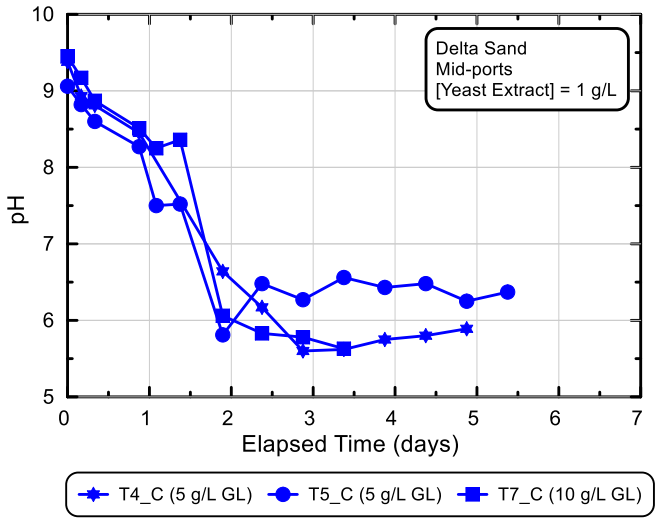

Figure 6.8. Comparison of pH measurements in time from mid-port locations in columns which received solutions with 1 g/L yeast extract and varying glucose concentrations (T4, T5, and T7).

Figure 6.9 presents pH measurements in time from mid-port locations in all columns which received solutions with 5 g/L glucose and varying yeast extract concentrations (T3 through T6). As shown, pH reduction rates varied as a function of supplied yeast extract concentrations as expected following previous batch experiments. Interestingly, all columns appeared to achieve immediate pH reductions with differences in pH trends between columns appearing to be more so related to the slope of pH reductions. In contrast to batch experiments wherein yeast extract variations resulted in large differences in pH reduction timing, however, differences between the lowest 0.2 g/L yeast extract column (T3) and the highest 5 g/L yeast extract column (T6) were much more subtle and resulted in a timing difference of approximately one day. Although these results confirmed that yeast extract concentrations can be used to control reaction rates when up-scaled to more representative soil columns, a wider range of yeast extract concentration differences may need to be considered in future experiments, if a broader range of reaction rates is desired.

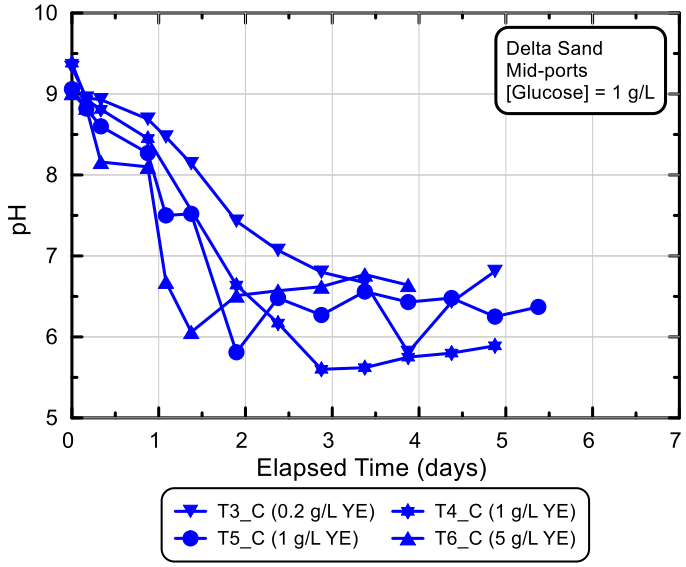

Figure 6.9. Comparison of pH measurements in time from mid-port locations in columns which received solutions with 5 g/L glucose and varying yeast extract concentrations (T3 through T6).

Figure 6.10 presents pH measurements in time from mid-port locations in all columns, which received solutions containing 5 g/L glucose, 1 g/L yeast extract, and varying NaCl concentrations (T4, T5, T8). As shown, the addition of 1 g/L NaCl appeared to have minimal impacts on observed fermentation activity in time. This result was consistent with previous batch experiments, which suggested that stimulated bacterial communities can tolerate NaCl concentrations of less than 5 g/L without detectable changes in fermentation activity.

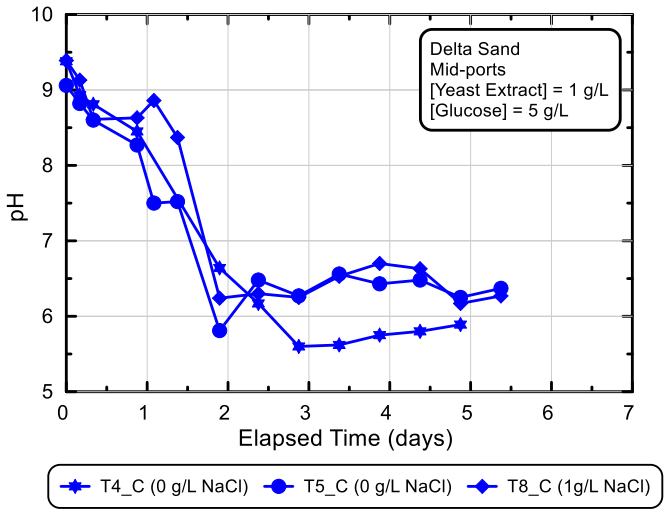

Figure 6.10. Comparison of pH measurements in time from mid-port locations in columns which received solutions with 5 g/L glucose, 1 g/L yeast extract, and varying NaCl concentrations (T4, T5, T8).

6.2.5 Comparison of Glucose Changes in Time Due to Treatment Solution Variations

Figure 6.11 presents glucose concentration measurements in time from mid-port locations for all biotic columns. As shown, a spectrum of glucose degradation rates was achieved. All columns exhibited similar initial lag times in activity between 1 to 1.5 days with significant glucose

degradation occurring over a duration of about 2 days after that. Some initial concentration offsets were also observed between columns, however, again likely due to solution mixing.

