Influence of Algae on Soil Saturated Hydraulic Conductivity: An *in situ* Treatment Option for Reducing Infiltration Beneath Unlined Algae Cultivation Ponds

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Dedication

For my mother – an eternal fount of inspiration and encouragement

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Abstract

Commercial production of algal biofuels is currently limited by high capital costs, including the cost of installation and maintenance of plastic pond liners, which mitigate seepage of cultivation fluids and control the release of salts and nutrients into the subsurface beneath outdoor algae cultivation ponds. However, studies of animal waste settling lagoons show that underlying soils ranging from sands to clay loams can exhibit reduced hydraulic conductivity within days to weeks after construction, reducing the need for plastic liners. The mechanisms of the hydraulic conductivity reductions, or "soil sealing", are physical rearrangement of soil particles, buildup of fines, and the accumulation of microorganisms and their metabolic products within pore spaces.

In this study, laboratory-scale soil column experiments investigated a new application for old technology by using fluids that are low-cost and readily available in algae biofuel production to reduce the saturated hydraulic conductivity (K_S) of soils of varying textures by promoting physical and microbial pore clogging mechanisms. Three fluid treatments were supplied to a fine sand soil 1) a nutrient solution used in commercial algae cultivation (NS), 2) the nutrient solution with glycerol added (NSG) and 3) algae (*Scenedesmus dimorphus*) growing in the nutrient solution (NSA). Relatively small reductions of K_S by NS (44-63%) indicate that the culture broth alone was insufficient to promote soil clogging. Larger reductions (77-94%) were seen by addition of the carbon substrate glycerol to the nutrient solution, indicative of enhanced bacterial growth. However, the K_S values produced by the NSG varied widely over time. The magnitudes of KS reductions by NSA were also large (84-95%) and remained stable.

The large, stable K_S reductions provided by algae, seen in the fine sand soil, were similar to those for a loamy sand (96-99%) and a loam soil (98%). The approximately two-order-of-magnitude K_S reductions imply that, like organic fines in animal waste holding ponds, algae can also significantly contribute to reductions in K_s of soils. This new technology appears to be successful in reducing the K_S of native soils and suggests the technology could be applied as an alternative to plastic liners.

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List of Abbreviations and Symbols

AD	Anaerobic digestion
bbl	Barrels of petroleum liquids
BBL	Billion barrels of petroleum liquids
CSPE	Chlorosulfonated polyethylene
GCL	Geosynthetic clay liner
GHG	Greenhouse gas
GWP	Global warming potential
Δh	Hydraulic head gradient
HDPE	High-density polyethylene
K_S	Saturated hydraulic conductivity
LEA	Lipid-extracted algae
LCA	Life cycle assessment
MBC	Microbial biomass carbon
MMbbl	One million barrels
NER	Net Energy Ratio
NS	Nutrient solution
NS+A	Algal broth (*Scenedesmus dimorphus* algae growing in nutrient solution)
NS+G	Glycerol amended nutrient solution
PBR	Photobioreactor
PVC	Polyvinyl chloride
Q	Flow or discharge rate
RD	Renewable diesel
RFS	Renewable Fuel Standard
RPP	Reinforced polypropylene
SSF	Saccharificattion-fermentation
TOC	Total organic carbon
TIC	Total inorganic carbon

Chapter 1: Introduction and Literature Review

Meeting the World's Energy Needs

Energy security, resource depletion, and climate change are some of the critical challenges facing the modern world. Economic growth in emerging economies such as China, the Middle East, and India are projected to account for 85% of the total increase in global liquid fuel consumption from 87 MMbbl/d in 2010 to 98 MMbbl/d in 2020 and 119 MMbbl/d in 2040 (DOE/EIA, 2014). Production of tight oil and shale gas and persistent high prices have added to liquid fuel reserves in recent years, and large reserves have allowed fossil fuels to surpass other energy sources, providing more than 80% of the world's liquid fuel demands over the last 25 years (IEA, 2013). However, burning fossil fuels releases enormous amounts of greenhouse gases into the atmosphere resulting in climate change concerns as atmospheric CO_2 levels have increased by 40% since the year 1750 (Hartmann et al., 2013).

The growing rate of the world's fuel consumption calls for economically efficient and environmentally sustainable fuel production (DOE/EIA, 2014). Biofuels have the potential to mitigate sustainability issues and have been given considerable attention as alternatives to petroleum in recent years. In fact, the US Energy Independence and Security Act of 2007 instituted the Renewable Fuel Standard (RFS), dictating that at least 36 billion gallons of renewable fuels be added to transportation fuel sold in the U.S. by year 2022 (Energy Independence and Security Act of 2007, 2012). The RFS also establishes greenhouse gas (GHG) emissions reduction thresholds for the biofuels produced.

First- and Second-Generation Biofuels

Thus far, liquid biofuels produced in the US only account for 7% of total transport fuel produced and are primarily corn-derived in the form of bioethanol, representing 94% of the total liquid biofuel produced in 2012; the remaining 6% of biofuels produced are biodiesels (USDA, 2013). While soy and corn feedstocks have provided the US with a measure of domestic energy security and a decrease in CO_2 emissions, drawbacks to using conventional agricultural crops for liquid fuel production include excessive fresh water consumption, extensive fertilizer

and pesticide application, land degradation, biodiversity loss, and potential disruption of global food prices (Kumar & Babu, 2013; Benson et al., 2014; Chisti, 2007). Grain demand is projected to nearly double in the next 10 years and increase by 70% by year 2050 as populations of developing countries grow (International Food Policy Research Institute, 2011). The demand for grain, the land and water required for its growth, and increasing food prices challenge the production of first-generation biofuels derived from conventional agricultural crops, and there is still perceived competition between land use for food and fuel crops (Kumar & Babu, 2013). There have also been concerns that emissions from indirect land use change have not been accounted for in GHG emission analyses of these crops, and that there may be fewer GHG benefits from these fuels over traditional fossil fuel resources than originally thought (Searchinger et al., 2008).

In addition to biomass derived from oil crops, cereals, starch and sugar crops, and cellulose crops (first-generation biofuel feedstocks), solid energy crops can produce methanol and ethanol utilizing the entirety of the plant (Sims et al., 2006). Second-generation biofuels focus on feedstock from nonfood biomass such as the non-edible residuals of food crops, forestry products, and municipal solid wastes (de Boer et al., 2012). They have the advantage of being cheap and abundant as well as significantly reducing net CO_2 production (Naik et al., 2010). Second-generation fuels produce less waste and better utilize abandoned or degraded land compared to first-generation biofuels (Kumar & Babu, 2013). There is, however, a lack of economic and scalable biorefining technology to produce a meaningful amount of these fuels proportional to overall liquid fuel needs (Naik et al., 2010).

Microalgae: The Third Generation Biofuel

A sustainable biomass feedstock must be easy to grow and produce high yields, providing a carbon neutral, environmentally sustainable, economically viable fossil fuel replacement that does not compete with food crops for resources (Biomass, Research & Development Technical Advisory Committee, 2007). Its production should utilize as much of the existing infrastructure for storage and distribution of liquid fuels as possible.

Microalgae has become one of the most promising feedstocks for commercial production of biofuels, containing more accessible forms of stored carbon than the lignocelluloses used for cellulosic biofuels (National Research Council, 2012). Lipid contents greater than 50% have been described in many species and periods of exponential growth rates allow cells of some microalgae strains to double every few hours, resulting in orders of magnitude higher productivity than first- or second-generation vascular-plant derived biofuels (Leite et al., 2013; Sheehan et al., 1998; Chisti, 2007). A review by Ho et al. (2014) compared yields of leading biofuel feedstocks, citing microalgae as capable of yielding 20 tons of oil per hectare, five times that of oil palm, the highest yielding oil crop. The National Renewable Energy Laboratory estimated that 200,000 hectares of microalgae could easily produce one quad of fuel (~1.7 MMbbl crude oil or ~8 billion gallons of gasoline contains the energy equivalent of one quad); 200,000 hectares is less than 0.1% of the climatically viable land area in the United States (Sheehan et al., 2008). In addition to smaller land area requirements, algal biofuels also have the potential to reduce CO_2 emissions from petroleum transport fuels by 30% with a net positive energy recovery and a smaller water footprint (Chisti & Yan, 2011; Chisti, 2008; Xu et al., 2011; Zhu et al., 2015).

Microalgae Production

Microalgae are an attractive feedstock option, but microalgae for biofuels have yet to be produced economically on an industrial scale. The culture methods currently used in algae farming, both for high-value products and in pilot-programs for biofuels, such as open raceway ponds, can be traced back its beginnings (Greenwell et al., 2010). Borowitzka (2013a) attributes the early uses of algae as a fuel to Harder and von Witsch in 1942, but also states that most of the early research in algae cultivation throughout the 1950s and 1960s was for wastewater treatment, nutritional substances, and nutraceuticals. In the 1970s- 1980s, a surge of research into microalgae as a fuel source was sparked by the 1970s oil crisis (Sheehan et al., 1998). However, after oil prices fell, interest dissipated.

Due to government policy mandates to develop renewable fossil fuel alternatives, microalgae is being studied because of its rapid growth rates, high aerial lipid production yields,

and capacity for GHG fixation (Alam et al., 2012; Galvrilescu & Chisti, 2005). However, commercial production of microalgae feedstock for biofuels is still in initial stages and is prohibitively expensive with current technologies. Research by aquaculturists, engineers, biologists, and chemists is being conducted in nearly every step of the production process in order to develop environmentally and economically sustainable commercial practices. Microalgae biofuel production generally follows a process of strain selection, cultivation, harvesting, dewatering, lipid extraction, and, finally, conversion to fuel (Boruff et al., 2015). As this research aims to reduce costs associated with commercial algae cultivation, regardless of intended products, strain selection and downstream processes will be briefly discussed first.

Strain Selection

"Algae" is a generic term used to describe 200,000 to 800,000 diverse species appearing throughout the kingdoms Protista, Chromista, and Plantae (Maity et al., 2014; Leite et al., 2013). Microalgae is a single or associated polyphyletic cells, mostly autotrophs, that convert CO_2, sunlight, nitrogen, and phosphorous into biomass with variable proportions of lipids, carbohydrates, proteins, and micronutrients (Leite et al., 2013; Benson et al., 2014). So far, over 3000 species of microalgae are found to be oil producing (Sheehan et al., 1998). The quantity of lipids and carbohydrates produced, and therefore the amount and types of biofuel that can be produced, varies by species. Differing cell characteristics and fuel-production pathways have different requirements for downstream processing. Species with high lipid contents are targeted for production of biodiesel. The lipids can be extracted from the biomass and used as a "drop-in" fuel after refining. Species abundant in sugars and carbohydrates can be fermented to create bioethanol. Other types of fuels that can be produced from microalgae include biobutanol, biogasoline, methane, hydrogen, and electricity.

Photoautotrophically-cultivated strains are currently favored, relying on sunlight or artificial light to produce lipid contents ranging from 5-68% (Sharma et al., 2011). The genera *Chlorella* sp. and *Chlorococcum* sp. are of particular interest for production of biodiesel, bioethanol, and hydrogen while *Haematococcus pluvialis* (red microalgae) and *Neochloris oleoabundans* (green microalgae), and marine *Nannochloropsis* sp. are also potential options

for the production of biodiesel (Maity et al., 2014; Abu-Ghosh et al., 2015). *Scenedesmus* sp. has also been recognized for its potential for both biodiesel and methanol production as well as a source of valuable carotenoid (Gerardo et al., 2015; Maity et al., 2014). Many different features must be assessed in selecting efficient and tolerant algal strains for commercial production of biofuels such as nutritional requirements (N, P, and K), pH, Eh, temperature, salinity, and irradiance (Maity et al., 2014). Research is still ongoing in microalgae strain selection, biology, ecology as well as genetic and metabolic manipulation to choose high lipid yielding, resilient species that can be cultivated at a large scale at reasonable prices and that will yield themselves to efficient harvesting and processing (Greenwell et al., 2010; Rodolfi et al., 2008; Smith & McBride, 2015).

Downstream Processes

Once a strain is selected and cultivated, the biomass is harvested and microalgal oils are extracted from the biomass and converted to fuel. A variety of methods for harvesting, dewatering, lipid extraction and conversion have been practiced on smaller scales, but commercial scales of many processes have yet to be established due to high energy requirements (de Boer et al., 2012). The first step once the microalgae have been cultivated is to collect the biomass (harvesting) and concentrate the algal cells, separating them from the growth medium (dewatering). This is often accomplished by flocculation, sedimentation, filtration, and/or centrifugation (Grima et al., 2004; Halim et al., 2012; Wijffels & Barbosa, 2010). This step can account for up to 20-30% of the total biomass production cost (Lee et al., 2015). Once the cells have been condensed, they are often dried, a very energy intensive process, and pretreated to disrupt the cells in preparation for efficient lipid extraction (Lee et al., 2015; Lardon et al., 2009). The ease of cellular disruption is also species dependent (species such as *Botryococcus braunii* excrete oils) reiterating the need for research into highly productive microalgae strains requiring the least energy for processes such as flocculation and cell lysing (de Boer et al., 2012; Wijffels & Barbosa, 2010). Chemical extraction uses solvents or supercritical fluids to break down the cell walls; issues with this occur due to the health and environmental issues associated with some solvents and the expenses associated with benign

solvent options (Halim et al., 2012; Wijffels & Barbosa, 2010). Mechanical extraction methods include mechanical press, ultrasound, or microwave-assisted extraction (Mubarak et al., 2015). Once extracted from the cells, the lipids are separated from the cell debris, remaining water, and residual solvents. The isolated lipids are then converted, usually by transesterification for biodiesel production, to fuels and by-products (Halim et al., 2012). Each of these downstream processes must be optimized in commercializing microalgal biofuel production.

Commercial Products

Until recent years, commercial algae cultivation has primarily been limited to high-value algal products, relying on only a few species including *Dunaliella salina*, *Spirulina* spp., and *Chlorella* spp. to produce food products, nutrition supplements, cosmetics, fine organic chemicals, plastic precursors, and biofertilizers (Spolaore et al., 2006; Boruff et al., 2015). Microalgae have been cultivated for the poultry, livestock, and aquaculture industries as feed for fish, mollusks, echinoderms, crustacean larvae, etc. (Del Campo et al., 2007; Muller-Feuga et al., 2000; Sharma et al., 2011; Reitan et al., 1997). *Dunaliella salina* has been mass cultured for β-carotene; other algae species as sources of antioxidants, dietary fiber, minerals, and proteins (Greenwell et al., 2010; Kuda et al., 2005). Rothlisberger-Lewis et al. (2016) also suggest using lipid-extracted algae residue (LEA) for animal feed and/or soil amendment as their study showed that LEAs have potential to sequester organic carbon and lessen agricultural GHG emissions. Microalgae are also some of only a few natural sources of a variety pigments (Del Campo et al., 2007).

Soratana et al. (2014) completed a life-cycle environmental impact study from different scenarios of microalgal biodiesel production and co-product allocation, finding the best environmental impact and highest total income utilized many co-products: bioethanol from LEA, biomethane for electricity, heat from simultaneous saccharification-fermentation (SSF) residues, land-applied material from SSF residue anaerobic digestion (AD) solid digestate, recycling the nutrients from SSF residue AD liquid digestate, and CO_2 recovered from the SSF process. Sharma et al. (2011) also indicates that the coupling of biofuel production with high-

value products would contribute greatly to the economic feasibility of commercial microalgal biofuel production.

Cultivation

Conditions

A flurry of research into selecting highly productive algal strains for biofuel production is currently ongoing. A productive algal strain must exhibit both high cell content of the biofuel feedstock (e.g. triglycerides for of biodiesel) and a high growth rate. These qualities must also be achieved reliably and economically enough to be commercially produced. Borowitzka (2013b) gives an overview of the influence of temperature sensitivity, efficiency of inorganic carbon uptake, pH and O_2 tolerance, salinity requirements, and morphology on algae growth rates and productivity. While these characteristics are largely species-dependent, environmental factors and cultivation techniques play a large role in optimizing production.

Microalgae can be grown on non-arable lands in freshwater, wastewater, saline waters, at high temperatures, and over a broad range of pH conditions (Maity et al., 2014; Benson et al., 2014). The range of optional growth conditions as well as utilization of CO_2 and nutrients makes microalgae an ideal candidate for a sustainable renewable fuel source. Additionally, pairing cultivation of microalgal biomass with wastewater treatment plants and industrial CO_2 sources, such as combustion power plants, has an added benefit of bioremediation, lessening reliance on freshwater resources and sequestering GHG emissions (Bajhaiya et al., 2012; Batan et al., 2010).

Bioreactors

Microalgae are primarily cultivated either in open-culture systems such as lakes, ponds, and lagoons or in highly controlled photobioreactor (PBR) systems. There are several different configurations of open-pond systems for microalgae cultivation. Circular and especially raceway ponds have been the most popular types over the years, as they are relatively inexpensive and easy to construct (Leite et al., 2013). Raceway ponds are shaped like a horse track and can be made from compacted earth, brick, cement, polyvinyl chloride (PVC), glass fiber, or polyurethane. Large paddlewheels generate flow for continuous recirculation of the culture

medium allowing it to interact with the atmosphere in shallow (10- 50 cm deep) ponds for temperature regulation and mixing (Jorquera et al., 2010). Circular ponds are mixed with a rotating arm that becomes ineffective at too large of pond areas, making raceway-style configurations preferable for scaled-up production.

Photobioreactors are closed-culture systems composed of transparent tubes, plates, or bags through which the algal broth is continuously circulated. These systems have large surface area-to-volume ratios maximizing the culture's exposure to light. Carbon dioxide is fed into the PBR, and the broth must be aerated to remove excess oxygen. PBRs may also need to be cooled to keep from overheating. Closed culture allows for optimization of culture conditions to suit a species' specifications. Photobioreactor systems permit photoautotrophic, heterotrophic, or mixotrophic algae growth with protection from contamination, lower evaporation, and greater control of the overall system, permitting culture of nearly any desired algae strain with greater productivity per unit area over open-culture systems. However, disadvantages include oxygen accumulation, bio-fouling, inferior durability and longevity of materials, substantial energy requirements for temperature regulation, and difficulty in scaling up production volumes (Mata et al., 2010).

The debate whether cultivation is superior in open-pond systems or closed PBRs is ongoing. Numerous authors indicate that while PBR cultivation allows higher productivity per unit area and eradicates many engineering and biological control problems encountered in open-pond systems, cultivation in PBRs is prohibitively expensive for the production of low-value products such as biofuel (Benemann, 2013; Carvalho et al., 2006; Tredici, 2004; Leite et al., 2013; Jorquera et al., 2010). This is due to large capital investment and operational costs (Richardson et al., 2012; Richardson et al., 2014; Kiran et al., 2014; Kirrolia et al., 2013; Mata et al., 2010). A study by the National Renewable Energy Laboratory in 2010 estimated costs of $9.84 and $20.53 per gallon of microalgal biodiesel for open-pond and PBR systems, respectively (Davis et al., 2011). Sawaengsak et al. (2014), who also found production costs in PBRs to be more than twice as high as in raceway ponds, echo these results. Additionally, Weschler et al. (2014) found the initial energy demand of PBRs to be fourteen times higher than the energy required for the same volume produced in a raceway pond. Historical

precedence of open-pond microalgae culture is likely to continue through the upscaling required for microalgal biofuel production due to the lower expense and energy requirements in comparison to photobioreactors (Borowitzka & Moheimani, 2013). While pond designs and operation procedures have been widely studied, they have yet to be optimized for the microalgae species being considered for biofuel manufacture.

Location

As research continues to provide insight into realistic production potential, estimations of land requirements for commercial-scale microalgal biofuel production become possible. Borowitzka and Moheimani (2013) use productivity estimates to calculate the land area needed to produce about 0.5% of US requirements at a productivity of 100,000 bbl lipids per year. The plant would require 2.5- 7.0 km^2 of pond space and a total land area of 5-14 km^2. In 2011, Wigmosta et al. estimated that production of 220 x 10^9 L/yr (~1.38 BBL/yr) of oil, 48% of US petroleum imports for the transportation section, would require 5.5% of the coterminous US land area (Wigmosta et al., 2011).

Clearly large areas of land are required if microalgal biofuels are to become a relevant renewable energy resource. Fortunately, this land does not have to be suitable for agriculture. As long as the land is flat, even marginal land or deserts are suitable for cultivation (Borowitzka & Moheimani, 2013). However, siting potential locations for microalgae production facilities does require consideration of climate conditions including irradiance, temperature, precipitation, and evaporation; geography and geology such as land slope, land use, land cover, and soil workability are also important in selecting potential locations (Boruff et al., 2015). Additionally, for cost and environmental benefits, transportation and utility infrastructure as well as nearness to CO_2, nutrient, and water sources should also be considered (Venteris et al., 2014; Borowitzka & Moheimani, 2013; Davis et al., 2014; Slegers et al., 2015).

There have been several studies developing potential location selection priorities. A recent study by the Pacific Northwest National Laboratory applied a spatiotemporal assessment tool to prioritize promising locations for several algae strains using species-specific growth rate estimates, fresh and brackish water availability, soil properties, and proximity to infrastructure

(Venteris et al., 2014). This study found that the strains considered would exhibit maximum productivity along the Gulf Coast region, with highest values on the Florida peninsula. Sites meeting all of their selection criteria were found along southern Texas (although cultivation of freshwater species would be limited due to the saline groundwater), Arkansas, and Louisiana. Their analysis echoed similar conclusions drawn from the US Department of Energy's Biomass Program's national resource assessment which applied the same biomass assessment tool to identify high production potential in portions of California, Arizona, Texas, the Gulf Coast region, and the southeastern seaboard (Davis et al., 2012). A previous assessment by Wigmosta et al. (2011) had also recommended focusing on the Gulf Coast, the eastern seaboard, and the Great Lakes as these areas optimize microalgae production while reducing land use and freshwater consumption. A study by Yang et al. (2011) found the low evaporation of the southeastern US would provide for a lower water footprint with suitably high solar radiation and temperature to foster microalgae growth; their study favored Florida, Hawaii, and Arizona for high productivity with low water footprints. Murphy & Allen (2011) also noted the productivity potential of Texas, Florida, and Arizona based on the photosynthetic efficiency, longer growing season, and available land.

Assessments of Current Production Practices

Numerous life cycle assessments (LCAs) have been conducted to determine the energy efficiency, GHG emission reductions, and costs associated with microalgae biofuels and to compare them to other fuels. Most LCAs have thus far been based on laboratory or theoretical data as the microalgae biomass industry is still in nascent stages relative to the corn and soy bioethanol or petroleum diesel production industries that have matured with technology and infrastructure development (Passell et al., 2013). LCA studies in the literature have shown mixed results as to the energy efficiency and emission reduction capacity of microalgae biofuels in comparison to these better-established industries. A pond-to-pump life cycle assessment by Batan et al. (2010) yielded a net energy ratio (NER) (energy in/energy out) below 0.93 for microalgae biodiesel. Values less than 1 are considered favorable. The study also found 5% lower net GHG emissions of microalgae biodiesel compared to soy biodiesel and much more favorable than conventional diesel, avoiding 75 g CO_2-equivalent emissions per MJ of energy

produced, and production also resulted in net avoidance of N_2O. The notional production system described in a comparative LCA study by Campbell et al. (2011) resulted in GHG emission reductions compared to ultra-low sulfur fossil diesel and canola diesel with reductions of 63.1 to 108.8 g/t/km but with the possibility of higher prices depending on production system. Passell et al. (2013) published an LCA on cultivation and harvesting data from a low yield (3 g/m^2/day) commercial algae producer using two hypothetical scenarios: a base case of a 1000 m^2 production area and a scaled-up case with a 101,000-m^2 production area. The base case resulted in an unfavorable NER of 33.4, and the scaled-up scenario with 1.37 (values below 1 are favorable). The base and scaled-up cases showed global warming potentials (GWP) of 2.9 and 0.18 kg CO_2-equivalent, respectively. The authors attribute the unfavorable NER and GWP of algal biofuel, when compared to petroleum and soy diesels that have NERs of 0.18 and 0.8 and GWP of 0.12 and 0.025 respectively, is the low productivity (other sources report order of magnitude higher cultivation productivies) and the small cultivation area. However, in a future case scenario with productivity of 50 g/m^2/day with yields of 0.75 kg oil/kg dry mass, NER becomes 0.64, much more favorable and comparable to soy diesel.

In Kadam's (2001) study, flue gases from coal-firing electricity production were used as a CO_2 source for algae production, and algal biomass was co-fired with coal for electricity. The study found the combination beneficial, reducing greenhouse gas potential, fossil energy consumption, particulates, as well as SO_X and NO_X reductions. In 2009, Lardon et al. found higher energy costs of cultivation, harvesting, and oil extraction for algae biomass than traditional energy crops. Sander and Murthy (2010) conducted pond-to-pump analyses and reported NERs >1 and GHG emissions both greater and lesser than those for conventional gas across a range of analyses depending on the steps used in processing the algal biomass. Clarens et al. (2010) published a study stating that energy, GHG, and water use are all higher for microalgae cultivation than for canola, corn, and switch grass but that coupling production with waste CO_2 and wastewater utilization reduces those problems. Clarens et al.'s follow-up study (2011) showed net positive energy balances for combinations of production processes joined with waste CO_2 and wastewater nutrients with lower land use impacts. The consensus appears to be that high-efficiency production technology (high growth and yield strain selection) and

utilizing natural and waste resources result in the lowest environmental and economic impacts relative to more traditional fuels (Soratana et al., 2012).

A theme that becomes apparent in the literature is that environmental and economic costs of biofuel production tend to be more favorable when microalgae is produced using renewable energy such as solar or wind and when utilizing nutrients from wastewater and CO_2 from industrial sources. Furthermore, once the hydrocarbons for biodiesel manufacture have been extracted, the remaining biomass can be processed for methane production using an anaerobic digester. The effluent generated after anaerobic digestion can then be fed back into the algae cultivation system as a source of nutrients (Leite et al., 2013). The literature also affirms the energy used to manufacture and transport materials necessary to build cultivation facilities as an economic and environmental obstacle to commercial production.

Burdens Associated with Cultivation Pond Liners

The algal biofuel industry is still in a nascent stage, developing technologies from laboratory-scale and pilot projects into cost-effective commercial-scale production. However, growth is currently limited by high capital investment and operational costs (Han et al., 2015). Similar to the design of sanitary landfills, many of the current commercial algae plant designs propose installation of plastic or clay liners beneath cultivation ponds to mitigate water loss due to seepage, and to control the release of salts and nutrients into the subsurface or groundwater. Geomembranes are flexible synthetic liners made from plastics such as high-density polyethylene (HDPE), chlorosulfonated polyethylene (CSPE or Hypalon), plasticized PVC, and flexible polypropylene. These materials create impermeable barriers with very low effective permeability ($<10^{-12}$ cm/s), high longevity, and resistance to chemical and ultraviolet light degradation (Ng, 2008).

