The impact of K-lactobionate and *Bacillus subtilis* on soil water retention in sandy loam soils

by

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Abstract

Due to a combination of expanded agricultural production, global warming, and depleting water resources, semi-arid agricultural regions of the world have come under increased water stress. Such water stress results in less freshwater available for crop production and impacts the economic foundations of rural agricultural areas. One such area is the western part of Kansas. Western Kansas is an agriculturally dependent region that produces much of the nation's crops. This area is located on top of the Ogallala aquifer, which is the water source for the large-scale irrigation farming that occurs in the area. However, due to its overuse, the aquifer has been in the process of depletion, with some areas reporting 100-foot drops in areas of pumpable water. This has spurred a need for water saving techniques. One such method is through improving soil water retention by adding amendments to the soil. Two amendments that show promise for retaining soil moisture and reducing water use requirements are *Bacillus subtilis* and K-lactobionate. *B. subtilis* is a Gram-positive bacteria that can wet soil through production of a biosurfactant molecules as secondary metabolites. K-lactobionate is a chemical compound produced from mixing lactobionic acid and KOH, and lactobionate derivatives are potentially available as a biproduct from the dairy industry. In this thesis, four studies were performed, with the goal of determining if K-lactobionate-*B. subtilis* amendments can be used to improve water penetration in soils, reduce runoff, and reduce water loss by increasing water retention during drought like conditions. First, water penetration and aggregation test of the two amendments in sandy loam soil was performed, which revealed that addition of a combination of K-lactobionate and *B. subtilis* reduced water penetration times to increase water infiltration into treated soils, and also caused the formation of large soil aggregates. *B. subtilis* and K-lactobionate was also studied as a means to reduce water evaporation in sandy loam soils through evaporation

experiments. It was found that the addition of K-lactobionate with and without *B. subtilis* to the soil produced less evaporation and high water rention, while *B. subtilis* alone produced similar evaporation and water retention as unamended soil. Finally, an experiment testing the growth of *B. subtilis* when using K-lactobionate as a substrate showed that adding K-lactobionate could stabilize *B. subtilis* cells in otherwise nutrient-poor environments, suggesting that it could enhance the application of *B. subtilis* amendments while in soil or when added to irrigation water for distribution. Overall, the two paired amendments showed promise as a potential way to prevent water losses in cropland. Future studies in non-sterile environments more representative of cropland are needed as a next step to continue to evaluate the potential of these soil amendments.

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Chapter 1 - Background

1.1 Introduction

Population growth, changing diets due to income and social changes, and expanded economic activity have given rise to increased food demand (Keating 2014). While only 20% of the world's cropland is irrigated, it is responsible for 40% of the world's agricultural output, making it disproportionately important to crop security (Fernandez-Cirelli 2009). Irrigation is especially important for arid areas, as the lack of precipitation means crops must be fed through flood irrigation (Qian 2022) or utilizing underground water resources (Dłużewski, 2017). Such methods are the predominant way of overcoming water scarcity in arid regions in modern agricultural production (Jabeen 2022). The use irrigation in arid regions has given way to salinization of cropland (Guan 2019), economic instability due to the increasing financial burdens of farming in water scarce regions (Blanco-Gutiérrez 2013) and increasing depletion of groundwater (Xia 2019). These problems have necessitated a need for water management in arid areas (García-Garizábal 2017), and as irrigation is responsible for 90% of water usage in low density agricultural areas (Altes 2023), finding ways to reduce irrigation water use will be critical to achieving better water management.

Another factor driving an interest in water management of irrigation is water scarcity in arid and semi-arid regions, with such interest generating a 93.1% increase in scientific studies investigating effects of water stress in arid regions (Morante-Carballo 2022). Currently, 35% of the world population suffers from water scarcity, with semi-arid and aird regions being noted as being particularly susceptible (Kahil 2016). Increasing prevalence of droughts have reduced grain production around 10% in the past 50 years (Wang 2022). This has led to issues ranging from a constant need to redrill wells to be deeper (Shao 2017) and increasing concerns about water quality,

as industrial activities contaminate increasingly scarce water sources (Gomes et al 2018). More widely, the increasing frequency and magnitude of drought is also tied to socio-economic instability, as water scarcity has led to increases in protests and conflicts in water starved areas (Ide et al 2021). Water scarcity is also tied to negative health outcomes such as higher infant mortality, an effect of lowered nutrient intake from lower crop production in arid regions (Rocha et al, 2015).

Despite the issue of water usage being widely researched, water use inefficiency still exists. It is estimated that non-revenue water, defined as water lost due to unplanned usages (such as firefighting) or failures in transportation (leaks and overflows) can account between 3%-55% of water used by water infrastructure (Babić et al, 2014). Irrigated agriculture also has water use losses, with an estimated global average water use efficiency for agriculture around 55% (Hoogeveen et al, 2019). Without intervention, this is a problem that will likely only get worse., Studies in Egypt have shown that climate change would result in an 8% increase in evapotranspiration, resulting in an increase in water use to maintain agricultural production at existing levels (Elnashar et al 2022). Water use inefficiency in irrigated agriculture is also exacerbated by factors such as improper land management, transportation losses, overuse of fertilizer, and water losses from the soil after the water is applied (Sharma et al, 2015).

1.2 Western Kansas and Irrigated Farming

The western area of Kansas is a large agricultural producer, representing a major producer of Kansas's corn and wheat production, as well as an overwhelming percentage of Kansas's beef and milk supply (KDA, 2022). This can be seen in terms of corn, of which the top five producing counties in KS lie on top of the high plains aquifer (KDA, 2022). Furthermore, corn represents half of Kansas crop production in terms of bushels produced (KDA, 2022).

Irrigation systems are an important part of the Kansas agricultural landscape, where 61% of all agricultural value was from irrigated farms (Rogers et al, 2004). The majority of this water is drawn from the Ogalala aquifer, where agricultural uses account for 90% of water withdrawals (Reyes 2020). The rate of withdrawal has outstripped the rate of regeneration, with some areas estimated to lose a meter of saturated thickness a year (Allen et al 2016). This loss is estimated to reduce the amount of irrigated area in the high plains aquifer by about 24% (Deines et al, 2019). These conditions in turn have spurred an interest in reducing water usage by policy changes, technological innovation, and management practices (Steiner et al, 2021). Retaining water moisture can play a critical role in certain water saving techniques such as deficit irrigation (Geerts and Raes 2009; Fang et al, 2015) where water is strategically limited at certain stages of plant growth (Geerts and Raes 2009; Fang et al, 2015)

In southwest Kansas, improvements in water use efficiency for corn production would be notable. Corn is the single largest consumer of water per acre-feet of irrigated land, three times more than the next highest consumer, sorghum (Aguilar 2017). Corn roots do not extend very far into the ground, with root density being on average three times higher in the first foot of soil than in the second foot of soil (Li et al 2002). Modeling has also shown that root density tends to cluster near the stalk, and that a large portion of the water uptake occurs within the first 20cm/0.66 ft beneath the ground (Benjamin et al 2006). This makes topsoil water conservation particularly important to southwest Kansas.

1.3 K-lactobionate

One potential soil amendment that has been studied for reducing water loss in soils is K-lactobionate (Kallenbach et al, 2019). Interest in K-lactobionate exists not only because of its potential as a soil amendment, but also because it can be produced from food waste (Kallenbach

et al, 2019). Waste from the food industry has become an increasing problem in the US, with an estimated 50% increase in food related waste since 1974, corresponding to 150 trillion kcal per year (Hall et al, 2009). Dairy industry wastes, particularly whey, have potential to be valorized for the production of K-lactobionate (Panesar et al, 2007). The dairy industry had 30% growth between 1991 and 2010 (Gillespie et al, 2014), produced an estimated 125 million tons of milk in the US in 2014 (Hemme et al, 2014). Lactose is a naturally occurring chemical within milk and other dairy products (Silinikove et al, 2015). It can be chemically or biologically converted into lactobionic acid (Cardoso et al, 2019), which has a host of potential applications including as an anticoagulant, an oxidizer, a skincare agent, and biodegradable surfactant (Minal et al, 2017). Thus, the production of lactobionic acid has been increasingly seen as a potential way to valorize and dispose of the 1.6 billion tons of whey waste produced by the dairy industry each year (Narala et al, 2022).

Food waste is also an environmental issue. For example, Europe was estimated to produce 88 million tons of food waste, equivalent to 186 Mt CO_2-eq, with the dairy and meat industry being noted as significant contributors (Scherhaufer et al, 2018). Whey itself is also considered an environmentally destructive pollutant when discarding it by either dumping it into fields or discharging it into nearby bodies of water (Smithers, 2008). Due to whey having a biochemical oxygen demand 175 times higher than typical effluent, whey can be particularly destructive to water ecosystems, and as such many countries such as the US and Canada have introduced legislation regulating whey discharge (Smithers 2008). This in turn has increased costs for dairy producers by introducing the need for treatment for whey produced in diary processing (Palmeiri et al, 2017).

Multiple laboratory methods have been shown to exist to convert whey to lactobionic acid utilizing a variety of bacteria and enzymes (Abuhena et al, 2022). While the production rate varies depending on the conversion method used, final concentrations can reach as high as 400 g/L of lactobionic acid (Alonso et al, 2013), with production rates going as high as 27 g/L/hr (Gutiérrez, L. F. et al, 2012). Such production rates reach the theoretical threshold of being economical for large scale production, which is concentrations approaching 50 g/L and production rates near 1 g/L/hr (Yang et al, 2007). However, concerns exist in terms of scale up, as both purification steps, and maintaining proper pH and temperature ranges at large scale still need to be considered (Alonso et al, 2013).