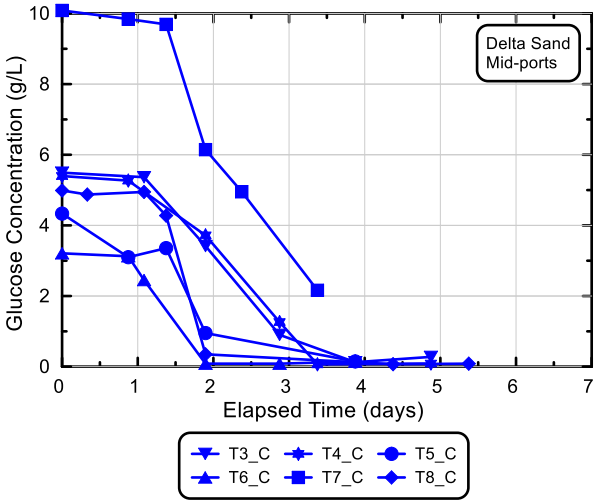

Figure 6.11. Comparison of glucose concentration measurements in time from mid-port locations in all biotic columns.

Figure 6.12 presents glucose concentration measurements in time from mid-port locations for all biotic columns with 1 g/L yeast extract and varying glucose concentrations. As shown, columns with initial glucose concentrations of 5 g/L achieved near full degradation of the glucose supplied between 2 and 4 days. In contrast, the 10 g/L glucose column (T7) experienced only partial utilization of supplied glucose before the solution completely gelled and prevented further sampling. Interestingly, this column had a similar time lag in initial glucose degradation activity (near 1.5 days) as all other 5 g/L glucose columns but exhibited a slightly steeper glucose degradation slope when compared to these columns, which was expected given the higher concentrations. Similar to earlier pH trends, this result again suggested that the use of 10 g/L glucose afforded minimal benefits when compared to 5 g/L glucose.

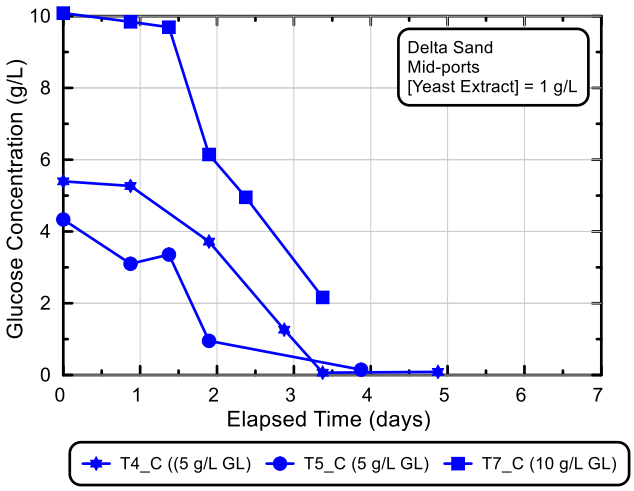

Figure 6.12. Comparison of glucose concentration measurements in time from mid-port locations for all biotic columns containing solutions with 1 g/L yeast extract and varying glucose concentrations.

Figure 6.13 presents glucose concentration measurements in time from mid-port locations for all biotic columns with 5 g/L glucose and varying yeast extract concentrations. Similar to previous pH trend observations, glucose degradation rates were proportional to supplied yeast extract concentrations. While in the highest 5 g/L yeast extract column (T6) full glucose degradation was observed after 2 days, in the lowest 0.2 g/L yeast extract column (T3), full degradation was observed near 3.5 days. This was similar to the ≈1 day offset observed in pH trends, and again suggested that while yeast extract may be used to alter reaction rates, such differences may be much less dramatic in columns when compared to batch experiments.

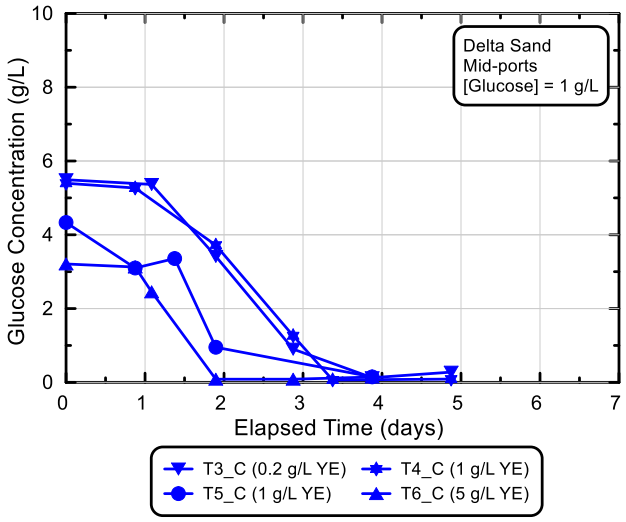

Figure 6.13. Comparison of glucose concentration measurements in time from mid-port locations in biotic columns containing solutions with 5 g/L glucose and varying yeast extract concentrations.

Figure 6.14 presents glucose concentration measurements in time from mid-port locations for biotic columns with 5 g/L glucose, 1 g/L yeast extract, and varying NaCl concentrations. As shown, similar to the previous pH responses, glucose degradation trends exhibited minimal differences between columns with and without 1 g/L NaCl. Interestingly, more significant differences were observed between the replicate columns (T4, T5) suggesting that any degradation variations related to NaCl additions were not detectable over inherent variability between columns and variations related to glucose assay measurements.

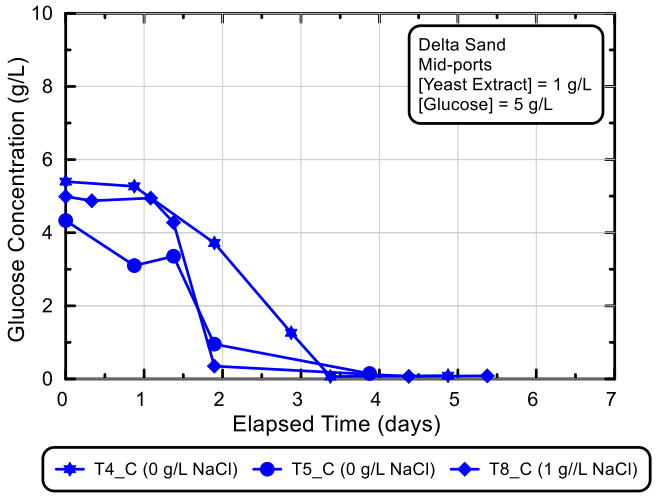

Figure 6.14. Comparison of glucose concentration measurements in time from mid-port locations in biotic columns containing solutions with 5 g/L glucose, 1 g/L yeast extract, and varying NaCl concentrations.