The cost of installation and maintenance of plastic pond liners is a significant obstacle to achieving commercial algae production cost targets, and the emissions associated with their manufacture contradict the aforementioned goal for third-generation biofuels of economic practicability and environmental sustainability of industrial-scale production. Pond liners represent the “single largest cost impact” to a commercial microalgae production facility, more

than doubling the pond cost (Davis et al., 2012). Huntley et al. (2015) also argues that cost is one of the greatest impediments to commercial-scale production, a sentiment echoed by Bosma et al. (2014) who claim a tenfold reduction in production costs is necessary for commercial production of algae for biofuels.

Numerous cost estimates for lining commercial production ponds can be found in the literature. Techno-economic analysis by Rogers et al. (2014) and Coleman et al. (2014) found synthetic lining of cultivation ponds to contribute 24% of capital costs (for a notional array of 6,000 0.8-hectare raceway ponds [i.e., total pond space of 4800 ha] and production of 1000 bbl/day) and 75% of capital costs (405 hectare of pond space), respectively. Another comprehensive techno-economic analysis by Beal et al. (2015) on the actual production of 100-ha production facilities concluded the largest contributions to capital costs were pond construction, namely the Hypalon liner used in one case and reinforced polypropylene (RPP) liners used in others. Two of the ponds were lined with an unnamed material equivalent to clay, and costs were determined to be ~\$3/m^2. The Hypalon liner cost \$30/m^2, and the RPP liners were \$13/m^2. The study further estimates a \$12.8 million cost for thirty years of operation (Beal et al., 2015). Lundquist et al. (2010) discusses pros and cons of lining materials, citing capital costs of \$136,000/ha for a clay lined pond and \$277,000/ha for a plastic lined pond. The authors propose that the cheapest option is simply to forgo pond liners all together, depending on the clay content of the onsite soil (Lundquist et al., 2010). In order for microalgae biofuels to realize their full potential as a commercial fossil fuel alternative, cost barriers must be minimized.

In a LCA by Stephenson et al. (2010), a raceway pond built from concrete blocks, which they considered "the most effective material in terms of both cost of construction and operation if freshwater algae are to be cultivated", was analyzed in respect to the fossil-energy requirements as well as global warming potential. These authors found the PVC lining of the raceway pond accounted for 13% of the fossil energy required and 10% of the GWP. This represents the third largest contributor in the cultivation process after the electricity required to operate the paddlewheel and application of fertilizer (contributions of these two processes could potentially be reduced dramatically using renewable energy and waste resources). Canter

et al. (2014) completed a LCA describing the energy use and GHG emissions per MJ of renewable diesel (RD) associated with infrastructure used to construct a microalgae growth and processing plant. In the baseline plant, based on the design on Lundquist et al. (2010) in which the ponds were lined with HDPE liners, the highest GHG emissions per MJ RD were attributed to the plastics used for the HDPE pond liners, the polypropylene geotextile cloth that goes under the liners, and the plastics used to make the inoculation pond cover and water pipes. In the case of unlined ponds, a 39% decrease in GHG emissions was found relative to that of the baseline plant infrastructure.

Alternatives to Synthetic Pond Liners

Hydraulic conductivity is a characteristic of a porous medium that describes fluid movement through the porous medium in response to a potential, i.e., a pressure or head gradient. Hydraulic conductivity can generally be thought of as the resistance of a porous medium to fluid flow; low hydraulic conductivity means a greater resistance to fluid flow. While many of the current commercial algae production plants install plastic pond liners as a low hydraulic conductivity barrier to diminish leakage potential, plastic liners are not a regulatory requirement for microalgae cultivation in many states (Davis et al., 2012). The high costs associated with installation and maintenance of lining open-pond microalgae cultivation facilities calls for exploration of alternatives. Substitutes for plastic-lined ponds include geosynthetic clay liners (GCLs), compacted clay liners, and unlined ponds.

Pond seepage can be prevented using geomembranes or GCLs. GCLs are manufactured by bonding a layer of bentonite-clay between two sheets of geomembrane. While the easy installation of GCLs achieves adequately low hydraulic conductivity to water of 1×10^{-10} cm/s, the liner can be vulnerable to puncture or tearing, may experience reduced efficiency due to swelling, and has economic and environmental issues similar to geomembranes related to manufacture of the materials (Bouazza, 2002).

Compacted clay has historically been used as a hydraulic barrier in a variety of containment systems such as those for industrial or municipal wastes, hazardous wastes, animal waste lagoons, landfills, solar ponds, aquaculture ponds, evaporation ponds, etc. Clay

soil liners can be constructed to varying thickness and permeability using heavy machinery and equipment. Drawbacks to the installation of a clay liner include the cost of material and transport, difficulty and time of installation, inability to clean if the system becomes contaminated, risk of desiccation or cracking due to freeze-thaw and wet-dry cycles, and the difficulty of making repairs. Costs of compacted clay liners typically range from $5 to $22 per square meter depending on desired thickness, size, and location of facility (Daniel, 1993).

Forgoing a liner altogether is ultimately most cost effective (Lundquist et al., 2010). An unlined pond constructed by Sapphire Energy demonstrated the feasibility of algae cultivation without a liner (Rogers et al., 2014). Prior to that, similar productivities were found in both lined and unlined ponds as part of the Aquatic Species Project, which tested unlined ponds for two years finding no observable leakage (Weissman et al., 1989; Weissman & Tillett, 1992). Some authors have proposed limiting construction of unlined ponds to locations with naturally low hydraulic conductivity due to high clay content in the soils, or soils that could achieve sufficiently low hydraulic conductivity values by reworking or simple, low-cost engineering to create a barrier against infiltration (Venteris et al., 2014; Lundquist et al., 2010). While forgoing a traditional liner would reduce costs, determining an acceptable site for a cultivation facility would become more dependent on soil and environmental conditions (Mata et al., 2010). Considering the soil characteristics of favorable potential production areas, namely the Gulf Coast region and southwestern US, it may be impractical to constrain potential facility locations only to sites with soils containing significant clay content, as sandy soils are common in much of these regions (Davis et al., 2012; Venteris et al., 2014).

In-Situ Solutions

In lieu of only targeting soils with inherently low saturated hydraulic conductivities, the development of low conductivity soil conditions by engineering in situ could provide an alternative for plastic or clay liners that would improve profitability and maintain water quality and quantity. Venteris et al. (2014) investigated application of a plugging model by Cihan et al. (2006) that estimates saturated hydraulic conductivity reductions from a soil's original value. The model was based on data from studies of soil sealing beneath animal waste lagoons.

Venteris et al. (2014) wanted to apply the model to prioritize potential facility locations based on soils that the plugging model showed could exhibit reduced hydraulic conductivity. However, the model appeared insensitive to the original soil characteristics, so the authors believed it was not useful for their assessment of potential facility locations. Nonetheless, the authors did note that the plugging model was also insensitive to the type of organic material and called for further investigation into soil plugging, particularly by organic waste and algae, as a simple in-situ engineering option for reducing hydraulic conductivity of soils in locations of potential algae cultivation facilities.

Examples from Animal Waste Holding Ponds

Regulations governing animal waste ponds and lagoons vary by state, with some state agencies regulating seepage rates or maximum saturated hydraulic conductivity (K_S) of the pond liner material (Parker et al., 1999). For soil liners containing most types of wastes, saturated hydraulic conductivities around 1×10^{-7} cm/s are desired in order to prevent seeping of nutrients and contaminants from the pond into the subsurface (Daniel and Benson, 1990). Numerous laboratory-scale and field-scale studies have demonstrated rapid development of low-conductivity "seals" within the soils beneath animal waste settling ponds regardless of soil texture or type of animal waste (Davis et al., 1973; Chang et al., 1974; Hills, 1976; DeTar, 1979; Culley & Phillips, 1982; Miller et al., 1985; Rowsell et al., 1985; Barrington et al., 1987a,b; Maulé et al., 2000; Cihan et al., 2006).

As family farms expanded into large-scale factory farms, manure management became a significant dilemma for farmers. The industry adapted by turning to manure lagoons to store and stabilize biological wastes. Concerns over infiltration of wastewater into the subsurface and subsequent groundwater contamination prompted a flurry of research since the mid-1960s into mitigating flux from the ponds. Thanks to these investigations, it is now well established that soils effectively create a sealing layer by infiltration of animal manures and other organic liquids, wherein the soil acts as a screen, accumulating an impermeable mat of fines and organic material contained in the animal waste at the soil surface. The low hydraulic conductivity of this sealing layer reduces infiltration by multiple orders of magnitude, regardless

of soil texture or manure type, to levels much lower than even that of compacted clay liners (Tyner & Lee, 2004).

Some investigators were able to conduct their examinations of seepage rates and soil sealing in actual lagoons, both newly built and reclaimed. Others constructed pilot-scale model lagoons for monitoring. Some authors used soil columns to conduct their experiments on a laboratory-scale. Only a few of the important works relating to animal waste pond sealing and the mechanisms that govern it will be summarized here.

Davis et al. (1973) observed a decrease in the hydraulic conductivity of a newly constructed dairy manure pond from an initial value 1.0×10^{-3} cm/s as water infiltrated the soil to 6.0×10^{-6} cm/s as liquid dairy manure was added. Robinson (1973) reported seepage rates from an unlined cattle manure digestion pond filled in alluvial soil containing layers of clay loam, silty clay loam, and clay. An initial seepage rate of 1.3×10^{-4} cm/s was recorded after the pond had been filled with water for one week. This rate decreased to 6.5×10^{-6} cm/s within three months (likely within three weeks based on dilution of surrounding groundwater), further reducing to 3.5×10^{-6} cm/s within six months. Hills (1976) constructed pilot model lagoons in loam, silt loam, sandy loam, and clay loam soils with variable depths, compacted soil thickness, and additives to investigate the effects of these parameters on the infiltration rate of dairy manure. Within three months, all lagoons had reached infiltration rates of approximately 8.8×10^{-7} cm/s. Sealing was hypothesized to occur due to physical filtration of solids at the soil-manure interface and reinforced by the excretions of microorganisms. The depth of sealing by biological processes was a function of the hydraulic gradient. The role of additives in this clogging study was deemed insignificant. Miller et al. (1985) monitored infiltration rates from a manure storage pond built in coarse textured sand with gravel layers. Within 90 days, the pond had effectively self-sealed, reaching an infiltration rate of less than 10^{-6} cm/s.

Investigations were also performed to understand the mechanisms driving the observed changes in hydraulic conductivities. Travis et al. (1971) measured the infiltration rates of cattle feedlot lagoon water into undisturbed soil columns of four soils: two alluvial loam soils, a silty clay loam soil, and a clay loam soil. They first established steady-state infiltration by application of 0.01 N $CaSO_4$. Upon introduction of lagoon water, the infiltration rate first decreased

abruptly in all soils, and then gradually decreased to zero. The authors also measured the amount and type of salt accumulation finding an abundance of Na, NH_4, and K ions, which they credit for being at least partially responsible for the infiltration reductions by dispersion of soil particles. Chang et al. (1974) packed soil columns with loam soil, sandy soil, silty clay soil, or play sand and installed them along the bottom of a dairy manure-holding pond. Columns were recovered after an initial two-day application of well water and then 3, 7, 17, 29, and 64 days after wastewater was added to the pond. The quick initial reduction in hydraulic conductivity in all soils was attributed to the entrapment of particulates in the soil pore space. The hydraulic conductivity further decreased over time. Water movement was no longer detectable under laboratory conditions in all but the sand column within 30 days. The authors also investigated the mechanism of sealing which they attributed primarily to entrapment of particulates in the pore space. However, they also determined from measurement of polysaccharide content that the soils were further sealed by the growth of slime-producing microorganisms and their products. The authors also noted that the extent of soil sealing was independent of soil texture; although texture did influence the time required to become effectively sealed. Culley and Phillips (1982) conducted laboratory-scale percolation tests on soil cores of sand, loam, and clay soils infiltrated with liquid cattle manure. They found that the hydraulic conductivity of all the soils had decreased to 3.5×10^{-6} cm/s within 5-10 days, suggesting that the seal itself controls the infiltration rate and not soil texture. Rowsell et al. (1985) conducted a laboratory study on soil cores composed of a sandy loam, a loam, and a clay soil infiltrated with screened liquid cattle manure, sterilized screened cattle manure, and salt solutions. The infiltration rates decreased rapidly, converging to 1×10^{-6} cm/s in all soils within 30 days. These authors credit physical blocking of pores at the soil surfaces by particulates within the manure. They propose that further sealing may also occur with time as a result of biological activity, but that neither biological activity nor soil dispersion had a substantial role in soil sealing. Barrington et al. (1987a,b) conducted laboratory column experiments on sand, loam, and clay soils infiltrated with liquid manure. The authors ascribed the approximately two orders-of-magnitude reductions found in all soil textures, regardless of the manure type, primarily to the seal created by the mechanical screening of solids at the soil-manure interface. They also mention that

biological activity in the soil may strengthen the seal by binding the solids to the soil particles. Maulé et al. (2000) investigated how deep within the soil the reduction of hydraulic conductivity occurs due to ponded swine manure by measuring the hydraulic conductivities at various depths within soil columns containing 9-33% clay. Before applying manure to the soil columns, the hydraulic conductivity values ranged from 1.3×10^{-4} cm/s to 3.0×10^{-6} cm/s. After applying hog manure, the hydraulic conductivity decreased rapidly to maintain values around 1.0×10^{-7} cm/s in all soils. The authors observed that most of the hydraulic conductivity reduction occurred near the soil-manure interface. A black layer had formed within two days of manure addition, and that the layer extended deeper into the column as time progressed at a rate of 0.3 mm/mo. Cihan et al. (2006) measured the sealing effectiveness in a sand, silt loam, and clay soil columns subjected to swine and dairy waste. In their experiments, a seal formed at the surface of the soil that reduced infiltration to rates of less than 10^{-6} cm/s within 60 days.

In summary, seal development is due to mechanical filtering and build-up of fines and organic material in the pore spaces between soil particles, like a coating forming on a screen, significantly reducing fluid flux, and biological sealing mechanisms serve to bind the trapped particles within the soils and strengthen the seal (Barrington & Broughton, 1988). The aforementioned studies used various types of animal wastes. However, the contents of the waste do not appear to control the clogging mechanisms since seal development is primarily attributed to pore clogging, and principal control over the efficacy is pore size distribution of the soil, where both physical re-arrangement of soil particles and microbial growth can provide additional pore clogging materials (Barrington et al., 1987a,b). Miller et al. (1985) and Rowsell et al. (1985) both agree that sealing of the porous media is insensitive to soil texture, because the sealing is the result of the physical clogging process, which, as shown by Culley and Phillips (1982), can occur in clay, loam, or even sandy soils. Maulé et al. (2000) demonstrate that sand percentages ranging from 20% to 70% can develop a seal with hydraulic conductivity of 1×10^{-7} cm/s. Tyner and Lee (2004) used a soil liner and seal model to show that flow restriction is not due primarily to the thickness of the liner or the soil hydraulic conductivity, but is dominated by the very low hydraulic conductivity of the sealing layer, claiming that the seal can have much

lower hydraulic conductivities than even compacted clay liners. A subsequent paper by Tyner et al. (2006) supported the predictions made from their model.

Column Experiments on Saturated Hydraulic Conductivity Reductions

Baveye et al. (1998) cites one of the earliest reports on the variability of the transmittance of water through porous media was given by Slichter in 1905, wherein the author noticed that flow through sands and gravels for extended experiment duration saw reductions in flow rate. An experiment by Green and Ampt was conducted in 1911 in an effort to understand time effects on flow through soil columns. The authors encountered an 80-fold reduction in flow rate within two weeks (Green & Ampt, 1911).

Since then, many laboratory and field studies have been conducted to investigate the mechanisms that produce porous media clogging, as the resulting reduction in hydraulic conductivity can significantly disrupt the efficiency of irrigation systems, biofilters, sand filters, landfill leachate collection systems, artificial groundwater recharge, wastewater disposal wells, drains, and many other natural and man-made fluid storage or transport systems. The disposal of liquid wastes in porous media is made especially difficult due to clogging (Davis et al., 1973; McIntyre and Riha, 1991; Thomas et al., 1966; Bouma, 1971). However, while clogging in many situations can be deleterious clogging of soils underlying artificial ponds and lakes such as beneath algae cultivation ponds, manure-holding lagoons, or landfills may be beneficial (Tollner et al., 1983). Manipulation of biological clogging in the subsurface has also been studied for applications in bioremediation, as it may be desirable for a substrate to be in contact with subsurface microbial communities for a given duration (Essa et al., 1996; Van Cuyk et al., 2001).

Many investigators choose to observe porous media clogging over time using column experiments, since there are relatively easy methods to determine the hydraulic conductivity by simultaneous measurement of flow rate and hydraulic head gradient using Darcy's law:

$$Q = K_S \cdot A \cdot \frac{\Delta h}{L} \qquad \textbf{(Equation 1)}$$

where Q refers to the rate of discharge, or flow rate ($L^3\ T^{-1}$); K_S denotes the hydraulic conductivity of the saturated material ($L\ T^{-1}$); A is cross-sectional area of the column (L^2); Δh is the hydraulic head gradient (L); and L is the distance between the two points where h is

measured (L) (Baveye et al., 1998). The hydraulic head *h* is the sum of the gravitational potential energy and the hydrostatic pressure. The hydraulic head gradient is a vector gradient between hydraulic head measurements over the distance between points of hydraulic head measurement. Column experiments are usually operated either with a constant hydraulic head gradient or by maintaining a constant flow rate. Although, a falling hydraulic head gradient has occasionally been used where neither Q nor Δh are held constant (Baveye et al., 1998).

A great number of column experiments on hydraulic conductivity reductions have been completed over the last hundred years e.g., Green & Ampt, 1911; Fireman & Magistad, 1945; Christiansen, 1944; Allison, 1947; McCalla, 1950; Gupta & Swartzendruber, 1962; Avnimelech & Nevo, 1964; Jones & Taylor, 1965; Thomas et al., 1966, 1968; Nevo & Mitchell, 1967; Ripley & Saleem, 1973; Rice, 1974; Hills, 1976; Frankenberger et al., 1979; Frankenberger & Troeh, 1982; Tollner et al., 1983; Okubo & Matsumoto, 1979, 1983; Ehlinger et al., 1987; Taylor & Jaffé, 1990a,b; Cunningham et al., 1991; Vandevivere & Baveye 1992a,b,c; Ragusa et al., 1994; Jennings et al., 1995; Seki et al., 1998; Stewart & Fogler, 2001; Bielefeldt et al., 2002; Seifert & Engesgaard, 2007; Francisca & Glatstein, 2010; Pavelic et al., 2011; Rühle et al., 2013; and a great many more. While the sheer number of column experiments having investigated clogging mechanisms and their manipulations in porous media is too vast to review thoroughly here. The long history and success of these studies justify the use of column experiments to investigate the potential clogging effects from the addition of nutrients, microorganisms, and algae to the soils in the study that will be presented here.

The plethora of these types of experiments cited in the literature, also allows for identification of trends that could be expected, as well as providing insight into best column operation practices, and a general understanding of some of the clogging mechanisms that can alter the saturated hydraulic conductivity of a soil.

Winterer (1922, 1923) conducted some of the earliest column experiments. Since these authors found a pattern in the flow rate vs. time curves in an assortment of soils, the temporal reduction patterns found in the literature will be discussed first. Winterer (1922, 1923) determined that it is the nature of the flow rate to decline, increase sharply, and then decrease continuously until the end of the experiment. An important study by Allison (1947) also

determined a pattern to the change in permeability of soils over a long period of submergence in which the soils first exhibit a reduction to a minimum permeability attributed to structural changes from swelling and dispersal of soils upon submergence. Highly permeable soils are noted to encounter a minor decrease, while more impermeable soils can have a significant reduction. This is followed by a sharp increase in permeability, which he attributes to entrapped air due to wetting from above dissolving until a maximum is attained where all the gas bubbles have been removed. The remainder of the experiment shows steady reductions in the permeability. These reductions are ascribed to the dissolving of aggregates, deposition of fines, and biological clogging. The tapering of the tail end of the third phase is attributed to the contributions of different clogging and unclogging mechanisms such as the aggregation of particles and cells by microbial activities as well as reductions due to microorganisms feeding on organic materials. More thorough discussion of the temporal behavior of infiltration into packed soil columns can be found in McGauhey and Krone's (1967) based on early reports by Winterer (1922, 1923), Winterkorn (1942), Allison (1947), Christiansen (1944) and in Baveye et al.'s 1998 literature review of biological clogging.

Mechanisms of Porous Media Clogging

The reduction of saturated hydraulic conductivity is a physical process ensuing from accumulation of materials in voids, reducing the available pore space, or from changes in friction coefficients or fluid viscosity resulting in a reduced ability to transmit fluids (Davis et al., 1973; Baveye et al., 1998). Soil texture, structure, bulk density, aggregation, organic matter content, clay mineralogy, clay dispersion, microorganisms, and the volume of entrained air can all play a role in determining the rate and extent that the hydraulic conductivity of a soil is altered (Christiansen, 1944; Allison, 1947; Poulovassilis, 1972; Frankenberger et al., 1979; Shaw et al., 1985; Taylor & Jaffé, 1990a; Vandevivere & Baveye, 1992a,b,c; Rinck-Pfeiffer et al., 2000; Arnon et al., 2005; Francisca & Glatstein, 2010). The materials accumulated in the voids of porous media may originate from the influent fluid, the medium itself, or from microorganisms occurring in either the fluid or the medium. Just as Chang et al. (1974), Barrington and Broughton (1988), and others observed fluid flux reductions in the sediment beneath animal

waste ponds attributed to physical clogging mechanisms and reinforced by biological ones, the clogging of porous media and subsequent decline in hydraulic conductivity is generally attributed to coinciding effects of physical, chemical, and biological processes (McGauhey & Krone, 1967; Baveye et al., 1998; Rinck-Pfeiffer et al., 2000). Gette-Bouvarot et al. (2014) states, "Chemical processes can contribute to clogging, but the deposition and accumulation of suspended solids have been recognized as the primary cause of clogging in most sedimentary environments." These authors go on to say that the development of biofilms by microbiological processes can ultimately clog the pores remaining after mechanical clogging takes place.

Physical Clogging Mechanisms

Physical clogging in saturated porous media occurs when fine particles and colloids accumulate in or block pore spaces (Baveye et al., 1998). The physical mechanisms that contribute to clogging include the filling of pore spaces by entrapped gas bubbles, mechanical sieving of solid particles suspended in the infiltrating liquid, and degeneration of the soil structure (Vandevivere & Baveye, 1992a). The solid particles that reduce the pore space may be carried into the porous media by the infiltrating water, or be present as fine soil particles, colloids, or organic matter in the soil that may become mobilized during flow (Rebhun & Schwarz, 1968; Rice, 1974; Okubo & Matsumoto, 1983; Oberdorfer & Peterson, 1985; Shaw et al., 1985; Ragusa et al., 1994; Rinck-Pfeiffer et al., 2000; Skolasińska, 2006; Pavelic et al., 2011). These particles may become trapped in pore spaces or coated on grains, reducing effective porosity and permeability, primarily near the interface of the infiltrating fluid and the sediment or soil (McGauhey & Krone, 1967; Rice, 1974; Hills, 1976; Shaw et al., 1985; McDowell-Boyer et al., 1986; Pell & Nyberg, 1989; Schälchli, 1992; Rinck-Pfeiffer et al., 2000; Skolasińska, 2006).

Pavelic et al. (2011) suggest that physical clogging is more significant than other forms of clogging, especially when the source waters have high particulate concentrations. Several authors have attributed clogging of injection and extraction wells primarily to the accumulation of suspended particles (Oberdorfer & Peterson, 1985; Wang & Banks, 2006). Oberdorfer and Peterson (1985), in a study of the literature relating to the injection of sewage effluents, cite the primary cause of clogging is due to suspended solids contained in the injectant. An example

laboratory study by Jones and Taylor (1965) on septic tank effluent percolation through sands indicated an immediate large reduction in infiltration capacity attributed to the mechanical filtration of particulate matter in the infiltrating effluent. The particulate load may correlate with the quality of water infiltrating the medium as seen by Pavelic et al. (2011), who used waters of varying degrees of treatment with solid particulate concentrations ranging from 0.009 mg/L in potable water to 7.2 mg/L in the least remediated wastewater to infiltrate soil columns. The least remediated waters carried larger loads, resulting in greater degrees of initial clogging due to the mechanical filtration of particles in pore spaces of the soil. A column experiment by Rinck-Pfeiffer et al. (2000) also found that suspended particles remaining in the post-secondary treated water influent, despite having lower levels of suspended solids (around 3-4 mg/L), contributed to porosity and hydraulic conductivity reductions by filtration of the suspended particles onto aquifer matrix at the inflow end of the column during early stages of their experiment. Schälchli (1992) also states that concentration of the suspended load even affects the clogging and induced hydraulic gradient of a riverbed.

McDowell-Boyer et al. (1986) and Baveye et al. (1998) give thorough descriptions of the processes of filtration of variable-size suspended particles infiltrating a porous medium. When the suspended particles are either larger or proportionate to the grain size of the porous medium, the particles are unable to penetrate the surface, instead adhering to the grain surfaces at the inlet to form a filter cake. Straining of suspended particles at the infiltrative surface of a porous medium results in an ever-tightening mat developing on a soil surface that increasingly restricts fluid infiltration as the trapped particles themselves become the filter. According to McGauhey and Krone (1967), “This phenomenon will in fact largely be dominant if only a portion of the suspended particles are larger than the pore openings.” This process greatly reduces the overall hydraulic conductivity. Particles smaller than that of the pore openings of the media may be mechanically filtered out within the filter cake or after penetrating the surface of the porous medium. Suspended particles are carried through pore openings until they become lodged in smaller channels or pore throats. Accumulation and orientation of fine materials in pore spaces can reduce permeability by forming meniscus-shaped bridges between grains that block access to larger flow channels (Skolasińska, 2006).

This case can also greatly decrease the hydraulic conductivity of the porous medium, also creating a finer filter as accumulations of particles occurs. Physical clogging by suspended particles in the influent, if the particles are much smaller than the pore spaces of the medium, may not markedly reduce the saturated hydraulic conductivity. However, in addition to becoming physically lodged in the pore spaces, physicochemical interactions between the porous medium and particles suspended in the infiltrating water can bring the two close enough together that the particles can attach to the grain surface by electrostatic and Van der Waals' forces (Fehr et al., 1992; Goldenberg et al., 1993; Skolasińska, 2006). Accumulations of fines on the grain surface can also reduce the hydraulic conductivity of the porous medium, depending on the portion of the pore space removed. These changes affect the hydraulic conductivity and fluid dynamics of the system.