1.4 Previous Research Biological and Organic Soil Amendments

Agricultural water usage plays an important role of the food-energy-water nexus in Kansas, accounting for 84% of non-domestic water use. As such, reducing overall water usage must account for water use in agriculture. One such method is to use soil amendments. Interest in soil amendments include the use of chemicals treatments to prevent phosphorus runoffs (Serrenho et al, 2012), utilizing organic compounds to reduce heavy metal bioavailability in soils (Malik et al, 2021), and increase water retention in soils (Malik et al, 2023). Generally, amendments can be broken into organic amendments, such as animal waste, biochar, and composts, and inorganic amendments such as clay particles and certain chemicals such as Phosphorus (Palansooriya 2013). Bacterial soil amendments have long been investigated for biocontrol and biofertilizer applications. For example, *Pseudomonas fluorescens* is known to produce cyclic lipopeptides which have antifungal and biosurfactant properties (Nielsen and Sorensen, 2003), which in turn antagonize plant pathogens such as *Pythium ultimum*, *Fusarium oxysporum*, and *Phytophthora*

cryptogea (Fenibo et al, 2019). Bacterial soil amendments can also offer advantages such as long-term and sustained function in the soil and lessened environmental risk compared to traditional methods such as pesticides and other chemicals (Gutierrez et al, 2022). Organic amendments, although widely used, have their disadvantages, such as risking the introduction of harmful pathogens into the soil (Goss and Goorahoo, 2013). One potential organic amendment utilized to increase soil water retention is biochar. Biochar is an amendment derived from biomass such as wood waste, animal waste, and sewage, making it widely available in all parts of the world (Duku et al, 2011). Biochar as a material is defined by its varied pore sizes and its hydrophobicity, a characteristic due to the presence of aliphatic compounds on the surface of the biochar. When mixed with sandy soils it was found to increase water retention, which was attributed to it reducing the average pore size in the soils. However, other studies have indicated problems with biochar, and found no significant effect, which was presumed to be because of the hydrophobicity of the biochar and increasing drainage in certain soils (Li et al, 2021). Compost is another organic amendment to soil that can be utilized to increase water retention. It was found that incorporating composts in the upper 15-30 cm of loam, sandy loam, and clay can produce increases in water retention. Unlike biochar, this was attributed to higher porosity of the soil after application (Kranz et al 2020).

Bacterial soil amendments have recently received interest beyond biocontrol and biofertilizer applications, and have been investigated for providing improvements in soil water retention. One species of bacteria that could increase soil water retention is *Bacillus subtilis,* a Gram-positive bacteria that produces a panel of biosurfactants, most notably Surfactin. Surfactin molecules have both hydrophobic and hydrophilic characteristics that allow it to transition hydrophobic surfaces to hydrophilic surfaces (Ohyama et al, 2014). *B. subtilis* has the potential

to increase soil water retention both through biosurfactant production and through the production of EPS, which may drive soil aggregation and further retain water.

In a study utilizing clay and sandy soils, it was found that the presence of *B. subtilis* reduced evaporation and water loss, especially at the initial stages (Zheng et al, 2018). The study noted that EPS was identified in the soils with *B. subtilis* present, and that EPS had a high-water storage capacity, potentially hundred times its dry weight (Carminati et al, 2017), acting as a storage site for water in the soil. The presence of EPS was also theorized to increase capillary action within the soil, which could serve to increase soil water retention as well (Zheng et al, 2018). In another set of experiments at similar scale, *B. subtilis* was tested on loamy soils and the evaporation rates studied (Gutierrez et al, 2022). Specifically it found that the addition of *B. subtilis* increased the evaporation in the fast evaporation stage but then had a slower evaporation in the slow evaporation stage. Overall, this led to less water lost compared to the control, making *B. subtilis* a promising soil amendment to conserve water. The results of these tests found an increase in evaporation initially and then lower evaporation as time went on. This was attributed to the production of Surfactin, which transitioned hydrophobic soil to hydrophilic soil. This in turn aided capillary action resulting in a faster drying out of the top layer, but limiting evaporation in later stages when water became scarce (Gutierrez et al, 2022).

Another feature that makes *B. subtilis* promising as a potential widespread soil amendment is its scalability. *B. subtilis* was produced at a $1*10^9$ CFU/ml scale with a pilot bioreactor of 3000L at 32°C, a pH of 7.5, and agitation at 250 rpm (Abuhena et al, 2022). Such results were achieved using a growth media of 20 g/L molasses combined with 2 g/L urea (Abuhena et al, 2022). Another benefit of *B. subtilis* is its ability to sporulate. Previous studies have shown that *B. subtilis* when sporulated can retain 10% spore viability after 40 years when

kept in water at 4°C, as well as having 100% spore viability after 1 year (Uhlrich et al, 2018; Watson Int, 2022). Such capacity could potentially allow *B. subtilis* to be stored on site and mass produced when needed. This also introduces the possibility of seeding the ground with sporulated *B. subtilis* and then using K-lactobionate or other nutrients in the soil to germinate the bacteria, reducing the amount of time the soil needs to be colonized with *B. subtilis*. Another promising feature of *B. subtilis* as an amendment is the potential for it to be widely spread through irrigation systems. Spread of pathogenic bacteria from contaminated irrigation water to croplands is an already observed phenomenon (Akinde et al, 2016), and the utilization of bacteria being added to oil well water is utilized for oil recovery in soils (Patel et al, 2015).

Addition of Lactobionate compounds can also improve soil water retention. In a study combining cations paired with lactobionic acid, there was an average 37% increase in available water (Kallenbach et al, 2019). There were a variety of mechanisms proposed for this, including increasing SOC, increased bacterial activity, and cation induced soil aggregation (Kallenbach et al 2019). In drier soils, it was shown that the cation of the metal-lactobionate complex was the primary driver of soil water absorption (Korshidi et al, 2016). There is also the potential that the lactobionate intrinsically increased soil water retention through the addition of extra soil organic carbon, which has been shown to increase available water capacity by about 1.16% for every 1% increase in soil organic carbon (Minasny and McBratney, 2018).

A final area of focus relevant to these studies is related to transport of *B. subtilis* in soil, which can be is driven by processes such as chemotaxis, quorum sensing, and is influenced by the physical and hydrodynamic properties of the soil (Englelhardt et al, 2022). The bulk density of the soil also impacts bacterial transport, as higher densities resulted in less bacterial transport in lower parts of the soil (Van Elsas et al, 1991). Bacterial penetration in soil also tends to be

shallow, as testing with *E. coli* leeched slowly through course soils such as sandy loam, found little movement (Hossain et al, 2006). Although bacteria have means of self-transport, studies have shown they can at most move a few millimeters on their own (Unc and Goss, 2003). Thus, bacteria rely on the movement of water to propagate in soils (Unc and Goss, 2003). Chemotaxis, the movement of bacteria through the use of various appendages in response to various chemicals, further modifies bacterial movement. This form of movement requires a "flooded" state, as bacteria have difficulty traversing air gaps. Therefore, dry soil can reduce the movement of bacteria in soil (Van Elsas et al, 1991). There are other methods of bacterial movement such as sliding, twitching, or "hitchhiking". However, due to the lack of organics, this does not readily occur in sterile soils (Yang and van Elsas, 2018). Bacterial movement is also dependent on soil texture, with coarser soil with large air bubbles and/or dense packing limiting bacterial movement (Yang and van Elsas, 2018). Overall, significant transport of bacteria within soil requires connected water pathways between soil particles (Griffin and Quail, 1968). One such means of creating better pathways is through soil aggregation, which can help water flow through soils which in turn can carry beneficial bacteria such *B. subtilis*, to deeper areas.

1.5 Thesis goals

The goal of this thesis is to investigate the feasibility of combining *B. subtilis* with K-lactobionate as well as to investigate synergistic effects the two may have for an improved soil amendments that increases soil water retention in sandy loam soils, as well as a potential means to recycle whey byproducts from dairy production. To accomplish these goals, a set of three experiments were performed to test the applicability and feasibility of the amendments, specifically:

- Characterize changes in water penetration time in sandy loams soils after treatment *B. subtilis* and K-lactobionate amendments (Chapter 2).
- Characterize changes in soil water retention after treatment *B. subtilis* and K-lactobionate amendments (Chapter 3).
- Investigate the growth of *B. subtilis* when K-lactobionate is used as the growth substrate in an otherwise nutrient poor environment (Chapter 4).

Chapter 2 - Soil Aggregation and Penetration

2.1 Introduction

Soil aggregation is a process in which soil particles flocculate, cement, or rearrange themselves (Duiker et al, 2003). It is an important component of soil structure and is key to sustainable soil management (Lehman et al, 2020). Aggregation is the result of soil organic carbon (SOC), ionic bridging, biota, and clay and carbonates present in soil (Bronick & Lal, 2005). Soil aggregation is important to microbial diversity as it regulates water, air, and nutrient movement by determining pore connectivity in soil (Carson et al, 2010; Ebrahimi & Or, 2016). Soil aggregation is also tied to water infiltration and retention (Franzluebbers 2002), soil fertility, and cation uptake (Bedel et al, 2018; Guo et al, 2019; Li et al, 2016). Water infiltration plays an important role in crop production, as decreased penetration rates are tied to lower growth as well as higher fungi and weed growth, which further harm crop production (Nagy et al, 2018). Infiltration also affects agricultural water budget, as it impacts the soil's water intake (Bittelli, 2010). Infiltration rates are impacted by a variety of factors including compaction, presence of large aggregates, soil type, organic matter, and soil water content (Souza et al, 2021). Lactobionate-based salts have been previously identified as a potential soil aggregator with mechanisms of soil bridging from bivalent cations, and by interactions with bacteria in the soil. With this background, the goal of this study was to investigate the effect of K-lactobionate and *B. subtilis* treatments on soil aggregation and water infiltration in sandy loam soil.