6.2.6 Changes in Soil Column Electrical Conductivities

Figure 6.15 presents electrical conductivity measurements in time obtained from abiotic columns with and without added NaCl (0 g/L or 1 g/L) as well as biotic experiments with and without added NaCl (0 g/L and 1 g/L) with supplied yeast extract (1 g/L) and glucose (5 g/L). As shown, when columns were initially saturated with de-ionized water, column electrical conductivities were near 0.3 mS/cm. Immediately after introducing colloidal silica solutions, however, electrical conductivities increased immediately with initial values near 0.6 mS/cm for columns without NaCl and slightly higher initial values near 0.7 to 0.8 mS/cm for columns with 1 g/L NaCl. During the 6 day monitoring period, abiotic columns had relatively stable electrical conductivity values which only gradually increased by ≈0.15 mS/cm. In contrast, large increases in column electrical conductivities were observed after 1 day in the biotic column containing 1 g/L NaCl (T8) and after 1.5 days in the biotic column not containing any added NaCl (T5). After between 1.5 and 3.5 days,

near stable electrical conductivity values were observed in biotic columns with an overall increase near ≈0.65 mS/cm observed in the column without NaCl (T5) and an overall increase near ≈0.7 mS/cm observed in the column with 1 g/L NaCl (T8). While these results clearly showed that column electrical conductivities increased during the biologically-mediated process, it was unclear if such increases were related to ionic strength increases resulting from glucose fermentation activity, or other electrical conductivity changes related to the formation of bonds between silica colloids and gelation progression.

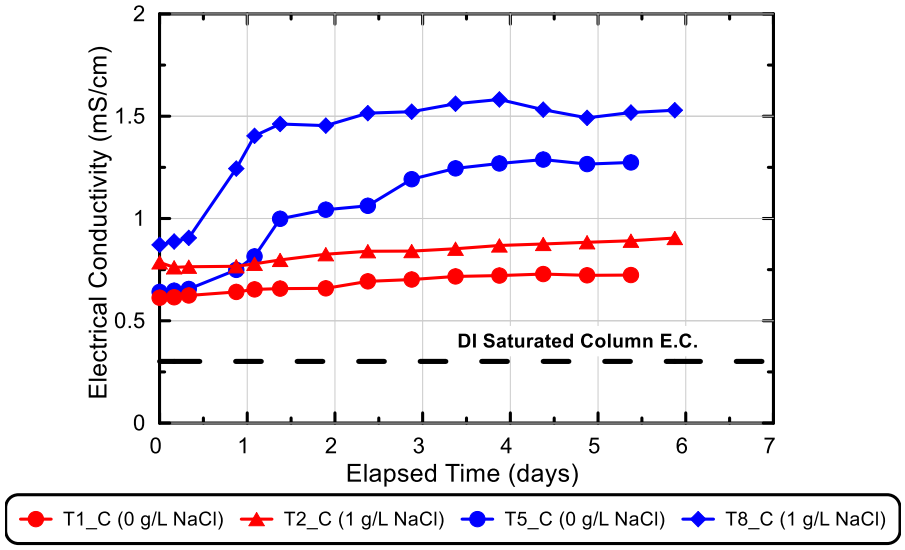

Figure 6.15 Soil column electrical conductivity measurements in time obtained for select abiotic (T1, T2) and biotic (T5,T8) columns with and without 1 g/L NaCl. Red trendlines are reflective of abiotic columns which contained no added glucose or yeast extract. Blue trendlines are reflective of biotic columns which contained solutions with 1 g/L yeast extract and 5 g/L glucose supplied.

In order to better understand the mechanism of electrical conductivity increases in biotic columns, electrical conductivity measurements were directly compared to changes in solution pH values. **Figure 6.16** presents soil column electrical conductivity measurements versus solution pH values for abiotic columns with and without added NaCl (0 g/L or 1 g/L) as well as biotic columns with supplied yeast extract (1 g/L), glucose (5 g/L), and with or without added NaCl (0 g/L and 1 g/L). As shown, pH values in abiotic columns decreased from 9.5 to between 7 and 8 over time, with only minimal increases in soil column electrical conductivities near ≈0.15 mS/cm. In contrast, biotic columns achieved large increases in soil column electrical conductivities over time between 0.65 and 0.7 mS/cm while at the same time pH value decreased. Although pH reductions and increases in electrical conductivities appeared to be well correlated in the biotic column without added NaCl (T5), in the biotic column with 1 g/L NaCl (T8), large increases in electrical conductivities were observed with minimal changes in pH values. The rapid increase in electrical conductivities in the column with added NaCl without significant pH changes suggested that measured electrical conductivity increases in soil columns may be more closely related to the gelation of solutions as opposed to changes in solution ionic strengths from the glucose fermentation process.