Fine materials in a soil may be mobilized by drag/lift forces, be washed out of one zone, and become lodged in smaller pore spaces down-gradient (Pavelic et al., 2011). The larger particles, when no longer supported collapse. Where the fine particles become lodged, the effective porosity, and therefore the hydraulic conductivity, becomes reduced (West et al., 1992). Pavelic et al. (2011) also concluded from their experiments involving sandy and loamy soils that the abundance of fines and smaller pore sizes in the loamy soil resulted in more effective physical clogging than in the sandy soil. However, the physical clogging attributed to small-scale transport of fines was likely exacerbated by the repacking of the soils into columns, disturbing the soil's natural structure (Pavelic et al., 2011; Lewis & Sjöstrom, 2010). Soil structure can also be disturbed by the breakdown of soil aggregates due to prolonged saturation of a soil, which reduces the cohesion of soil particles, potentially freeing fines to be mechanically redistributed down-gradient (Baveye et al., 1998). Cycles of wetting and drying, or prolonged drought, can similarly break up aggregates and affect soil hydraulic conductivity.

Another physical clogging mechanism relates to air bubbles. Gases can be trapped in pore spaces during the wetting of soil, if the medium is not perfectly saturated, or can be released by the dissolution of gases present in the influent solution (Gupta & Swartzendruber, 1962). Gases entrapped in porous materials have been shown to occupy between 2% and 26% of the pore space (Fayer & Hillel, 1986; Williams & Oostrom, 2000; Heilweil et al., 2004). Perfect

saturation of repacked soils is very difficult to achieve, and is typically accomplished by wetting under a vacuum and/or under an atmosphere of CO_2 (Baveye et al., 1998; Lewis & Sjöstrom, 2010). Wetting under a vacuum achieves most efficient saturation and removal of gas bubbles. However, the process is considered too time consuming and difficult to apply in many cases and may cause a disturbance in soil texture (Christiansen, 1944). Therefore, common convention in laboratory column experiments is to saturate soil gradually from below (Lewis & Sjöstrom, 2010).

Chemical Clogging Mechanisms

Baveye et al. (1998) review how the electrolyte concentration, organic composition of influent, pH, redox potential, and mineralogical composition of the porous media interact to result in changes in soil swelling properties as well as precipitation, dissolution, and dispersion of materials that effect the hydrodynamic characteristics of the soil. However, since primary clogging mechanisms are related more to physical and biological processes, discussion of chemical effects on the hydraulic conductivity of porous media will only be discussed in reference to specific experiments and to the influence of microorganisms on the precipitation/dissolution on the chemistry of clogging products.

Biological Clogging Mechanisms

While biological processes represent the other main clogging mechanism in saturated porous media, these processes can also be very complex. Biological clogging greatly affects and is affected by physical and chemical clogging processes. The term “bioclogging” describes the filling of pore spaces through the accumulation of microorganisms, their metabolic products, and/or gaseous byproducts, resulting in reduction of the porosity and hydraulic conductivity of a porous media (Ivanov & Chu, 2008). Microorganisms in the subsurface or infiltrating waters can utilize nutrient and energy sources to grow, reproduce, and excrete metabolic products, thus altering the pore configuration (Frankenberger et al., 1979).

The types of microorganisms that may be involved in processes of bioclogging depend on the environment. Various types of eukaryotes have been associated with clogged porous media. Okubo and Matsumoto (1983) identified ciliates in the clogged pore space of an

experimental soil column. Ragusa et al. (1994) attribute a marked reduction in hydraulic conductivity (22%) of a soil sample to inoculation by benthic green algae due to development of an algal/bacterial mat. Battin and Sengschmitt (1999), Ibisch et al. (2009), and Gette-Bouvarot et al. (2014) are some of the other authors to report the contributions of algae to clogging in soils. Seki et al. (1998) determined that rapid bacterial growth results in faster reductions of hydraulic conductivity compared to fungal clogging.

Microorganisms, like other particulate matter in soils, can be removed from fluid flow by straining, sedimentation, entrapment, and adsorption to reduce the hydraulic conductivity. However, many studies largely attribute hydraulic conductivity reductions in porous media to the production of biofilms (Baveye et al., 1998). Early microbiologists viewed microorganisms as freely suspended, planktonic cells; however, in natural environments, many microorganisms tend to associate with surfaces and other cells in order to reside in advantageous microenvironments, rather than being swept away into less favorable ones (Donlan, 2002; Watnick & Kolter, 2000). Donlan (2002) describes how Van Leeuwenhoek first discovered biofilms when studying tooth surfaces, noticing that some microorganisms tend to surface-associate and produce extracellular polymeric substances, creating biofilms. Donlan goes on to define a biofilm as "an assemblage of microbial cells that is irreversibly associated (not removed by gentle rinsing) with a surface and enclosed in a matrix of primarily polysaccharide material." In fact, biofilms are primarily composed of extracellular materials, which can account for over 90% of the dry mass, is responsible for adhesion of the biofilm to surfaces, and enfolds the microorganisms (Flemming & Wingender, 2010). Biofilms can form on many surfaces including soil and aquifer materials, living tissues, indwelling medical devices, water system pipes, and natural aquatic systems.

To form a biofilm, bacteria must approach a surface, or other cells residing on a surface, closely enough to attach. Flow hydrodynamics help govern the ease of attachment by formation of a thin boundary layer of low-flow velocity around a surface, outside of which flow is more turbulent. Cells in flowing liquid behave as particles, so formation of a thinner hydrodynamic barrier due to high linear velocity around the surface make it easier for cells to traverse and attach; too great of velocities make attachment difficult due to shear forces

(Rijnaarts et al., 1993; Zheng et al., 1994). In addition to flow dynamics, other properties of the cell and the aqueous and porous media play roles in attachment as reviewed by Donlan (2002). Characklis et al. (2009) found preferential attachment of cells to rougher surfaces due to the lower shear forces and higher surface area. Smaller particle sizes are associated with larger specific surface area available for colonization. However, despite the availability of surface area in their experiments with very fine sand, Vandevivere and Baveye (1992c) did not find higher bacterial counts in sand of a small particle size. Rittmann (1993) also states that grain size is not the governing factor for microbial colonization. Rather, grain size, size distribution, and surface roughness all play a role in the available surface area and pore space for which stable biofilms can form. The nutrient availability of the medium also plays a role in cell attachment, as bacteria will preferentially attach to a nutritive surface in nutrient poor environments, but no such preference is observed in a nutrient rich environment (Watnick & Kolter, 2000).

Once the cells find a suitable surface to attach to, the bacteria use flagella or pili for locomotion about the surface until they find other bacteria to form or enlarge a microcolony of bacterial cells (O'Toole & Kolter, 1998; Pratt & Kolter, 1998; Watnick & Kolter, 1999). Most bacterial communities are stabilized by a matrix of extracellular polymeric substances (EPS) produced by the bacteria. These products tend to be sticky oozes that some bacteria produce more than others. There are different types of EPS that bacteria can secrete and some are more "sticky" and more stable than others are. These substances play an important role in bioclogging as they are what bind cells, and sometimes other incorporated particles, to one another to form a "film". Biofilms are not motionless heaps of bacterial cells, nor are the films homogenous on the surface of solid particles. Instead, biofilms form a complex, heterogeneous network of microcolonies of bacterial cells, encased in a matrix of exopolymeric substances, separated from other colonies by interstitial voids (Lewandowski, 2000). The microcolonies of different species may be separate or intermingle, as cells migrate from one colony to another, changing the structure of the biofilm. Motile cells can also detach from the biofilm, probably influenced by cell-to-cell signaling, as discussed by Xie et al. (2000) and Davies et al. (1998). However, detachment of cells encased by exopolysaccharides is difficult and may require production of an exopolysaccharide lyase, such as that shown for a starved *P. fluorescens* by

Allison et al. (1998), that degrades the exopolysaccharide for consumption and allows motility and detachment of cells from the biofilm.

Numerous types of bacteria have been shown to contribute to clogging in a variety of aqueous settings and to varying degrees. Sometimes clogging occurs by accumulation of cells in discrete colonies (Vandevivere & Baveye, 1992a; Thullner et al., 2002). Other types of bacteria produce large quantities of pore-filling EPS (Ivanov & Chu, 2008). Vandevivere and Baveye (1992b) tested different strains of bacteria, finding that larger impacts on saturated hydraulic conductivity were achieved by slime biofilm producing strains of bacteria. Environments with carbon and nutrient availability, the presence of an electron acceptor, and adequate temperature encourage the growth of microorganisms and production of EPS, and biofilm accumulations are also governed by the rate of cell adsorption, desorption, detachment, and filtration (Thullner, 2010; Kanmani et al., 2014).

Vandevivere and Baveye (1992a) tested the assumption that bacterial reduction of saturated hydraulic conductivity is strictly an anaerobic process. Their experiment on sand columns percolated by a nutrient solution and inoculated by obligately aerobic *Arthrobacter* sp. found hydraulic conductivity reductions up to four orders of magnitude within approximately one week. They determined the clogging was due to the formation of a thick bacterial mat at the inlet end of the column, and when the formation of the mat was prevented, clogging rates were much slower. This study confirmed that indeed aerobic bacteria could also achieve significant bioclogging and that bioclogging is not necessarily dependent on development of biofilms but can also be achieved by accumulation of bacterial cells. Their analysis indicated bioclogging due to *Arthrobacter* sp. cells assembling into three-dimensional aggregates that adhere to pore walls by exopolymeric linkages, adsorption, or due to mechanical filtering of the cell aggregates in pore throats.

In summary, studies of clogging mechanisms demonstrate the potential to manipulate soil clogging in natural soils of varying textures due to physical clogging by the filtering and redistribution of fine sediments or organic materials, the washing of those fine particles and colloids into smaller pore spaces, the development of microbial cell aggregates and biofilms, the incorporation of fine materials into those biofilms, the role of extracellular polymeric

substances, as well as several other potential processes contributing to clogging. Additionally, literature reviews of column experiments on soil clogging show that clogging is primarily a surface phenomenon, occurring within the upper most soil depths or at the soil-water (or other infiltrating fluid) interface (McGauhey & Krone, 1967; McDowell-Boyer et al., 1986; Baveye et al., 1998). This surficial nature is due to mechanical straining of fine particles and colloids, which can create an ever-tightening mat on the infiltration surface as suspended particles, both mineral and organic, are filtered out by the tightening pore spaces of the porous media or accumulating particle filter. Organic mats also tend to accumulate at the inlet end of columns as evidenced by the highly cited works of Gupta and Swartzendruber (1962) and Vandevivere and Baveye (1992a,b). In their column experiments, the zone near the inlet has commonly been observed to dominate the reduction in hydraulic conductivity by buildup of bacterial cells and biofilms due to nutrient availability as soil columns were injected with nutrient. Zhao et al. (2009) demonstrated the clogging of surface sediments by the accumulation of biomass in pores and creation of clogging mats. Zhao et al. found that while biofilm growth contributed to the clogging process, it was organic solids suspended in wastewater that dominated observed clogging, and that the effects were primarily limited to the upper 15 cm of the medium. These two mechanisms have commonly been observed in column studies of hydraulic conductivity reductions to interact with one another, often with the biological clogging processes serving to strengthen and reinforce mechanical clogging (Rebhun & Schwarz, 1968; Okubo & Matsumoto 1982; Shaw et al., 1985; Rinck-Pfeiffer et al., 2000).

Can Algae Cultivation Ponds Be Built Without Engineered Liners?

Rationale

Microalgae's rapid growth rates, high aerial biomass yield, high energy content per unit mass, capacity for GHG fixation, and small land requirements make microalgae a promising feedstock for commercial production of biofuels over other terrestrial bioenergy feedstocks. Algae production has historically been restricted to smaller-scale production for high-value commercial products such as nutritional supplements, fine organic chemicals, and cosmetics. Microalgae for biofuel have yet to be produced economically on an industrial scale due to high capital investment and operating costs. For this reason, research is ongoing in nearly every step

of the production process to develop technologies that maximize production and optimize environmental benefits while minimizing costs.

This research project addresses one of the largest contributors to the high capital cost of commercial microalgal biofuel production – the installation and maintenance of plastic pond liners, which can account for at least 20%-35% of capital costs (Davis et al., 2012; Coleman et al., 2014; Rogers et al., 2014). Similar to the design of sanitary landfills, many commercial algae plant designs propose installation of plastic liners beneath cultivation ponds to mitigate water loss due to seepage and control the release of salts and nutrients into the subsurface or groundwater. The cost of installation and maintenance of plastic pond liners is a significant obstacle to the economic viability of commercial algal biofuel production, and the emissions associated with their manufacture contradict one of the primary purposes of bioenergy production – environmental sustainability. In order for microalgae biofuels to realize their full potential as a commercial fossil fuel alternative, cost barriers must be minimized. Since, as of yet, a pond liner is not a technical regulatory requirement for algae ponds, forgoing a pond liner altogether may be the ideal option for reducing infrastructure-related emissions and capital costs, if permitted by local soil conditions as well as local and state environmental regulations.

While many natural soils have sufficient clay contents to be used as a lining material, targeting only locations with soils with large clay contents or naturally low saturated hydraulic conductivities would severely limit the potential facility locations otherwise suitable for production. The development of infiltration-resistant soil conditions by engineering in situ could provide a satisfactory alternative for plastic liners that would improve profitability and maintain pond water quantity and groundwater quality.

Numerous laboratory-scale and field-scale studies of animal waste settling ponds have demonstrated that, regardless of the type of animal waste held in the ponds, underlying soils ranging from commercial-grade “play sand” to clay loams can exhibit reduced hydraulic conductivity within days to weeks after construction, potentially eliminating the need for plastic liners (Davis et al., 1973; Chang et al., 1974; Hills, 1976; Culley & Phillips, 1982; Miller et al., 1985; Rowsell et al., 1985; Barrington et al., 1987a,b; Maulé et al., 2000; Tyner et al., 2006; Cihan et al., 2006; SNTC, 1993). The mechanisms of the hydraulic conductivity reductions, or

“soil sealing”, are physical rearrangement of soil particles, trapping of fine soil particles and organic material, and accumulation of microorganisms and their metabolic products within soil pore spaces. These processes serve to plug the pore spaces within soils, thus reducing the volume or connectivity of pore spaces available for fluid flow.

As described above, Cihan et al. (2006) measured sealing effectiveness in soils of varying textures under application of swine and dairy wastes. The authors found that over time, the sealing of each soil converged to a similar reduced infiltration rate, regardless of soil texture or contents of the waste. They developed a model to predict sealing rates and applied it to their data and data from previous studies. The model indicated that once a seal was developed in a soil, the original soil hydraulic conductivity had little impact on the reduced infiltration rate. The model was also insensitive to the type of waste.

Venteris et al. (2014) conducted a spatiotemporal assessment for selecting potential locations for future algae cultivation facilities for biofuel production in the US. In their study, the authors “sought to identify sites where natural soil conditions would minimize water losses or were favorable for reduction of conductivity through simple, low-cost engineering approaches.” To do this, they used estimates for saturated hydraulic conductivity under natural and engineered conditions to determine locations in the US with either naturally low saturated hydraulic conductivity soils or soils that could achieve low saturated hydraulic conductivity by either compaction or simple engineering. They also applied Cihan et al.’s (2006) waste sealing model to predict values for hydraulic conductivity reductions by engineering soils in situ by plugging the pore space with organic matter. However, since the model appeared insensitive to the original soil conditions, they decided it provided little value to site discrimination for locating sites for new algae cultivation facilities. Nonetheless, the idea to use engineered soils with low hydraulic conductivity as a basis for algae production site selection proposes a new application for an old technology. Perhaps simple, economical engineering techniques such as the in-situ sealing of soils beneath animal waste holding ponds could be applied to the soils beneath algae production ponds to reduce the saturated hydraulic conductivity and eliminate the need for expensive plastic pond liners.

Goal

The goal of this project is to investigate the potential of low-cost, readily available fluids in algal biofuel production as in-situ soil treatment technologies to stimulate soil pore clogging and reduce the saturated hydraulic conductivities of soils beneath algae production facilities.

Hypothesis

The mechanisms responsible for sealing soils beneath animal waste holding ponds, i.e., redistribution of soil particles, filtration of suspended particles in the infiltrating waters, and/or enhanced bacterial growth, can be stimulated in soils beneath algae production facilities to achieve significant reductions in the soil saturated hydraulic conductivity.

Objective

Determine the magnitude of hydraulic conductivity reductions by application of the nutrient media used as for algae culture, organic carbon substrates, or the algal culture itself to soils of various textures.

Approach

Studies from animal waste holding ponds and from similar industries have shown that soil pores may become clogged by various physical, chemical, or biological mechanisms such as physical rearrangement of soil particles, filtration of suspended solids within the infiltrating wastes, and buildup of microbes and their metabolic products within pore spaces of soils. These mechanisms can result in orders-of-magnitude reductions of saturated hydraulic conductivity of soils underlying animal waste ponds, often achieving suitably low saturated hydraulic conductivity values (~10^{-7} cm/s) to be protective of underlying groundwater and reduce the reliance on pond liners.

This study attempted to stimulate these pore clogging mechanisms in soils that are found in locations selected for commercial algae production. A series of laboratory-scale soil column experiments were conducted to investigate the potential for reducing the hydraulic conductivity of these soils by application of various fluids treatments. Multiple soil textures and origins were investigated. The treatments tested were limited to low-cost, readily available fluids in algal biofuel production.

Phase I of the study tested whether the nutrient solution for algae culture would stimulate microbial growth to promote biological clogging and reduce of the saturated hydraulic conductivities of two local loam soils with their existing microbial communities. The experiment also incorporated varying soil textures by mixing sand additions with the loam soils to evaluate if texture significantly affected the physical rearrangement of soil particles, leading to additional mechanical clogging of soils. Changes to the soils' saturated hydraulic conductivities were monitored over the duration of infiltration. In the experiments of Phase II, glycerol, a by-product of biodiesel production, was added to the nutrient solution and applied to one of the local loam soils to potentially enhance microbial growth and biological clogging. These two treatments, the nutrient solution and the glycerol amended nutrient solution, were also supplied to fine sand soil from the site of an algae testbed facility in Texas, as was a third treatment consisting of an algae broth composed of the green algae *Scenedesmus dimorphus* growing in the nutrient solution, similar to composition of the ponds of a production facility. Phase III of this study further examined the saturated hydraulic conductivity reductions found in the Texas fine sand by the buildup of algae cells suspended in the broth. The algal broth treatment was also tested in a loamy sand from a co-located algae production and wastewater treatment facility in California as well as the local loam soil from Phase I and II.

Chapter 2: Materials and Methods

A series of laboratory-scale soil column experiments were conducted to observe changes in saturated hydraulic conductivity in soils representing a range of soil textures sampled from locations selected for commercial algal biofuel production (Figure 1) in response to application of various solution treatments utilizing fluids that are low-cost and readily available in algal biofuel production (Table 1).

In the preliminary phase of column experiments, two local loam soils were chosen to test sealing capacity by infiltrating with the nutrient solution for algae cultivation. Soil columns were permeated with two liquids in sequential order: (1) a soil water simulant (5 mmol/L $CaCl_2$) and (2) a nutrient solution by Guillard (1987) used in freshwater algal production facilities. The first solution enables quantification of mechanical clogging due to particle rearrangement and microbial growth, and the second solution enables quantification of additional biological clogging resulting from enhanced microbial growth associated with nutrient uptake. In the second phase of the study, glycerol was added to nutrient solution to test the sealing potential of adding of a carbon substrate to the nutrient solution to encourage microbial growth and subsequent biological clogging in one of the local loam soils. The local soil test was followed by a set of experiments on a fine sand soil sampled from the site of an outdoor raceway system test bed in Corpus Christi, Texas. This experiment simultaneously compared the effects of four solution treatments on the saturated hydraulic conductivity of the fine sand soil: the soil water simulant, the nutrient solution, the nutrient solution amended by glycerol addition, and an algal broth composed of the green algae *Scenedesmus dimorphus* growing in the nutrient solution. Phase III investigated the magnitude of hydraulic conductivity reductions by application of the algal broth treatment to a loamy sand soil from the Delhi County Water District's microalgae wastewater treatment plant in Delhi, California where algal biofuel production in conjunction with wastewater treatment is being studied. The algae treatment was also tested on the local loam soil of Phases I and II in this phase of the study.

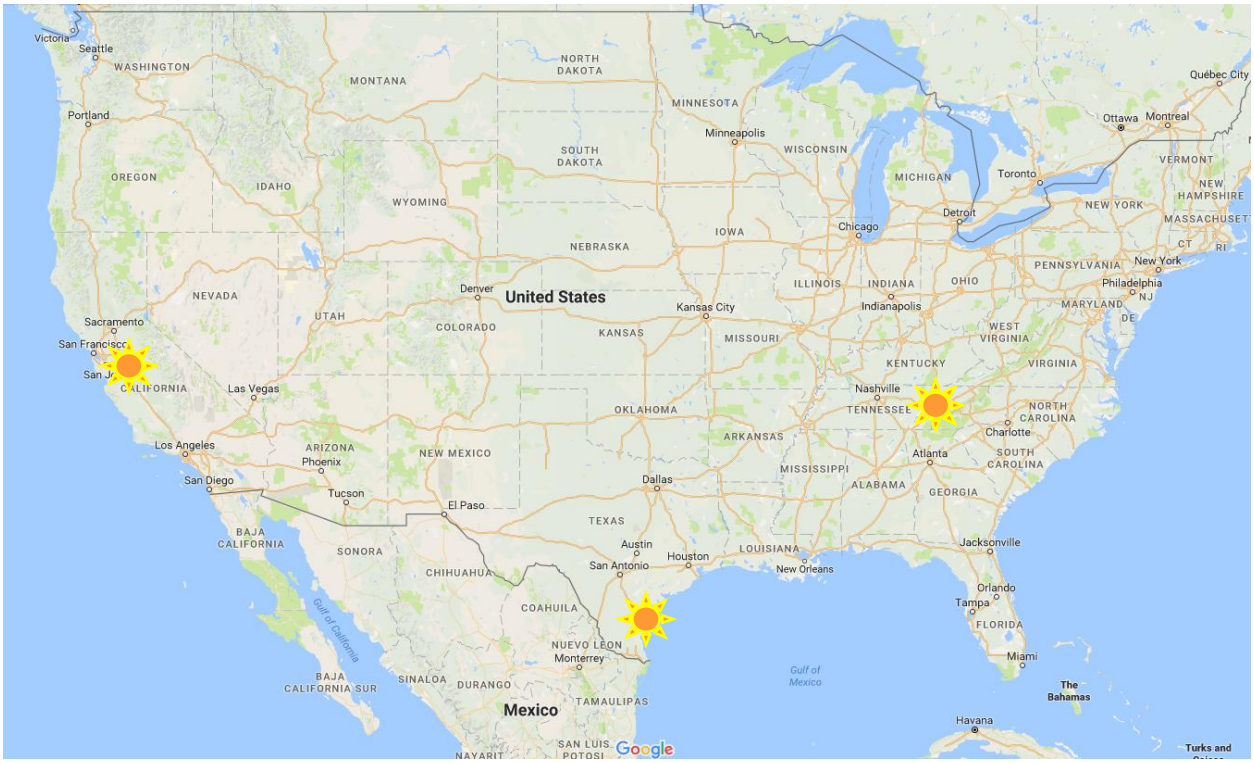

Figure 1: Locations of Soil Samples Tested [indicated by sun symbols] (Google Maps, 2017)

Table 1: Overview of the Different Phases of the Study

Experiment	Column Name	Soil	Soil Origin	Treatment	What was studied?
Phase I: Preliminary Experiments	FB+C	Freel's Bend Loam	Oak Ridge, Tennessee	Control (5 mmol/L $CaCl_2$)	1) Will soil particle rearrangement or natural microbial growth significantly alter K_S? 2) Can the nutrient solution used for algae cultivation enhance microbial pore clogging to reduce K_S? 3) Does soil texture have a significant affect?
	FB+NS			Nutrient Solution	
	FBS+C	Freel's Bend Loam mixed with sand		Control (5 mmol/L $CaCl_2$)	
	FBS+NS			Nutrient Solution	
	WB+C	Walker Branch Loam	Oak Ridge, Tennessee	Control (5 mmol/L $CaCl_2$)	
	WB+NS			Nutrient Solution	
	WBS+C	Walker Branch Loam mixed with sand		Control (5 mmol/L $CaCl_2$)	
	WBS+NS			Nutrient Solution	
Phase II: Scoping Experiments	FB+NSG	Freel's Bend Loam	Oak Ridge, Tennessee	Nutrient Solution + Glycerol	1) Will adding a carbon substrate to the nutrient solution result in greater K_S reductions? 2) Do these treatments have a similar effect on K_S in a fine sand soil? 3) Can algae significantly contribute to soil pore clogging?
	FB+NSG Rep				
	TX+C	Texas Fine Sand	Corpus Christi, Texas	Control (5 mmol/L $CaCl_2$)	
	TX+C Rep				
	TX+NS			Nutrient Solution	
	TX+NS Rep				
	TX+NSG			Nutrient Solution + Glycerol	
	TX+NSG Rep				
	TX+A			Algae in Nutrient Solution	
	TX+A Rep				
Phase III: Hydraulic Conductivity Reductions by Algae	CA+C	California Loamy Sand	Delhi, California	Control (5 mmol/L $CaCl_2$)	1) Does the algae treatment have a similar effect on K_S of other soils?
	CA+C Rep				
	CA+A			Algae in Nutrient Solution	
	CA+A Rep				
	TN+C	Freel's Bend Loam	Oak Ridge, Tennessee	Control (5 mmol/L $CaCl_2$)	
	TN+C Rep				
	TN+A			Algae in Nutrient Solution	
	TN+A Rep				

Soils

The two loam soils were sampled from different locations on the Oak Ridge National Laboratory Reservation in Oak Ridge, TN in order to maximize the likelihood of having a productive bacterial strain for biological clogging. The first soil is from the Etowah series (Soil Survey Staff, 2016) and was sampled from Freel's Bend (FB) on the Clinch River. The second was obtained adjacent to the Clinch River's Walker Branch (WB) and is of the Fullerton soil series (Soil Survey Staff, 2016). To evaluate if soil texture significantly affected the sealing mechanisms stimulated by the treatment solutions, multiple textures were created in the loam soils by additions of either 0% or 40% acid-washed, autoclaved Iota standard quartz sand (d_{50} = 224.64 µm) (Unimin Corp.). The microbial communities of the soils remained intact, but the sand was sterilized by autoclave to prevent microbial contamination. The Texas (TX) soil of Phase II was sampled from the site of an outdoor raceway system test bed in Corpus Christi, Texas. It is classified as the Galveston and Mustang fine sand soil (Soil Survey Staff, 2016). Phase III tested a soil sampled from the Delhi County Water District's microalgae wastewater treatment plant in Delhi, California (CA) and the Tennessee (TN) Freel's Bend loam. The CA soil is classified the Delhi loamy sand (Soil Survey Staff, 2016).

