Comparative transport of *E. coli* and *Legionella* in 2-Dimensional Porous Media Tank

by

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ABSTRACT

The study was to analyze the extent of bacterial transport in a two-dimensional tank under saturated conditions. The experiments were done in a 2-D tank packed with 3,700 in^3 of fine-grained, homogenous, chemically inert sand under saturated conditions. The tank used for transport was decontaminated by backwashing with 0.6% chlorine solution with subsequent backwashing with chlorine-neutral water (tap water and $Na_2S_2O_3$) thus ensuring no residual chlorine in the tank. The transport of bacteria was measured using samples collected from ports at vertical distances of 5, 15 and 25 inches (12.7, 38.1 and 63.5 cm) from the surface of the sand on both sides for the 2-D tank. An influent concentration of 10^5 *CFU/*mL was set as a baseline for both microbes and the percolation rate was set at 11.37 inches/day using a peristaltic pump at the bottom outlet. At depths of 5, 15 and 25 inches, *E. coli* breakthroughs were recorded at 5, 17 and 28 hours for the ports on the right side and 7, 17 and 29 hours for the ports on the left sides, respectively. At respective distances *Legionella* breakthroughs were recorded at 8, 22 and 35 hours for the ports on the right side and 9, 24, 36 hours for the ports on the left side, respectively which is homologous to its pleomorphic nature. A tracer test was done and the visual breakthroughs were recorded at the same depths as the microbes. The breakthroughs for the dye at depths of 5, 15 and 25 inches, were recorded at 13.5, 41 and 67 hours for the ports on the right side and 15, 42.5 and 69 hours for the ports on the left side, respectively. However, these are based on visual estimates and the physical breakthrough could have happened at the respective heights before the reported times.

This study provided a good basis for the premise that transport of bacterial cells and chemicals exists under recharge practices.

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LIST OF ABBREVIATIONS

Abbreviations	Full Forms
ASU	Arizona State University
ATCC®	American Type Culture Collection
C	microbe concentration
°C	Degrees Celsius
CDC	Centers for Disease Control and Prevention
CFT	colloid filtration theory
CFU	colony forming unit
D	Hydrodynamic dispersion coefficient
DI	Deionized
DLVO	Derjaguin and Landau 1941, Verwey and Overbeek 1948
DNA	deoxyribonucleic acid
dsDNA	double stranded deoxyribonucleic acid
°F	Degrees Fahrenheit
g	gram(s)
g/L	grams per liter
gal	gallon(s)
mg/L	milligram per liter
hr(s)	hour(s)
hh : mm	hour : minute
in^3	cubic inches
in/day	inches per day
in/hr	inches per hour
L	liter(s)
MHOS	micromho(s)
MHOS/cm	micromhos per centimeter
mL	milliliter(s)
mL/min	milliliters per minute
mm	millimeter(s)
μμ	Microns
nm	nanometer(s)
ND	not detected
NOP	National Organic Program
NTU	Nephelometric Turbidity Unit
PBS	phosphate buffer saline

Abbreviations	Full Forms
RPM	revolutions per minute
S	attached microbe concentration
t	Time
TDS	Total Dissolved Solids
TNC	Too numerous to count
TSA	Tryptic Soy Agar
TSB	Tryptic Soy Broth
U.S.	United States
WHO	World Health Organization
x	Distance
v	interstitial microbe velocity
ρ_b	dry bulk density
ε	bed porosity
5L	5 inch, Left Side
5R	5 inch, Right Side
15L	15 inch, Left Side
15R	15 inch, Right Side
25L	25 inch, Left Side
25R	25 inch, Right Side

1. INTRODUCTION

1.1 Motivation and significance

The concern over the purity of water and the transport of contaminants in a closed system has led to a lot of studies in the specific field. Transport of contaminants is a huge issue in water systems, especially when large communities are attached to them. Contaminant transport in groundwater is a concern for communities that are dependent on groundwater and perform artificial ground water recharge. Many studies directed towards the analysis of surface and groundwater systems have shown presence of *E. coli* and shown its potential for transport and movement in soil. Thus transport studies for bacteria gain more prominence in systems that perform artificial recharge and are dependent on groundwater as their primary source. It is estimated over 150 million Americans directly depend on groundwater for their water supply (Hynds et al., 2014). In an adjacent study, 15% of the groundwater samples collected from all over the US, showed fecal contamination.