2.2 Materials

Sandy Loam soil was taken from Kansas State University experimental fields south of Manhattan, Kansas. The soil was then sieved through a 2000 mm sieve to remove any large organic components, homogenize soil particles sizes present in the samples, and remove any

other clods that could impact soil characteristics and drain times. Two soil samples were sieved, one sample from the top foot of soil, the second sample was taken 1 foot below the surface. *B. subtilis* (Strain 1A1135, Bacillus Genetic Stock Center) was prepared by culturing on an agar petri dish (Gutierrez et al, 2022). Plates were then put into an incubator for 48 hrs, and then refrigerated at 4 °C. *B. subtilis* was later grown in liquid culture by inoculating a colony from the agar into a test tube filled with 2 ml of Tryptic Soy Broth (TBS). The liquid was then placed in a shaker set to 31°C and left to grow for 24 hours. K-lactobionate was prepared at 0.17M, based on previous studies indicating K-lactobionate provided improved soil water retention (Kallenbach et al, 2019). K-lactobionate was prepared by mixing 35.8 g of lactobionic acid in 100 ml of water, then in a fume hood, slowly adding in 5M KOH with a pipette until a pH reader indicated a pH of 7. 200 ml of ultrapure water was then added, followed by 17 ml of this mixture to a 50 ml tube. 34 ml of ultrapure water was then added, resulting in a 0.17M mixture of K-lactobionate (Fasching et al, 2016).

2.2.1 Water Penetration Test Procedure

The water penetration test procedure was a modified version of the single ring infiltrometer test (Maryland Department of the Environment, 2009). A 600 ml volume of the sandy loam top soil was completely dried to remove any soil water content. Soil was sterilized to prevent bacteria from impacting the added amendments by putting it into a 1000 ml griffin beaker and baking in an oven at 104 °C for 24 hours. The soil was then left at 30 °C for 24 hours. The soil was then heated to 104 °C for 2 hours and cooled down again to 30 °C for 24 hours. This step was repeated twice. Finally, the soil was left overnight at 55°C. For drying, the soil was put in an oven at 105°C for 24 hours right before use. Nine soil masses from the same soil sample were then weighed, ranging from 19-20.3 g. Soil was added to a series of 9 clear 0.8-inch diameter

acrylic tubes cut to a height of 4 inches. Tubes were aligned vertically, then 6 ml of ultrapure water was added and the time for the water to drain to soil level was recorded. The samples were then dried at 104 °C for 24 hours.

A solution of *B. subtilis* was prepared by taking *B. subtilis* after culture in TSB solution and centrifuging it. The TSB was then poured out and the cells were resuspended into ultrapure water vortexed for 30 seconds. In 3 separate wells in a 96 well plate container, 100 microliters of the liquid were added to measure the OD_{600}. From the average, the liquid was diluted to an OD_{600} of 0.1, equivalent to a density of $1x10^7$ cells/ml. Then in 3 replicate test tubes, 0.34 ml of the diluted bacterial solution was added, along with 5.7 ml of water.

In three control soil samples, 6 ml of ultrapure water was added, in three test soil samples, 6 ml of the bacterial solution was added (resulting in a $1.67x10^5$ cells/g soil tube), and in three test soil samples, 6 ml of 0.17M K-lactobionate (0.018g-Lactobionate/g soil) with bacteria solution was added. The inoculated soil was left to incubate for 24 hours at 31°C in an oven. Afterwards, the samples were dried again at 104°C for 24 hours. The dried soil was set up and 6 ml of ultrapure water was added to record penetration time for each. Statistical significance was determined using a t-test comparing samples before and after amendment.

2.2.2 Characterization of Soil Aggregation

Soil from the penetration test was removed from the cylinders and placed into different plastic boats. Three sieves were then prepared, a 2000mm sieve, a 1000mm sieve, and a 250mm sieve. They were placed on top of each other going from 2000 mm to 1000 mm to 250 mm sieve sizes. Soil samples were filtered by pouring the contents of the weighing boat onto the top 2000 mm sieve and then shaking it in a circular motion for 30 seconds above the 250 mm sieve. The 2000 mm and 1000 mm sieve were then taken off and the 250 mm sieve was then shaken in a circular

motion. The residue on top of each sieve, as well as the residue that passed through the 250mm sieve were weighed. The resulting weights were then normalized into a fraction of the total weight of soil added and compared.

2.3 Penetration Test Results

The average penetration time of the ultrapure water in sandy loam soil was found to be 31 seconds for untreated soil, 15 seconds for soil treated with *B. subtilis*, and 8 seconds for soil treated with K-lactobionate and *B. subtilis* together, as shown in Figure 2.1. Of the results, untreated soil and the K-lactobionate combined with *B. subtilis* treated soil were found to be statistically different from the control (P = 0.005), with the treated soil showing a 75% decrease in penetration time. *B. subtilis* also showed lower penetration times, but the difference was not statistically significant compared the water control (P = 0.07).

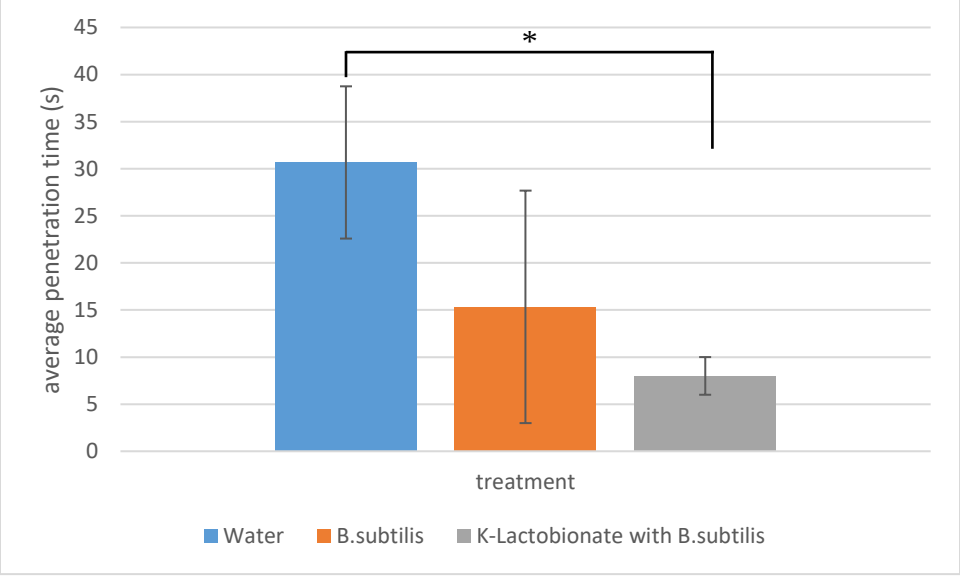


Figure 2.3.1 Average penetration time of ultrapure water in treated sandy loam soil

To validate that the increased penetration in the soil amended with *B. subtilis* and K-lactobionate was a function of the amendments and not the soil itself, the penetration of the sandy loam soil before and after treatment in individual soil samples was then recorded. The penetration time of the soil before amendments were added was found to be on average 33 seconds. This was statistically greater than the penetration time after treatment, with a reduction of about 75% with treatment. These results were statistically significant (P = 0.04).

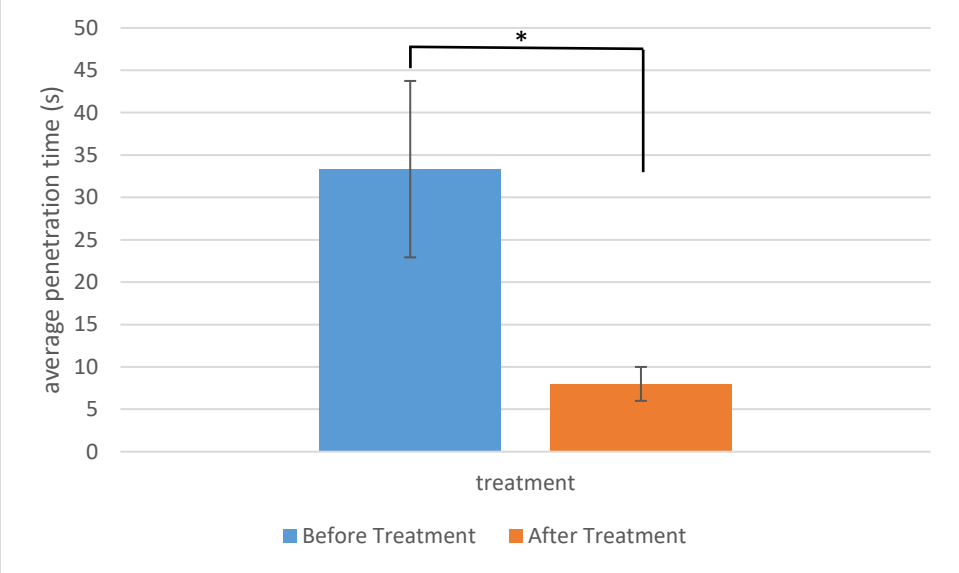


Figure 2.3.2 Average penetration time of ultrapure water in individual sandy loam soil samples before and after treatment of *B. subtilis* and K-Lactobionate (P = 0.04)

2.3.2 Aggregation Test Results

The aggregate fractions of untreated soil are shown in Figure 2.3 and were found to be 37% aggregates between 0.25mm and 1mm in size, and 63% aggregates less than 0.25mm. The aggregate fractions of soil treated with *B. subtilis* were found to be 39% aggregates between 0.25 mm and 1mm in size, and 61% aggregates less than 0.25 mm. The aggregate fractions of soil treated with *B. subtilis* and K-lactobionate were found to be 33% aggregates between 0.25 mm and 1 mm in size, and 61% aggregates less than 0.25 mm, and 5% aggregates larger than 5 mm.