Soil Characterization

Prior to experimentation, soil samples were sieved to <2 mm. Particle size distribution, gravimetric water content, soil pH, carbon, nitrogen, microbial biomass carbon, and total iron oxides were determined in the lab (Table 2). Field bulk density measurements were obtained from National Resources Conservation Services web soil surveys (Table 2) (Soil Survey Staff, 2016). The Buoycous hydrometer method was used for particle size analysis (Gee and Or, 2004). Soil pH was determined by shaking a 2:1 ratio of 5 mmol/L $CaCl_2$ solution to soil solids then measuring the pH of the supernatant after centrifugation. Total C and N were measured by combustion on a LECO TruSpec CN analyzer (LECO Corp.). Total organic C (TOC) was determined by the standard method of removing total inorganic C (TIC) by treating the soil with 3 mol/L HCl for 1 hour and rinsing three times before repeating analyses on LECO to quantify TOC. Soil TIC was computed from the difference between total soil C (untreated soil) and TOC. Microbial biomass carbon (MBC) was quantified using the chloroform fumigation extraction

Table 2: Soil Characterizations

Soil	Particle Size Distribution			Textural Classification	Field Moist Bulk Density (g/cm^3)	Gravimetric Water Content (%)	pH ($CaCl_2$)	Nitrogen (%)	Carbon (%)	MBC (mg/g soil)	Iron Oxides (mg/L)
	Sand (%)	Clay (%)	Silt (%)								
FB	41.20	13.84	44.96	Loam	1.30 – 1.45	21.77, 31.93*	6.04	0.20	1.63	0.18	Not measured
WB	46.84	10.71	42.45	Loam	1.45 – 1.55	22.25	Not measured	0.19	3.10	0.23	Not measured
TX	90.09	6.78	3.11	Fine Sand	1.40 – 1.70	18.97	8.14	Below detection	0.27	0.03	0.86
CA	83.72	10.28	6.00	Loamy Sand	1.55 – 1.70	6.91	4.30	0.01	0.33	0.02	4.15

*Phase III experiment

method where the C concentration is calculated from the difference in C between samples that have been fumigated and the non-fumigated samples, and dividing the C concentration by the extraction efficiency k, estimated as 0.45 (Vance et al., 1987; Beck et al., 1997). Total Fe oxides were obtained by dithionate-citrate-bicarbonate method (Loeppert & Inskeep, 1996) and analyzed using inductively coupled plasma mass spectrometry (ELAN-6100, Perkin Elmer Corp.).

Apparatus

A total of eight soil columns were assembled for the preliminary experiments. Four columns were constructed with each type of soil, of which two replicate columns were filled with 100% soil. The other two contained 60% soil mixed with 40% Iota quartz sand. Transparent schedule 40 PVC pipe, 2.0 cm in diameter and 6.7 cm in length were packed 6.0 cm deep, leaving a saturated headspace of 0.7 cm above the soil for solution to pond. A 1/16 in inner diameter tube adapter served as the column outlet. A glass wool filter was inserted into the outlet and overlain by a thin layer of sand to prevent the loss of fine soil particles. The columns were packed with damp soils by dividing the required mass of soil to achieve the desired bulk density into three portions and adding each portion to the column in even fragments, gently tamping down each portion using five taps of a plastic rod to achieve uniform packing. The outside of each column was covered with aluminum foil to prevent light entry and remained covered for the duration of the experiment. The columns were then saturated slowly from beneath with soil water simulant (5 mmol/L $CaCl_2$) over a period of five days and a pond of soil water simulant was allowed to develop above the soil surface. The saturated column was then allowed to rest for two days to facilitate dissolution of entrapped air and allow suspended particles to settle. In this and subsequent experiments, all parts interacting with the soil or solution were sterilized by autoclave (STERIS) on the 30-minute cycle at 121˚C before use to prevent microbial contamination.

The permeameters used for the Phase II experiments on TX soils and in the Phase III experiments were constructed from 6 in long by 1 in diameter schedule 40 316L stainless steel pipe fixed at the ends with threaded caps of the same material. Outlets were created by drilling and tapping the cap to fit a 1/8 in inner diameter tube adapter. The soil was supported by a laser-cut flow diffuser overlain by 316 stainless steel wire cloth with 0.0015 in openings and a

0.0257 in opening screen to prevent channelized flow and loss of fines. The columns were packed to a depth of 11 cm with damp soils by dividing the required mass of soil into ten portions and adding each portion to the column in eight even fragments, gently tamping down each portion using five taps of a plastic rod to achieve uniform packing. The mass of soil added was chosen in effort to approximate bulk density values reported in the soil survey (Table 2) (Soil Survey Staff, 2016), but adjusted to permit gravity flow and achieve initial flow rates measurable within fraction collection system. The columns were then saturated slowly from beneath with soil water simulant (5 mmol/L $CaCl_2$) over a period of six days and then allowed to stand for two days to facilitate dissolution of entrapped air and allow suspended particles to settle within the 4 cm ponded head of solution above the soil.

Solutions

The soil water simulant was prepared by dissolving granular calcium chloride in Milli-Q (MQ) water to make a 5 mmol/L $CaCl_2$ solution. The nutrient solution was prepared from stock solutions and stored at 4˚C according to the recipe given in Appendix A, the constituents and concentrations of which are listed in Table 3. To prevent microbial contamination, both solutions were sterilized in an autoclave (STERIS) at 121˚C on the 30-minute cycle. The solutions were allowed to thermally equilibrate to 20˚C before use in the experiments. In Phase II, glycerol was added to the nutrient solution at 11.36 mL glycerol per liter of nutrient solution. This contributed carbon from glycerol to the nutrient solution at a concentration equal to 3% of the soil carbon for the Freel's Bend soil. Since the TX soil had a much lower C content than FB, the glycerol additions would have been minute. Therefore, the same proportion of glycerol to nutrient solution was used in the TX columns for consistency between experiments. The soil water simulant, nutrient solution and the glycerol amended nutrient solution were sterilized by autoclaving and allowed to reach a thermal equilibrium. In the TX experiments, these solutions were buffered using either 5 mol/L KOH or 0.5 mmol/L HCl to the pH of the soil (Table 2). The soil water simulant was also buffered in the CA and TN experiments of Phase III.

For the algal suspension of Phases II and III, the green algae *Scenedesmus dimorphus* was grown in nutrient solution until a dense batch culture was achieved. The nutrient solution for algal culture and to prepare the algal broth was made according to the recipe in Appendix A**,**

Table 3: Nutrient Solution Composition

Component	Concentration (µmol/L)
NH_4Cl	49.5
$Na_2B_4O_7 \cdot 10\ H_2O$	50.0
KCl	99.9
$MgSO_4 \cdot 7\ H_2O$	150.0
$CaCl_2 \cdot 2\ H_2O$	250.0
$NaHCO_3$	150.0
$Na_2SiO_3 \cdot 9\ H_2O$	105.6
$NaH_2PO_4 \cdot H_2O$	36.2
$NaNO_3$	882.4
Na_2EDTA	11.7
$FeCl_3 \cdot 6\ H_2O$	11.7
$CuSO_4 \cdot H_2O$	1.1×10^{-2}
$ZnSO_4 \cdot 7\ H_2O$	1.5×10^{-2}
$CoCl_2 \cdot 6\ H_2O$	8.4×10^{-3}
$MnCl_2 \cdot 2\ H_2O$	1.8×10^{-2}
$NaMoO_4 \cdot 2\ H_20$	5.7×10^{-3}
Thiamine HCl	586.6
Biotin	2.1×10^{-3}
Vitamin B12	4.1×10^{-4}

sterilized in an autoclave, and allowed to cool before use. A fresh algal broth with culture density of 5 x 10^5 cells/mL was made every second day by adding an aliquot of dense batch culture to sterilized nutrient solution. On the days that fresh broth was not made, the previous day's broth was diluted with nutrient solution back to a density of 5 x 10^5 cells/mL. To simplify measuring the cell concentration of the batch culture or broth each day for dilution, the relationship between cell density and optical density was established. Cell densities were measured by Benchtop FlowCAM (Fluid Imaging Technologies). Optical density was measured at 750 nm using a Multiskan FC Microplate Photometer (Thermo Scientific). Cell density (cells/mL) was plotted versus optical density for serial dilutions of the batch culture (Appendix B). The target broth density 5 x 10^5 cells/mL corresponded to a target optical density of 0.0126. The following dilution equation could then be used dilute the batch culture:

$$V_1 \times S_1 = V_2 \times S_2 \qquad \textbf{Equation 2}$$

where V_1 is the desired volume of algal broth, S_1 is the target optical density 0.0126, V_2 is the volume of algae culture required, and S_2 is the optical density of the algae measured in the batch reader. This equation is also used to dilute the previous day's broth. However, S_2 becomes the optical density of the previous day's broth rather than the batch culture. The algae broth, stored in the clear plastic supply bottle as it is delivered by a Mariotte bottle to the columns, was kept in suspension by constant gentle agitation using a stir plate to keep the cells evenly dispersed throughout the solution (Figure 2).

Experimental Procedure

The flow experiments involved application of solutions supplied by a Mariotte device to maintain a constant head within the solution pond above the soil surface. Flow was vertical, from top to bottom. Solution exited the column outlet into a fraction collector connected below (Figure 2). The fraction collector collected effluent for a set period, adjusted for each column depending on how quickly the permeating solution flowed through the column. The measured volume of effluent over the collection time determined the flow rate. The flow rate, head values, and column dimensions were used to calculate the saturated hydraulic conductivity by rearranging Darcy's Law (Equation 1). Charting the resulting saturated hydraulic conductivity

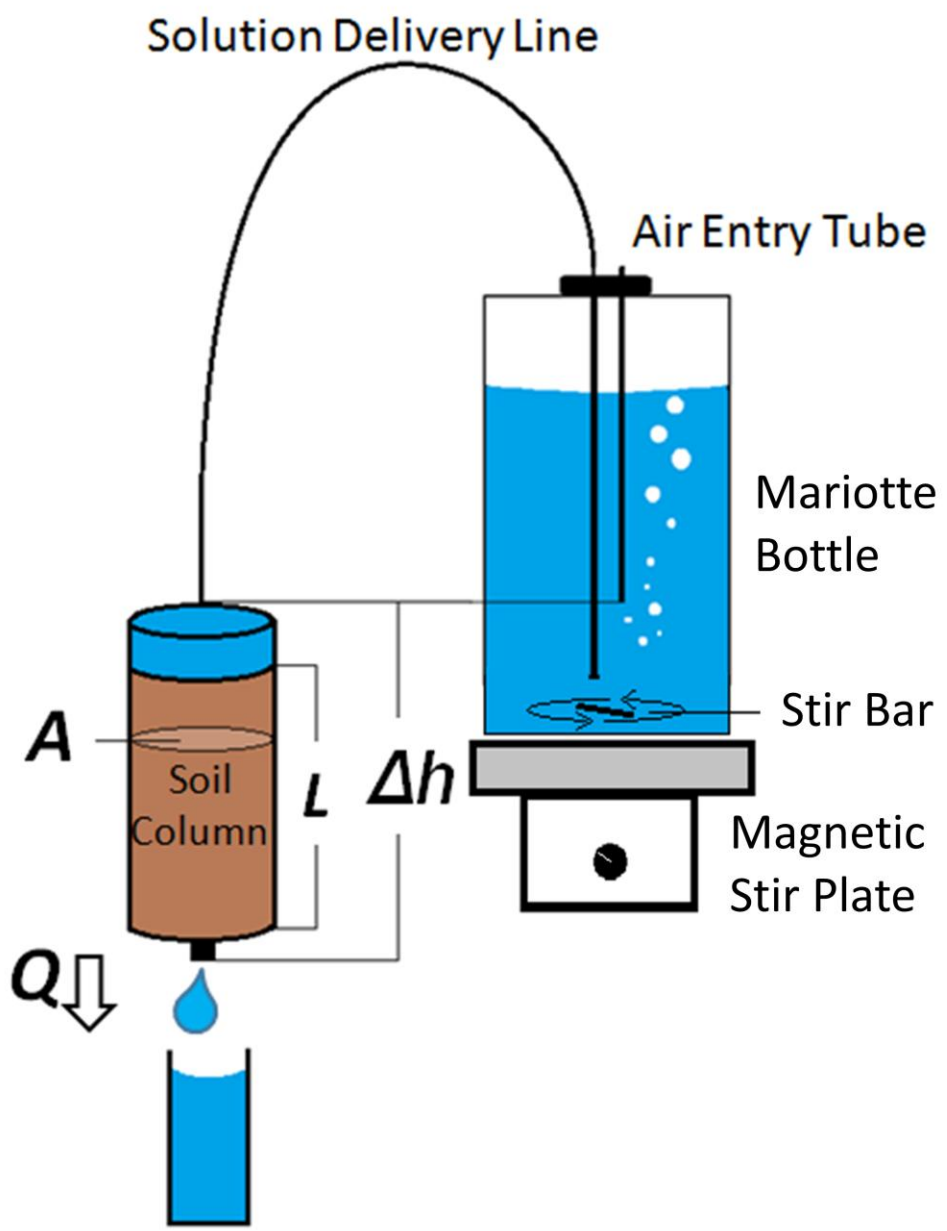

Figure 2: Column Experiment Design

values on a logarithmic scale versus time allows for comparison of the effects that the experiment parameters may produce on the soil saturated hydraulic conductivity.

High flow velocities and the limited number and capacity of collection tubes carried by the fraction collectors made it difficult to measure flow rate and maintain an adequate volume of solution in the supply bottle overnight, particularly during the early stages of the experiment. Therefore, the solution supply line and outlet/fraction collector connections were clamped at the end of each day to stop flow and unclamped to resume flow each morning.

The pH of column effluents was measured twice daily on fresh samples. Following the flow experiments on the TX soil columns of phase II and the Phase III columns were allowed to drain. Grab samples were taken at depths of 1, 3, and 8 cm from the top of the soil column and stored in airtight containers at -80°C to be preserved for focused ion beam scanning electron microscope imaging and other solid-phase analyses of the extent and mechanisms of the saturated hydraulic conductivity reductions.

Chapter 3: Results and Discussion

Phase I: Preliminary Experiment

The initial phase of experiments was designed to answer the following questions: 1) What changes to saturated hydraulic conductivity occur in repacked soil columns due to particle redistribution and natural microbial growth? 2) Will the nutrient solution used for algae culture enhance microbial growth and reduce saturated hydraulic conductivity? 3) Does soil texture significantly affect pore clogging by either of these mechanisms?

To answer these questions, soil columns were constructed from two similar loam soils and were permeated with two liquids in sequential order: 1) a soil water simulant (5 mmol/L $CaCl_2$) and 2) a nutrient solution by Guillard (1987) used in freshwater algal production facilities (Table 3). Infiltration by the first solution enabled quantification of mechanical clogging due to particle rearrangement and microbial growth, and the second solution enabled quantification of additional biological clogging resulting from enhanced microbial growth associated with nutrient uptake. Vandevivere and Baveye (1992a,b) established that diverse bacterial strains, differing in metabolic characteristics and products, can alter the hydraulic conductivity of porous media. Numerous studies since the late 1940s indicate two to three orders-of-magnitude reductions of saturated hydraulic conductivity of soils can be credited to growth of such bacteria (Thullner, 2010). Therefore, the two similar loam soils were sampled from different locations on the Oak Ridge National Lab Reservation in Oak Ridge, TN to maximize the likelihood of having a productive strain for bacterial clogging.

Pavelic et al. (2011) ascertain that physical clogging is more significant in sandy soils than in loamier soils; however, biological clogging becomes more significant in finer textured soils due to higher specific surface area. This is consistent with Vandevivere et al. (1995) who determined that a given level of biomass produces greater clogging effects in fine-textured materials than in coarser materials. To do determine if texture significantly influenced the capacity of the treatments to alter hydraulic conductivity in these soils, multiple soil textures were investigated by constructing columns from a mixture of 60% soil (by weight) and 40% sterilized Iota quartz sand. Laboratory measurements were made for particle size distribution,

gravimetric water content, soil pH, carbon, nitrogen, microbial biomass carbon (MBC), and total iron oxides (Table 2).

Soil columns were slowly saturated from beneath with soil water simulant and a pond of soil water simulant was allowed to develop above the soil surface. Flow commenced by connecting the solution pond to a Mariotte device to maintain a constant head within the pond (Figure 2). Flow was vertical from top to bottom through the soil column. The flow rate, Q (cm^3/s), was calculated from the volume of effluent solution over time. Darcy's law (Equation 1) was used to calculate saturated hydraulic conductivity, K_s (cm/s), from the flow rate, column dimensions, and the hydraulic gradient drop in the column. Results are presented as changes in K_S over time. Ideally, K_S would decrease over time with various treatments including nutrient solutions, algae, etc., as described in the experiment objectives.

One pair of columns was constructed for each soil and texture, i.e. one pair of 100% Freel's Bend soil columns and one pair of 60% soil columns. One column of each pair was randomly selected to be used as a control. Control columns received only soil water simulant for the duration of the experiment. The test column was infiltrated with soil water simulant for ~50 hours to allow particles to redistribute within the soil column and natural microbial growth to take place. Infiltration by soil water simulant in test columns is referred to as the pretreatment phase. The influent supply was then switched to the nutrient solution for ~50 hours to enhance microbial growth. Infiltration by the treatment solution will be called the test phase. Flow rates were monitored throughout the experiment and used to calculate K_S. The resulting K_S values from the preliminary experiments are charted versus time by column pairs in Appendix C.

For the preliminary set of experiments, changes in K_S are calculated from the maximum K_S value recorded during the first half of the experiment and the last K_S value recorded at the end of the experiment (Table 4). The maximum K_S value was chosen partly due to the observed trend in K_S over time (Appendix C) where an initial K_S increase occurs shortly after flow commences, reaching a maximum K_S value before transitioning to more gradual K_S changes throughout infiltration by the soil water simulant. This inflection point likely results from a change in dominant mechanisms controlling fluid flow within the pore space. The mechanisms

that govern the initial changes in K_S observations in repacked soil columns include out-washing of fines and dissolution of entrapped air. These processes can increase the volume and connectivity of pore spaces available to transmit fluids, resulting in an initial increase in K_S, before pore clogging mechanisms begin to reduce them, resulting in decreasing K_S values (Allison, 1947; Okubo & Matsumoto, 1983).

Table 4: Preliminary Experiment Results

Column	Packed Bulk Density (g/cm^3)	Change in K_S (% Decrease)
FB+C	1.068	81
FB+NS	1.045	87
FBS+C	1.206	30
FBS+NS	1.272	49
WB+C	1.225	74
WB+NS	1.062	32
WBS+C	1.400	42
WBS+NS	1.392	52

To simplify discussion of the results of these and subsequent experiments, each soil column will be referred to by a moniker consisting an abbreviation of the soil sampling location followed by an abbreviation for the solution it received, i.e., *+C* denotes control columns and *+NS* is used for columns receiving nutrient solutions. Columns containing a mixture of soil and sand are indicated by adding the letter *S* after the soil abbreviation, e.g., WB+NS denotes the column containing 100% Walker Branch soil infiltrated with nutrient solution, and WBS+NS

denotes the column containing 60% Walker Branch soil mixed with 40% sand infiltrated with nutrient solution.

In the 100% Freel's Bend soil column pair (Figure C.1), application of only the soil water simulant in FBC resulted in an 81% decrease in K_S by the end of the experiment. Whereas in the test column, infiltration of FB+NS by the soil water simulant followed by nutrient solution resulted in an 87% reduction of K_S. However, in the Walker Branch soil column pair (Figure C.2), application of nutrient solution resulted in smaller decreases in K_S (32%) in the test column than the soil water simulant in the control column (74%). The combined FB and WB results suggest that the nutrient addition approach is not effective.

To determine if the same results could be expected in soils with different textures, additional textures were created in these soils by addition of 40% sand, and the experiment was repeated (Figures C.3 & C.4). The sand additions resulted in less significant K_S reductions (Table 4). Unlike Pavelic et al.'s (2011) findings where the authors determined that physical clogging was more significant in sandy soils than in loamier ones, this does not appear to be the case in this study as the clogging in the coarser columns was less pronounced than in the finer columns of 100% loam soil. Other literature supports the premise that finer soils tend to clog more effectively by both physical and biological mechanisms. Physical clogging can be more apparent in columns composed of fine-grain soil due to redistribution of fines, subsequent collapse of soil structure when grains become unsupported by fine grain out-washing, and smaller pore spaces for straining fine-grain particles (West et al., 1992; Baveye et al., 1998; Lewis & Sjöstrom, 2010; Pavelic et al., 2011). The larger surface areas per volume of soil for microbes to colonize may produce greater biological clogging effects in finer soils according to Characklis et al. (2009). However, subsequent research has shown that grain size is not the governing factor for microbial colonization (Vandevivere & Baveye, 1992c; Rittmann; 1993). Rather, grain-size distribution, surface roughness, and nutrient availability all play a role in biological clogging mechanisms. In this study, the less significant clogging in coarser texture could be due to larger pore size distributions that would not clog as easily by the redistribution of out-washed fines. This difference could also be an artifact of repacking the soils or be due to replacing 40% of the soils containing their native microbial community with sterilized sand,

reducing the original number of microbes at the onset of the experiment relative to their 100% soil counterparts. Therefore, in subsequent experiments, the potential for solution treatments to clog soils of varying textures will be investigated using the natural texture differences between the different types of soils sampled.

Application of soil water simulant in the control columns showed the potential for K_S reduction due to rearrangement of soil particles within the columns. Three of the four column pairs in this experiment indicated a slightly greater decline in the saturated hydraulic conductivity in columns receiving nutrient solution as opposed to columns infiltrated only by soil water simulant. However, these K_S reductions do not indicate substantial additional clogging by enhanced microbial growth and are not great enough to support application of nutrient solution alone to achieve significant reductions of the saturated hydraulic conductivity or support pond sealing.

The *Ks* reductions seen in this preliminary experiment are not as dramatic as the 2-3 orders-of-magnitude or larger reductions achieved by numerous previous investigators through enhanced microbial activity (Allison, 1947; Mitchell & Nevo, 1964; Gupta & Swartzendruber, 1962; Okubo & Matsumoto, 1979; Frankenberger et al., 1979; Taylor & Jaffé, 1990a; Cunningham et al., 1991; Vandevivere & Baveye, 1992a,b,c; Seki et al., 1996; Holm, 2000; Seifert & Engesgaard, 2007). However, this may be because this set of experiments was different from those cited. These studies all included a carbon source in the infiltrating solution, whereas the nutrient solution for algae cultivation used in these preliminary experiments lacked a carbon source. Therefore, Phase II repeated the experiments with the addition of a carbon source to the nutrient solution. Replicates were also added in the subsequent experiments for greater confidence in observed results.

Phase II: Scoping Experiments

Thullner (2010) reviewed biological clogging in saturated porous media flow systems, noting that many studies optimized growth conditions to promote bioclogging by enhancing microbial activity. This was commonly achieved by adapting the pore water composition to provide a suitable carbon source and ensure nutrient (phosphorus, sulfur, iron, calcium, and most importantly nitrogen) availability. Some of the easily degradable carbon sources

investigated in those experiments included glucose, acetate, methanol, sucrose, dextrose, propylene glycol, and others (Thullner, 2010). Since supplying nutrients alone in the Phase I experiments did not result in significant reduction of the saturated hydraulic conductivity, the second phase of experimentation considered addition of the carbon substrate glycerol to the nutrient solution to enhance microbial activity and, as a result, more effectively reduce the saturated hydraulic conductivity by biological clogging of the loam soil.

Cell growth and lipid productivity of microalgae can also be amplified by heterotrophic growth using various carbon substrates. However, this benefit can be offset by the cost of organic carbon sources, which are typically much higher than that of the other nutrients required for cultivation (Liang et al., 2009). Glycerol was selected as a carbon source for this study because it would not present a financial burden. In fact, crude glycerol is a by-product of biofuel production; generating 10% (by weight) glycerol in production processes (Johnson & Taconi, 2007). Due to increased biofuel production in recent years, global production of crude glycerol surged from 200,000 tons in 2003 to over 2,000,000 in year 2011 (Ciriminna et al., 2014). The glut created, as the current market for crude or refined glycerol cannot accommodate the surplus, has incited pressure on biodiesel producers to explore new applications for crude glycerol by-product or at least methods of disposal (Johnson & Taconi, 2007). Liang et al. (2009) found that low dose applications for glycerol around 1-2% (v/v) improved biomass production and culture with 2% glycerol yielded higher lipid contents for *Chlorella vulgaris*. Sandhya and Ali (2015) tested different carbon substrates for their effects on bacterial EPS production finding glycerol to yield the greatest production of EPS.

Tennessee Loam Soil with Glycerol Amended Nutrient Solution

In the first experiment of Phase II, a pair of soil columns was packed with Freel's Bend soil and permeated with two liquids in sequential order: 1) soil water simulant (5 mmol/L $CaCl_2$) and 2) glycerol amended nutrient solution. The soil water simulant enabled quantification of mechanical clogging due to particle rearrangement. The addition of glycerol to the nutrient solution investigated the use of a carbon substrate to potentially enhance microbial growth and aid in biological clogging.

As in the preliminary set of experiments, the soil water simulant permeated the replicate pair of columns for approximately 50 hours of flow. Just as in the Phase I columns, pretreatment with soil water simulant resulted in a small initial increase to a maximum K_S. After this inflection point was reached, the soil water simulant displayed steady reductions of K_S. The column influents were then switched to the glycerol amended nutrient solution. Unfortunately, one replicate column failed early in the experiment due to the pond of solution draining unexpectedly when the solution supply line became disconnected. The K_S values versus time for the successful column, FB+NSG, are plotted along with the 100% Freel's Bend columns (FB+C and FB+NS) from the Phase I experiment in order to visually compare the effects of the carbon substrate addition (FB+NSG) to the nutrient solution treatment (FB+NS) and the control column (FB+C) which received only soil water simulant (Figure 3).

In FB+NSG, the K_S value under pretreatment was reduced from the maximum value of 9.8×10^{-3} cm/s to 3.5×10^{-3} cm/s over the ~50 hours of soil water simulant flow. Once the column was switched to the nutrient solution plus glycerol, a rapid order-of-magnitude reduction in K_S occurred within the first 37 hours of nutrient and glycerol application. This appears on the K_S versus time chart as a steeper slope immediately after the influent solution was changed from soil water simulant to nutrient solution with glycerol. The reaction of FB+NSG to the treatment solution is noticeably different from that of the FB+C and FB+NS data (Table 5). In the preliminary experiment columns, infiltration by nutrient solution did not significantly differ from the trend displayed by soil water simulant during the pretreatment phase of FB+NS or FB+C. As the reduction of K_S by the addition of glycerol appeared more dramatic than the preliminary columns, flow was allowed to continue. Over the next 150 hours, K_S decreased by another order of magnitude in FB+NSG before tapering off.