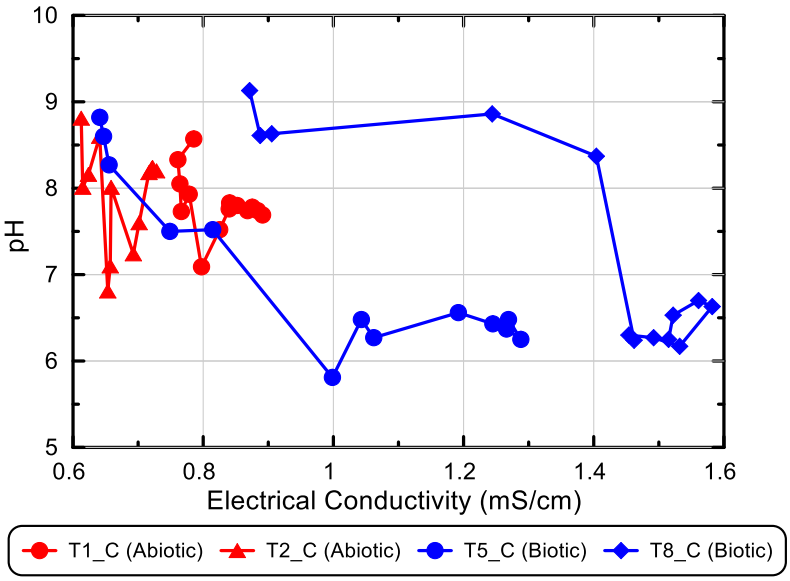

Figure 6.16. Soil column electrical conductivities versus solution pH measurements for select abiotic (T1, T2) and biotic (T5,T8) columns with and without 1 g/L NaCl. Red trendlines are reflective of abiotic columns which contained no added glucose or yeast extract. Blue trendlines are reflective of biotic columns which contained solutions with 1 g/L yeast extract and 5 g/L glucose supplied.

6.2.7 Changes in Soil Column Shear Wave Velocities

Figure 6.17 presents soil column shear wave velocities (V_s) in time for all columns as measured by bender element sensors at mid-height. As shown, prior to treatment, all columns had initial shear wave velocities between 90 and 110 m/s. Following colloidal silica injections, however, all columns experienced small reductions in shear wave velocities over the 5-day monitoring period. Although it was unclear why soil shear wave velocities decreased in time in both abiotic and biotic columns, it was hypothesized that such decreases may have been related to the relaxation of applied vertical stresses from top caps over time (abiotic and biotic columns) and/or the swelling of supplied colloidal silica solutions during gelation (biotic columns).

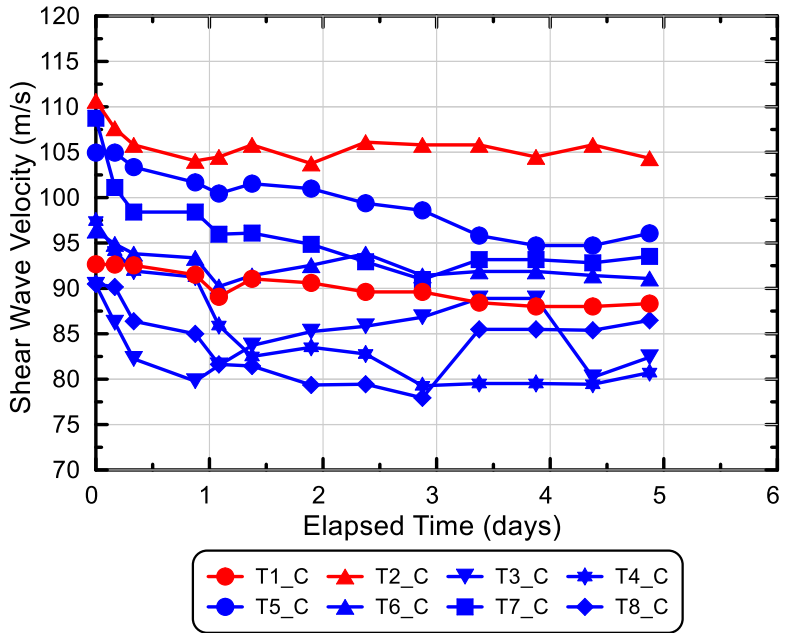

Figure 6.17. Soil column shear wave velocities (V_s) in time for all columns as measured by bender element sensors at mid-height.

In order to better compare trends in soil shear wave velocities between biotic and abiotic columns, changes in shear wave velocities in time were considered instead. **Figure 6.18** presents changes in soil column shear wave velocities in time for all columns. As shown, both abiotic columns exhibited shear wave velocity reductions in time that were near 5 m/s over the 5-day monitoring period and likely reflected the effects of vertical stress relaxation over time. When considering biotic columns, however, shear wave velocities appeared to reduce more significantly in time for most columns with shear wave velocity reductions in time that were between 4 and 18 m/s. While the larger shear wave velocity reductions in biotic columns were unexpected, such reductions may have resulted from the swelling of colloidal silica solutions, which could have decreased

interparticle contact stresses and thus decreased small-strain shear moduli values despite also imparting a small tensile strength. Similar shear wave velocity reductions have also been observed in other past studies involving sands treated with abiotic colloidal silica solutions (Spencer et al. 2008), again owing to expected reductions in interparticle contact stresses.

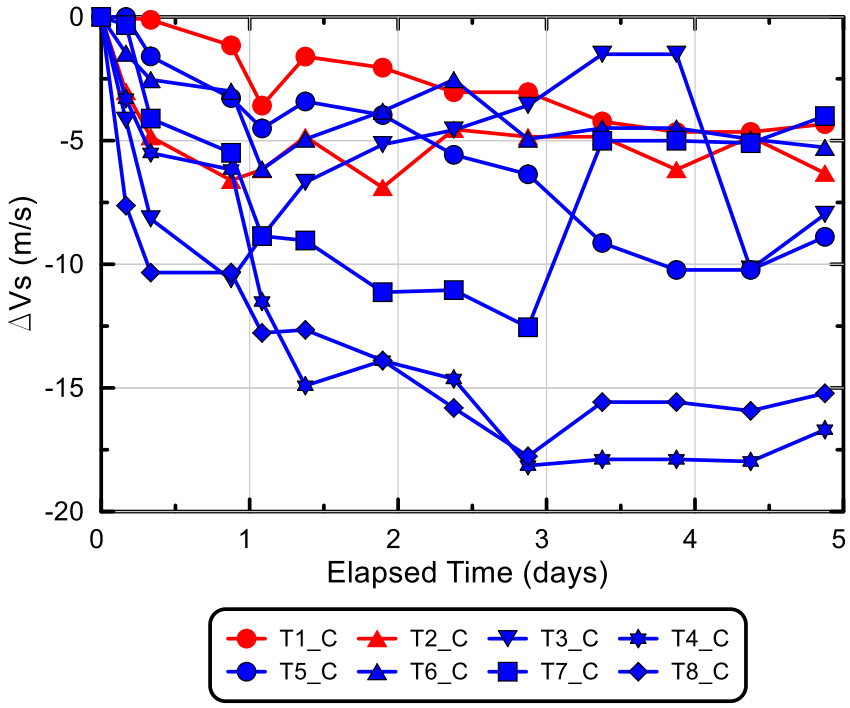

Figure 6.18. Changes in soil column shear wave velocities (V_s) in time for all columns as measured by bender element sensors at mid-height.

