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Soil Sealing by Algae: An Alternative to Plastic Pond Liners for Outdoor Algal Cultivation

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Abstract

Commercial production of algal biofuels is currently limited by high capital costs, particularly costs of synthetic pond liners that minimize seepage of cultivation fluids, salts, and nutrients into the subsurface beneath outdoor algae cultivation facilities. Even some profitable nutraceutical companies bear the high costs of pond liners. However, studies of animal waste holding ponds and aquaculture facilities have shown that underlying soils can effectively "self-seal", potentially eliminating the need for plastic liners. Here, we explored the potential for self-sealing with algae to provide an alternative to synthetic pond liners in unlined algae cultivation ponds. Laboratory-scale soil column experiments investigated soil clogging by algae, a topic rarely discussed in the literature. Columns composed of fine sand, loamy sand, and loam soil displayed reductions in saturated hydraulic conductivity of the entire soil column of up to two orders of magnitude in response to infiltration by the alga *Scenedesmus dimorphus* suspended in algal culture nutrient solution at a density of 5×10^8 cells L^{-1}. Scanning electron microscope imaging showed a dense algal layer on the surface of each soil column, thickly coating mineral grains with algal material and filling pore spaces. The algal coating was absent in samples at more than 3 cm depth in the soil columns, and measurements of chlorophyll content also confirmed that algal cells were mostly confined to the soil surface. In this study, the application of an algal suspension contributed significantly to soil sealing. This new application of biological sealing technology should be studied further to determine its utility and durability as an alternative to plastic liners, perhaps in conjunction with soil compaction to achieve hydraulic conductivities beneath algal production ponds that are protective of groundwater quality.

Keywords

Pond liner; soil sealing; schmutzdecke layer, pore clogging; infiltration; hydraulic conductivity; unlined ponds

1. Introduction

Global microalgae biomass production is about 20,000 dry tons per year, with 90% of the production for food and nutraceuticals (Benemann et al., 2018). However, microalgae are also an attractive biofuel feedstock option due to rapid growth rates, high areal biomass yield, high energy content per unit mass, reduced land and water footprints, and capacity for greenhouse gas fixation (Galvrilescu & Chisti, 2005; Alam et al., 2012; Maity et al., 2014; Benson et al., 2014). Despite this potential, microalgal biofuels have yet to be produced economically on a commercial scale due to high capital investment and operating costs (Han et al., 2015).

For open pond or raceway algae cultivation systems, as opposed to closed photobioreactors, synthetic pond liners are often used to mitigate potential water loss due to seepage and to prevent the release of salts and nutrients into the subsurface or groundwater. Liners may be required by local or US state permits for some algae cultivation applications. Technological and economic analyses have found that synthetic pond liners can constitute between 24%-75% of capital costs for algal biofuels, depending on the scale of production (Davis et al., 2012; Rogers et al., 2014; Coleman et al., 2014). It is acknowledged that liners are economically unfeasible for commodity-scale products that have low or no profit margin, but technological and economic assessments continue to include liner costs because too many uncertainties remain regarding the ability of underlying soils to effectively contain pond waters (Davis et al., 2016; DOE/EIA, 2014; USDOE, 2016). Moreover, emissions associated with synthetic pond liner manufacturing and

transportation can offset greenhouse gas emission reduction benefits of algae as a feedstock, with overall 39% higher greenhouse gas emissions in lined ponds as compared to unlined ponds (Canter et al., 2014).

Unlined ponds have been used for cultivation of algae in many aquaculture applications (Tucker et al. 2008); however, concerns remain about potential leakage for some applications such as liquid biofuel production. For example, similar productivities were found in both lined and unlined ponds as part of the US Department of Energy-funded Aquatic Species Project, which tested unlined algae ponds for two years finding no observable leakage (Weissman et al., 1989; Weissman & Tillett, 1992). Some authors have proposed limiting construction of unlined ponds for algae to locations with soils that have naturally low hydraulic conductivity due to high clay content in the soils (Venteris et al., 2014; Lundquist et al., 2010). Although many natural soils have clay contents sufficient for use as a lining material, targeting only locations with clayey soils or naturally low hydraulic conductivities would severely limit options for potential open pond facility locations. The development of infiltration-resistant soil conditions in situ could provide a satisfactory alternative for synthetic liners that would improve profitability and maintain pond water quantity and groundwater quality.

An example of in situ mitigation of soil seepage is found in animal waste holding ponds. As family farms expanded into large-scale factory farms, the industry turned to manure lagoons to store and stabilize biological wastes. Over time, an abundance of laboratory- and field-scale studies of animal waste settling ponds have established that, regardless of the soil texture or the type of waste, soils underlying waste ponds develop low-hydraulic conductivity seals within days to weeks after construction, often eliminating the need for synthetic 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; SNTC, 1993; Maulé et al., 2000; Tyner et al., 2006; Cihan et al., 2006). Aquaculture production also relies on unlined ponds. Ponds are often located in clayey and silty soils, and compaction may be used to minimize seepage (Stone & Boyd, 1989; Chumnanka et al., 2015; Tucker et al., 2017). For soils underlying most ponds, saturated hydraulic conductivities around 1 x 10^{-7} cm s^{-1} are desired in order to prevent seepage of nutrients and contaminants from the pond into the subsurface (Daniel & Benson, 1990), although greater values may be allowed by some states (Parker et al., 1999).