To prevent an outbreak of a pandemic via ingestion of contaminated water, accurate remediation and risk assessment studies must be conducted. The prediction/estimation of the transport of bacteria in saturated systems can aide in the process of better reclamation techniques. Microbial transport is a complex phenomenon. This, although studied vastly, leaves research gaps that need filling. Packed, saturated media offer a good substitute for ground water movement as it elucidates the effects of deposition kinetics and advection. The extent of removal of the microbes via filtration and physical removal gives an estimate of the extent of transport. *E. coli* is a well-known

and widely accepted indicator of fecal pathogens. The presence of *E. coli* generally indicates fecal contamination. The motivation for the study was based on this very fact.

Legionella, a common, well-known water-borne pathogen is understudied and there exist a lot of research gaps about its potential for transport. Legionellosis has been on the rise country-wide ever since its discovery in 1976 (Hicks et al., 2011). *Legionella* has been the part of the contaminant candidate list (CCL) 3(2009) and 4(2015) released by the United States Environmental Protection Agency (US. EPA). It is known to seek sanctuary amidst biofilms, amoeba and ciliate hosts. Studies about the presence of *Legionella* in reclaimed water are seriously understudied and the information gap hence formed is one of the main motivations of this study.

1.2 Goals of this study

The goal of this study is to establish a better understanding of the transport of bacteria in a saturated two dimensional confined matrix with supervised flow parameters. In this project, we have investigated transport for E. *coli*, a non-reactive inert dye (fluorescein Na- salt) and *Legionella*. E. *coli* is a widely accepted indicator in spite of it having cons as an indicator. There are multiple cases where it has been noted that the pathogens have out-survived the indicator, one reason why we need better predictive methodologies. The study was done in a two-dimensional granular porous media packed tank. The concentration of microbes or dye in the samples collected from the ports at 5 inches, 15 inches and 25 inches will help us establish a breakthrough curve. Breakthrough would indicate the time when the microbe and the tracer (dye, fluorescein diacetate) reach a

specific port hence identifying the transport. The data hence received could be used to predict better the transport of pathogens that are potential hazards to public health. The main goals could be listed as,

1. Measure and establish the transport and breakthrough of *E. coli* via experimentation.
2. Measure and establish the transport and breakthrough of *Legionella* via experimentation.
3. Measure the transport and breakthrough of dye (fluorescein-Na salt) via experimentation.

2. LITERATURE REVIEW

2.1 *E. Coli* background information

2.1.1 Characteristics and morphology.

E. coli or *Escherichia coli* belong to the family Enterobacteriaceae (*Escherichia coli*: pathogen safety data sheet- infectious substances, 2012). It was discovered by Theodore Escherich in 1884. *E. coli* belonging to the Enterobacteriaceae family, showcases all the major traits of the bacteria from this family:

1. Ferments Glucose.
2. Reduces Nitrates to Nitrites.
3. It is gram-negative and rod-shaped.
4. It is motile (flagellated strains). (Sanders & Brophy-Martinez, 2007)

The diameter of *E .coli* varies from 0.25-1.0 μm and the length is measured to be around 2 μm. These are facultative anaerobes, non-spore forming generally motile fecal coliforms that do not generate enterotoxins. (*Escherichia coli*: pathogen safety data sheet-infectious substances, 2012)