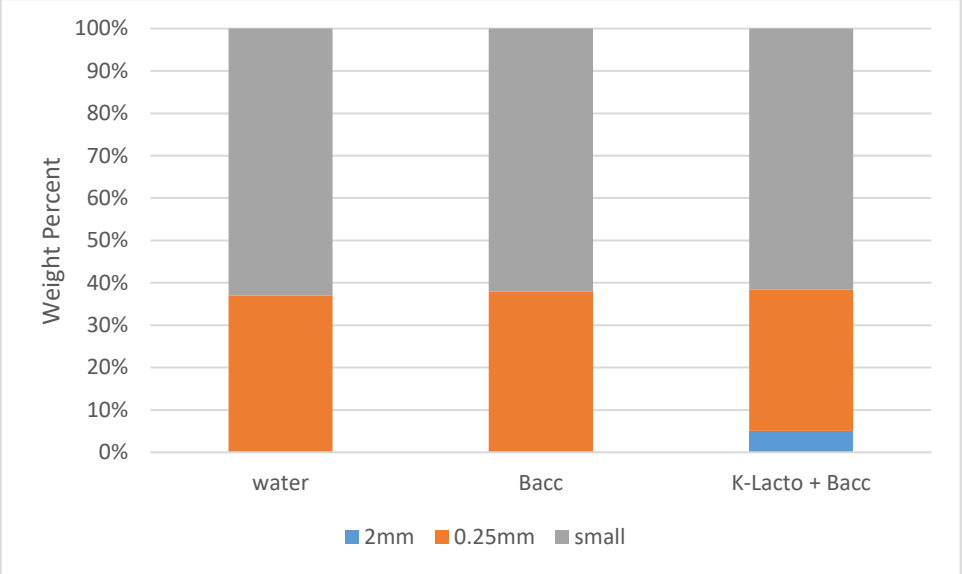


Figure 2.3.3 Comparison of sandy loam aggregate fractions under various amendments. Leftmost figure is aggregate faction when only exposed to water, middle figure is aggregate faction when soil is treated with *B. subtilis*, rightmost figure is aggregate faction when soil is treated with K-lactobionate and *B. subtilis*.

2.5 Discussion

The penetration test revealed that *B. subtilis* alone did not statistically decrease penetration time into the soil, nor produce a change in aggregate composition in the soil. However, addition of K-lactobionate with *B. sutbtilis* did provide a decrease in penetration time that was statistically significant from the control and also caused large aggregate formation. The functions of the soil responsible for water infiltration are bulk density and the porosity. Specifically, larger pore size and greater pore continuity impact the flow of water into soil, especially in the top 10 cm (Kutílek, 2004). Larger pore sizes are tied to greater water infiltration, which is why sandy soils tend to have greater penetration than clay soils (Martinez et al, 2018). Furthermore, aggregate stability plays a large role in water infiltration of soil. Weak aggregates can be broken down by

the impact of water on the soil, forming a seal on the top layer that impedes water flow (Lado et al, 2004). Bacteria, potentially including *B. subtilis*, can help with the stabilization through the formation of exopolysaccharides that act as binding agent for soil aggregates (Foster, 1981). Experiments done with the addition of EPS from *Bacillus* strains on clay and silt soils found aggregate formation (Martin, 1946). This was further reinforced by another study that found a positive correlation between adding nutrients to soils with known aggregating bacteria, and larger aggregate values (Caesar-TonThat et al, 2008). This suggests a possible mechanism for aggregation here, as *B. subtilis* supplemented with K-Lactobionate may produce EPS to help form aggregates that increased the pore size of the soil. However, it was also found that excessive EPS production could hinder infiltration by clogging soils (Mitchell & Nevo, 1964). As such, EPS production from *B. subtilis* supplimented with K-lactobionate remains a potential, but unproven mechanism of creating aggregates in soil, and requires further research to validate.

Cations introduced into soils also have the potential to cause aggregation. Notably in a 2.5-year study, Gypsum was found to help fields keep water retention and lower penetration resistance by decreasing the amount of exchangeable, soluble sodium cations, which was tied to aggregate breakdowns (Valzano et al, 2001). This is due to cations bridging the humic substances coating soil particles, binding them together (Wershaw, 1999). Further studies with potassium ions also noted the potential of the ion to increase aggregate stability in humic soils (Huang et al, 2016). However, this is not a consistent result as a study investigating K levels on clay soils in Switzerland revealed lower aggregate stability with higher K levels (Farahani, 2018). This was explained as a function of monovalent ions causing dispersion of clay particles, as well as reducing hydraulic conductivity of soils through the increase in diffusion layer thickness, as well as adding water repulsive forces to the soil particles (Farahani, 2018). Another

explanation for increased aggregation is the presence of lactobionic acid, which has five hydroxyl groups and polar carboxyl groups that potentially enable hydrogen bonding and soil aggregation in clay (Martin et al, 1955). This reinforces the original research by Kallenbach that theorized increased aggregate formation with the introduction of K-lactobionate in the presence of bacteria (Kallenbach 2019).

2.6 Conclusion

Two treatments utilizing *B. subtilis* and *B. subtilis* combined with K-lactobionate were investigated for in changes in aggregation in sandy loam soil. In both penetration and aggregate formation, the addition of *B. subtilis* to sterilized and dry soil did not differ significantly from the control. However, the presence of K-lactobionate and *B. subtilis* showed a statistically significant, 75% decrease in penetration time, corresponding with the formation of large aggregates. One potential mechanism is the presence of lactobionic acid hydrogen bonding with soil particles to drive soil aggregates. Another possible mechanism for aggregation is production of bacterial EPS, a known binding agent causing the production of larger aggregates, which could be induced both by the drying conditions and perhaps from the presence of K-lactobionate as a nutrient source. These results demonstrate the potential utility of K-lactobionate and *B. subtilis* together as a soil amendment to preserve water through inducing better water infiltration. Future studies should focus on identifying concentrations of K-lactobonate that best promote water penetration as well as testing the combination in non-sterile environments more representative of cropland.

Chapter 3 - Soil water retention in sandy soils with the addition of K-lactobionate and *B. subtilis*

3.1 Introduction

Sandy loam soils, prevalent in SW Kansas, have shown in past experiments to have better water retention rates when *B. subtilis* is added as an amendment (Arraya et al, 2017; Gutierrez et al, 2022). Such increases in water retention are important for reducing water usage for agricultural production. Traditionally, the timing and quantity of crop irrigation varies depending on region, soil quality, and the amount of rain during the growing season. This is especially crucial during mid-growth crop production, when water is needed the most. As irrigation is a large source of groundwater depletion, methods of reducing irrigation are key to reducing freshwater consumption.

Soil water content (SWC), the volume of water within a volume of soil, is intrinsically tied to irrigation scheduling (Bitelli, 2011), and is a measure of water retention in the soil. SWC is also important from an economic perspective, as increased SWC improves land value, creating economic value for farmers. Unpublished research from the 2023 NRT capstone course at K-State utilized Ricardian regression to provide an economic analysis of land values, and estimated that every 1% increase in available water content will produce a 7% increase in land rental value. Thus, average increases in SWC of even 0.05%, could improve land value of sandy soils by up to 35%. One method of reducing irrigation demand is to condition the soil to retain moisture, avoiding water loss from of evaporating or drainage. Therefore, keeping SWC high is important to reducing water waste, as the longer soil SWC remains above certain thresholds, irrigation water will not be utilized. K-lactobionate is a potential source of increasing SWC, as studies have shown that applying K-lactoboinate at 0.042g-lactobionate per gram of soil helps reduce

water loss (Kallenbach, 2019). *B. subtilis* is another amendment that can potentially reduce water evaporation and thus keep SWC high, also reducing the need for irrigation. Surfactin, the soil wetting agent from *B. subtilis*, is optimally produced in slightly acidic condition between 6.5-6.8 pH, 30 °C, and 90% aeration (Mawgoud et al, 2014). Current estimation of western Kansas soils puts average aeration at roughly 25% of soil volume (soil management, 2024), while soil pH tends to be in this optimal range (Araya et al, 2021). The soil temperature in late spring through the summer generally correlates to a soil temperature between 20-30°C (Zhao et al 2021). These metrics suggest the K-lactoboinate and *B. subtilis* could be effective as water-retaining soil amendments when used in the western Kansas area, which motivate the investigation of the combined effect of these two amendments on soil water retention in representative sandy loam soils.

3.2 Materials and Methods

3.2.1 K-lactobionic acid preparation

K-Lactobionate was prepared by mixing 35.8 g of lactobionic acid in 100ml of water in a fume hood, slowly adding in 5M KOH with a pipette until a pH reader indicated a pH of 7. Then 200 ml of ultrapure water was added. 17 ml of this mixture was added to a 50 ml tube, and 34 ml of ultrapure water was added resulting in a 0.17 M mixture of K-lactobionate (Fasching et al, 2016). Previous unpublished research indicated that 0.17 M K-lactobionate would produce a high carrying capacity of *B. subtilis*, thus enabling cell densities sufficient for soil wetting.