Initially, FB+NSG exhibited about half an order-of-magnitude reduction of K_S by the soil water simulant, indicative of physical rearrangement of soil particles. Infiltration with glycerol amended nutrient solution displayed a more rapid rate of reduction that resulted in a more than two orders-of-magnitude reduction in K_S. This is attributed to physical rearrangement of soil particles and microbial growth that was probably enhanced by the addition of a carbon substrate within the nutrient solution. The results of FB+NSG indicate a greater decline in the

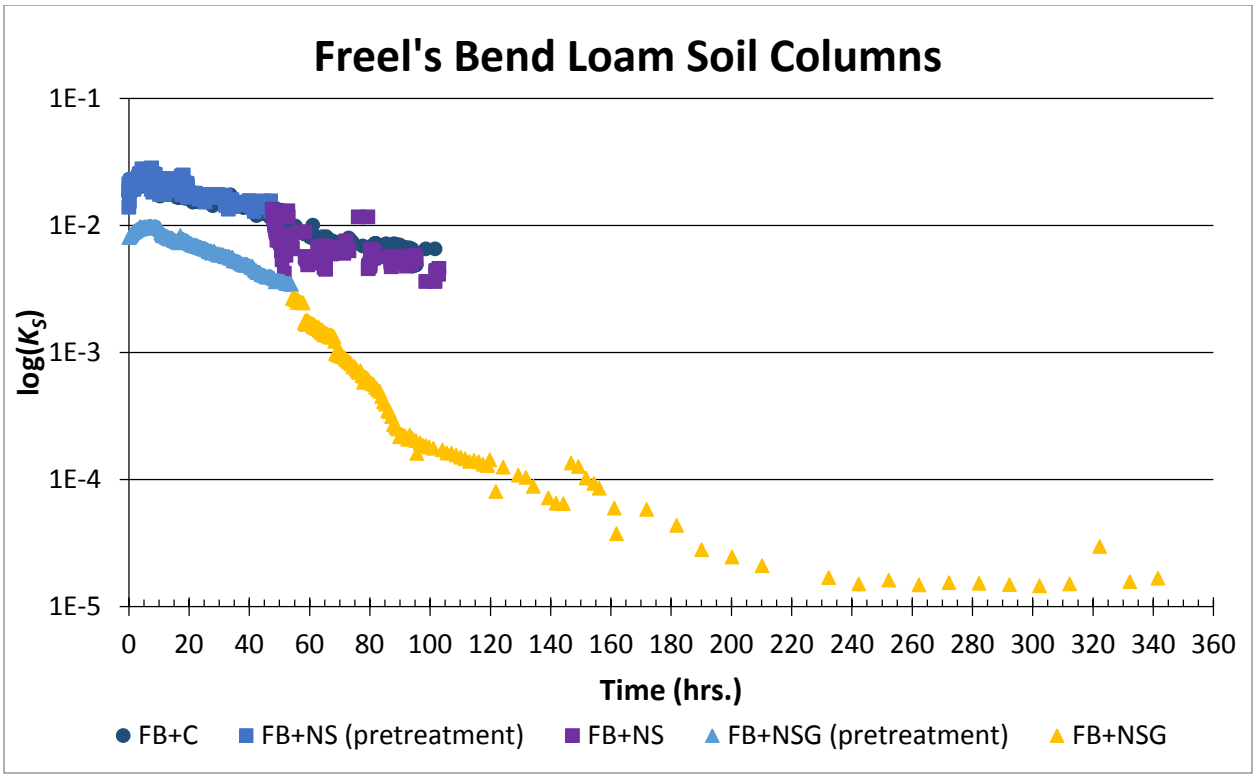

Figure 3: Glycerol Amended Nutrient Solution and Phase I Freel's Bend Columns

Table 5: Results of Phase II Glycerol Amended Nutrient Solution on Freel's Bend Loam Soil

Column	Bulk Density (g/cm^3)	Change in K_S (% Decrease)
FB+NSG	1.036	99
FB+NSG Rep	1.034	96

saturated hydraulic conductivity under application of nutrients and a carbon source than by soil water simulant or nutrient supply alone. These findings support the idea that microbial growth may be enhanced by the availability of carbon and nutrients in the pore water leading to bioclogging and multiple orders-of-magnitude *Ks* reductions and agree with the results of the numerous studies finding similar reductions of K_S by enhanced microbial activity after addition of a carbon source (Allison, 1947; Mitchell & Nevo, 1964; Gupta & Swartzendruber, 1962; Okubo & Matsumoto, 1979; Frankenberger et al., 1979; Taylor & Jaffé, 1990a; Cunningham et al., 1991; Vandevivere & Baveye, 1992a,b,c; Seki et al., 1996; Holm, 2000; Seifert & Engesgaard, 2007).

Comparison of Solutions on K_S of Corpus Christi Sand

To validate the strong K_S reduction exhibited by the Freel's Bend loam soil after addition of glycerol to the nutrient solution, two replicate sets of experiments were conducted on soil columns of a fine sand soil sampled from the site of an outdoor raceway system test bed in Corpus Christi, Texas. In each replicate set, four soil columns were constructed to compare the effect of four different solutions simultaneously: soil water simulant, the nutrient solution, the glycerol amended nutrient solution, and the contents of the algae ponds themselves, i.e., an algal broth of the green algae *Scenedesmus dimorphus* growing in the nutrient solution.

Numerous laboratory-scale and field-scale studies of animal waste settling ponds have shown that, regardless of the soil texture or the type of biological waste, low-hydraulic conductivity seals develop within the soils below these ponds (Davis et al., 1973; Chang et al., 1974; Hills, 1976; DeTar, 1979; Culley & Phillips, 1982; Miller et al., 1985; Rowsell et al., 1985; Barrington et al., 1987a,b; Maulé et al., 2000; Cihan et al., 2006). Many of these studies investigated the mechanisms of the soil sealing, and found that the initial, predominant sealing mechanism is the filtration of suspended solids at the soil-water interface (Chang et al., 1974; Hills, 1976; Culley & Phillips, 1982; Rowsell et al., 1985; Barrington et al., 1987a,b). Soils of these experiments range in textures from clay loams to play sands, but straining and bridging of suspended fines carried in the waste were shown to seal the soils regardless of texture. Suspended particles larger than the pore spaces at the infiltrative surface of a soil result in an

ever-tightening mat developing on a soil surface that increasingly restricts fluid infiltration as the trapped particles themselves become the filter for finer and finer particles (McGauhey & Krone, 1967). The accumulation and orientation of fine materials smaller than the pore spaces that penetrate the filter cake or soil surface can form meniscus-shaped bridges between grains that block flow channels (Skolasińska, 2006). Microorganisms, like other particulate matter soils, can be removed from fluid flow by straining, sedimentation, entrapment, and adsorption to reduce the hydraulic conductivity.

The authors of these animal waste soil sealing studies also agree that biological processes can reinforce the mechanical sealing and further reduce the soil hydraulic conductivity as the organic materials of the waste break down and fuel microbial growth and production of biofilms (Hills, 1976; Chang et al., 1974; Culley & Phillips, 1982; Rowsell et al., 1985; Barrington et al., 1987a,b). However, while most of the biological sealing in these studies was attributed to bacterial cells and biofilms, bacteria are not the only microorganisms to produce polysaccharides and other polymers. Algae also excrete large amounts of polysaccharide; EPS was found to compose 20-60% of the total organic matter of some green algae (Lewin, 1956). The vast majority of microorganisms, both prokaryotic (bacteria) and eukaryotic (algae) tend to produce EPS and live and grow in polymicrobial aggregates forming flocs, biofilms, or mats (Wingender et al., 1999).

Ragusa et al. (1994) investigated benthic algae and bacteria as low-cost seepage control option for irrigation channels. Their study attributed a marked reduction in hydraulic conductivity (22%) of a soil sample to inoculation by benthic green algae due to development of an algal/bacterial mat within the first few millimeters of irrigation soil in their columns. Battin and Sengschmitt (1999) pointed out the role of algae in clogging in a large river system, suggesting that algae may mechanically block void spaces or fuel bacterial clogging of the riverbed. Gette-Bouvarot et al. (2014) evaluated the respective influences of sediment particle deposition and biofilm growth on the decreased permeability in two infiltration basins used for groundwater recharge. Their results showed considerably reduced permeability due to clogging of the top sedimentary layer in the two basins. The highest reduction of permeability attributed to the development of a dense algal mat on the soil surface of sediment. The authors also

associated greater clogging with algal biomass than the contribution by bacterial biomass or physical filtration of particulate organic matter on the surface.

Each column was first infiltrated with soil water simulant (5 mmol/L $CaCl_2$) in order to quantify mechanical clogging due to particle rearrangement. The infiltrating solution was changed in three of the four columns. One randomly chosen column continued infiltration by the soil water simulant for the duration of the experiment as a control (TX+C). The same solutions tested on the Freel's Bend soil, nutrient solution and nutrient solution amended with glycerol, were supplied to TX+NS and TX+NSG, respectively. These treatments encourage changes to K_S by mechanical clogging and biological clogging from enhanced microbial growth. The fourth column (TX+A) received an algal broth composed of the green algae *Scenedesmus dimorphus* suspended in the nutrient solution to quantify mechanical and/or additional biological clogging associated with the algal biomass. Mechanical clogging could occur due to particle rearrangement and a buildup of algal cells in the pore space. Additional biological clogging may result from the algae's biological activities as well as enhanced microbial growth due to the addition of nutrients and a carbon substrate in the form of decaying algae cells. Since the experiment was repeated in two replicate sets, the columns of second set are distinguished by adding the abbreviation *Rep* to the end of the column moniker, e.g., TX+NS Rep refers to the column of the replicate set containing the Texas fine sand soil infiltrated by nutrient solution as its test solution.

After an initial equilibrium was established by infiltration of the soil water simulant (Figure 4a), the test columns were all supposed to be switched to their secondary solutions. TX+NS and TX+NSG were switched to their respective test solutions after ~80 hours of flow with soil water simulant. TX+A should also have been switched at ~80 hours along with the other test columns. However, the algae batch culture had yet to reach a suitable cell density from which to make the 5×10^5 cells/mL algal broth, so the pretreatment phase of TX+A was allowed to continue while the algae batch culture grew. Once the batch culture became dense enough, the infiltrating solution was switched to algal broth. Infiltration by soil water simulant in TX+A occurred for a total of 131 hours. After switching to the secondary solutions, flow continued

until combined pretreatment and test flow totaled ~200 hours for each column (~120 hours of infiltration by test solutions in TX+NS and TX+NSG and ~75 hours for TX+A).

In the replicate set of columns, each column was infiltrated with the soil water simulant for ~106 hours while K_S values equilibrated (Figure 5a). The pretreatment phase was followed by application of the secondary solutions for ~300 hours. However, TX+NS Rep was stopped a bit earlier (after 260 hours) due limited supply of nutrient solution.

The charts of K_s versus time presented in Figures 4a and 5a tend to show slight changes in K_S immediately upon the onset of flow. After these initial adjustments, K_S remained rather stable, increasing slightly throughout the pretreatment phase of this experiment with values varying only from 2.4 x 10^{-3} to 4.3 x 10^{-3} cm/s in the first set of TX columns and from 2.4 x 10^{-3} to 6.8 x 10^{-3} cm/s in the replicate set. Generally, there was very little difference between the overall trends or values of K_S between the columns due to infiltration of the soil water simulant. The processes driving the variable behavior of K_S observed in the pretreatment phase of these experiments, when columns were inundated with soil water simulant, may be related to the formation or dissolution of entrapped gas, the out-washing or redistribution of fines, or a combination of these or other processes that affect the temporal behavior of K_S in column studies as reviewed by Baveye et al. (1998). However, the mechanisms of these changes were not investigated in the experiments presented here because the magnitudes of these changes are not substantial.

The goal of this study is to investigate the feasibility of achieving low saturated hydraulic conductivities by manipulating soil clogging mechanisms to potentially replace plastic pond liners. Therefore, because the secondary solutions promote dramatic changes to the saturated hydraulic conductivity, the secondary solutions become the focus of these and subsequent experiments. Therefore, the changes in K_S due to the treatment solutions (Table 6) reflect the difference in the last K_S values measured during the test phase of the experiment and K_0, where K_0 represents the saturated hydraulic conductivity value at the beginning of flow with the treatment solution (or equivalent time for continued flow with soil water simulant in control columns).

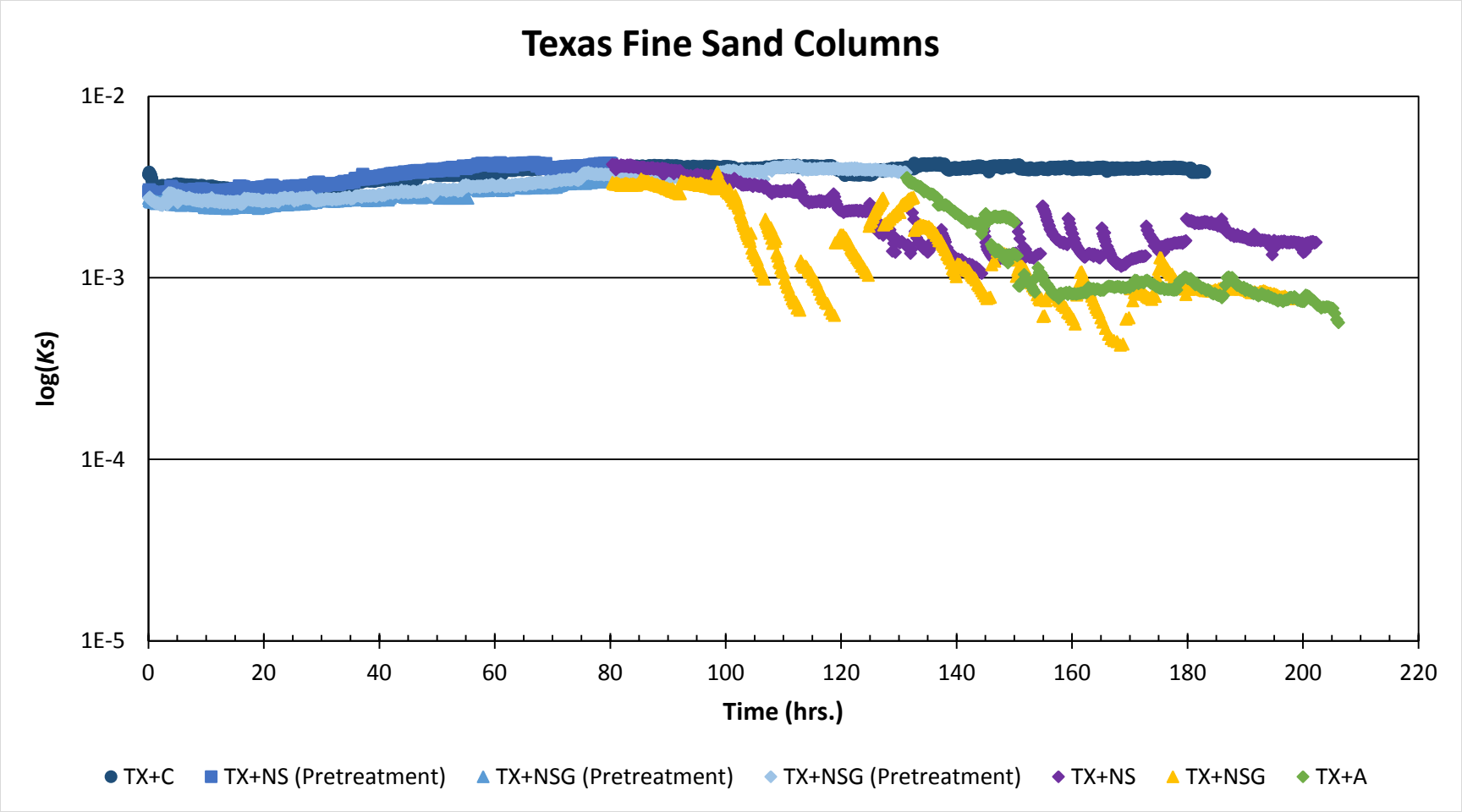

Figure 4a: Saturated Hydraulic Conductivity of Texas Fine Sand Columns

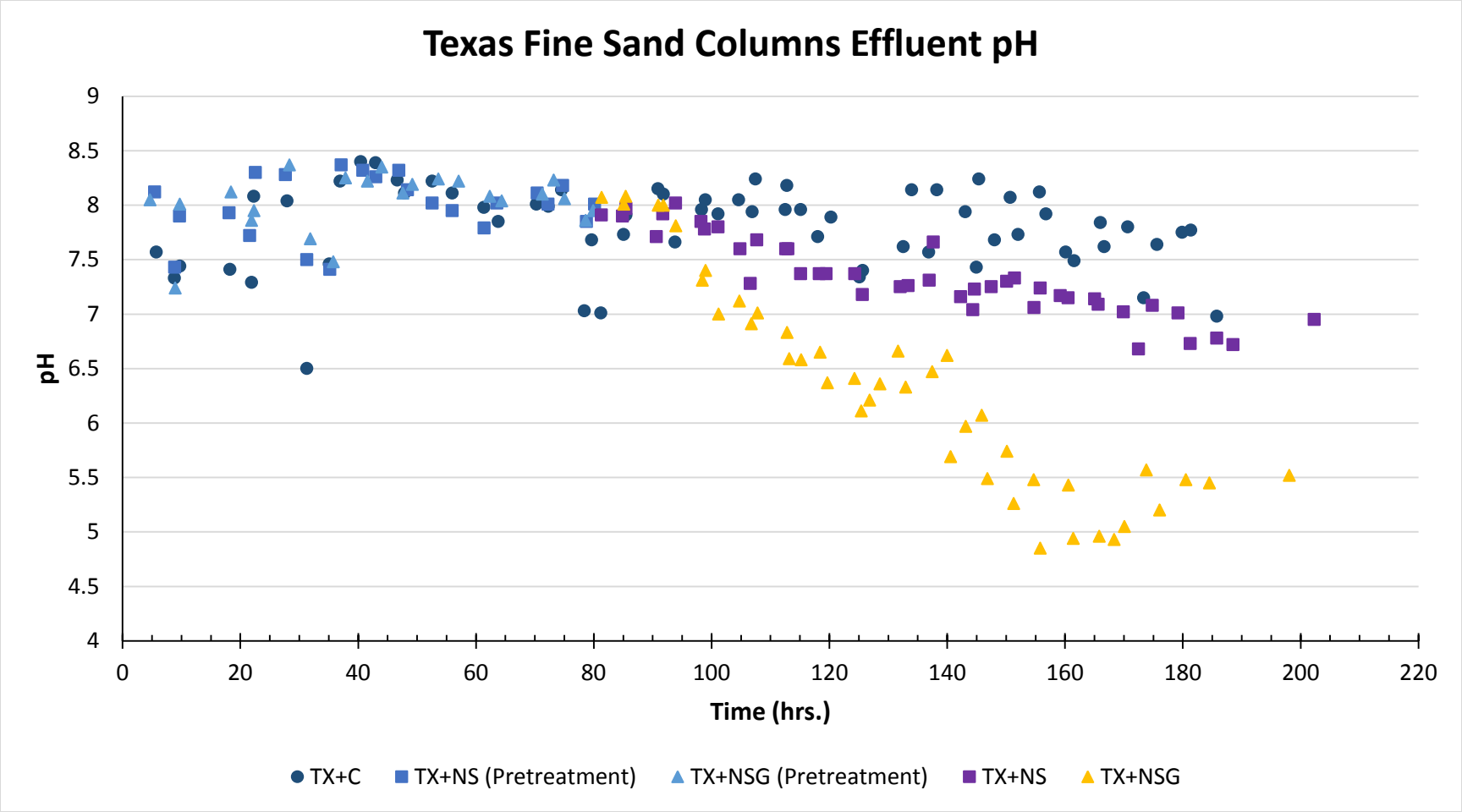

Figure 4b: Effluent pH of Texas Fine Sand Columns

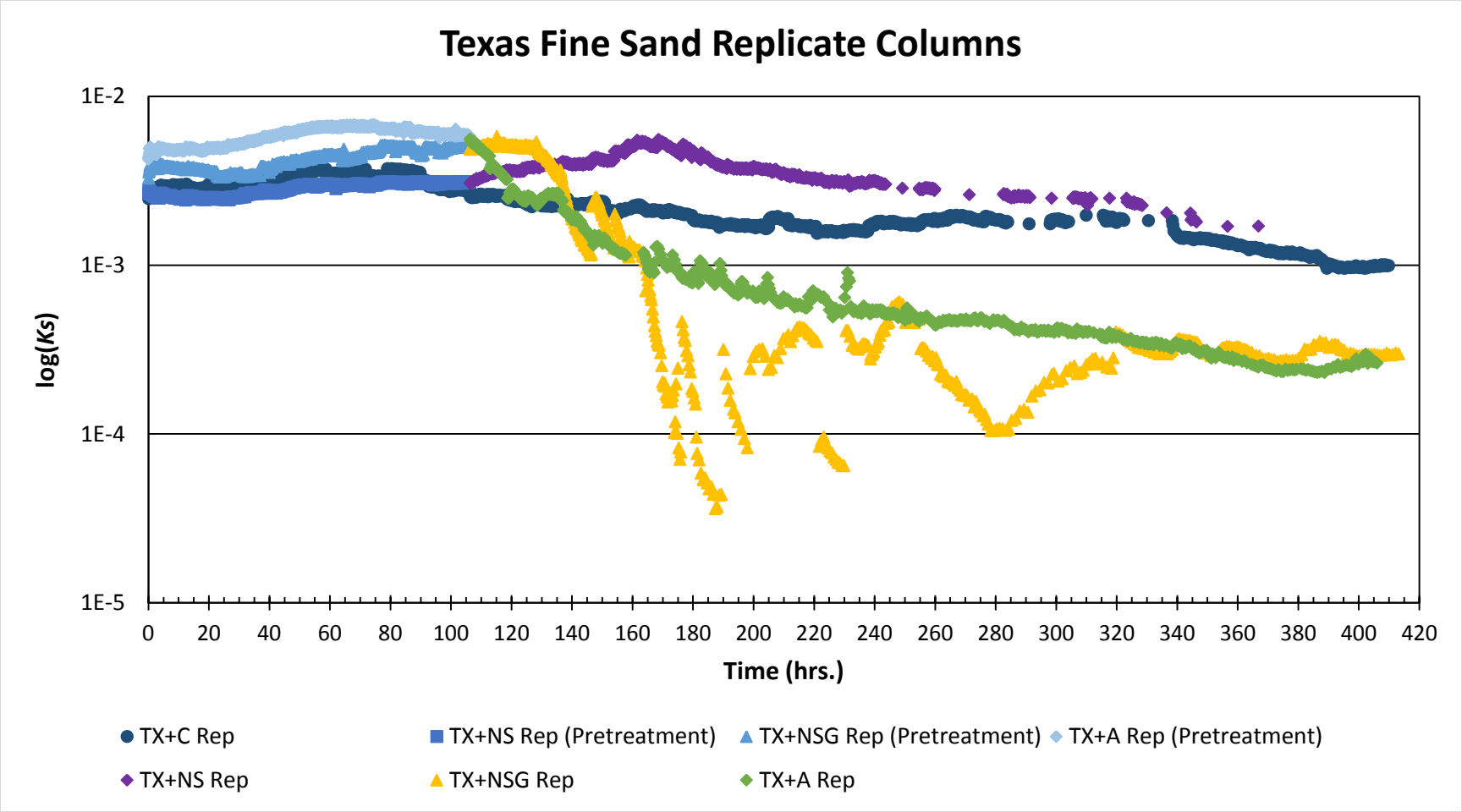

Figure 5a: Saturated Hydraulic Conductivity of Texas Fine Sand Replicate Columns

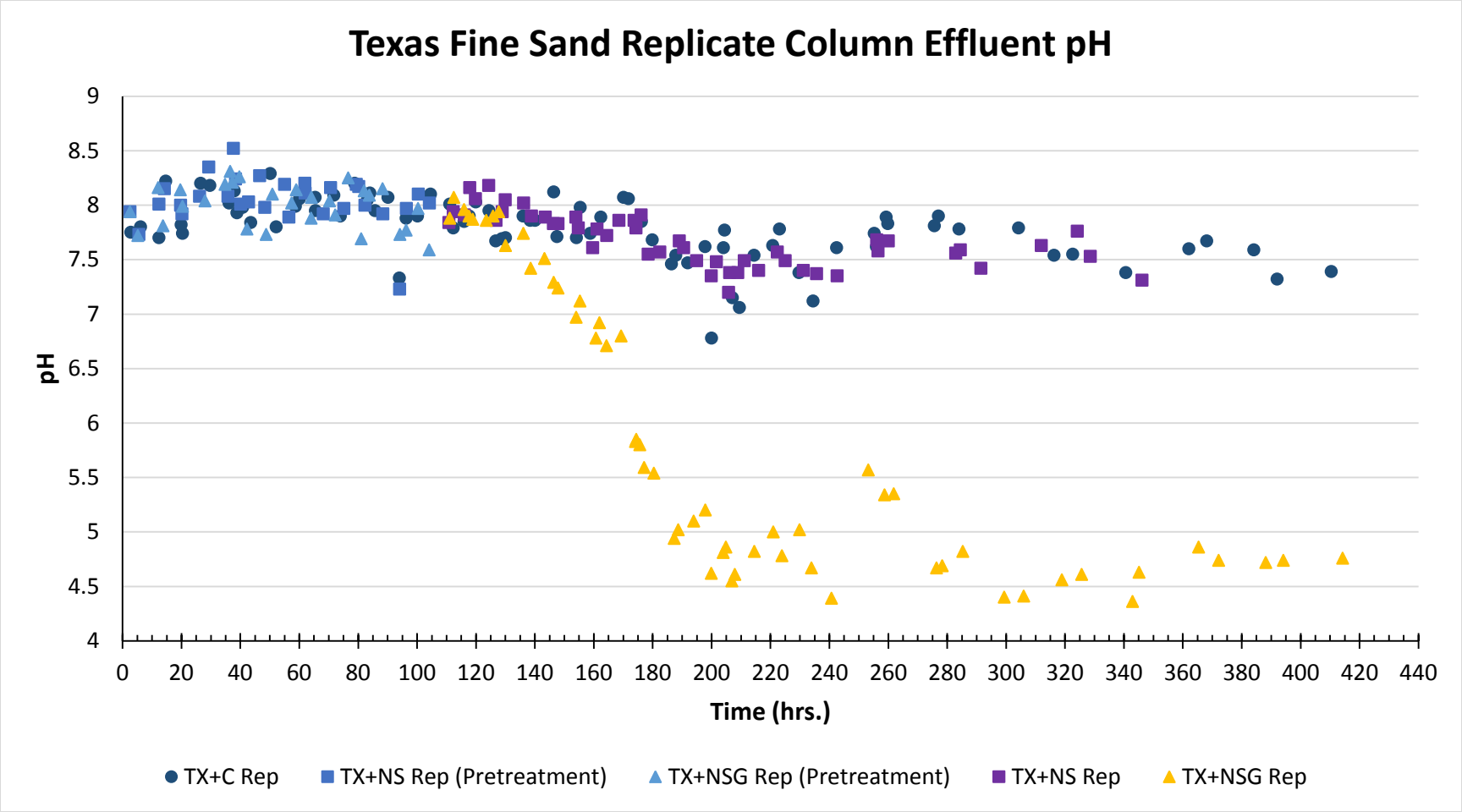

Figure 5b: Effluent pH of Texas Fine Sand Replicate Columns

Table 6: Results of Phase II Scoping Experiments on Texas Fine Sand Columns

Column	Packed Bulk Density (g/cm^3)	Change in K_S (% Decrease)
TX+NS	1.294	63
TX+NSG	1.300	77
TX+A	1.300	84
TX+NS Rep	1.300	44
TX+NSG Rep	1.299	94
TX+A Rep	1.299	95

In TX+NS (Figure 4a), application of the nutrient solution at first resulted in a steady decline in K_S over the first 31 hours of infiltration from K_0 of 4.22 x 10^{-3} cm/s to 3.01 x 10^{-3} cm/s. However, from this point, reductions in K_S occurred throughout the flow period each day followed by recovery of K_S when flow was discontinued in the overnight hours. At the end of the experiment, flow was continuous for a 22-hour period and the rate at which K_S declined was stable. A maximum reduction of 75% was observed about halfway through the nutrient solution infiltration period when K_S reached a minimum value of 1.06 x 10^{-3} cm/s. However, from the onset of the experiment to the final reading, values for K_S were reduced by 63%. The same periodicity of K_S was observed for TX+NSG, under periodic flow of the glycerol amended nutrient solution after a period of fairly stable decline over the first 18 hours of flow. Wherein, K_S experienced steep declines during flow periods, but seemed to recover somewhat during the rest periods. The maximum reduction from K_0 observed reached almost two orders of magnitude at 87%. However, sustained decline during the continuous flow period at the end of the experiment resulted in a final reduction of 77% from 3.35 x 10^{-3} cm/s to 7.67 x 10^{-3} cm/s. Although flow in TX+A was also stopped each night, K_S did not recover as significantly during

the resting periods as was observed in the columns infiltrated by the nutrients and glycerol. However, the overall magnitude of reduction was similar. The rate of K_S reduction by algae was much more stable over the first 26 hours of flow, after which point a leveling-off occurred reaching a final minimum value of 5.66 x 10^{-3} cm/s, an 84% reduction from the K_0 value of 3.55 x 10^{-3} cm/s.