Some animal waste pond-sealing studies were conducted in actual or pilot-scale lagoons; however, many authors used laboratory-scale soil columns to test for successful sealing and to provide insights into the mechanisms of soil clogging by animal waste. There is a long history of using laboratory-scale column studies for observations of porous media clogging. Advantages of this approach are manageable column sizes and volumes of infiltrating solutions, flexibility in simulating various environmental and field situations, and the relative ease of methods to determine hydraulic conductivity (McGauhey & Krone, 1967; McDowell-Boyer et al., 1986; Baveye et al., 1998). The literature indicates that clogging is primarily a surface phenomenon, occurring within the uppermost soil depths or at the soil-liquid interface, and is primarily the result of physical straining of suspended fine particles and colloids and the buildup of microorganisms and their products within soil pores. In fact, management of a variety of effluents using slow sand filtration relies on the buildup of microbes and their by-products in the surface in a "schmutzdecke layer" to enhance filtration of harmful bacteria and facilitate water recycling (McNair et al., 1987; Livingston & Slack, 2014).

Although bacteria are the most widely discussed microorganisms in literature on biological clogging of porous media, bacteria are not the only microorganisms capable of clogging pores.

The majority of microorganisms, both prokaryotic (bacteria) and eukaryotic (algae) tend to produce substantial amounts of extracellular polysaccharides and live and grow in polymicrobial aggregates forming flocs, biofilms, or mats (Wingender et al., 1999). Nonetheless, few investigators have examined the role of algae in clogging porous media.

Ragusa et al. (1994) investigated benthic algae and bacteria as a low-cost seepage control option for irrigation channels. In their study, the authors inoculated a fine sandy loam soil with algae and bacteria. They attributed the greater than 80% reduction in hydraulic conductivity over 16 days to the development of an algal/bacterial mat within the first few millimeters of inoculated soil columns and the associated production of extracellular polysaccharides by the algae and bacteria within this layer. Battin and Sengschmitt (1999) pointed out the role of algae in clogging the riverbed of 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 of 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 due to the development of a dense algal mat on the surface of the soil. The authors also associated greater clogging with algal biomass compared to buildup of bacterial biomass or physical filtration of particulate organic matter on the surface.

The burden of pond liners to the profitability and sustainability of algae biomass production, and in particular, algal biofuel production, calls for investigations into alternatives to replace synthetic liners. Perhaps the simple, economical techniques used for the in situ sealing of soils could be applied to algae production ponds to reduce saturated hydraulic conductivity and eliminate the need for expensive synthetic pond liners in many locations. Here, we use laboratory-

scale columns to study soils of various types to determine the magnitude of reductions in hydraulic conductivity in response to application of standard algal culture medium, thereby determining the extent to which algae can enhance clogging of porous media.

2. Materials and Methods

2.1 Soils

Two soil types were collected from locations where algal biofuel production is occurring, plus one additional soil type (Table 1). The Texas (TX) soil was sampled from the site of an outdoor raceway system test bed in Corpus Christi, Texas, USA. This soil is classified as the Galveston and Mustang fine sand (Soil Survey Staff, 2016). The California (CA) soil was sampled from the Delhi County Water District's microalgae wastewater treatment plant in Delhi, California, USA where algal biofuel production in conjunction with wastewater treatment is being studied. The CA soil is classified as the Delhi loamy sand (Soil Survey Staff, 2016). The Tennessee (TN) soil is a loam corresponding to the Etowah series (Soil Survey Staff, 2016) and was sampled from Freel's Bend on the Clinch River located on the Oak Ridge National Laboratory Reservation in Oak Ridge, TN, USA.

Prior to experimentation, soil samples were sieved to <2 mm. Particle size distribution, gravimetric water content, soil pH, carbon (C), nitrogen (N), and microbial biomass carbon were determined in the lab (Table 1). Field bulk density measurements were obtained from National Resources Conservation Service's web soil surveys (Table 1) (Soil Survey Staff, 2016). The Bouyoucos hydrometer method was used for particle size analysis (Gee & Or, 2004). Soil pH was determined by shaking a 2:1 ratio of 0.005 mol L^{-1} $CaCl_2$ solution to soil solids for 2 hours, then measuring the pH of the supernatant after centrifugation. Total C and N were measured by

combustion on a LECO TruSpec CN analyzer (LECO Corporation, St. Joseph, MI, USA). Total organic C (TOC) was determined by the standard method of removing total inorganic C (TIC) by treating the soil with 3 mol L^{-1} 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 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 which is estimated as 0.45 (Vance et al., 1987; Beck et al., 1997).

2.2 Soil Columns

Permeameters were constructed from 15.2 cm long by 2.54 cm 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 0.32 cm inner diameter tube adapter. The soil was supported by a laser-cut flow diffuser overlain by 316 stainless steel wire cloth with 0.004 cm openings and a 0.065 cm opening screen to prevent channelized flow and loss of fines. All parts interacting with the soil or solution were sterilized by autoclave (STERIS Corporation, Mentor, OH, USA) to prevent microbial contamination. The columns were packed to a depth of 11 cm by dividing the required mass of soil into ten portions and adding each portion to the column in even fragments, gently tamping down each portion using a plastic rod to achieve uniform packing. Columns were packed to approximate field bulk density values (Table 1) (Soil Survey Staff, 2016), but were adjusted as needed to permit gravity flow. The columns were then saturated slowly from beneath with a soil water simulant (see below) over a period of six days and then allowed to stand for two days to facilitate dissolution of entrapped air. To simulate an algal pond, a 4-cm head of solution was ponded directly above the soil surface, and the bottom outlet was clamped shut.