3.2.2 Soil inoculation and evaporation

The procedure for soil inoculation, saturation, and evaporation described by Gutierrez (2022) was followed. The experiment and data collected is from an unpublished experiment done by Gutierrez in 2023. Columns (19.07 cm^3) were packed with 40 g of wet sandy loam soil to a bulk density of 2.10 g/cm^3 and a porosity of approximately 0.32 for all samples. The soil was

packed densely to achieve consistent bulk density, which is necessary to get accurate results (Gutierrez et al, 2022). Another 40 g sandy loam soil sample, taken from the soil used was completely dried and weighed to find the dry mass of the samples. Columns were used for the control (only water), *B. subtilis* (Strain 1A1135, Bacillus Genetic Stock Center) (n=3), K-lactoboionate (n=3), and for KOH-lactobionic acid combined with *B. subtilis* (n=3). Inoculation was done by diluting the bacteria to an OD_{600} 0.1 and inoculating to obtain a final cell density of 5 X 10^5 cell/g of soil. The columns were placed in an incubator for 24 hours and then in water for 48 hours to saturate the columns. After saturation, the columns were placed in an oven for drying. The oven was set to 30°C and incubated for 72 hour evaporation periods. Weights were taken during packing, after saturation, and after evaporation trials, all the data was then used to tabulate the SWC of the samples before and after the experiment. SWC can be defined in one of two ways, as a gravimetric SWC or volumetric SWC. They are tabulated through the following methods (Kirkham, 2014).

Equation 3.1 – Volumetric SWC

$$\theta_V = \frac{V_W}{V_T}$$

Equation 3.2 – Gravimetric SWC

$$\theta_G = \frac{M_{Wet} - M_{Dry}}{M_{Dry}}$$

Where θ_V is the volumetric SWC, V_w is the volume of water, V_t is the bulk volume of the wet soil, θ_G is the gravimetric SWC, M_{wet} is the mass of the wet soil and M_{dry} is the mass of soil after being completely dried. θ_V can be related $\theta_G$through the following equation:

Equation 3.3 – Relation between volumetric and gravimetric SWC

$$\theta_V = \theta_G * \rho_b$$

Where ρ_b is the bulk density which is defined as the mass of dry soil per unit bulk volume. In the experiment, results are presented in volumetric SWC, calculated utilizing Equation 3.3 after calculating the gravimetric SWC.

3.3 Results

The results (Fig. 3.3.1) revealed that K-lactobionate and *B. subtilis* paired with K-lactobionate produced significantly less water loss during the 72 hour drying period. K-lactobionate lost 4.69 g of water compared to the 7.76 g of water lost in the control (P= 0.030). K-lactobionate paired with *B. subtilis* also produced a statistically significant difference with a water loss of 6.63 g compared to the 7.76 g lost in the control (P = 0.02). There was also differences in initial volumetric SWC and final volumetric SWC levels between the 4 amendments (Figs. 3.3.2 and 3.3.3). The initial and final SWC of K-lactobionate alone was 0.544 cm^3 water/cm^3 soil and 0.306 cm^3 water/cm^3 soil respectively. The initial and final SWC of K-lactobionate paired with *B. subtilis* was 0.539 cm^3 water/cm^3 soil and 0.201 cm^3 water/cm^3 soil respectively. Both were statistically different than the control initial SWC of 0.495 cm^3 water/cm^3 soil and final SWC of 0.1 cm^3 water/cm^3 soil ($P < 0.05$). *B. subtilis* alone did increase the initial SWC, however the increase was not significantly different than the control, and equivalent levels of SWC were noted after the evaporation period. Finally, the change in soil water content (ΔSWC) was calculated from the preceding data (Fig. 3.3.4). Consistent with the prior figures, K-lactobionate only produced a statistically-significant ΔSWC of 0.239 cm^3 water/cm^3 soil and K-lactobionate and *B. subtilis* paired together produced a statistically-significant ΔSWC of 0.338 cm^3 water/cm^3 soil (P= 0.029 and P= 0.02 respectively). *B. subtilis* did not produce a statistically significant difference from the control.

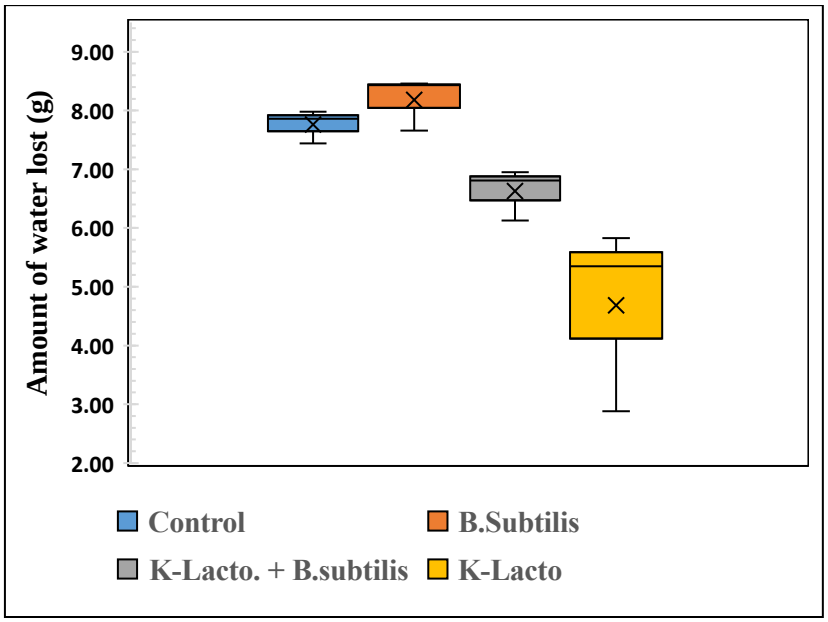


Figure 3.3.1 Water loss in soil after 72 hrs of drying

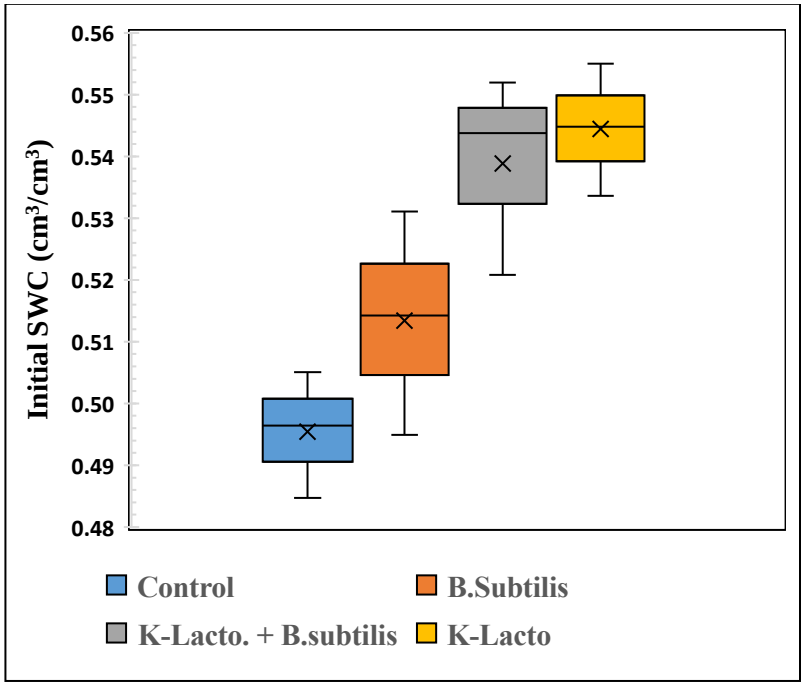


Figure 3.3.2 Average of the initial volumetric SWC with different amendments

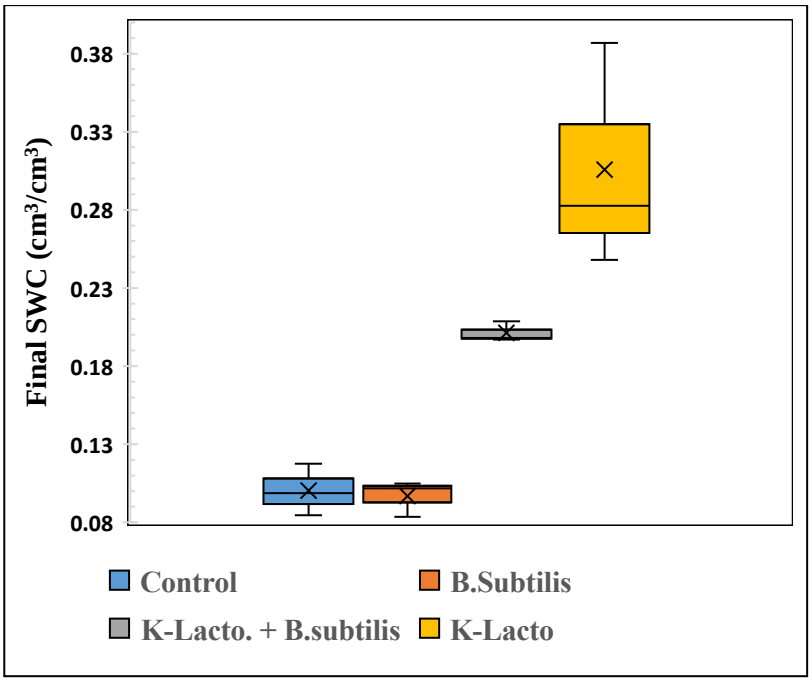


Figure 3.3.3 Average final volumetric SWC with different amendments

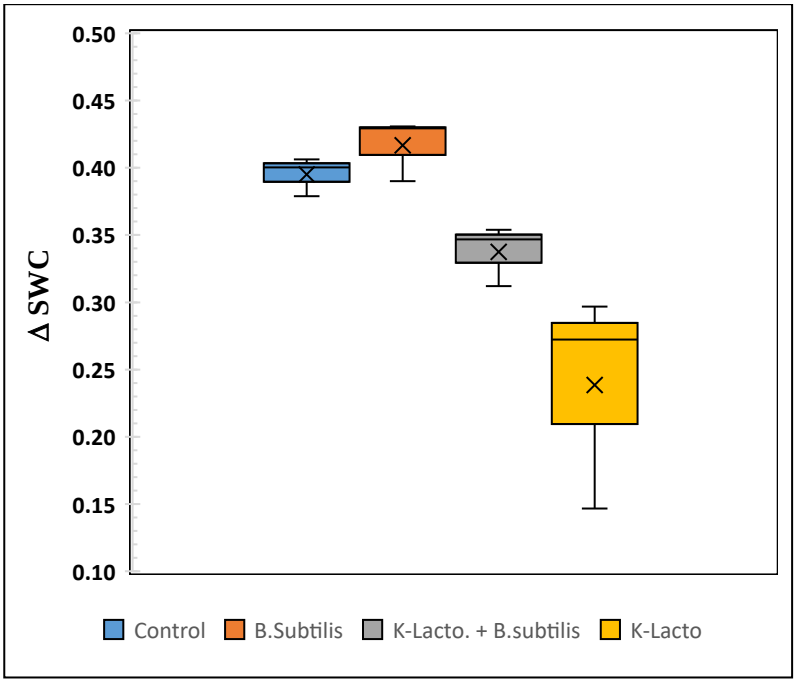


Figure 3.3.4 Average change in volumetric SWC over 72 hour drying period with different amendments.