The replicate set of columns displayed similar results to the first set, with the exception of TX+NS Rep (Figure 5a). This column experienced a subtle, increase in K_S over the first 62 hours of nutrient solution flow from K_0 of 3.04 x 10^{-3} cm/s to a maximum value of 5.54 x 10^{-3} cm/s. After this maximum was reached, the saturated hydraulic conductivity reduced steadily to a final value of 1.70 x 10^{-3} cm/s, a 44% reduction from K_0. As in the first replicate, application of the glycerol amended nutrient solution resulted in a more dramatic, albeit fluctuating, reduction in K_S. A nearly two orders-of-magnitude reduction from K_0 of 4.85 x 10^{-3} cm/s to a minimum of 3.61 x 10^{-5} cm/s occurred within the first 82 hours; however, this minimum was not sustained. In effort to control these fluctuations, columns were operated continuously for the second half of the experiment. Under continuous infiltration, K_S decreased from a relative peak at 140 hours to a relative minimum at ~170 hours. Towards the end of the experiment, a more sustained K_S resulted in an overall final reduction of K_0 by 94%. Infiltration by algae in TX+A once again exhibited similar magnitudes of reductions as the nutrient plus glycerol addition, however far more greatly sustained. Episodes of minor K_S recovery are evident during the portion of the experiment in which infiltration was discontinuous (the first 130 hours of algae flow). However, the following 170 hours of infiltration by algae shows a stable reduction by 95% from the K_0 value of 5.59 x 10^{-3} cm/s to a final K_S of 2.6 x 10^{-4} cm/s.

Measurements of effluent pH were taken each day of flow, usually twice daily, on fresh samples (Figures 4b and 5b). The pH behavior of TX+C and TX+C Rep remained constant over the duration of the experiment. Unlike the other three treatments, the pH of the algal broth was not adjusted prior to column application. The rapid rate of algal growth causes its own daily variations of pH, independent of changes that may occur within the soil column; therefore, pH measurements for TX+A and TX+A Rep are not shown. In TX+NS and TX+NS Rep, pH values exhibit a slight reduction. TX+NSG and TX+NSG Rep exhibit far more dramatic decline

in effluent pH values, likely from formation of HCO_3^- as organic carbon decomposes and produces CO_2 indicative of significant microbial activity.

The approximately half-order of magnitude reductions achieved by infiltration of nutrient solution, like in the Phase I experiments on loam soils, did not appear to consistently or significantly reduce the saturated hydraulic conductivity of these columns and, therefore, do not appear indicative of enhanced microbial growth. This is consistent with the small decreases observed in the pH of the column effluents. Therefore, application of nutrient solution alone does not appear to be an effective treatment method for the sealing of the loamy soils of the Phase I experiments or of the Texas fine sand soils in Phase II experiments.

However, the addition of glycerol to the nutrient solution resulted in the greatest K_s reductions of both soils, reaching approximately two orders of magnitude in FB+NSG and in TX+NSG Rep. It appears that these reductions are the result of enhanced microbial growth due to the addition of nutrients and a carbon substrate in the glycerol amended nutrient solution. The decreasing pH values that occur with the transition from the $CaCl_2$ solution to nutrients and glycerol support this conclusion. However, K_S values varied wildly in both TX+NSG and TX+NSG Rep, with strong recoveries in K_S values during discontinuous flow.

Previous studies of biofilm morphologies under fluid flow suggest causes for the K_S recoveries observed. As the biofilms grow due to nutrients and carbon availability, they reduce the available pore space for fluid flow, which could inhibit the supply of fresh solution (Characklis, 2009). The accumulation of biofilm in the pore space can also increase hydrodynamic shear stress, which can result in sloughing of biofilm and spontaneous recovery of K_S (Mitchell & Nevo, 1964; Okubo & Matsumoto, 1979; Rittman, 1982; Taylor & Jaffé, 1990a; Dupin & McCarty, 2000). The large recoveries observed during the earlier times of the solution treatment application, in which the supply line was clamped and flow stopped overnight, could be the result of the intermittent static and dynamic flow. The biofilms may have preferentially grown under static conditions and sloughed when flow was commenced, increasing the shear stress. Another possibility is that, under static conditions, the microbes depleted the carbon and nutrient supply, leading microbes to die and detach from the biofilm. Then, nutrient and carbon supplies were restored when flow was commenced again and biofilm growth could

recover to again cause K_S reductions. However, no investigations were made to identify the mechanisms behind the variations in flow in this study.

Infiltration by algae produced a dense algal mat on the surface of the soil. The algal broth treatment also achieved similar magnitudes of K_S reduction as the glycerol amended nutrient solution, approaching two orders of magnitude in both replicates, with far less variability in K_S over time. If the large K_S reductions by glycerol and nutrient solution result in biological clogging that diminishes during the rest periods, while the columns receiving algal broth only exhibit very minor recoveries in K_S during rest periods, perhaps the main clogging mechanism in the algae columns is different than that of the glycerol and nutrients columns.

Many of the studies previously described cited large reductions of K_S due to the mechanical straining of suspended fine sediments, organic particles, or microbial cells and the build-up of a dense, low-conductivity layer at the soil-water interface (Chang et al., 1974; Rice, 1974; Hills, 1976; Culley & Phillips, 1982; Rowsell et al., 1985; Shaw et al., 1985; Barrington et al., 1987a,b; Pell & Nyberg, 1989; Schälchli, 1992; Vandevivere &Baveye, 1992a,b; Rinck-Pfeiffer et al., 2000; Skolasińska, 2006; Zhao et al., 2009). The column and field studies by Ragusa et al. (1994), Battin and Sengschmitt (1999), Gette-Bouvarot et al. (2014) on infiltration reductions by algae credited reductions in infiltration capacities to the algal mat observed on the porous media surfaces in their studies.

Phase III: Hydraulic Conductivity Reductions by Algae

While the K_S in the columns infiltrated by glycerol amended nutrient solution achieved the lowest minimum K_S values in the Phase II experiment on fine sand soils from Texas, these reductions were highly inconsistent throughout the course of the experiment, intermittently dropping and recovering K_S over time. Their K_S values eventually converged with the stably reduced K_S values sustained by infiltration of the algal broth. Therefore, Phase III of this study focused on the most successful of the Phase II treatments – algae. Phase III explored the sealing potential of the *Scenedesmus dimorphus* algal broth in two soils: 1) a loamy sand from the site of a wastewater treatment plant in Delhi, California that is currently a pilot program for algal biofuel production co-located with wastewater remediation and 2) the local loam soil from Freel's Bend in Oak Ridge, Tennessee used in Phase I and Phase II.

Four soil columns were constructed per soil, such that a replicate pair of experiments was conducted with two soil columns per replicate: a control column and test column infiltrated with the algal broth. As in the previous experiments, soil columns were permeated with the two liquids in sequential order. In the pretreatment phase, the soil water simulant (5 mmol/L $CaCl_2$), infiltrated all columns in order to establish an equilibrium value for saturated hydraulic conductivity. After equilibrium was established, the supply of infiltrating solution of one column from each replicate pair was switched to a test solution composed of 5 x 10^5 cells/mL *Scenedesmus dimorphus* algae growing in nutrient solution. The pretreatment solution enabled quantification of mechanical clogging due to particle rearrangement. The application of algal cells suspended in nutrient solution investigated mechanical clogging by the filtering of suspended algal cells and accumulation of biomass in pores as well as additional biological clogging that may occur due to algal activity or by decaying algal cells being used as a substrate for enhanced microbial activity. In the Phase III experiments, values of K_S are plotted over time where T=0 occurs at the start of application of algal broth to the columns in order to "zoom in" on the effects of these treatments (Figures 6 and 7).

California Loamy Sand

The soil water simulant permeated all four columns for a duration of approximately 85 hours of flow to allow K_S to equilibrate, after which time the influent was replaced with the algal broth composed of the green algae *Scenedesmus dimorphus* in one column of each replicate pair (CA+A and CA+A Rep). In both algae columns (Figure 6), a marked reduction of K_S began immediately upon application of the algal broth to the columns. This stable rate of decline was observed over the first 30 hours of flow in both columns before leveling off at equilibrium values. For CA+A Rep, application of the algal broth resulted in a 97% reduction from the K_0 value of 3.0 x 10^{-3} cm/s (Table 7). CA+A experienced a second, smaller period of K_S reduction at 85 hours before leveling off again towards the end of the experiment. In CA+A, K_S experienced an overall reduction of 99% from the K_0 value of 2.3 x 10^{-3} cm/s.

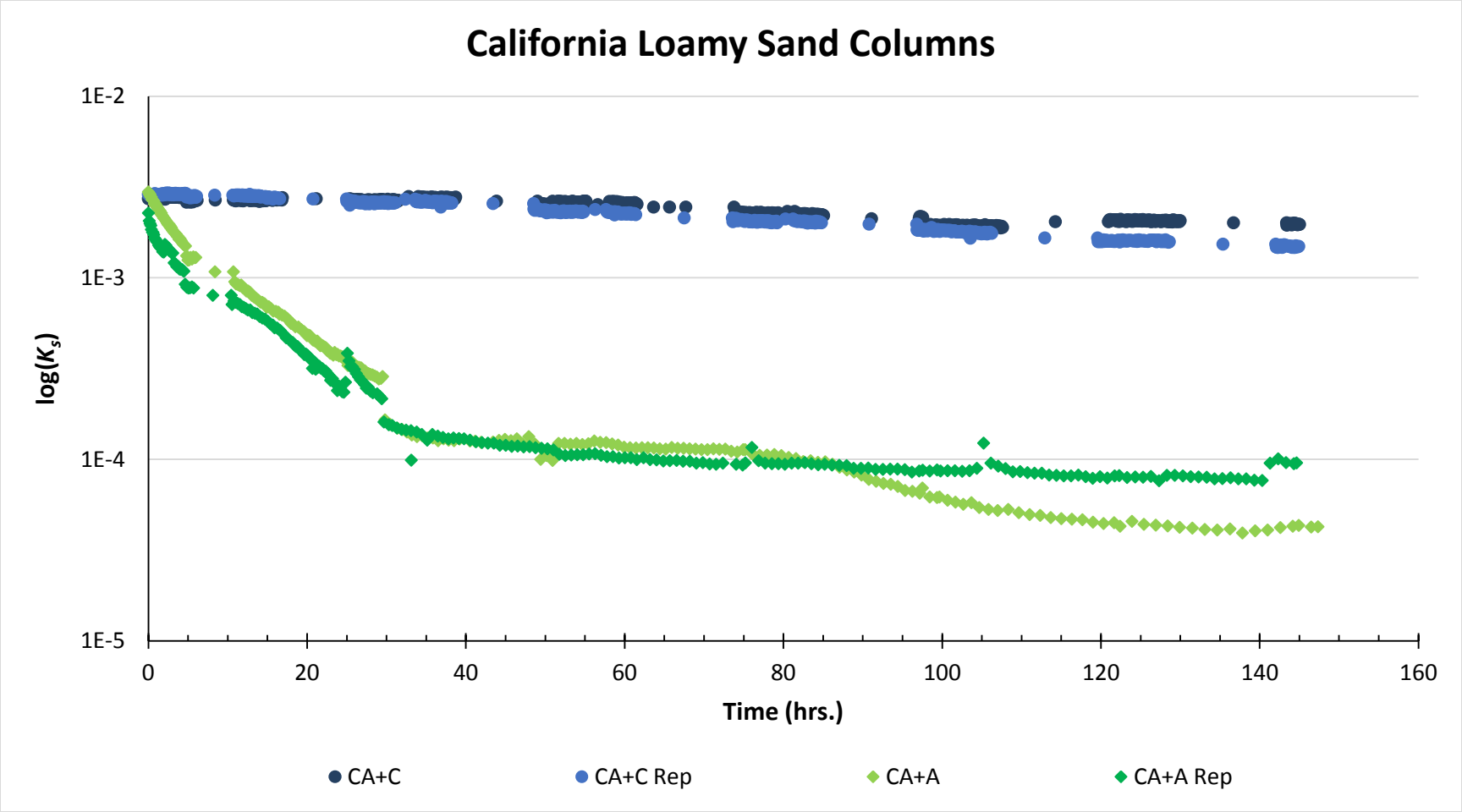

Figure 6: Saturated Hydraulic Conductivity Reductions of California Loamy Sand Soil

After flow through the column was discontinued, the column was allowed to drain. Grab samples were taken at depths of 1, 3, and 8 cm. When the column was deconstructed, a dense algal mat was observed at the soil-pond interface.

Table 7: Results of Phase III Experiments on California Loamy Sand and Tennessee Loam Soil Columns

Column	Packed Bulk Density (g/cm^3)	Change in K_S (% Decrease)
CA+A	1.700	99
CA+A Rep	1.700	96
TN+A	1.150	98
TN+A Rep	1.150	98

Tennessee Loam Soil

In the second set of experiments, the soil water simulant pretreated two replicate pairs of columns for approximately 120 hours of flow to allow K_S to equilibrate and for the batch culture of *Scenedesmus dimorphus* to reach a suitable concentration from which to make volumes of the diluted algal broth. The algal broth was supplied to the test columns for ~255 hours. Similar reductions in K_S were observed in both FB+A and FB+A Rep of about 98%, just as was observed for the algae columns of the California loamy sand soils in the previous experiment (Table 7). The test columns showed K_0 values of 9.6 x 10^{-4} cm/s and 1.1 x 10^{-3} cm/s in FB+A and FB+A Rep, respectively. While the most dramatic decline in the California soils occurred by an order of magnitude over the first 30 hours, the Tennessee loams exhibited a less sharp trend in reduction of K_s, declining by an order of magnitude over the first 34 and 54 hours in FB+A and FB+A Rep, respectively (Figure 7).

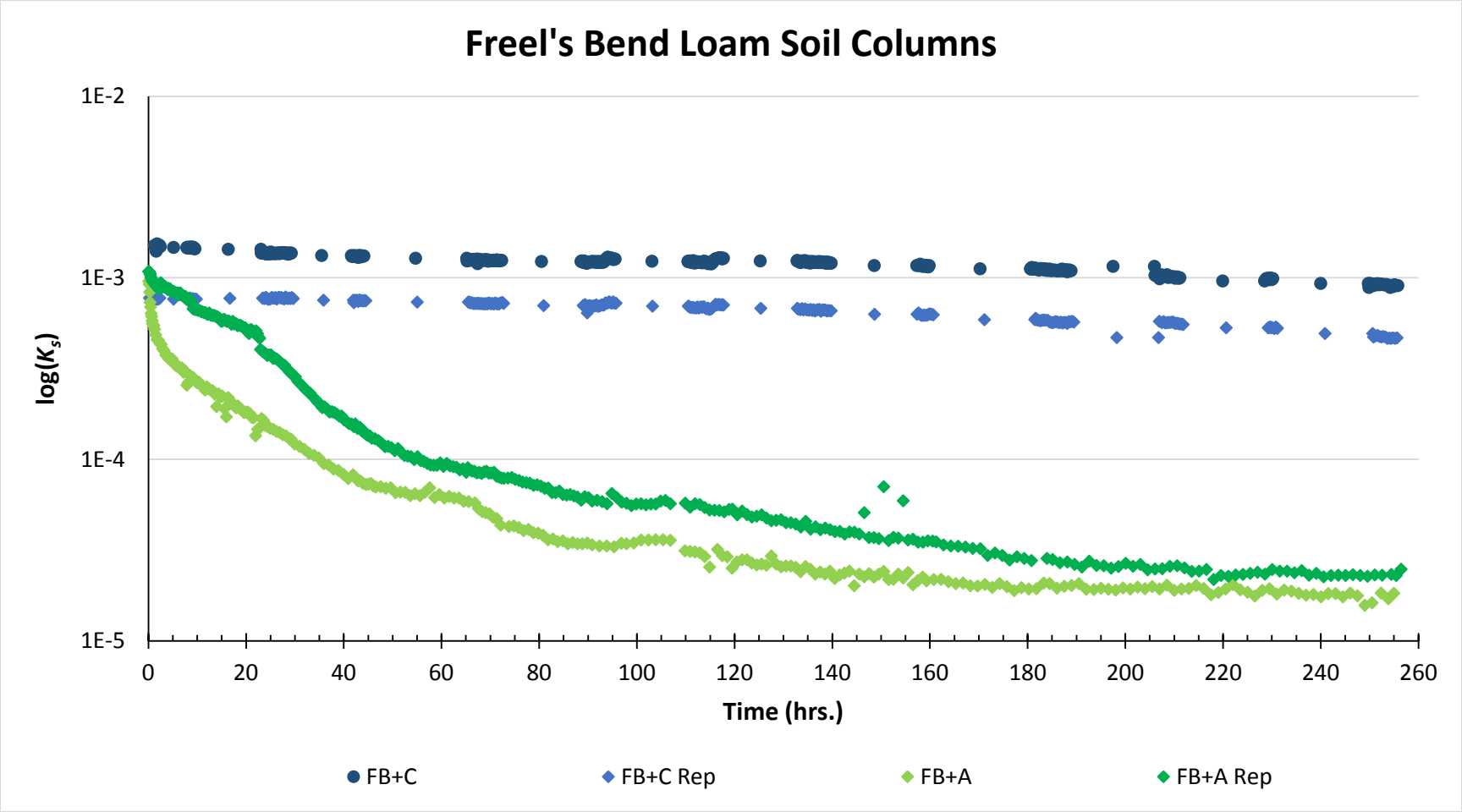

Figure 7: Saturated Hydraulic Conductivity Reductions of Tennessee Loam Soil

The nearly two order-of-magnitude reductions in saturated hydraulic conductivity in the Freel's Bend columns confirm the observations made for both the Texas and California soils. A dense mat of algae also developed in each of the algae columns of the Tennessee soils (Figure 8).

Focused Ion Beam Scanning Electron Microscopy

After the flow experiments of Phases II and III, the columns of TX, CA, and TN soils were allowed to drain before being destructively sampled in 1 cm increments with depth below the soil surface. For the columns receiving the algal broth treatment solution, the algal mat that developed at the soil surface was also sampled. Samples were stored at -80° C before being viewed under Zeiss Auriga 40 focused ion beam scanning electron microscope (FIB-SEM) (Zeiss Group).

The following FIB-SEM images (Figures 9-14), taken of CA+A column samples, were selected to illustrate the mechanism of soil clogging in the algae treated columns - the buildup of algal materials within the pore spaces, especially at the soil surface where dense algal mats formed in each algae treated column. At depths greater than 1 cm below the soil/solution interface little algal material was visible when the soil sample was collected. In the FIB-SEM images, the soil grains and void spaces are clearly visible and no cellular materials were observed (Figures 9 and 10). However, in the upper 1 cm of soil, some algae were visible during sample collection. In the FIB-SEM images, a thin film of algal materials coated soil grains, connecting the grains to one another and blocking interstitial spaces (Figure 11). Cell colonies could also be identified (Figure 12). FIB-SEM images of the algal mats at the soil surface revealed that soil grains were coated with a thick layer of algal material that also filled the interstitial spaces that would otherwise be available for fluid flow (Figures 13 and 14).

Figure 8: Algal Mat at FB Soil-Solution Interface

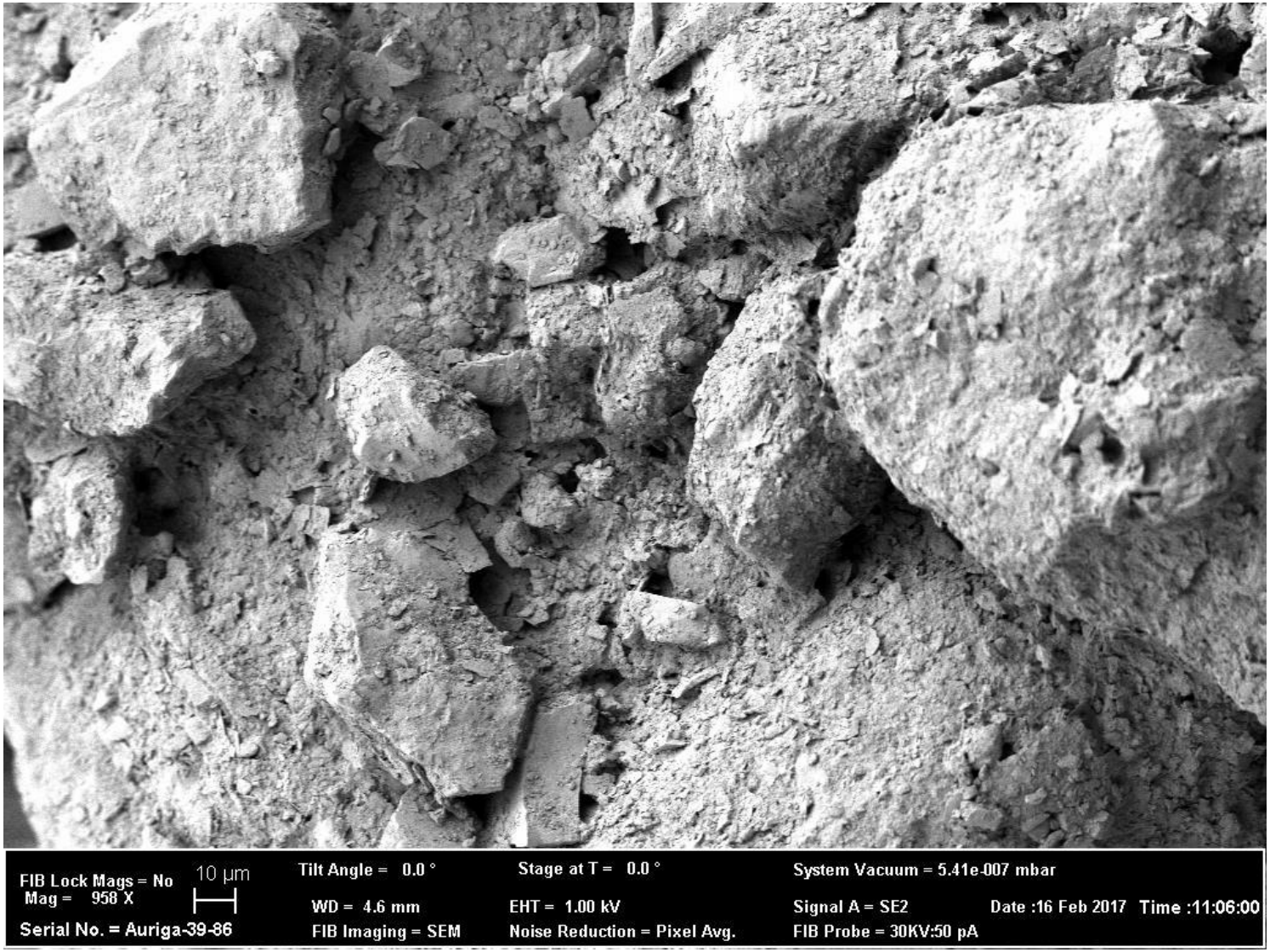

Figure 9: "Zoomed-out" FIB-SEM image of CA+A column soil grains (1-2 cm depth)