2.3 Solutions

Soil columns were permeated with two liquids in sequential order: (1) soil water simulant and (2) an algal broth composed of the green algae *Scenedesmus dimorphus* growing in a WCL-1 nutrient media (Table 2) (Guillard, 1987), a standard culture medium used in freshwater algal studies (Orona-Navar et al., 2017, Purkayastha et al., 2017). The soil water simulant was 0.005 mol L^{-1} $CaCl_2$ buffered to the pH of the soil (Table 1) using either 5 mol L^{-1} KOH or 0.0005 mol L^{-1} HCl as required. To prevent microbial contamination, all solutions were sterilized as described above.

A dense algal culture was grown in sterile nutrient solution. Every day, the nutrient solution was used to dilute aliquots from the dense algal culture to a density of 5 x 10^8 cells L^{-1}. This dilute algal culture was then applied to the soil columns, as discussed below. Dilution volumes were calculated using the relationship between measured optical density of the starting culture at 750 nm (Schoepp et al., 2014) using a Multiskan FC Microplate Photometer (Thermo Scientific, Waltham, MA, USA) and the target optical density of 0.0126 associated with a cell density of 5 x 10^8 cells L^{-1} as measured by Benchtop FlowCAM (Fluid Imaging Technologies, Yarmouth, ME, USA). The algal culture is stored in a transparent bottle, and constant gentle agitation using a stir plate was used to evenly disperse the cells in suspension (Figure 1). The algal bottle was placed at constant room temperature (20° ± 2° C) with a 14-hour photoperiod of 100 µmol m^{-2} s^{-1} photosynthetically active radiation.

2.4 Experimental Procedure

For each of the three soils (CA, TN, and TX), two replicate pairs of soil columns were constructed. Two control columns for each soil received only soil water simulant for the duration of the experiment (CA+C, TN+C, and TX+C). The other two replicate columns were permeated in sequential order with 1) soil water simulant and 2) algae in the nutrient broth (CA+A, TN+A, and TX+A). The soil water simulant solution enabled quantification of mechanical clogging due to natural microbial growth and particle rearrangement. The second solution enabled quantification of additional clogging and subsequent hydraulic conductivity reductions resulting from the application of algal cells suspended in nutrient solution.

Flow commenced by connecting the solution pond at the top of the columns to a Mariotte device to maintain a constant head within the pond (Figure 1) and opening the bottom outlet (Klute and Dirkson, 1986). The system is a steady-state constant head device and flow was through the

soil column from top to bottom. Solution exited the bottom outlet into a fraction collector below. The flow rate, head values, and column dimensions were used to calculate the saturated hydraulic conductivity with Darcy's Law (Equation 1):

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

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) measured over the column length, L (L). Results are presented as changes in K_S over time (t).

2.5 Statistical Analysis

Statistical testing using R (R Core Team, 2013) was completed to validate the changes in soil saturated hydraulic conductivity observed in the column flow experiments. Changes in K_S (Table 3) were grouped by soil type (TX, TN, CA) and by treatment (control vs. algae). Two-way analysis of variance (ANOVA) was used to study the relationship between the change in hydraulic conductivity and the two independent variables (soil type and treatment), with significance level α=0.05.

An ANOVA with replication was performed to determine if there was significant interaction between soil and treatment. A second ANOVA without replication was performed to determine if soil type or treatment applied more greatly influenced the change in K_S observed in the experiment. An unpaired Welch Two Sample t-test was chosen for determining if the changes in K_S of the algae treated columns are significant compared to the control columns.

2.6 Scanning electron microscope and chlorophyll analyses of column soils

Following the experiments, each column was allowed to drain before sub-samples were taken at depths of 0, 3, and 8 cm from the top of the soil column and stored in airtight containers

at -80°C. A Zeiss Auriga 40 scanning electron microscope (SEM) (Zeiss Group, Oberkochen, Germany) was used for post-flow imaging of soil samples. Samples were frozen at -80 °C, attached to an aluminum stub with conductive carbon tape, and then allowed to air dry. Just prior to imaging, the samples on the stubs were sputtered with a thin layer (~15nm) of gold in an SPI sputter coater (Structure Probe Inc., West Chester, PA, USA). Samples were then imaged in a Zeiss Auriga SEM operating at either 1kV or 5kV using the standard SE detector.

Chlorophyll *a* was extracted from soil samples in acetone as follows. Soils were placed in 50 ml centrifuge tubes which were filled with 10 ml of 90% acetone and placed in an ice bath in a sonicator for 7 minutes at 60 Hz amplitude. Samples were kept on ice in the dark for 30 minutes before centrifuging at 5000 rpm for 5 minutes. The fluorescence of the supernatant was measured before and after acidification with 0.1 N HCl using a 10-AU fluorometer (Turner Designs, San Jose, CA) (Arar & Collins, 1997). The chlorophyll concentration was calculated from the measured extract fluorescence by Equation 2:

$$C = \frac{F \cdot \frac{r}{r-1} \cdot (F_b - F_a) \cdot V_{ext} \cdot D_f}{M_s} \qquad \textbf{(Equation 2)}$$

where C is the chlorophyll *a* concentration in the extract solution analyzed, F is the response factor for the sensitivity setting (F = 1.0427), r is the before-to-after acidification ratio of a pure chlorophyll *a* solution (r = 1.9517), F_b and F_a are the fluorescence of a sample extract before and after acidification, V_{ext} is the extract volume, D_f is the dilution factor, and Ms is the oven-dry mass of the soil sample.