6.2.8 Relationship between Glucose Degradation and Solution pH Reductions

Figure 6.19 presents a comparison of solution glucose concentrations and pH values measured for samples obtained from all batch and column experiments over time. As shown, two general trends can be observed for experiments with either 5 g/L glucose or 10 g/L glucose supplied. Regardless

of glucose concentration magnitudes, however, as glucose concentrations decreased, progressive decreases in solution pH values were observed. In both 5 g/L and 10 g/L glucose experiments, reductions to from pH values near 9.5 to values between 5 and 6 were achieved after degradation of near 3 g/L glucose. As reactions proceeded toward completion, some small increases in pH were observed for 5 g/L glucose experiments likely reflecting the limited ability of pH measurements to measured increasingly gelled solutions. While it appeared that experiments with 5 g/L glucose could achieve minimum pH values within the targeted range of 5 to 6 for most experiments, 10 g/L glucose experiments achieved similar final values, although behaviors near reaction completion could not be tracked accurately due to the gelation of solutions before full glucose degradation. These results suggested that when colloidal silica solution conditions are relatively well known, similar relationships may be used to indirectly estimate glucose concentrations from measured pH values.

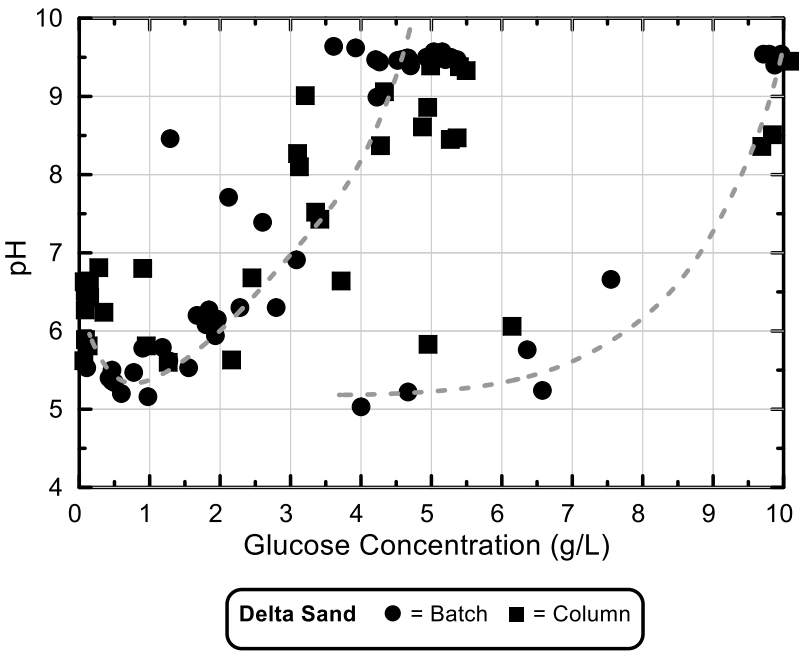

Figure 6.19. Comparison of solution glucose concentrations and pH values measured for samples obtained from all batch and column experiments over time.

6.2.9 Soil Column Post-Treatment Properties

Table 6.2 presents a summary of post-treatment properties for all columns including initial and final pH values, initial and final shear wave velocities, initial and final hydraulic conductivities (k), and measured unconfined compressive strengths (UCS) for all columns. As shown, all biotic columns achieved large pH reductions as well as decreases in shear wave velocities and hydraulic conductivities, with some columns achieving measurable unconfined compressive strengths. In contrast, abiotic columns exhibited smaller pH decreases, more minimal shear wave velocity and hydraulic conductivity reductions, and no measurable unconfined compressive strengths. While these results confirmed that colloidal silica gelation had measurable impacts on the post-treatment engineering properties of biotic soil columns, comparisons of properties between columns were examined further.

Table 6.2 Summary of Soil Column Post-Treatment Properties

Column	**$pH_{initial}$**	**pH_{final}**	**$V_{s,initial}$ (m/s)**	**$V_{s,final}$ (m/s)**	**$k_{initial}$ (m/s)**	**k_{final} (m/s)**	**UCS (kPa)**
T1	9.04	8.24	92.7	88.3	5.2×10^{-3}	4.4×10^{-3}	-
T2	9.18	7.69	110.7	104.3	2.2×10^{-3}	1.7×10^{-3}	-
T3	9.33	6.81	90.4	82.4	4.6×10^{-3}	5.9×10^{-5}	26.9
T4	9.38	5.89	97.4	80.7	8.1×10^{-3}	1.3×10^{-4}	33.5
T5	9.06	6.37	105.0	96.1	3.7×10^{-3}	1.0×10^{-4}	-
T6	9.01	6.64	96.4	91.1	6.8×10^{-3}	1.0×10^{-4}	19.0
T7	9.45	5.63	108.7	93.5	6.6×10^{-3}	1.5×10^{-4}	11.9
T8	9.39	6.27	90.5	86.5	4.3×10^{-3}	7.3×10^{-5}	-

As shown in **Table 6.2**, select biotic columns had measurable unconfined compressive strengths ranging between 11 and 34 kPa after treatment. This was much lower than expected, given that

UCS values between 30 and 80 kPa have been measured for soils treated with 5% colloidal silica solutions in past studies (Gallagher et al. 2002). While such measurements confirmed that gelled colloidal silica could impart a small increase in tensile strength on treated soil specimens, variations between columns and the relatively small UCS magnitudes measured likely resulted from a variety of different disturbances. In particular, (1) UCS tests were performed after final hydraulic conductivity measurements and may have been disturbed by high injection pressures and fluid flow, (2) prior to UCS tests, electrical conductivity probes were removed from select columns and resulted in voids which likely further disturbed columns, and (3) the extrusion of treated soils from acrylic columns after treatment likely induced further disturbances. For these reasons, the obtained unconfined compressive strength values were not believed to be fully representative of column specimens, however, such measurements were still able to qualitatively confirm the presence of gelled colloidal silica solutions within columns.