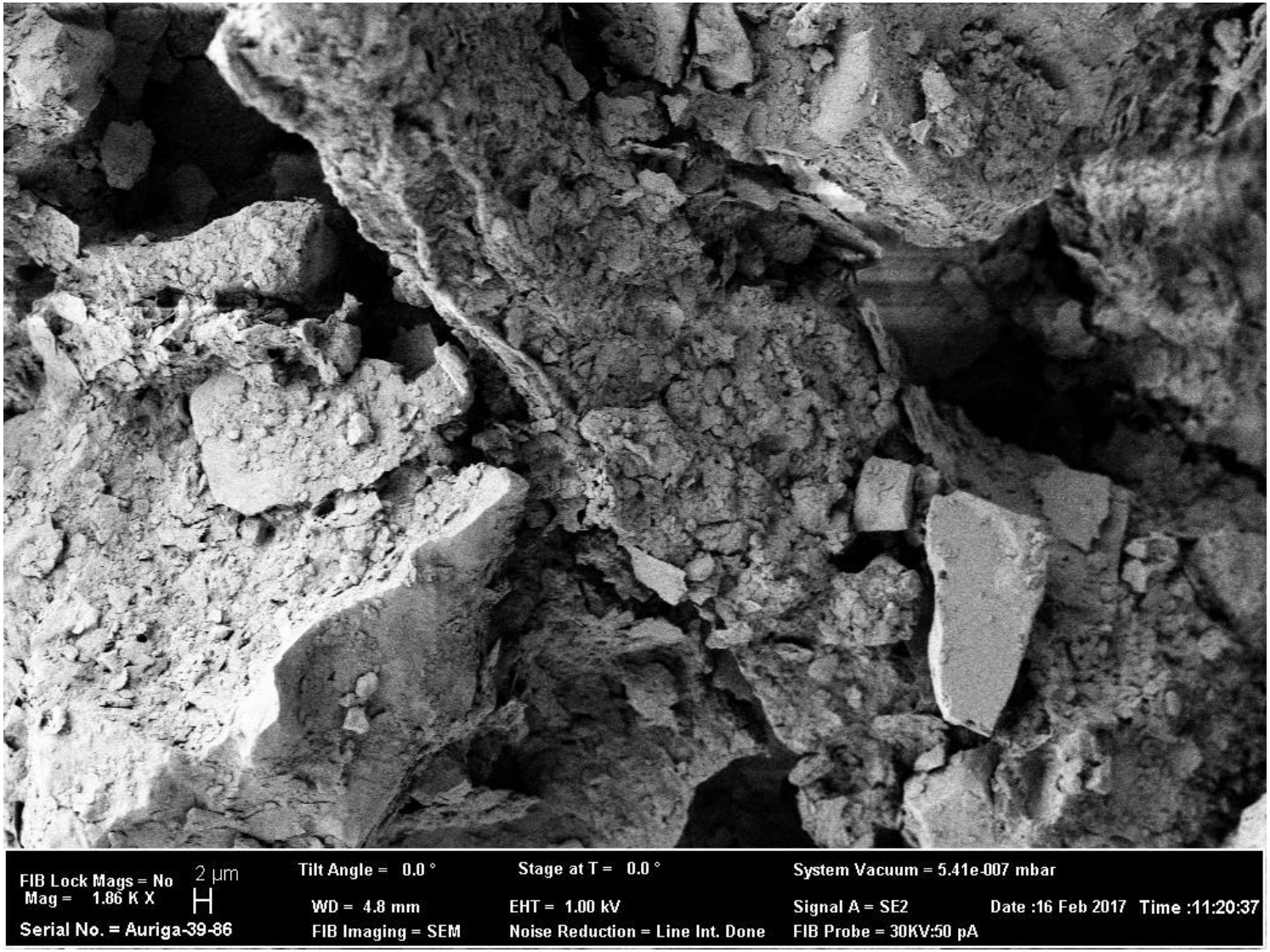

Figure 10: "Zoomed-in" FIB-SEM image of CA+A column soil grains (1-2 cm depth)

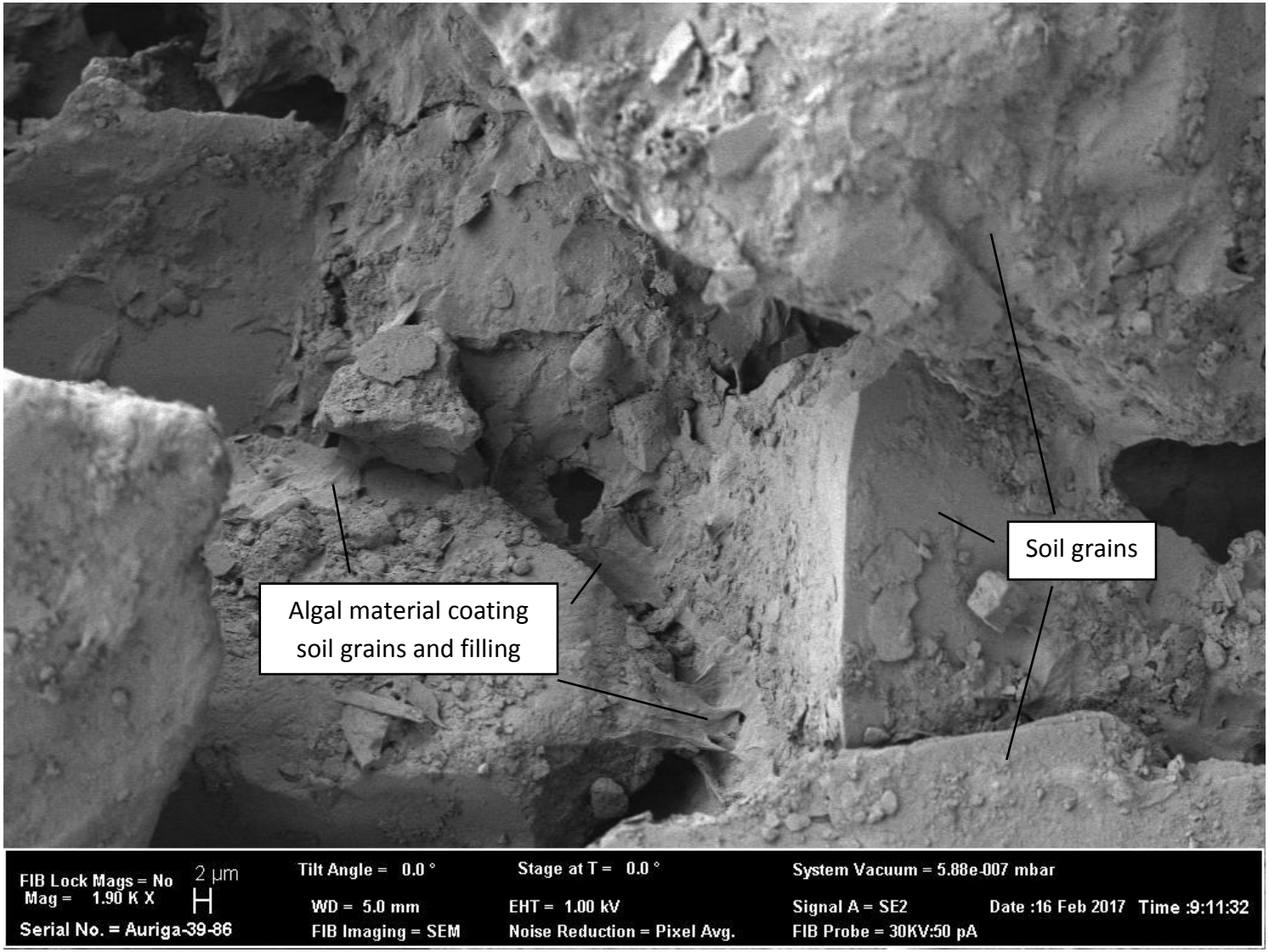

Figure 11: FIB-SEM image of CA+A column soil grains and interstitial spaces with algal material (0-1 cm depth)

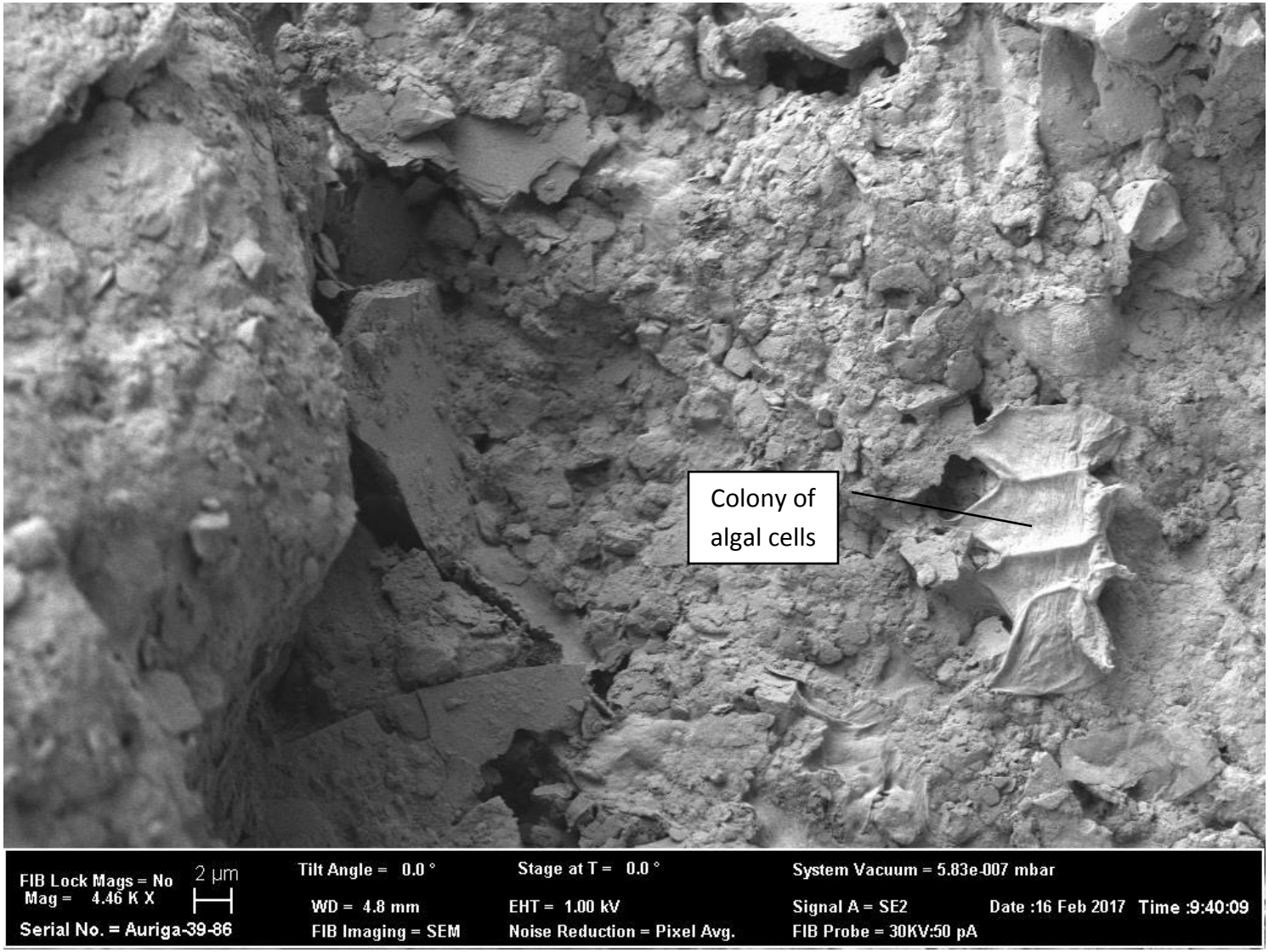

Figure 12: FIB-SEM Image of CA+A column soil with algal cells and materials (0-1 cm depth)

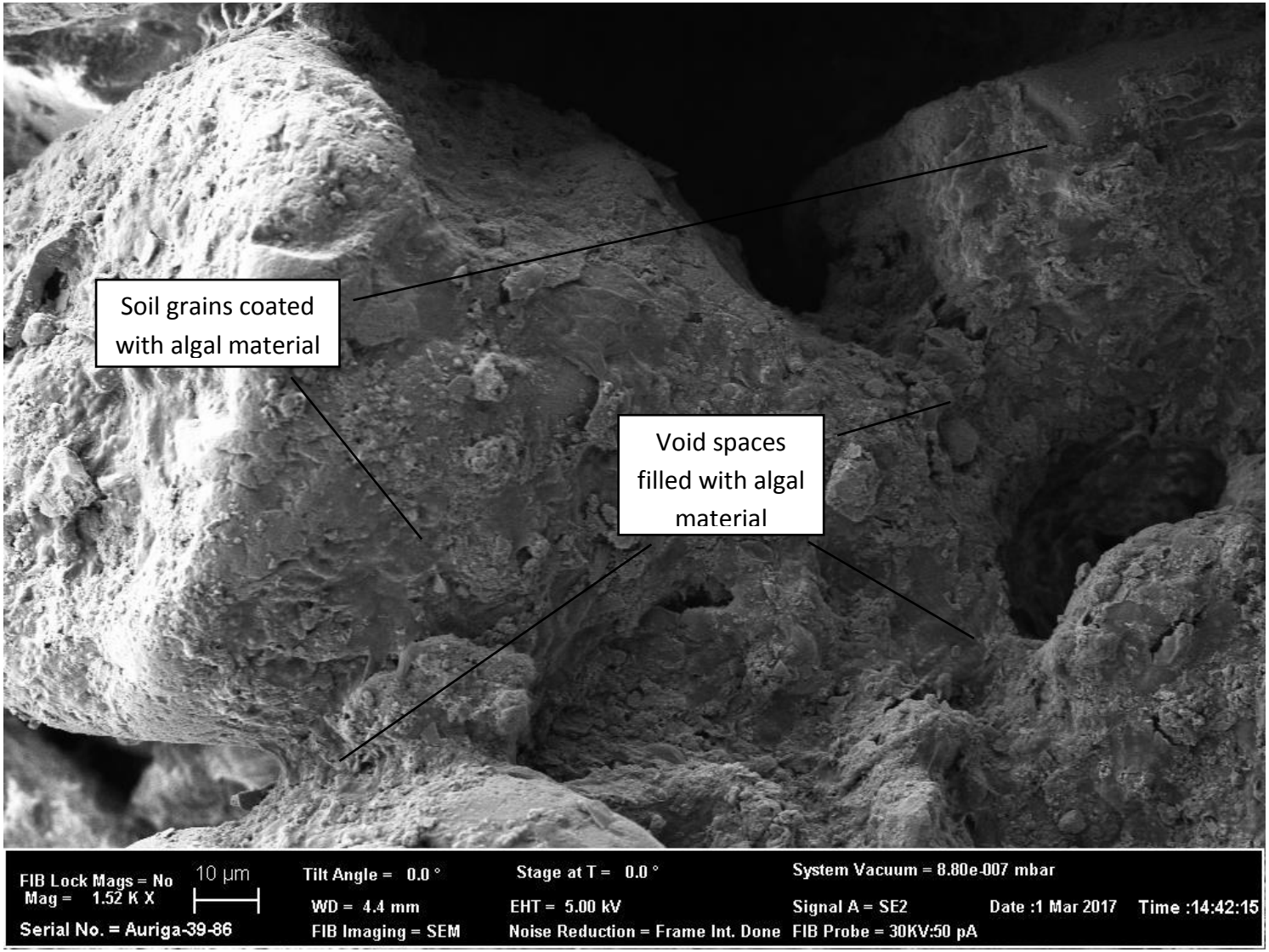

Figure 13: FIB-SEM Image of CA+A column algal material coating and connecting grains within algal mat (0 cm depth)

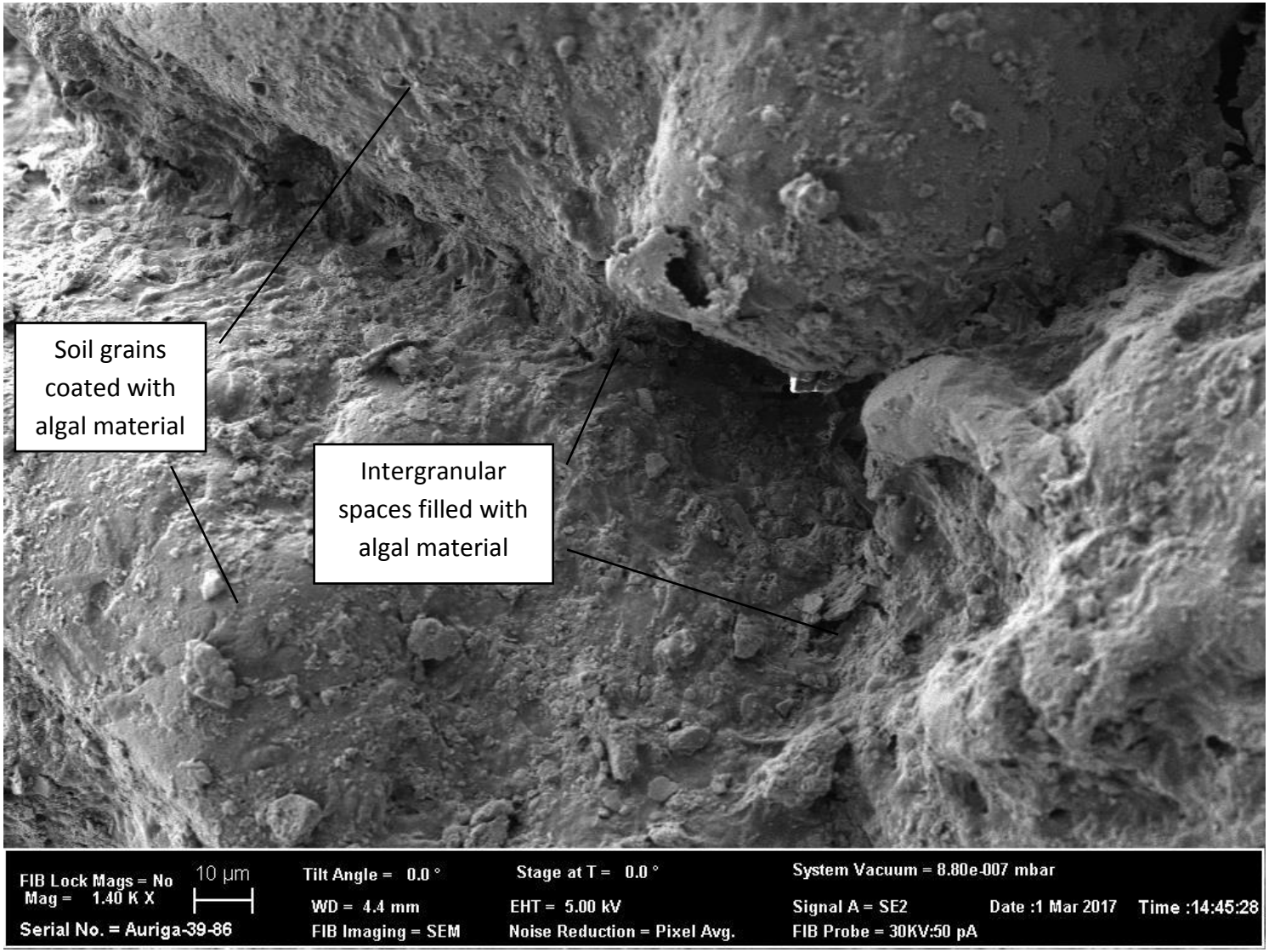

Figure 14: FIB-SEM image of CA+A column algal material coating grains and interstitial spaces within algal mat (0 cm depth)

Statistical Analyses

Saturated hydraulic conductivity is a complex characteristic of porous media that describes fluid movement through a medium and can generally be thought of as the resistance of a porous medium to fluid flow. There are many factors that can contribute to changes in K_S within a repacked soil column: soil characteristics, the packed bulk density of the soil column, bulk density changes resulting from soil compaction due to the hydraulic head or redistribution of fine soil particles, etc. To validate the results of this study, these factors were investigated by statistical analyses to determine if they significantly influenced the saturated hydraulic conductivity reductions of the columns receiving solution treatments. The soil column experiments of the preliminary tests on FB and WB soils were not included, as the experiments were not replicated. The experiments on TX soils of Phase II and the experiments of Phase III on CA and TN soils were replicated (Table 8) for greater confidence in the interpreted results of these experiments.

Influence of Factors Affecting K_S and Bulk Density

To determine if the treatment applied to the soil column or the type of soil tested more greatly influenced the changes in hydraulic conductivity observed in the experiment, a two-way analysis of variance (ANOVA) with replication was performed to determine if there was interaction between the two factors soil and treatment (Table 9). The null hypothesis of this ANOVA is that if the F-value equals zero, the two factors do not interact. The ANOVA resulted in an F-value for the interaction term of 0.01; the hypothesis was accepted. The soil and treatment do not interact.

The second two-way ANOVA excludes interactions of the two treatments (Table 10). In this ANOVA, the null hypothesis is that if the F-value equals zero, both factors equally affect the change in K_S. The F-value for soil is close but not equal to zero. This implies that the differences in soils do affect K_S, but not greatly. The F-value for the treatment factor is much greater than zero. Therefore, the K_S reductions are significantly affected by the treatment solution.

Plotting the K_S reductions (Table 8) of the control and algae treated columns of by treatment visually displays the different magnitudes of K_S reductions resulting from the two

Table 8: Changes to K_s and Bulk Density

Soil	Treatment	K_S Reduction (%)	Δ Bulk Density (%)
TX	Control	6.623	21.749
TX	Control	60.834	22.358
TN	Control	39.744	-1.283
TN	Control	39.680	-0.477
CA	Control	27.671	-0.064
CA	Control	48.140	0.836
TX	Algae	84.041	24.08
TX	Algae	95.292	27.116
TN	Algae	98.091	0.054
TN	Algae	97.711	-0.194
CA	Algae	98.572	2.211
CA	Algae	95.782	2.11

Table 9: ANOVA with Replication Statistics

Factor	K_S Reduction	Δ Bulk Density
Soil	$F(2,6) = 0.198$, $p = 0.82578$	$F(2,6) = 798.983$, $p = 5.35E^{-8}$
Treatment	$F(1,6) = 34.438$, $p = 0.00108$	$F(1,6) = 13.513$, $p = 0.010$
Interaction	$F(2,6) = 0.010$, $p = 0.99013$	$F(2,6) = 2.076$, $p = 0.207$

Table 10: ANOVA without Replication Statistics

Factor	K_S Reduction	Δ Bulk Density
Soil	F(2,8) = 0.263, p = 0.775369	F(2,8) = 624.92, p = $1.64E^{-9}$
Treatment	F(1,8) = 45.765, p = 0.000143	F(1,8) = 10.65, p = 0.0115

different solution treatments (Figure 15). Plotting the K_S reductions by soil type shows little difference between the resulting changes in K_S due to the different soils treated (Figure 15).

The initial packed bulk densities (Tables 6 and 7) and the final post-flow bulk density measurements of each column were used to calculate the change in bulk density that occurred over the duration of the flow experiments (Table 8). Positive values indicate soil compaction to generate a higher post-flow bulk density. Negative values indicate a lower bulk density than the initial packed bulk density that might be attributed to the out-washing of fines. To determine if soil type or treatment affected the change in bulk density in the soil columns the two two-way ANOVAs were performed. The first two-way ANOVA with replication (Table 9) evaluates whether these factors interacted to change the bulk density of the soil columns. The F-value for the interaction term for the bulk density ANOVA is 2.076 with a p-value of 0.2065. Since this number is not equal to zero, there is interaction between soil type and treatment in changing the bulk density. However, this number is not significant, suggesting that this interaction is fairly minor.

The second two-way ANOVA excludes interactions of the two treatments (Table 10). In this ANOVA, the hypothesis is that if the F-value equals zero, soil and treatment equally affect the change in bulk density. However, the F-value for soil is much greater than zero. This means that the type of soil significantly affects the change seen in the bulk density. Since the F-value for treatment is 10.65, which is relatively close but not equal to zero, treatment has a minor but significant effect on the change in bulk density during the experiments. This becomes apparent by examining this information visually by box plots (Figure 16).