3.4 Discussion

From the data, the presence of K-lactobionate, both with and without *B. subtilis*, provided a significant increase in initial SWC after the saturation period. This could be due to K-

lactobionante or a biosurfactant produced by *B. subtilis* decreasing the soil hydrophobicity. *B. subtilis* only also increased the initial water content marginally, perhaps also due to biosurfacant production. Each of these increases are noteworthy, as it indicates that less water would be lost due to runoff immediately after an irrigation event. The trends agree with literature that utilizes artificial surfactants in sandy soil, which resulted in a change of soil water content from anywhere between 4-38% (Oostindie et al, 2008). However, this only explains the initial higher SWC.

After the evaporation period, there was no meaningful difference in the residual water content of the control and the column only amended with *B. subtilis*, however the column with both *B. subtilis* and K-lactobionate, and K-lactobionate on its own, exhibited a final water content three to four times as high as the control. From the results in Ch. 2 (Fig. 2.3), the most notable difference between the columns containing K-lactobionate and the other columns was the presence of larger aggregates. Larger aggregates have been shown to retain water better than smaller aggregates, especially in scenarios where the soil experiences high suction, usually present in near dry soils (Tamboli et al, 1964). Thus, a likely explanation for these findings is that larger aggregates present in soils with K-lactobionate were able to hold water during the drying period due to the greater internal soil porosity from the larger aggregates (Tamboli et al, 1964).

An unexpected result here was the lack of statistical difference in water loss between *B. subtilis* amended soils and control soils. This is contradictory to previous research that indicated that the addition of a *B. subtilis* amendment would reduce water loss (Gutierrez et al, 2022). More recent and currently unpublished data has identified that *B. subtilis*-mediated wetting may be dependent on the forms of inorganic nitrogen in the soil, specifically the nitrate (NO_3^--N) and ammonia (NH_4^+-N) levels in the soil. Since the soil used here was taken at a different time and from a different location from the samples used in from Gutierrez *et al*. (2022), the differences in

inorganic nitrogen forms and levels are a possible explanation as to why *B. subtilis* was not as effective in this experiment. It is also likely that the *B. subtilis* strain used here (Strain 1A1135) did not produce the same levels of biosurfactant as the strain used in prior studies (ATCC 6051). *B. subtilis* 1A1135 was modified from *B. subtilis* strain 168 which is deficient in Surfactin production, but able to produce other biosurfactants. The data also indicated that K-lactobionate on its own was just as effective as K-lactobionate paired with *B. subtilis* at reducing water loss. This introduces the possibility of not needing to utilize *B. subtilis* to improve SWC during evaporation periods.

3.5 Conclusion

The noted increases in residual water content in sandy loam from K-lactobionate-based treatments show potential, as higher soil water content is important to crop productivity by reducing the amount of irrigation cycles that occur (Datta et al, 2017). These results are also promising in improving water savings, as the addition of K-lactobionate produced a noticeable reduction in soil water evaporation. The results show between 200-300% increases in final soil water content, which are in the same range to those found in other studies, despite utilizing less K-lactobionate. This is promising from an economic perspective and opens up potential studies to identify the ideal concentrations of K-lactobionate. Future research should investigate changes in SWC of sandy loam soils at larger scales, moving towards croplands that contain varied organic matter and microbial communities, which could modify results.

Chapter 4 - Growth of *B. subtilis* in K-lactobionate media

4.1 Introduction

Microbial communities play an important role in soil health. Whether through phytoremediation of toxins (Glick, 2010), improving plant growth through nitrogen fixation and production of plant hormones in the rhizosphere (Wang et al, 2014), or nutrient cycling in the soil (Wiryawan et al, 2022). Microbial communities can also be used to improve water retention in soils, including using *B. subtilis* in sandy loam soil to improve long term water retention (Gutierrez et al, 2022). Certain soils such as sandy loam soil are known for their poor water holding capacities as well as low nutrient content, making them inconducive to beneficial bacterial growth (Sutardi, 2022). One way to combat infertility and improve microbial growth in nutrient poor soils is through the use of organic soil amendments (Tang et al, 2016). One such amendment is K-lactobionate, a molecule produced from the lactobionic acid, which can be made from the waste of milk production, mixed with KOH (Kallenbach, 2019). Previously, K-lactobionate had been studied by mixing the K-lactobionate at a rate of 0.042g of lactobionate per gram of soil which had produced desirable results in terms of soil water retention (Kallenbach 2019). However, K-lactobionate as a bacterial growth accelerator has not been extensively studied, although it is known to be beneficial, specifically in preventing excessive swelling of mitochondria (Wollenman et al, 2017). Thus, *B. subtilis* and K-lactobionate are two amendments that may interact in a synergistic way, as K-lactobionate can be used as a nutrient source for the growth and survival of *B. subtilis*. The potential synergistic benefits of the two components as soil amendments motivate the study of the use of K-lactobionate for *B. subtilis* growth. Here, *B. subtilis* growth from K-lactobionate at two concentrations were chosen. In

addition to 0.17 M, the concentration used in Chapters 2 and 3, 0.5M K-lactobionate was also chosen, as this molarity is commonly made in known production methods (Fasching et al 2016).

4.2 Experimental Methods

B. subtilis (1A1135, Bacillus Genetic Stock Center) stocks were stored at -80°C in 25% glycol solution. Agar was prepared in an 8 ml plastic petri dish, prepared by heating 40g of agar solution in 1000 ml of ultrapure water and autoclaved at 122°C for 15 minutes. This agar solution was then left to cool to about 80°C, then poured into the petri dish in a biosafety cabinet and left to cool for 10 minutes. After the agar was solidified, *B. subtilis* was applied with an inoculating loop in a biosafety cabinet sterilized by 15 minutes of UV light. This agar plate was then left in a heated incubator set to 31°C for 24 hrs. In a biosafety cabinet, 2 ml of TSB in a test tube was inoculated with a single colony from the agar plate and grown in a shaker for 24 hrs at 31 °C. The solution was then centrifuged for 15 min at 4,400 rpm and the TSB was poured out while the bacteria remained on the bottom of the test tube. 2 ml of ultrapure water was added into the tube and the tube vortexed again to resuspend the bacteria in water. K-lactobionate was prepared by mixing 35.8 g of lactobionic acid in 100 ml of water, then in a fume hood, slowly adding in 5 M KOH with a pipette until a pH reader indicated a pH of 7. Then, 200 ml of ultrapure water was added resulting in a 0.5 M solution of K-lactobionate. 17 ml of this mixture was added to a 50 ml tube, and 34 ml of ultrapure water was added resulting in a 0.17 M mixture of K-lactobionate.

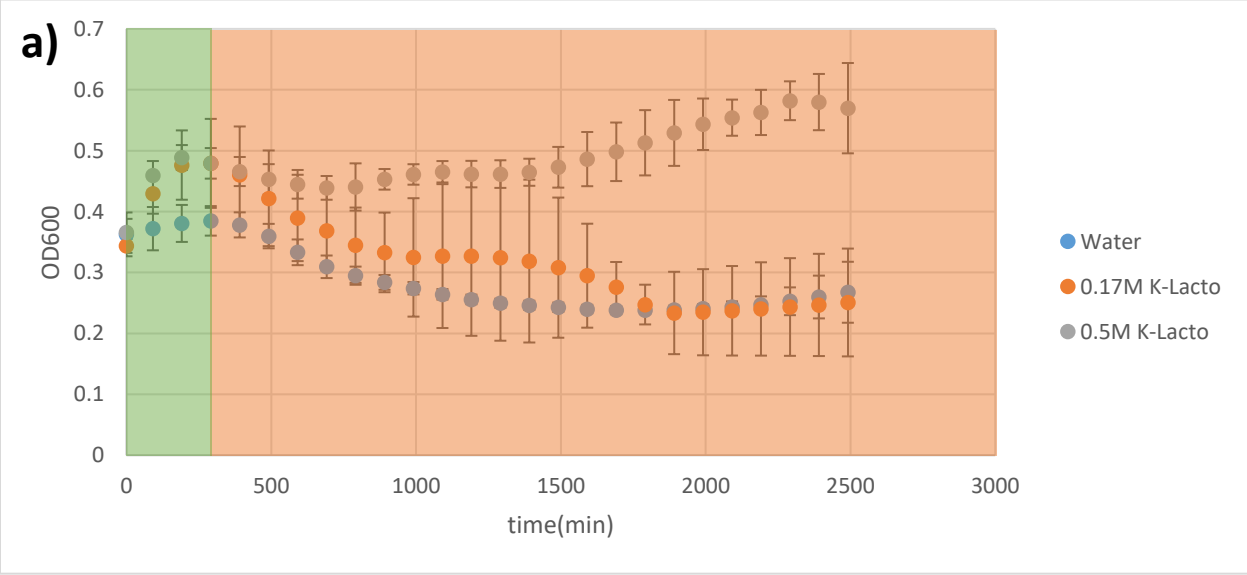
Optical density (OD) readers are a well-established tool for studying bacterial growth (Krishnamurthi, 2021). As *B. subtilis* can range from a few micrometers in size (Errington & van der Aart, 2020), they can be feasibly measured with OD_{600} measurements (Stevenson et al, 2016). In a clean 96 well plate, six wells were filled with 100 microliters of water and 100