Figure 6.20 presents both initial and final soil column hydraulic conductivity measurements for all columns. As shown, soil column hydraulic conductivities decreased between 1.5 and 2 orders of magnitude in all biotic columns that experienced colloidal silica gelation. These reductions were similar to hydraulic conductivity reductions observed in other past studies on sands using abiotic colloidal silica grouts (Persoff et al. 1999). While hydraulic conductivity reductions were expected following colloidal silica gelation, the magnitudes of these reductions will likely be controlled by supplied silica concentrations, a variable which was not considered in this study (all experiments contained 6% by mass colloidal silica solutions). When considering hydraulic conductivities measured in abiotic columns, final values exhibited only small reductions when compared to initial values due to incomplete gelation in these columns.

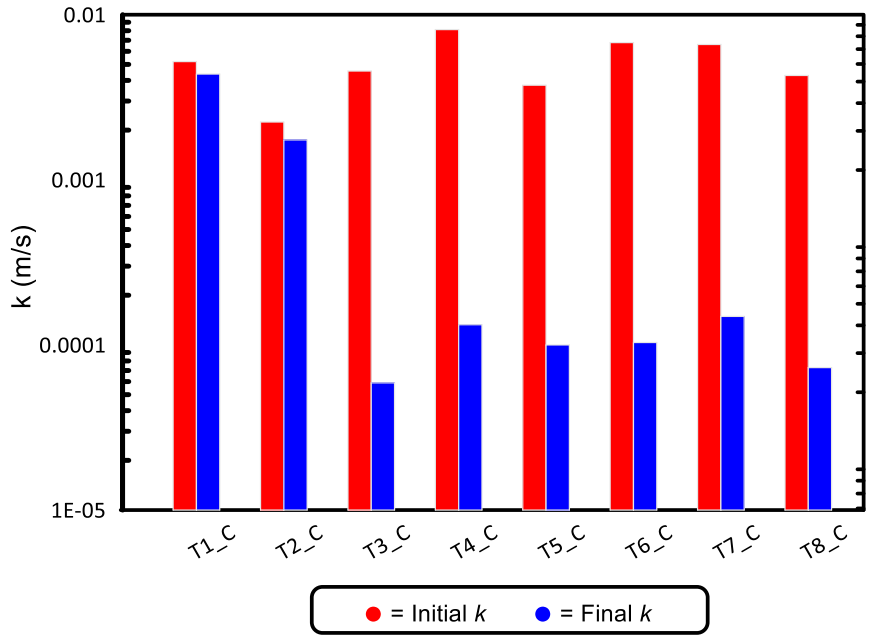

Figure 6.20. Initial and final soil column hydraulic conductivity measurements for all columns.

6.2.10 Batch Experiment Post-Treatment Properties

Table 6.3 presents a summary of post-treatment properties for all batch experiments including initial and final pH values and initial and final viscosities. As shown, all batch experiments receiving nutritive solutions (T3 through T8) achieved large pH reductions to final values near 5.5 along with large viscosity increases. In contrast, abiotic batch experiments achieved minimal pH changes with final viscosities that were similar to initial viscosities. Although soil concentrations were shown previously to impact reaction rates, these differences in supplied soil masses did not appear to affect post-treatment properties.

Table 6.3 Summary of Batch Experiment Post-Treatment Properties

Batch Experiment Treatment Solution	Delta Sand Concentration (g/L)	$pH_{initial}$	pH_{final}	Initial Viscosity (cP)	Final Viscosity (cP)
T1	0.05	9.50	9.58	≈1.6	1.6
T1	0.1	9.50	9.63	≈1.6	1.6
T2	0.05	9.50	9.42	≈1.6	1.8
T2	0.1	9.50	9.44	≈1.6	1.6
T3	0.05	9.50	5.62	≈1.6	4,800
T3	0.1	9.50	5.48	≈1.6	4,300
T4	0.05	9.50	5.54	≈1.6	6,400
T4	0.1	9.50	5.24	≈1.6	>20,000
T5	0.05	9.50	5.54	≈1.6	7,800
T5	0.1	9.50	5.52	≈1.6	>20,000
T6	0.05	9.50	5.34	≈1.6	920
T6	0.1	9.50	5.16	≈1.6	940
T7	0.05	9.50	5.31	≈1.6	>20,000
T7	0.1	9.50	5.51	≈1.6	>20,000
T8	0.05	9.50	5.26	≈1.6	7,900
T8	0.1	9.50	5.38	≈1.6	>20,000

Figure 6.21 presents final solution viscosity measurements for all batch experiments containing 0.1 g/L Delta Sand. As shown, all biotic batch experiments experienced large viscosity increases to values exceeding 900 cP. In contrast, abiotic experiments maintained viscosity values similar to initial conditions (≈1.6 cP) suggesting that detectable gelation did not occur in these solutions over the monitoring period (as expected).