3. Results

3.1 Column Flow Experiments

Initial equilibrium K_S values were quickly established by infiltration of the soil water simulant, following minor adjustments upon onset of flow. Results of the column experiments are presented as graphs of K_S in cm s^{-1} versus time (Figures 2-4), where $T = 0$ occurs at the start of application of algal culture to the columns and the corresponding time in the control columns receiving only the soil water simulant. The magnitude of changes in K_S due to pore clogging by algae (Table 3) reflect the reduction measured from K_0 ($T = 0$ h) to the final K_S measured at the end of six days of flow ($T = 144$ h).

For the soil columns containing the Texas fine sand (Figure 2), TX+A exhibited a decline in K_S values over the first 26 hours of flow, after which point the K_S values leveled off to reach a final, minimum value of 5.66×10^{-3} cm s^{-1}, an 84% reduction from the K_0 value of 3.55×10^{-3} cm s^{-1}. In the replicate column TX+A Rep, a stable reduction of 91% from the K_0 value of 5.59×10^{-3} cm s^{-1} to a final K_S of 2.6×10^{-4} cm s^{-1} was observed.

In the California loamy sand (Figure 3), both algae columns exhibited a decline in K_S immediately upon application of the algal broth to the columns. A constant 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 96% reduction from the K_0 value of 3.0×10^{-3} cm s^{-1} (Table 3). 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 2.3×10^{-3} cm s^{-1}.

The Tennessee loam soil columns (Figure 4) displayed similar K_S reductions of 98% in TN+A and a 96% reduction in TN+A Rep from K_0 values of 9.6×10^{-4} cm s^{-1} and 1.1×10^{-3} cm s^{-}

[1] in TN+A and TN+A Rep, respectively. While the most dramatic decline of K_S in the California soils showed an order-of-magnitude reduction over the first 30 hours, the Tennessee loams exhibited a gentler trend in reduction of K_S, declining by an order of magnitude over the first 34 and 54 hours in TN+A and TN+A Rep, respectively.

3.2 Statistical Analysis

The observed K_S reductions are significantly affected by the algal broth treatment (two way ANOVA without replication $F(1,8) = 45.76$, $p < 0.001$) (Table 3). The changes in K_S of the algae treated columns are significant compared to those observed in control columns; i.e., the two orders-of-magnitude reductions from the initial K_S values observed in the algae columns was statistically significant (unpaired Welch two sample t-test ($t(5) = 7.327$, $p < 0.001$). The contribution to K_S reductions by the type of soil tested was found to be non-significant ($F(2,8) = 0.263$, $p = 0.775$). There was no significant interaction between soil type and the algal culture treatment that resulted in the observed changes in K_S (two way ANOVA with replication, $F(2,6) = 0.010$, $p = 0.990$).

3.3 Scanning Electron Microscopy

At the end of the flow experiments, each soil column was sampled at depths of 0, 3, and 8 cm. When the algae columns were deconstructed for sampling, a dense algal layer was visually observed at the soil-pond interface in each column (Figure 5). No algae was visually observed at depths of 3 and 8 cm.

To examine the algal layer, SEM images were taken of the CA soil columns (Figure 6). No algal cells or materials were visible in the images from the control column, CA+C, at 0 cm depth, demonstrating the soil's loamy sand texture (Figure 6a). In the images from the CA+A at 3 cm depth (Figure 6b), a thin film of algal materials coated the grains of soil, connecting the grains to one another and partially filling some interstitial void spaces. Two dried algal cells are visible in

the image. At 0 cm depth, the CA+A column samples displayed a much thicker buildup of algal materials. The soil grains were extensively coated by algae, and the interstitial voids were partially or entirely filled (Figure 6c).

3.4 Chlorophyll Quantification

Chlorophyll *a* concentrations were calculated with respect to the mass of dry soil for all column samples taken at 0 cm and 3 cm depths (Figure 7). The chlorophyll *a* concentrations for the control columns were not displayed, as they were orders of magnitude lower than the treatment columns (the highest concentration of all control samples was 57 $\mu g\ g^{-1}$). In the columns receiving algae, chlorophyll *a* concentrations ranged from 195 to 63,000 ug g^{-1}, indicating that the added algae permeated into the columns. The chlorophyll *a* concentration at 0 cm depth was approximately two orders of magnitude higher than that observed at 3 cm depth, indicating that most of the algae resided in a concentrated layer near the solution-soil interface.

4. Discussion

In our experiments, we investigated the feasibility of algae to reduce the permeability of soils for application to outdoor algal production facilities. Small-scale laboratory columns were used to minimize the time and effort that would be necessary for full-scale field experiments, though the latter are also required before field deployment could be recommended. We used three soils including a fine sand, a loamy sand, and a loam from locations as diverse as California, Texas, and Tennessee. For all soils, adding live algae reduced the saturated hydraulic conductivity by 84-99% within a period of 6 days (Figures 2-4). The final K_S of the algae columns was 10^{-3} to 10^{-4} cm s^{-1} (Table 1). Other studies in non-algal systems found large reductions in K_S due to the mechanical straining of suspended fine sediments, organic particles, and microbial cells (Rowsell et al., 1985;

Barrington et al., 1987a,b; Vandevivere & Baveye, 1992; Skolasińska, 2006; Zhao et al., 2009). Similarly, Ragusa et al. (1994) found that inoculation by benthic algae and bacteria greatly reduced K_S (>80%) due to the development of a mat of algae and bacteria near the soil surface. Here, only small reductions in K_S were observed in control columns, likely representing the effects of natural microbial growth and soil particle rearrangement (Figures 2-4) (Allison, 1947; Pavelic et al., 2000). Statistical analyses confirmed that the algal treatment was responsible for the significant reductions in K_S observed.