microliters of the *B. subtilis* solution. Six wells were filled 100 microliters of 0.17M K-lactobionate solution and 100 microliters of *B. subtilis* solution. Finally, wells were filled 100 microliters of 0.5M K-lactobionate solution and 100 microliters of *B. subtilis* solution. Growth in well plates was measured with an Epoch2 Microplate Reader for 42 hrs at 31°C. Averaged results were tabulated by taking the average of n=5 independent trails, growth curves from individual trials are shown in Figures A.1 to A.3 in Appendix A. Error for averages was tabulated using standard deviation from all 5 trails. Student t-tests assuming unequal variances were used to identify statistical differences.

4.3 Results

Growth curves are shown in Figure 4.1a. From this data, *B. subtilis* growth in water, 0.17M K-lactobionate solution and 0.5M K-lactobionate were compared. The carrying capacity, defined as the OD_{600} reading at the end of the 2491 minute trial was found to be 0.2676, 0.2508, and 0.5698 respectively. Of these the 0.5 M and the water control were statistically different ($P< 0.01$), while 0.17 M and the water control were not statistically different ($P= 0.72$), as shown in Figure 4.1b. The overall change in OD_{600} signal over the 2491 minute period represented the overall change in bacteria levels in the well plate, shown in Figure 4.1c. The overall rate for water, 0.17 M solution, and 0.5 M solution was found to be -0.1372 hr^{-1}, -0.13411 hr^{-1}, and 0.2945 hr^{-1}. The 0.5 M solution was statistically different than the water control ($P < 0.01$), but the 0.17 M solution was not different than the water control ($P = 0.96$). The decay rate was defined as the change in OD_{600} signal over the 261 to 2491 minute period. This represented the rate at which the bacteria population changed in the solution after the first peak, which was found to around at 261 minutes in all samples. The decay rate for water, 0.17 M solution, and 0.5 M solution was found to be -0.1899 hr^{-1}, -0.3703 hr^{-1}, and 0.1424 hr^{-1}, as shown in Figure 4.1d. It is noted that after 261

minutes the water and 0.17 M bacterial populations decayed, however the 0.5 M solution was able to recover and reach higher bacterial populations after the first peak. 0.5 M soultions were statistically different ($P<0.01$) than the water control, but 0.17M soulutions were not different compared to the water control ($P= 0.17$). The initial growth rate was defined as change in OD_{600} signal from t = 0 to t = 261 minute period and represented the initial growth rate of the bacteria in the well plate. The overall rate for water, 0.17 M solution, and 0.5 M solution was found to be 0.3117 hr^{-1}, 1.884 hr^{-1}, and 1.5944 hr^{-1} respectively, as shown in Figure 4.1e. Both 0.17 M and 0.5 M were statistically different compared to the pure water control with $P = 0.03$ and $P<0.01$ respectively.



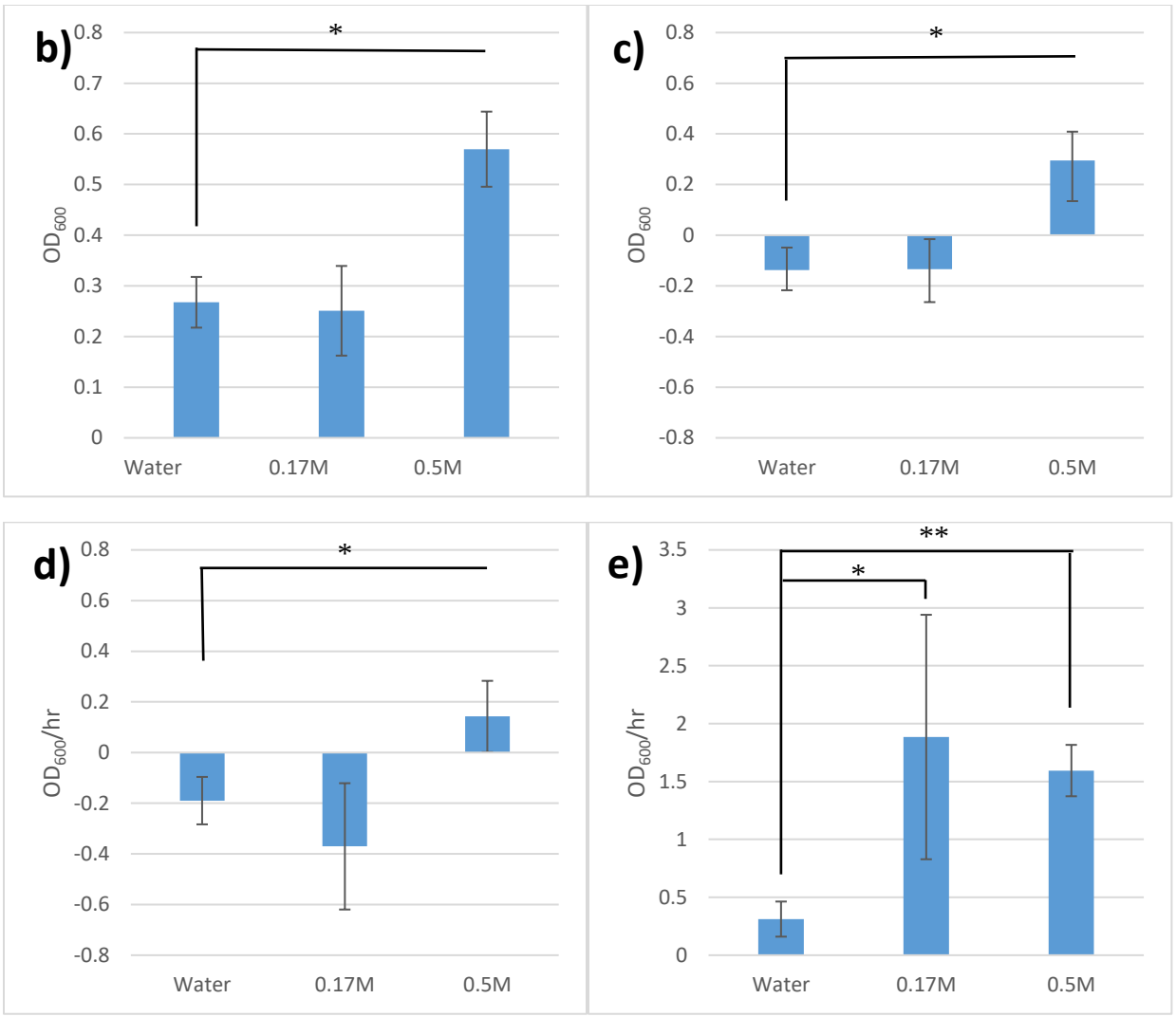


Figure 4.3.1 Comparison of *B. subtilis* growth in water, 0.17M K-lactobionate and 0.5M K-lactobionate. A) Growth trajectories generated from OD_{600} readings of *B. subtilis* during culture in the three media with green area representing initial growth, and orange area representing decay B) Comparison of the carrying capacity of *B. subtilis* in the three media (t=2491 min). C) Comparison of the overall population change of *B. subtilis* in the three media (t=0 to t=2491 min). D) Comparison of decay rates between t=261 min to t=2491 min. E). Comparison of initial growth rates between t=0 min to t=261 min.

4.4 Discussion

Initial *B. subtilis* growth rates were similar for both concentrations of K-lactobionate, but the carrying capacity was noticeably higher in the 0.5 M K-lactobionate solution as opposed to 0.17M K-lactobionate solution and the control. Higher growth rates and higher carrying capacity are expected results. Carbon sources from food byproducts that have been shown to generally

cause bacteria growth (Sánchez-Zapata, 2013). K-lactobionate is a sugar acid, which is a readily digestible carbon source for bacteria (Gunina & Kuzyakov, 2013), and *Bacillus* strains were shown to grow with even minimal additions of carbon (Mageshwaran, 2014). The higher growth rate corresponding to the higher K-lactobionate substrate concentration was also expected, as growth is directly correlated to substrate concentration (Kayombo et al, 2003). Cations, such as the potassium in K-lactobionate, have also been shown to promote cellulose production in certain strains of bacteria (Hong & Qiu, 2008), an important component of EPS production, which is important for bacterial proliferation (Schmid, 2015). More specifically, Potassium is a critical nutrient for the growth of *B. subtilis*, with potassium deficiencies in soil being shown to limit growth (Gundlach et al, 2017). The results suggest the potential of K-lactobionate as a nutrient source for *B. subtilis* in otherwise nutrient poor soils or in irrigation waters used for distribution. As shown in Fig 4.1a, the presence of 0.5M K-lactobionate solution was able to sustain *B. subtilis* over a 41.5 hr time period, which may be enough time for distribution into soils through irrigation water.