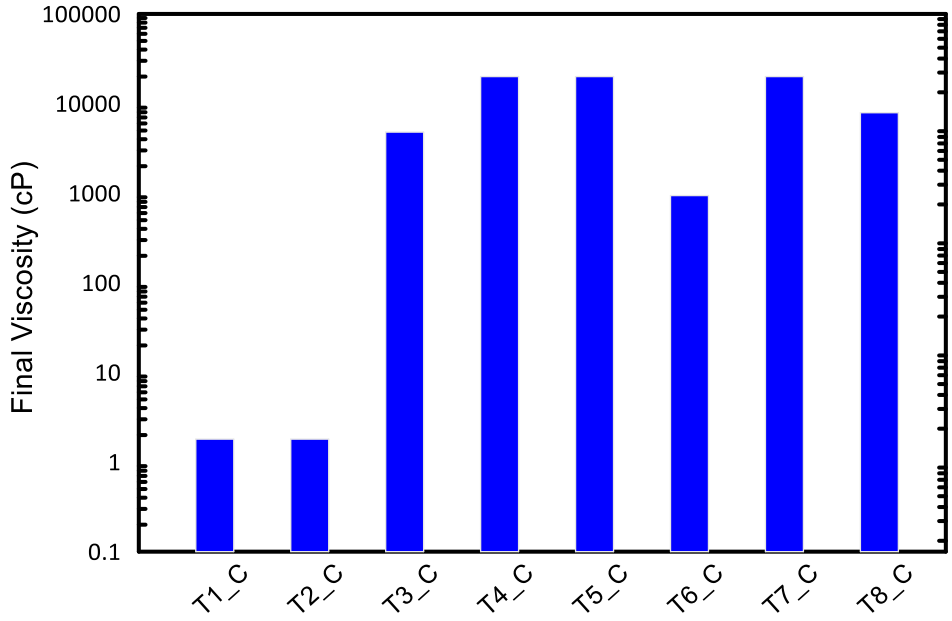

Figure 6.21. Final solution viscosity measurements for all batch experiments containing 0.1 g/L Delta Sand.

6.3 Conclusions

A series of soil column experiments were performed to up-scale treatment strategies developed in batch to more representative soil volumes. Column experiments allowed for verification of trends observed in batch experiments regarding the influence of treatment conditions as well as the investigation of new non-destructive monitoring methods and the examination of post-treatment engineering property changes. In all experiments, solutions were designed to enrich indigenous glucose-fermenting microorganisms following techniques developed in earlier chapters. When comparing soil column experiments to similar batch experiments, the pH reduction behavior of soil columns was found to be much less sensitive to changes in supplied treatment solutions most likely due to the presence of a much larger initial microbial density from added soils. Although variations in yeast extract were shown to influence reaction rates in columns as expected, interestingly, variations in supplied glucose and NaCl appeared to have minimal effects on

observed microbial activities. Consistent relationships between solution glucose concentrations and solution pH values further suggested that pH measurements may indeed be used to effectively and indirectly monitor glucose fermentation activity. At the end of experiments, all biotic columns and batch experiments achieved successful glucose fermentation and colloidal silica gelation with resulting increases in electrical conductivities in time, decreases in soil shear wave velocities in time, small measured tensile strengths, and hydraulic conductivity reductions between 1.5 and 2 orders of magnitude reflective of the presence of colloidal silica gels. Corresponding biotic batch experiments also achieved large increases in solution viscosities after treatment, which were reflective of successful gelation. In similar abiotic experiments, minimal changes were observed suggesting that such solutions can remain relatively unchanged in the absence of microbial fermentation. While more work is needed to further study the up-scaling of the bio-mediated colloidal silica gelation process, these results are encouraging and suggest that such a process can be completed in field-representative soils with the potential for a wide variety of important geotechnical applications.

Chapter 7: CONCLUSIONS

Colloidal silica grouting is an environmentally-friendly ground improvement method that can improve loose problematic granular soils through the formation of stiff colloidal silica gels within soil pore spaces. Most commonly in the conventional approach, various chemical accelerants are first added to alkaline colloidal silica suspensions, solutions are injected into the intended treatment zone, and gelation occurs over time resulting from the neutralization of negative colloidal surface charges and van der Waals attraction between colloids. Although the method holds significant promise for practical ground improvement applications including the mitigation of earthquake-induced soil liquefaction and modification of subsurface groundwater flow, the traditional colloidal silica grouting process has achieved limited adoption in engineering practice due to several key issues. In particular, challenges have been encountered with respect to: (1) effectively controlling grout gelation times and ensuring their stability when subjected to various field-representative subsurface conditions, and (2) successfully monitoring and verifying improvement of soils in-situ.

In order to address these limitations, a research study was completed to investigate the potential of bio-mediated processes to improve the control of colloidal silica gelation and the probability of treatment success for various subsurface conditions, as well as afford the opportunity for new process monitoring and verification methods. In this process, both augmented mixed acid fermenting *E. coli* cells and stimulated indigenous microorganisms were used to ferment supplied glucose to control colloidal silica gelation through both solution pH reductions and ionic strength

increases. Small-scale batch experiments were first performed to assess initial process feasibility and further evaluate the effect of various treatment and environmental factors on the fermentation activity of stimulated and augmented microorganisms. Following batch experiments, soil column experiments were performed to up-scale developed treatment techniques to more representative soil volumes and explore the ability of various non-destructive monitoring methods to track fermentation activity and gelation progression. From the results of this study, the following conclusions can be made:

- Abiotic batch experiments completed in the absence of microbial fomentation suggested that when solutions contain no added sodium chloride, gelation occurred most rapidly when pH values where between ≈5 and 6. As sodium chloride concentrations increased, however, the pH range over which gelation occurred most quickly broadened and included higher pH values. When solutions were adjusted to high pH values near 9.5 and contained no added NaCl, abiotic solutions did not exhibit gelation even after 120 days.
- When colloidal silica solutions included variations in supplied glucose concentrations, glucose appeared to control the magnitude of pH reductions rates in both stimulated and augmented experiments. Concentrations near 5 g/L glucose were shown to achieve final pH reductions to values within the targeted pH range (pH ≈ 5 to 6), however, as supplied glucose concentrations further increased to 10 g/L, final pH values did not exhibit substantial further decreases. This likely resulted from increased buffering of solutions by produced organic acids.
- When colloidal silica solutions included variations in supplied yeast extract concentrations, increases in yeast extract concentrations resulted in increases in pH reduction rates in both stimulated and augmented experiments.