We attributed the observed reduction in K_S to the development of a dense algal layer at the soil surface (Figure 5). The chlorophyll *a* measurements demonstrated that the majority of the algae biomass was present at the very top of the soil surface, with much lower chlorophyll *a* concentrations at 3 cm depth (Figure 7). The SEM images (Figure 6) showed that the pore spaces available for flow were greatly reduced by the buildup of biomass on soil surfaces and connecting grains. The comparison of Figure 6a (control column) with Figures 6b and 6c (algae columns) demonstrates that the pores were visibly filled in the algae column soils. Comparison of Figure 6c at 0 cm to Figure 6b at 3 cm shows more extensive pore filling at the surface compared to 3 cm depth.

Studies of soil pore clogging related to animal waste holding ponds established the development of a sealing layer at the soil surface (Chang et al., 1974; Rowsell et al., 1985; Maulé et al., 2000; Tyner & Lee, 2004; Tyner et al., 2006; Cihan et al., 2006). Soils act as a screen, accumulating an impermeable mat of fines and organic material at the surface, reinforcing the accumulation of microorganisms and their metabolic products within the pore spaces (Davis et al., 1973; Chang et al., 1974; Hills, 1976; Barrington et al., 1987a,b), which is a desirable feature in sand filtration systems (McNair et al., 1987; Livingstone & Slack, 2014). These mechanisms

reduced the volume and connectivity of pore spaces available for fluid flow, resulting in orders-of-magnitude reductions in the hydraulic conductivity of soils beneath animal waste ponds (to as low as 10^{-7} cm s^{-1}) (Culley & Phillips, 1982; Miller et al., 1985; Rowsell et al., 1985; Cihan et al., 2006; SNTC, 1993). Thus, clogging and sealing mechanisms greatly reduced the need for pond liners to protect underlying groundwater in many scenarios, including in aquaculture ponds (Stone & Boyd, 1989; Chumnanka et al., 2015; Tucker et al., 2017). Pore clogging has specifically been observed with algae (Ragusa et al., 1994; Battin & Sengschmitt, 1999; Gette-Bouvarot et al., 2014). Therefore, our results demonstrating the buildup of algal layers on the soil surface and the concomitant reduction in K_S are consistent with previous results in animal waste holding ponds and with algae. It is important to recognize that the measurements of K_S include the entire length of the soil column (Figure 1), even though the SEM observations and chlorophyll *a* concentrations demonstrate that clogging occurred primarily at the upper surface of the soil. In order to be protective of groundwater underlying algal production facilities, the reductions in hydraulic conductivity due to algal pore clogging would have to be greater than those we observed in this bench-scale column experiment, in which column bulk densities were relatively low in order to achieve measurable flow rates. However, some states have accepted values as high as 6.9 x 10^{-6} cm s^{-1}, while some states have lacked regulations (Parker et al., 1999). Future experiments should examine more realistic (i.e., higher) soil bulk densities to investigate the potential for stronger reductions in K_S.

Soil type only played a small role in the K_S reductions observed here. Waste pond studies demonstrated that sealing is equally effective regardless of the initial soil texture or the contents of the waste (Barrington et al., 1987a,b; Cihan et al., 2006). For example, manure applications effectively sealed soils to the same endpoint K_S on loam, sand, and silty clay soils (Chang et al.,

1974), and on sand, loam, and clay soils (Culley & Phillips, 1982; Rowsell et al., 1985). Thus, reductions in K_S after infiltration of organic materials were not entirely dependent on the initial soil texture (Cihan et al., 2006; Chang et al., 1974; Culley & Phillips, 1982; Rowsell et al., 1985; Maulé et al., 2000) suggesting that the recommendations in Venteris et al. (2014) to site algal production facilities only on locations with low K_S soils may be overly cautious.

5. Conclusions and Implications

Synthetic pond liners are one of the major contributors to the high capital costs of algae cultivation for biofuels, other bioproducts, and profitable nutraceuticals. Column studies were used to investigate the effect of algae on K_S (hydraulic conductivity) of soils to evaluate the potential as an in situ soil treatment technology to replace pond liners in algae production facilities. By treating three diverse soils with the contents of the production ponds, i.e., algae growing under standard culture and nutrient conditions, a dense algal layer developed at the soil-fluid interface on the surface of the soil columns. The algal layer appeared to be responsible for the approximately two orders-of-magnitude integrated reductions in K_S (i.e., through the entire length of the soil column) for all three soils. The final K_S was relatively insensitive to soil type, at least for the soils tested here.

This technology, i.e., using the algae itself as a sealant, appears to be successful in reducing saturated hydraulic conductivity, even in the relatively coarse soils tested in this study. However, these treatments alone did not achieve K_S values that would effectively inhibit all leaching into the subsurface. Greater reductions of K_S (~2x those achieved here) are needed to reach the target K_S value of approximately 10^{-7} cm s^{-1} to be sufficiently protective of the underlying groundwater. The small scale of the columns used in the present study did not allow for such large reductions in K_S,

leading to a significant volume of nutrient and algal solution permeating through the columns before sufficient clogging occurred. Before considering deployment, additional studies should be performed that consider two- and three-dimensional flow.

We noted above that replacing plastic pond liners with soil sealing technology would reduce capital costs of algae production. However, a techno-economic analysis that includes numerous additional factors would be needed to quantify the potential cost savings. In some systems partial lining might be required in erosional areas of algal ponds or raceways (Craggs et al., 2012, 2015; Davis et al., 2016), reducing potential cost savings. Moreover, operational expenses could be altered if unlined ponds have significant differences in water use, nutrient use, or crop protection, compared to lined ponds. If additional soil compaction is needed to further reduce hydraulic conductivity, that would constitute an operational expense. Some local permits or state regulations in the US may require plastic pond liners for some applications of microalgae, and a large body of evidence regarding the longevity of hydraulic conductivity reduction might be required for these regulatory agencies to modify their liner requirements. Nonetheless, unlined ponds are regularly used in aquaculture (Tucker et al., 2008) and are actively investigated in the biofuels industry (McBride & Merrick, 2014).