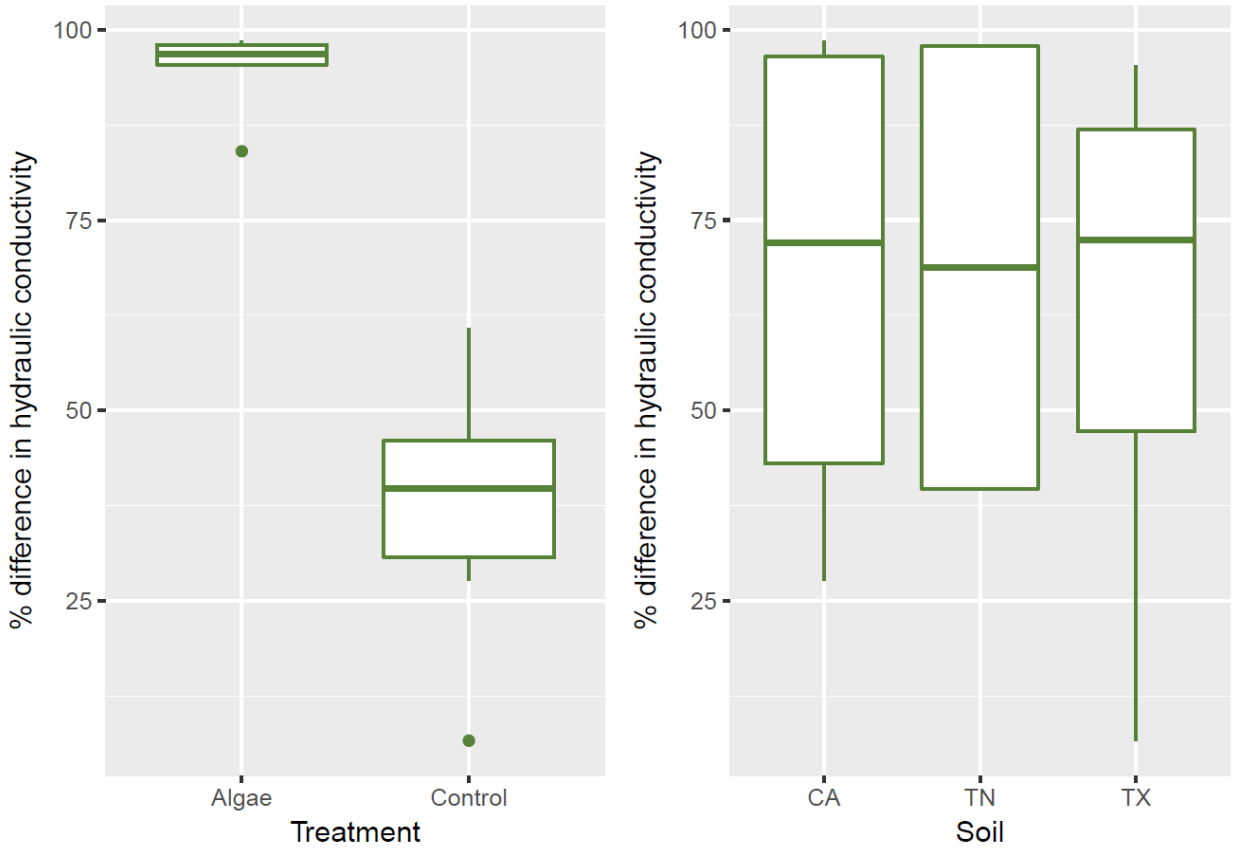

Figure 15: K_s Reductions by Treatment and Soil

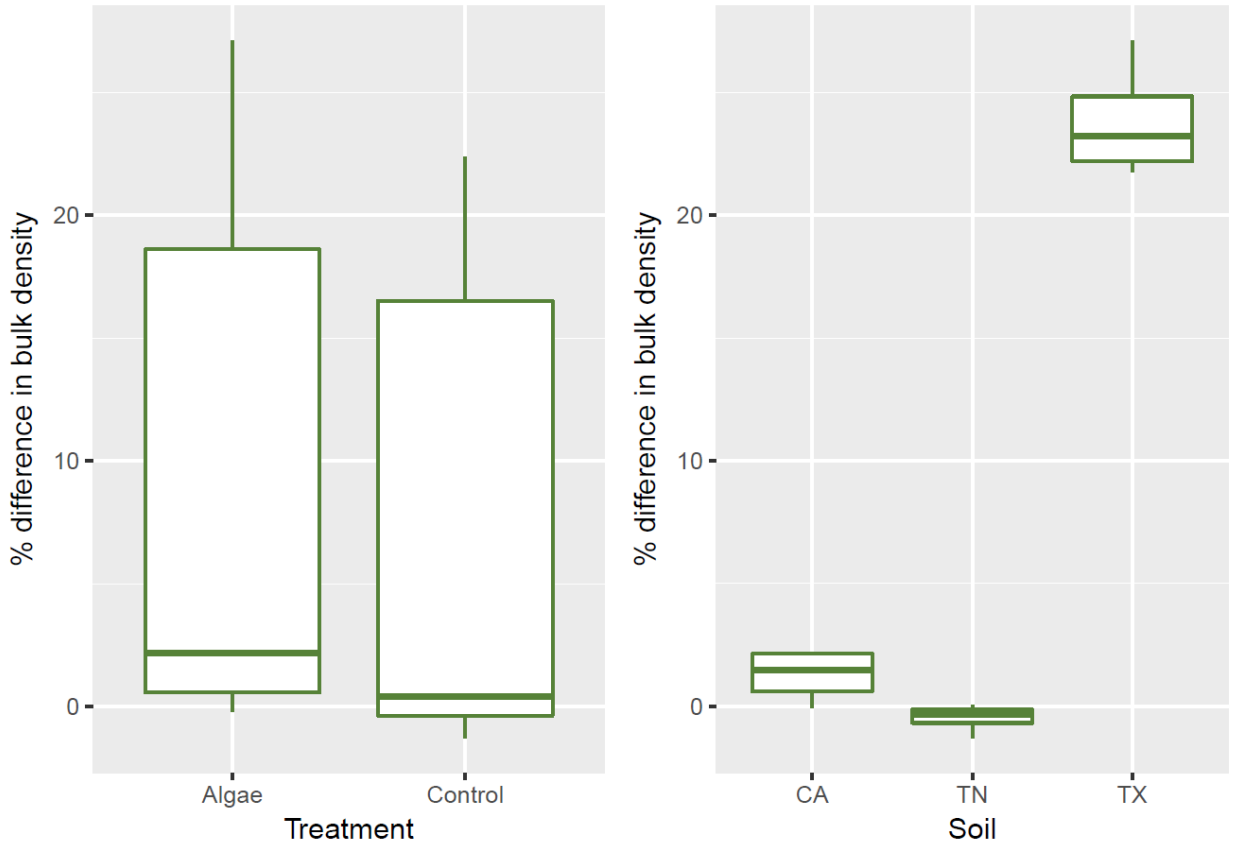

Figure 16: Change in Bulk Density by Treatment and Soil

Effect of Treatments on K_S and Bulk Density

Plotting the K_S reductions and changes in bulk density of the control columns and the algae treatment columns (Table 8) visually displays the differences between the resulting K_S and bulk density changes of the two different solution treatments (Figures 15 and 16). However, to determine if this difference in K_S of the control and algae treatments is statistically significant, first F-tests were performed to determine if the variances of the control group and the variances of the algae group are equal (Table 11). The results of the F-tests reveal there is a slight difference between the variances of the control and algae treatment groups for the changes in K_S. Therefore, an unpaired Welch Two Sample t-test was chosen to determine if the treatment's influence on K_S or on bulk density was statistically significant (Table 11). The results of the t-test indicate that the treatment does significantly affect the saturated hydraulic conductivity but does not significantly contribute to the changes in bulk density.

Table 11: Effect of Treatment on Changes in K_S and Bulk Density

Factor	Treatment	Mean Differences (%)	F-Test	T-Test
K_S	Algae	94.915	F(5) = 0.087, p = 0.018	t(5) = 7.327, p = $3.653E^{-4}$
	Control	37.115		
Bulk Density	Algae	9.230	F(5) = 1.222, P = 0.831	t(5) = 0.291, p = 0.777
	Control	7.187		

In summary, these statistics validate the results observed in this study. Changes in bulk density during flow are primarily due to differences in the soils tested, although treatment does have a small effect on this change as well. Most importantly, the treatment supplied to the soil columns controls the observed saturated hydraulic conductivity reductions. Neither soil type, nor the changes in bulk density, significantly contributes to the K_S reductions.

Chapter 4: Conclusions and Future Work

Plastic pond liners are one of the major contributors to the high capital costs of biofuel production. Column studies investigated the potential of applying readily available fluids in algal biofuel production as in-situ soil treatment technologies to reduce the saturated hydraulic conductivities of soils beneath algae production facilities. Application of nutrient solution proposed for culture media alone did not produce substantial clogging in the loam or fine sand soils tested. However, the addition of the carbon substrate glycerol to the nutrient solution resulted in significant enhancement of microbial growth resulting in maximum reductions of saturated hydraulic conductivity greater than two orders of magnitude in the loam and fine sand columns. By treating these three soils with the contents of the production ponds, i.e., algae growing in the nutrient solution, a dense algal mat developed on the surface of the soil columns, which appeared to be responsible for the approximately two orders-of-magnitude reductions achieved in all three soils: the loam, loamy sand, and fine sand soils.

This new technology appears to be successful in reducing the saturated hydraulic conductivity of native soils. However, these treatments alone do not achieve adequately low K_S values in the soils tested to replace plastic pond liners. The algae test columns produced an average volume of 3,809 cm^3 over the average flow duration. While this is significantly lower than the 24,953 cm^3 of soil water simulant that infiltrated the average control column, it still constitutes a significant volume of fluid infiltrating the soils. If the infiltration rate of the algae columns was scaled-up to production levels, it would correspond to a loss 33,402 m^3/d of cultivation fluids for a 10-acre pond. The cultivation fluids are a valuable water resource and composed not only of the algae itself, but also nutrients such as nitrogen and phosphorous. Loss of cultivation fluids would necessitate increase water consumption and nutrient application, driving up production costs and potentially contaminating the underlying groundwater, especially in the case of production with wastewater. In the case of the soils tested, pond liners would still be necessary.

Greater reductions of K_S (~2x those achieved) are needed in order to reach the target K_S value of approximately 10^{-7} cm/s and to be protective of the underlying groundwater. These findings suggest the technology should be studied further, perhaps in conjunction with soil

compaction during pond construction, to determine its utility and durability as an alternative to plastic pond liners.

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Appendices

Appendix A

Nutrient Solution Recipes

Stock Preparations for WCL-1 Nutrient Solution

Use at 1mL/L media. Store at 4˚C. Use these six stocks in addition to the five F/2 stocks plus vitamins to make WCL-1 media. WCL-1 is made using DI H_2O.

1) Dissolve 0.6625g NH_4Cl into 250 mL DI H_2O
2) Dissolve 4.7675g $Na_2B_4O_7 \cdot 10\ H_2O$ into 250 mL DI H_2O
3) Dissolve 1.8625g KCl into 250 mL DI H_2O
4) Dissolve 9.2425g $MgSO_4 \cdot 7\ H_2O$ into 250mL DI H_2O
5) Dissolve 9.19g $CaCl_2 \cdot 2\ H_2O$ into 250ml DI H_2O
6) Dissolve 3.15g $NaHCO_3$ into 250ml DI H_2O

Recipe for F/2

In use, they are 1 mL/L. Store at 4˚C

1. Dissolve 15 g $Na_2SiO_3{\cdot}9H_2O$ into 500 mL DI H_2O
2. Dissolve 2.5g $NaH_2PO_4{\cdot}H_2O$ into 500ml DI H_2O
3. Dissolve 37.5g $NaNO_3$ into 500ml DI H_2O

To prepare Working Stock Trace Metals:

In use 1 mL/L.

1. Dissolve 2.18g Na_2EDTA (Disodium Salt C10H14N2Na2O8.2H2O) into 500 mL DI H_2O
2. Add 1.58g $FeCl_3{\cdot}6H_2O$
3. Add 0.5ml of Primary Stock Trace Metals

To prepare 50 mL Primary Stock Trace Metals:

Refrigerate and save the Primary Stock Solution, it can be used for years

1. Dissolve 0.098g $CuSO_4{\cdot}H_2O$ into 10 mL DI H_2O
2. Dissolve 0.22g $ZnSO_4{\cdot}7H_2O$ into 10 mL DI H_2O
3. Dissolve 0.10g $CoCl_2{\cdot}6H_2O$ into 10 mL DI H_2O
4. Dissolve 0.18g $MnCl_2{\cdot}4H_2O$ into 10 mL DI H_2O
5. Dissolve 0.063g $NaMoO_4{\cdot}2H_2O$ into 10 mL DI H_2O

To prepare Working Stock Vitamins:

Cold filter sterilize using a 0.2 µm filter. In use 0.5 µL/mL media

1. Dissolve 20 mg Thiamine HCL into 100 mL DI H_2O
2. Add 1.0 mL of Biotin Primary Stock
   Dissolve 1.0 mg Biotin into 9.6 mL DI H_2O for Biotin Primary Stock (Store Frozen)
   Allows for ~11% H_2O crystallization

3. Add 0.1 mL B12 Primary Stock (1 mg/mL) (Store Frozen)
   Dissolve 1.0 mg B12 into 0.89 mL DI H_2O
   Allows for ~4% H_2O crystallization

Appendix B

Scenedesmus dimorphus Culture Cell Density vs. Optical Density

Table B.1: Cell and Optical Densities for *Scenedesmus dimorphus* Stock Culture Dilutions

Cells/mL	Average OD_{750}
726,190	0.0200
94,536	0.0050
191,694	0.0077
421,986	0.0107
559,674	0.0147

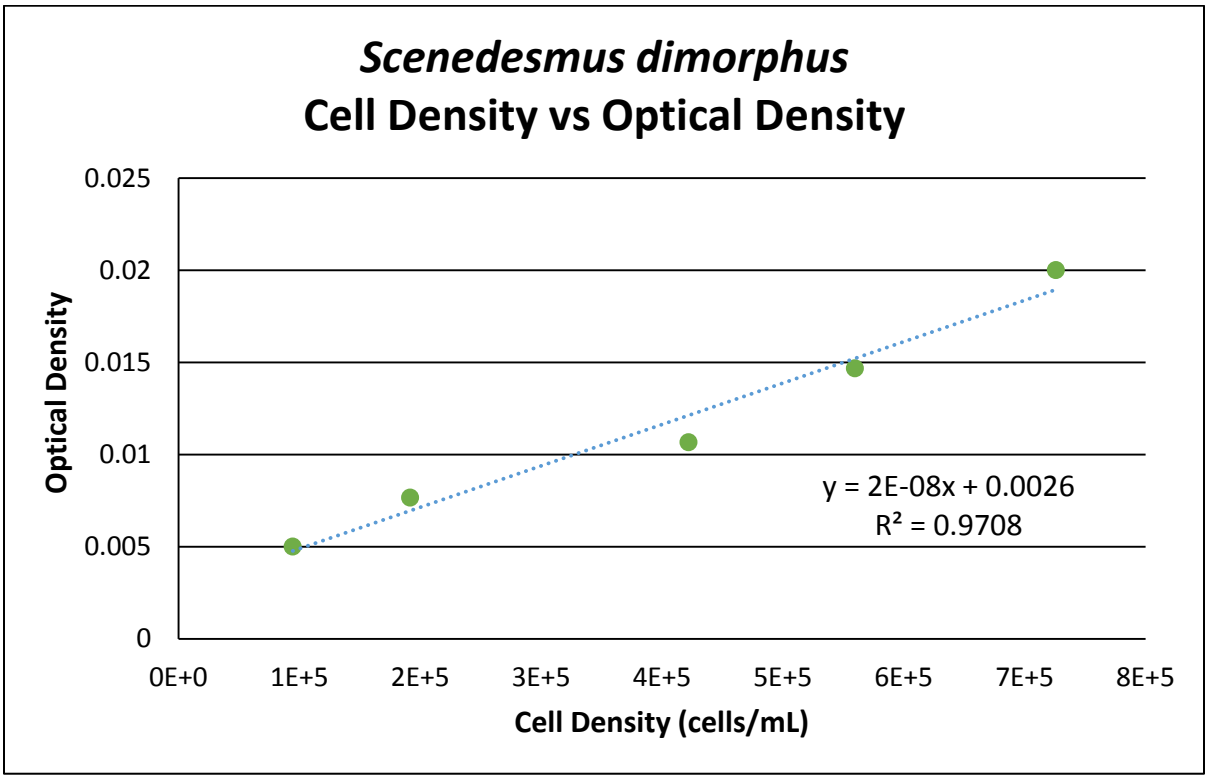

Figure B.1: Scenedesmus dimorphus Stock Culture Dilutions

Appendix C

Preliminary Experiment Charts

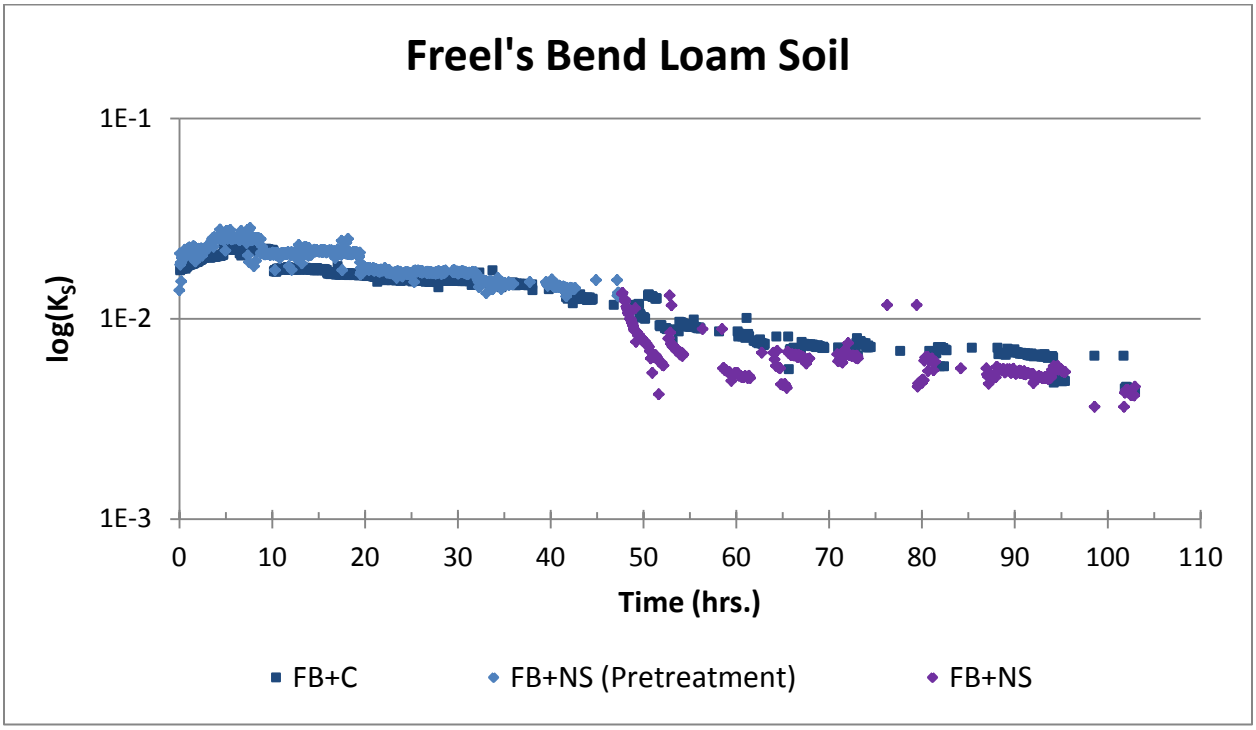

Figure C.1: Preliminary Experiment on 100% Freel's Bend Loam Soil

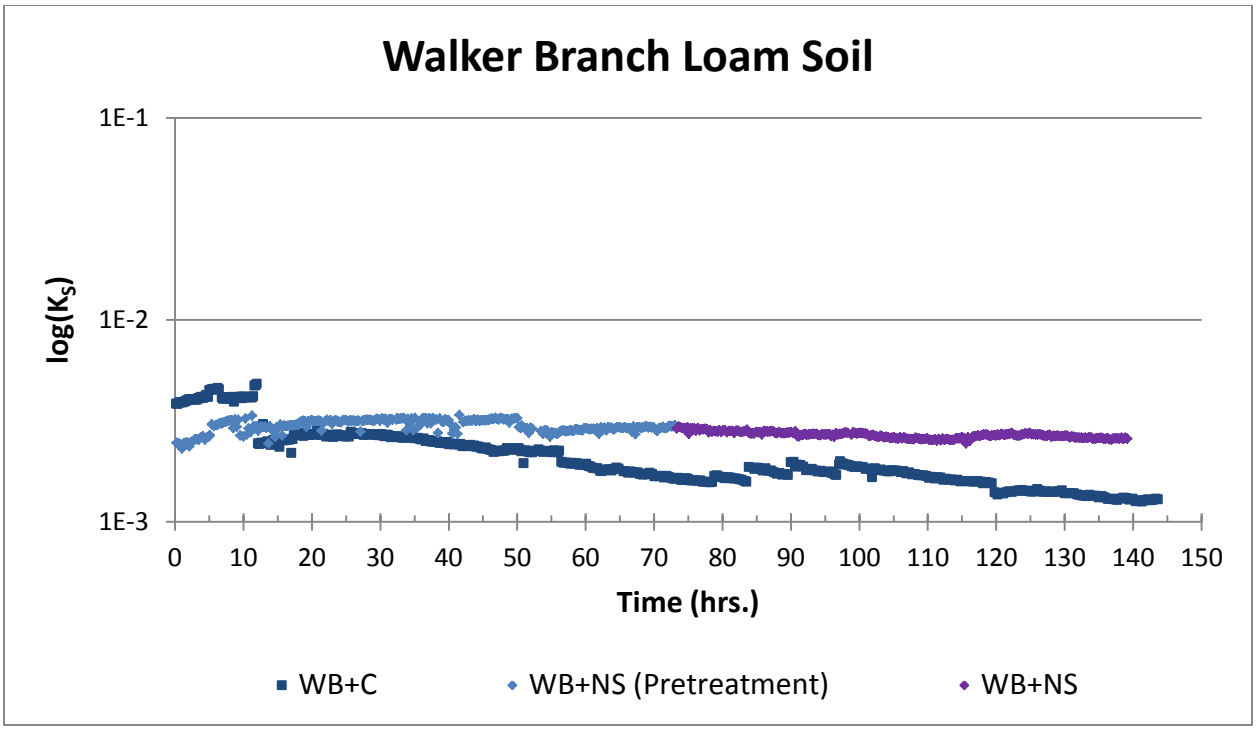

Figure C.2: Preliminary Experiment on 100% Walker Branch Loam Soil

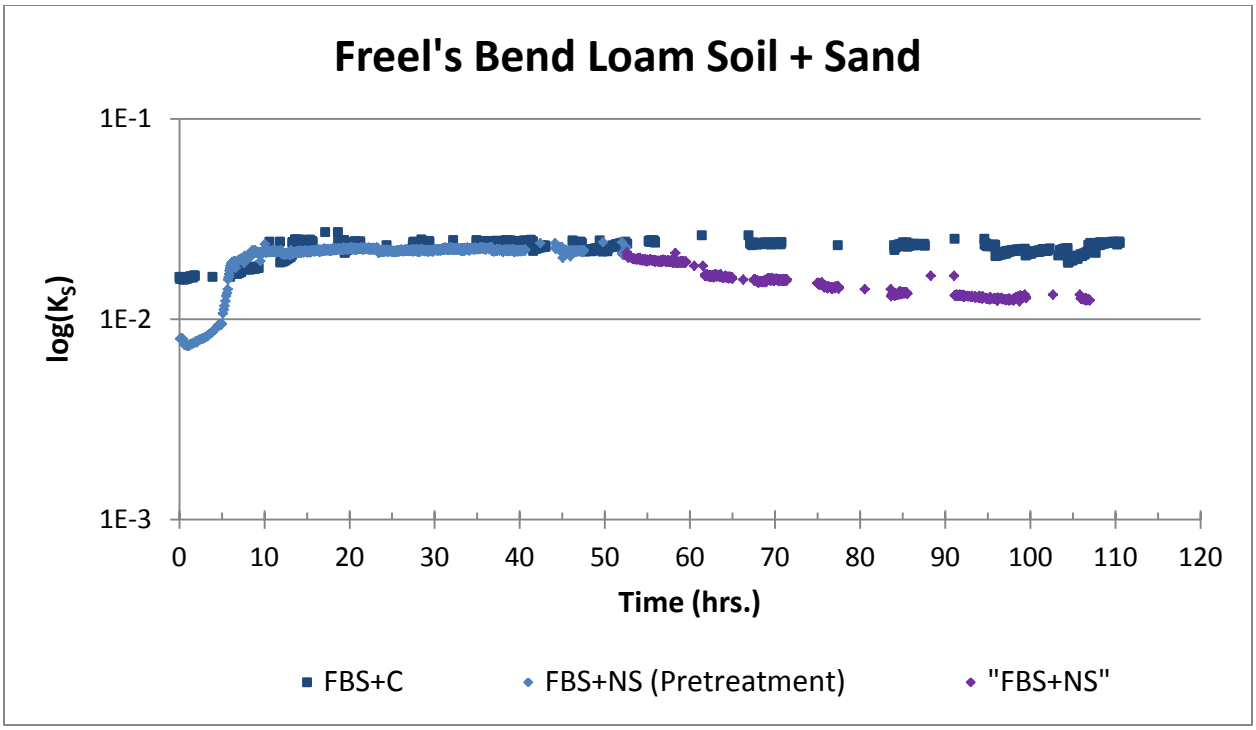

Figure C.3: Preliminary Experiment on 60% Freel's Bend Loam Soil Mixed with Sand

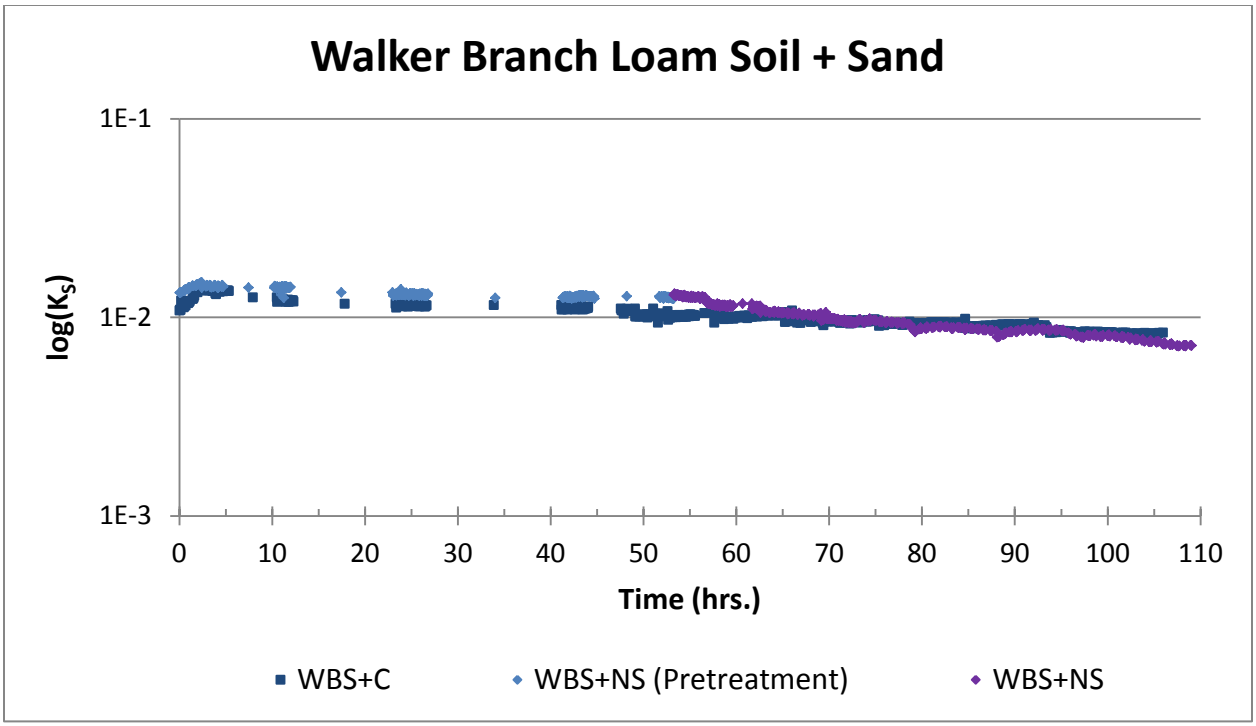

Figure C.4: Preliminary Experiment on 60% Walker Branch Loam Soil Mixed with Sand

Vita

Molly Brianne Pattullo was born on Fort Walton Air Force Base to Mary Jane and Brad Pattullo. Molly attended high school at the Arkansas School for Mathematics, Sciences, and the Arts (ASMSA) in Hot Springs, AR. It was during a high school research program at the University of Arkansas at Little Rock that Molly was first exposed to the geological sciences through a project on X-ray diffraction and its application for analyzing crystal structures and mineralogy of Mars analog soils. After graduating from ASMSA in 2008, Molly enrolled in the Craft and Hawkins Department of Petroleum Engineering at Louisiana State University and attended a freshman summer field course in geology. This experience eventually inspired Molly to study geology. She transferred to Arkansas Tech University in Russellville, AR in 2011. In 2012, she became an undergraduate research fellow for a project analyzing sedimentation patterns in a large lake in Central Arkansas used as a municipal water resource. She earned her B.S. in Geology in 2013. She then worked for a year as a pari-mutuel teller at a horse track and hospitality clerk at a hotel in her hometown. Molly got back to playing in the dirt in 2014 when she entered the graduate program in the Department of Earth and Planetary Sciences at the University of Tennessee. Molly graduated with her Masters of Science in Geology in May 2017.