- In stimulated experiments, increases in supplied soil mass to fluid volume concentrations resulted in increases in pH reduction rates likely due to larger initial cell densities. Successful stimulation in sterile experiments containing very low soil concentrations (0.05 g/L), however, suggested that microorganisms facilitating this process may be abundant in natural sands.
- In augmented experiments, unexpectedly high augmented *E. coli* cell densities near 10^8 cells/mL were required to reliably obtain sufficient fermentation activity, with large variations in pH reduction behaviors occurring at lower cell densities near 10^7 cells/mL. Although variations in activity may have resulted from cell preparation techniques, initial investigations suggested that per cell activities were relatively insensitive to changes in pelleting and rinsing techniques.
- When colloidal silica solutions included variations in supplied NaCl concentrations, increases in NaCl concentrations resulted in inhibition of both augmented and stimulated fermentation activity. While inhibition of augmented *E. coli* fermentation activity was detected at NaCl concentrations as low as 1 g/L, inhibition of stimulated fermentation activity was not observed until NaCl concentrations approached 10 g/L suggesting that stimulated microorganisms may be more tolerant of variations in salinity.
- In soil column experiments, successful glucose fermentation and gelation was obtained in all stimulated columns within 6 days, however, abiotic columns which received colloidal silica solutions without added yeast extract or glucose remained relatively unchanged.
- Electrical conductivity was shown to be able to effectively monitor gelation in time using embedded probes in soil column experiments. While electrical conductivities increased dramatically in time in biotic columns, minimal changes were observed in time in abiotic columns. When comparing electrical conductivity changes to corresponding pH changes at

similar times, the obtained relationships suggested that electrical conductivity increases may be primarily reflective of colloidal silica gelation as opposed to fermentation related aqueous chemical changes.

- Although all soil columns exhibited shear wave velocity (V_s) decreases in time due to vertical stress relaxation, biotic columns experienced V_s reductions that were significantly greater than similar abiotic control experiments. This was similar to observations from other past studies and suggested that V_s values in treated soils may decrease following colloidal silica gelation due to colloidal silica swelling and reductions in interparticle contact stresses.
- Although the characterization of post-treatment engineering properties was not extensively investigated in this study, soil columns treated with the biologically-controlled silica gelation process achieved hydraulic conductivity reductions up to 2 orders of magnitude and small unconfined compressive strengths (UCS) up to 34 kPa.

The results of this study confirmed that both augmented and stimulated glucose fermenting microorganisms can be used to successively control colloidal silica gelation. In the performed experiments, the kinetics of glucose fermentation and colloidal silica gelation were controlled by variations in supplied growth factors, fermentative substrates, microbial abundances, and the salinity of colloidal silica treatment solutions, among other factors. Moving forward, non-destructive monitoring methods including electrical conductivity, hydraulic conductivity, glucose concentration, and pH measurements may be used to effectively track both colloidal silica gelation and microbial fermentation activity. While shear wave velocity measurements were also promising, more work is needed to verify that such measurements can effectively track colloidal silica gelation via reductions in interparticle contact stresses. Although this research focused on the development

of a novel bio-mediated process for colloidal silica grouting, many open research questions remain. In particular, future work is needed to (1) further characterize the mechanical behavior of these biologically-improved soils, (2) explore the potential of alternative verification and monitoring approaches, (3) examine the effect of other chemical additives which may be able to control gelation speed and achieve other reaction by-products, (4) evaluate the effect of gas phases produced during fermentation on pore-fluid compressibility and undrained soil behaviors, and (5) quantify process advantages over traditional colloidal silica grouting techniques, including demonstrating improved spatial control in up-scaled experiments.

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APPENDIX 1

Glucose Assay Measurements

The following protocol describes the process used for aqueous glucose concentration measurements. This process was developed specifically for the colloidal silica process wherein continued microbial activity in time after sampling as well as colloidal silica gelation presented unique challenges.

Step 1: Sampling & Sample Stabilization

All samples were obtained using sterile serological pipettes (batch experiments) and/or syringes with sterile needles (soil columns). In order to inhibit further microbial activity after sampling and prevent continued glucose degradation in time, all samples were immediately stabilized in a 2M NaOH base solution and frozen. Depending on the expected maximum glucose concentrations (5 g/L or 10 g/L), 0.125 mL of collected samples were mixed with either 0.583 mL or 1.167 mL of 2 M NaOH solution. These high pH conditions resulted in both inhibition of microbial activity as well as dissolution of all colloidal silica gels present within samples (over time).

Step 2: pH Adjustment

Immediately prior to the glucose assay, additional HCl volumes were added to neutralize the pH of NaOH-stabilized samples. This was performed to further dilute samples and because the glucose assay uses an enzyme that is inhibited at pH values that are not near neutral. **Table A.1** presents a summary of all volumes added to complete the sampling and assay measurement process.

Step 3: Glucose Assay Measurements

An EnzyChromTM Glucose Assay Kit III (EGL3-100) (BioAssay Systems Inc.) was used for all glucose concentration measurements. The assay has a linear detection range of 0.3 mM to 2.0 mM, which was significantly lower than the glucose concentrations used in our experiments. For this reason, samples were diluted substantially during Step 1. The assay process involved the addition of a single colorimetric reagent, incubation for 30 min at room temperature, and measurements using a multiplate spectrophotometer at a wavelength of 565 nm. The full assay protocol can be found on the Bioassay Systems website.

Table A.1. Summary of Glucose Assay Sample Preparation

Glucose Assay Sample Preparation				
Sample Characteristics	**Step 1: Stabilization Process Immediately After Sampling**		**Step 2: Added Immediately Before Glucose Assay**	**Step 3: Sample Processed Using Glucose Assay Kit**
Maximum Glucose Concentration (mM)	*Sample Volume (mL)*	*2M NaOH Volume Added to Sample (mL)*	*1M HCl Volume Added to Sample (mL)*	*Maximum Glucose Concentration after Dilution (mM)*
27.75 (5 g/L)	0.125	0.583	1.167	1.63
55.51 (10 g/L)	0.125	1.167	2.333	1.91