Coupling algae pond-sealing technology with soil compaction during pond construction may, for example, provide a suitable alternative to plastic pond liners in a wide variety of potential algal production locations. The proposed technology, while encouraging, should be studied further at larger and more realistic scales to understand its full potential and its limitations.

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References

Alam, F., Date, A., Rasjidin, R., Mobin, S., Moria, H., & Baqui, A. (2012). Biofuel from algae-Is it a viable alternative? *International Energy Congress 2012, 49*, 221-227. doi:10.1016/j.proeng.2012.10.131

Allison, L. E. (1947). Effect of microorganisms on permeability of soil under prolonged submergence. *Soil Science, 63*(6), 439-450.

Arar, E. J., & Collins, G.B. (1997). Method 445.0: *In vitro* determination of chlorophyll *a* and pheophytin *a* in marine and freshwater algae by fluorescence. In US Environmental Protection Agency, *Methods for the determination of chemical substances in marine and estuarine environmental matrices, 2nd Edition*. (pp. 133-154). Retrieved from https://nepis.epa.gov

Barrington, S. F., Jutras, P. J., & Broughton, R. S. (1987a). Sealing of soils by manure. I. Preliminary investigations. *Canadian Agricultural Engineering, 29*(2), 99-103.

Barrington, S.F., Jutras, P.J., & Broughton, R.S. (1987b). Sealing of soils by manure. II. Sealing mechanisms. *Canadian Agricultural Engineering, 29*(2), 105-108.

Battin, T. J., & Sengschmitt, D. (1999). Linking sediment biofilms, hydrodynamics, and river bed clogging: Evidence from a large river. *Microbial Ecology, 37*(3), 185-196. doi:10.1007/s002489900142.

Baveye, P., Vandevivere, P., Hoyle, B. L., DeLeo, P. C., & de Lozada, D. S. (1998). Environmental impact and mechanisms of the biological clogging of saturated soils and aquifer materials. *Critical Reviews in Environmental Science and Technology, 28*(2), 123-191. doi:10.1080?10643389891254197

Beck, T., Joergensen, R. G., Kandeler, E., Makeschin, F., Nuss, E., Oberholzer, H. R., & Scheu, S. (1997). An inter-laboratory comparison of ten different ways of measuring soil microbial biomass C. *Soil Biology & Biochemistry, 29*(7), 1023-1032. doi:10.1016/s0038-0717(97)00030-8

Benemann, J. R., Woertz, I., & Lundquist, T. (2018). Autotrophic microalgae biomass production: From niche markets to commodities. *Industrial Biotechnology, 14*, 3-10.

Benson, D., Kerry, K., & Malin, G. (2014). Algal biofuels: Impact significance and implications for EU multi-level governance. *Journal of Cleaner Production, 72*, 4-13. doi: 10.1016/j.jclepro.2014.02.060

Canter, C. E., Davis, R., Urgun-Demirtas, M., & Frank, E. D. (2014). Infrastructure associated emissions for renewable diesel production from microalgae. *Algal Research-Biomass Biofuels and Bioproducts, 5*, 195-203. doi:10.1016/j.algal.2014.01.001

Chang, A. C., Olmstead, W. R., Johanson, J. B., & Yamashita, G. (1974). The sealing mechanism of wastewater ponds. *Journal (Water Pollution Control Federation), 46*(7), 1715-1721.

Chumnanka, N., Boyd, C.E., Viriyatum, R., Tunkijjanukij, S. (2015). Bottom soil characteristics, survival and production of shrimp in low-salinity, inland ponds in Alabama and Florida (USA). *Journal of Soils and Sediments, 15,* 671-682.

Cihan, A., Tyner, J.S., & Wright, W. C. (2006). Seal formation beneath animal waste holding ponds. *Transactions of the ASABE, 49*(5), 1539-1544.

Coleman, A. M., Abodeely, J. M., Skaggs, R. L., Moeglein, W. A., Newby, D. T., Venteris, E. R., & Wigmosta, M. S. (2014). An integrated assessment of location-dependent scaling for microalgae biofuel production facilities. *Algal Research, 5*, 79-94. doi:10.1016/j.algal.2014.05.008

Craggs, R., Sutherland, D., & Campbell, H. (2012). Hectare-scale demonstration of high rate algal ponds for enhanced wastewater treatment and biofuel production. *Journal of Applied Phycology, 24*, 329-337.

Craggs, R., Park, J., Sutherland, D., & Heubeck, S. (2015). Economic construction and operation of hectare-scale wastewater treatment enhanced pond systems. *Journal of Applied Phycology, 27*, 1913-1922.

Culley, J. L. B., & Phillips, P. A. (1982). Sealing of soils by liquid cattle manure. *Canadian Agricultural Engineering, 24*(2), 87-89.

Daniel, D. E. & Benson, C. H. (1990). Water content density criteria for compacted soil liners. *Journal of Geotechnical Engineering*, *166*(12), 1811-1830.

Davis, S., Fairbank, W., & Weisheit, H. (1973). Dairy waste ponds effectively self-sealing. *Transactions of the ASAE, 16*(1), 69-71.