4.5 Conclusion

In 96-well plate culture, the growth of *B. subtilis* in media with and without K-lactobionate was compared utilizing an 96-well plate reader. Two K-lactobionate solutions were tested (0.17M and 0.5M) and compared to a control containing ultrapure water only. Initial growth rates were five to six times higher with K-lactobionate present than in ultrapure water. The carrying capacity after 42 hours was roughly equivocal for 0.17 M K-lactobionate and ultrapure water, but final carrying capacity was higher with 0.5 M K-lactobionate than with 0.17 M K-lactobionate and ultrapure water. The K-lactobionate solution is less effective than nutrient rich media such as Tryptic Soy Broth (TSB), which typically results in OD_{600} readings as high

1.0 within a 24 hour growth period. Despite this, the evidence indicates that *B. subtilis* has metabolism for K-lactobionate, as indicated by the concentration-dependent initial growth rates and in the case of 0.5 M K-lactobionate, sustained cell population over the 40 hr test period. This produced the desired results as it showed that K-lactobionate could be utilized to support *B. subtilis* populations in nutrient poor soils or irrigation water. Using the higher concentration of 0.5 M K-lactobionate may further improve soil aggregation, water penetration, and water evaporation studies, similar to those performed in Sections 2 and 3 at lower K-lactobionate concentrations, by promoting higher levels of *B. subtilis* growth and survival and potentially providing higher levels of soil aggregation. Future work here would investigate the ability of K-lactobionate to promote *B. subtilis* growth in nutrient-poor soils.

Chapter 5 - Conclusions

This thesis provided an investigation on the effect of soil water retention when amending soils with *B. subtilis* combined with K-lactobionate. In Chapter 2, water penetration times were tested in sandy loam soils amended with *B. subtilis* and *B. subtilis* with K-lactobionate and compared to unamended control soils. It was found that *B. subtilis* with K-lactobionate resulted in a 75% reduction in penetration time compared to the control. This was attributed to the formation of larger soil aggregates, increasing surface pore sizes allowing water to flow into the soil at a higher rate than in the less aggregated soils. These results reflect positively on utilizing K-lactobionate as a means of saving irrigation water by reducing runoff. Future studies should focus on deciphering the mechanisms of soil aggregation from this amendment, which could be due to EPS production or from K-lactobionate hydrogen bonding with soil particles.

In Chapter 3, *B. subtilis* and K-lactobionate amendments were tested for increases in soil water retention in sandy loam soil during periods of evaporation. It was found that K-lactobionate amended soils, both with and without *B. subtilis*, resulted in a higher initial soil water content. Soil treated with K-lactobionate only and K-lactobionate with *B. subtilis* both had higher SWC after the evaporation period. These results are in line with other literature findings indicating that soils with larger aggregates were able to hold more water during evaporation. Overall, this would indicate that K-lactobionate combined with *B. subtilis* can potentially increase water infiltration, water retention in sandy loam soils, and could potentially improve crop production through reducing runoff. Further research at a field scale is necessary to verify if this holds true in non-laboratory conditions where plant matter, environmental pressures, and other bacteria may alter results.

In Chapter 4, *B. subtilis* growth from K-lactobionate was investigated. It was found that the initial growth rate of *B. subtilis* increased 5-6 times with the presence of K-lactobionate compared to the ultrapure water control. It was also found that the carrying capacity of the solution was twice as high compared to an ultrapure water control with 0.5M K-lactobionate during a 42 hour period, although the 0.17M K-lactobionate solution also had similarly high carrying capacities in shorter timeframes. The data suggests that K-lactobionate can serve as an effective promoter of *B. subtilis* growth in otherwise nutrient poor environments. Future studies here include measuring *B. subtilis* growth with K-lactobionate after amendment into non-sterile, sandy and sandy loam soils.

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Appendix A - ODE Results

Figure A.1. ODE Results of water + *B. subtilis*

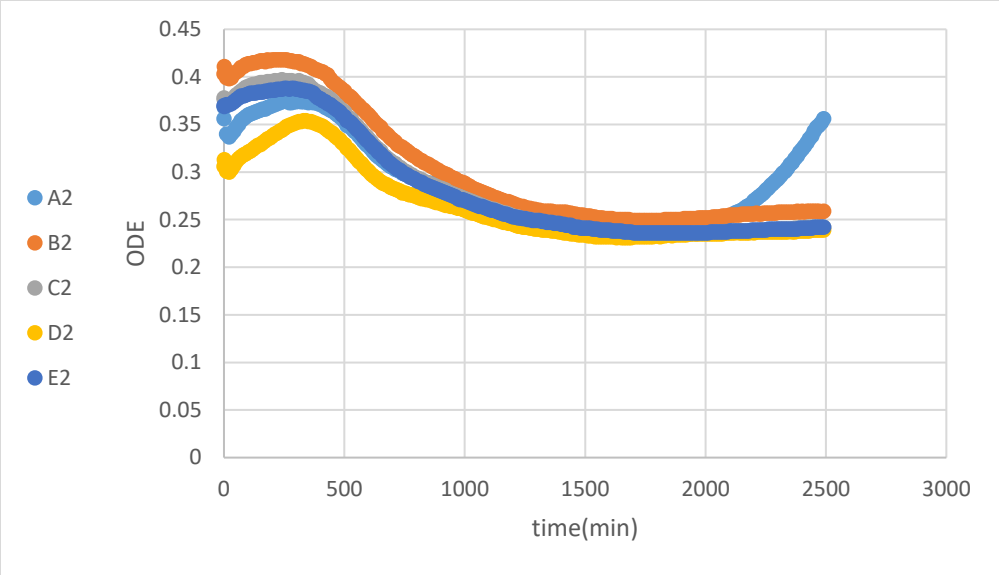


Figure A.2. ODE Results of 0.17M K-Lactobionate solution + *B. subtilis*

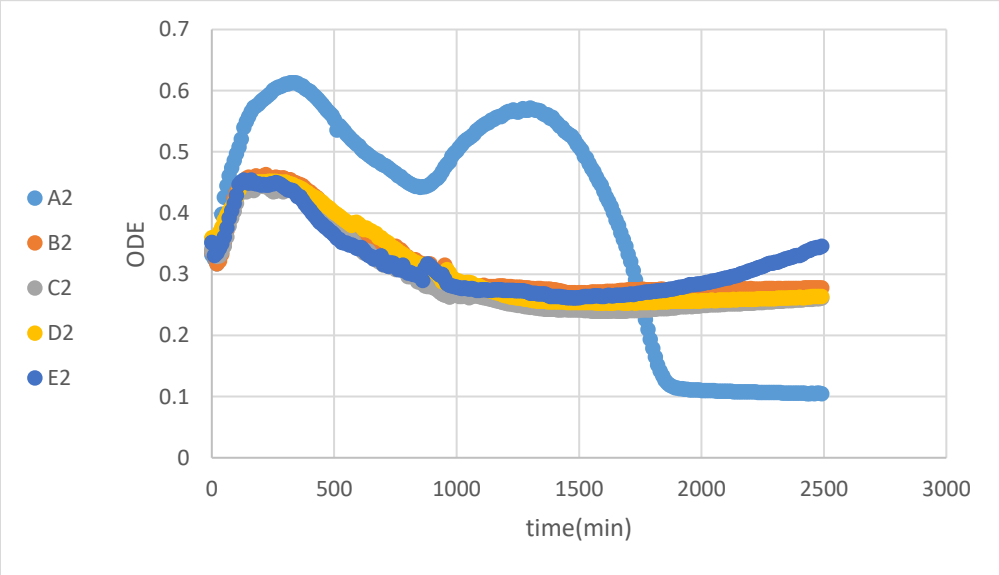
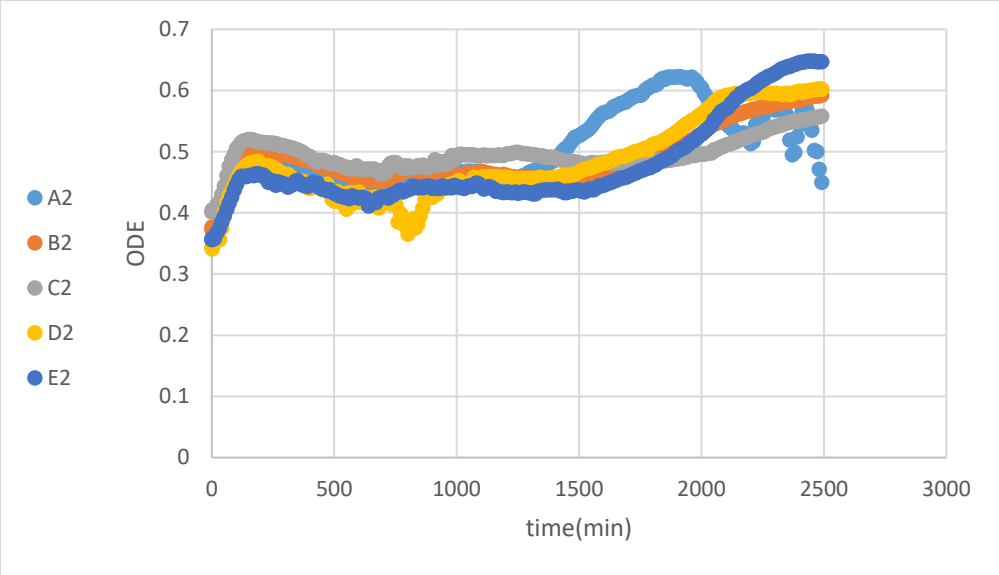


Figure A.3. ODE Results of 0.5M K-Lactobionate solution + *B. subtilis*


0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
ODE
A2
B2
C2
D2
E2
0
500
1000
1500
2000
2500
3000
time(min)