Davis, R., Fishman, D., Frank, E. D., Wigmosta, M. S., Aden, A., Coleman, A. M., . . . Wang, M. Q. (2012). Renewable diesel from algal lipids: An integrated baseline for cost, emissions, and resource potential from a harmonized model. ANL/ESD/12.4; NREL/TP-5100-55431; PNNL-21437.

Davis, R., Markham, J., Kinchin, C., Grundl, N., Tan, E.C.D., & Humbird, D. (2016). Process design and economics for the production of algal biomass: Algal biomass production in open pond systems and processing through dewatering for downstream conversion, NREL/TP-5100-64772. U.S. Department of Energy, Washington, DC.

DOE/EIA (US Department of Energy/Energy Information Administration) (2014). International energy outlook: World petroleum and other liquid fuels with projections to 2040. Report No. DOE/EIA-0484. Retrieved from: http://www.eia.gov/forecasts/aeo.

Gavrilescu, M., & Chisti, Y. (2005). Biotechnology - A sustainable alternative for chemical industry. *Biotechnology Advances, 23*(7-8), 471-499. doi:10.1016/j.biotechadv.2005.03.004

Gee, G. W., & Or, D. (2004). Particle size analysis: Hydrometer method. In J. H. Dane & G. C.C. Topp (Eds.) *Methods of soil analysis. Part 4. Physical methods.* SSSA Book Ser. 5 (pp. 278-283). Madison, WI: SSSA.

Gette-Bouvarot, M., Mermillod-Blondin, F., Angulo-Jaramillo, R., Delolme, C., Lemoine, D., Lassabatere, L., . . . Volatier, L. (2014). Coupling hydraulic and biological measurements highlights the key influence of algal biofilm on infiltration basin performance. *Ecohydrology, 7*(3), 950-964. doi:10.1002/eco.1421

Guillard, R. R. L. (1975). Culture of phytoplankton for feeding marine invertebrates. In W. L. Smith, M. H. Chanley (Eds.), *Culture of marine invertebrate animals*. (pp. 29-60) Plenum, New York, NY, USA: Springer.

Han, S. F., Jin, W. B., Tu, R. J., & Wu, W. M. (2015). Biofuel production from microalgae as feedstock: Current status and potential. *Critical Reviews in Biotechnology, 35*(2), 255-268. doi:10.3109/07388551.2013.835301

Hills, D. J. (1976). Infiltration characteristics from anaerobic lagoons. *Journal Water Pollution Control Federation, 48*(4), 695-709.

Klute, A. & Dirksen, C. (1986). Hydraulic conductivity and diffusivity Laboratory methods. In Klute, A. Ed., *Methods of Soil Analysis - Part 1 - Physical and Mineralogical Methods*, American Society of Agronomy, Madison, 687-734.

Livingston, P., & Slack, D. (2014). Management of the schmutzdecke layer in a slow sand filter to reuse drainage water from a greenhouse. *Advanced Materials Research*, *931-932*, 834-838.

Lundquist, T.J., Woertz, C., Quinn, N.W.T., & Benemann, J.R. (2010). A realistic technology and engineering assessment of algae biofuel production. Energy Biosciences Institute, University of California, Berkeley, CA, 1-178.

Maity, J. P., Bundschuh, J., Chen, C. Y., & Bhattacharya, P. (2014). Microalgae for third generation biofuel production, mitigation of greenhouse gas emissions and wastewater treatment: Present and future perspectives - A mini review. *Energy, 78*, 104-113. doi:10.1016/j.energy.2014.04.003

Maulé, C. P., Fonstad, T. A., Vanapalli, S. K., & Majumdar, G. (2000). Hydraulic conductivity reduction due to ponded hog manure. *Canadian Agricultural Engineering, 42*(4), 157-163.

McBride, R.C., & Merrick, D.S. (2014). Innovations in open pond algae agrciulture for biofuel production. *Industrial Biotechnology,* doi: 10.1089/ind.2013.1614

McDowell-Boyer, L. M., Hunt, J. R., & Sitar, N. (1986). Particle transport through porous media. *Water Resources Research, 22*(13), 1901-1921. doi:10.1029/WR022i013p01901

McGauhey, P. H., & Krone, R. B. (1967). *Soil mantle as a wastewater treatment system: Final report*. (Report 67-11). Sanitary Engineering Research Laboratory, College of Engineering and School of Public Health, University of California, Berkeley, CA.

McNair, D. R., Sims, R.C., Sorensen, D.L., & Hulbert, M. (1987) Schmutzdecke characterization of clinoptilolite-amended slow sand filtration. *Journal (American Water Works Association),79*(12)*,* 74-81.

Miller, M. H., Robinson, J. B., & Gillham, R. W. (1985). Self-sealing of earthen liquid manure storage ponds 1. A case-study. *Journal of Environmental Quality, 14*(4), 533-538.

Orona-Navar, A., Aguilar-Hernández, I., Cerdán-Pasarán, A., López-Luke, T., Rodríguez-Delgado, M., Cárdenas-Chávez, D.L., Cepeda-Pérez, E., & Ornelas-Soto, N. (2017). Astaxanthin from *Haematococcus pluvialis* as a natural photosensitizer for dye-sensitized solar cell. *Algal Research-Biomass Biofuels and Bioproducts, 26,* 15-24.

Pavelic, P., Dillon, P.J., Mucha, M., Nakai, T., Barry, K.E., & Bestland, E. (2011). Laboratory assessment of factors affecting soil clogging of soil aquifer treatment systems. *Water Research 45,* 3153-3163.

Purkayastha, J., Bora, A., Gogoi, H.K., & Singh, L. (2017). Growth of high oil yielding green alga *Chlorella ellipsoidea* in diverse autotrophic media, effect on its constituents. *Algal Research-Biomass Biofuels and Bioproducts, 21*, 81-88.

Parker, D. B., Schulte, D. D., & Eisenhauer, D. E. (1999). Seepage from earthen animal waste ponds and lagoons - An overview of research results and state regulations. *Transactions of the ASAE, 42*(2), 485-493.

Ragusa, S. R., Dezoysa, D. S., & Rengasamy, P. (1994). The effect of microorganisms, salinity and turbidity on hydraulic conductivity of irrigation channel soil. *Irrigation Science, 15*(4), 159-166.

Rogers, J. N., Rosenberg, J. N., Guzman, B. J., Oh, V. H., Mimbela, L. E., Ghassemi, A., . . . Donohue, M. D. (2014). A critical analysis of paddlewheel-driven raceway ponds for algal biofuel production at commercial scales. *Algal Research-Biomass Biofuels and Bioproducts, 4*, 76-88. doi:10.1016/j.algal.2013.11.007

Rowsell, J. G., Miller, M. H., & Groenevelt, P. H. (1985). Self-sealing of earthen liquid manure ponds. 2. Rate and mechanism of sealing. *Journal of Environmental Quality, 14*(4), 539-543.

Schoepp, N. G., Stewart, R. L., Sun, V., Quigley, A. J., Mendola, D., Mayfield, S. P., Burkart, M. D. (2014). System and method for research-scale outdoor production of microalgae and cyanobacteria. *Bioresource Technology, 166*, 273-281.

Skolasinska, K. (2006). Clogging microstructures in the vadose zone - laboratory and field studies. *Hydrogeology Journal, 14*(6), 1005-1017. doi:10.1007/s10040-006-0027-2

SNTC (South National Technical Center). (1993). Design and construction guidelines for considering seepage from agricultural waste storage ponds and treatment lagoons. *USDA*

Soil Conservation Service South National Technical Center Technical Note 716, 1-37.

Soil Survey Staff, Natural Resources Conservation Service, United States Department of Agriculture. Web Soil Survey. Available online at http://websoilsurve.nrcs.usda.gov/. Accessed 7/27/2016.

Stone, N.M & Boyd, C.E. (1989). Seepage from fishponds. Bulletin 599, Alabama Agricultural Experiment Station, Auburn University, AL.

Tucker, C. S., Hargreaves, J. A., & Boyd, C. E. (2008). Better management practices for freshwater pond aquaculture. In C. S. Tucker & J. A. Hargreaves (Eds.), *Environmental Best Practices for Aquaculture*. (pp. 151-226). Ames, Iowa, USA: Blackwell.

Tucker, C.S., Pote, J.W., Wa, C.L., Brown, T.W. (2017). Improving water-use efficiency for Ictalurid catfish pond aquaculture in Northwest Mississippi, USA. *Aquaculture Research 48,* 447-458.

Tyner, J. S., & Lee, J. (2004). Influence of seal and liner hydraulic properties on the seepage rate from animal waste holding ponds and lagoons. *Transactions of the ASAE, 47*(5), 1739-1745.

Tyner, J. S., Wright, W. C., & Lee, J. (2006). Lagoon sealing and filter cakes. *Transactions of the ASABE, 49*(2), 527-531.

US DOE (Department of Energy). (2016). Bioenergy technologies office multi-year program plan. US DOE Energy Efficiency & Renewable Energy, 258 pp., https://www.energy.gov/eere/bioenergy/downloads/bioenergy-technologies-office-multi-year-program-plan-march-2016

Vance, E. D., Brookes, P. C., & Jenkins, D. S. (1987). An extraction method for measuring soil

microbial biomass C. *Soil Biology & Biochemistry, 19*(6), 703-707. doi:10.1016/0038-0717(87)90052-6

Vandevivere, P., & Baveye, P. (1992). Saturated hydraulic conductivity reduction caused by aerobic bacteria in sand columns. *Soil Science Society of America Journal, 56*(1), 1-13.

Venteris, E. R., McBride, R. C., Coleman, A. M., Skaggs, R. L., & Wigmosta, M. S. (2014). Siting algae cultivation facilities for biofuel production in the United States: Trade-offs between growth rate, site constructability, water availability, and infrastructure. *Environmental Science & Technology, 48*(6), 3559-3566. doi: 10.1021/es4045488

Weissman, J. C., Tillet, D.M., & Goebel, R.P. (1989). Design and operation of an outdoor microalgae test facility final subcontract report (SERI/ STR-232-3569) Golden, C.O., USA: Solar Energy Research Institute.

Weissman, J.C., & Tillet, D.M. (1992). Design and operation of an outdoor microalgae test facility: Large-scale system results. In L. M. Brown & S. Sprague, *Aquatic species project report FY 1989-90* (32-56). Retrieved from http://www.nrel.gov/docs/legosti/old/4174.pdf

Wingender, J., Neu, T. R., & Flemming, H.-C. (1999). What are bacterial extracellular substances? In J. Wingender, T. R. Neu, & H.-C. Flemming (Eds.), *Microbial Extracellular Polymeric Substances*. (pp. 1-19). Berlin, Heidelberg, Germany: Springer. doi: 10.1007/978-3-642-60147-7

Zhao, L. F., Zhu, W., & Tong, W. (2009). Clogging processes caused by biofilm growth and organic particle accumulation in lab-scale vertical flow constructed wetlands. *Journal of Environmental Sciences, 21*(6), 750-757. doi:10.1016/s1001-0742(08)62336-0