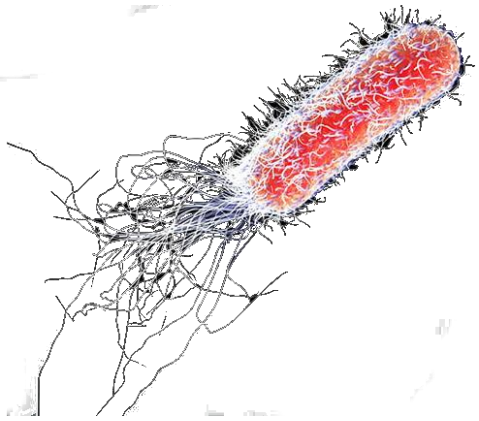


Figure 2.1 *E. coli* Bacteria

Source: *Biocote Limited*. Retrieved 2018, from https://www.biocote.com/blog/five-facts-e-coli/

2.1.2 *E .coli* as a microbial indicator

E. coli, although a naturally occurring soil bacteria, is used as an indicator organism for identifying fecal contamination of a water system. It is a fecal coliform bacterium that is present in the intestines of warm blooded animals and humans, the reason they can help identify if the water is fecally polluted. There have been 575,457 reported cases of water-borne diseases from the time 1971-2002, out of which 79 lead to deaths, 14% are caused by bacteria, 19% protozoa and 8% viral pathogens. (Wang et al., 2013). Since the presence of *E. coli* generally indicates fecal pollution, it is understood that other pathogens would also be present in the hence contaminated water, although *E. coli* cannot be considered an ideal indicator for those pathogens. The lifespan of *E. coli* is not very long outside the human body; hence it can be considered as a good indicator of recent pollution (Thompson et al., 2007). There are some contradictions to this theory though which claim the lifespan of *E .coli* to be a lot longer than estimated (Ishii et al., 2008). Their high number when they exist and relative ease of identification help them attain the tag of a widely accepted microbial indicator.

2.1.3 Transmission.

E. coli is found in intestines of warm-bodied organisms. (Vogt et al., 2005). Thus it is a good indicator of fecal contamination. The mode of transmission of *E. coli* is fecal-oral. *E. coli*, according to WHO, can be transmitted to human via ingestion of contaminated food, especially via the consumption of uncooked food (World Health Organization, 2016). The incubation period is estimated to be between 6 to 48 hours while the infectious dose is estimated at around 10^6 organisms. Once present in soil the *E. coli* can easily be washed down to the groundwater.

2.1.4 Presence of *E.coli* in soils and aquifers

E. coli, a member of the fecal coliform group, is identified as an indicator of fecal pollution. Their presence typically suggests fecal pollution. In studies conducted by the USGS, 1205 wells were analyzed. 1174 of these wells were found to be fecally contaminated. (USGS, 2006). Samples from 347 (29.6 % of the 1174 wells) tested positive for *E. coli.* The *E. coli* in the samples from the wells had a concentration ranging from 1-1200 CFU/100ml. This gives us the estimates that there is a good possibility of *E. coli* being present in groundwater. According to Ishii et al., 2006, the presence of *E. coli* in temperate soil varies seasonally.

Lithography plays a very important role in the presence of *E. coli* in aquifers (USGS, 2006).

Detection frequencies were higher for carbonate rocks, crystalline rocks, shale, undifferentiated sedimentary rocks as compared to unconsolidated materials, semi

consolidated sand or volcanic rocks. Crystalline rocks are primarily undifferentiated metamorphic rocks and schist; carbonate rocks are listed primarily as dolomite and limestone while unconsolidated materials are typically mixtures sand, gravel and clay/ alluvium. More than 50% of the wells sampled having carbonate rocks like the ones at parts of the Valley and Ridge, the Floridian and Piedmont and Blue Ridge aquifers, showed relatively higher coliform presence. (USGS, 2006) On the other hand, frequency of detection of coliforms in consolidated rocks is significantly low. About 20% of the wells tested positive. (USGS, 2006) It has seen in various studies that most principal aquifers have primarily unconsolidated materials, hence the choice of the material, gravel and heterogeneous sand. In carbonate rocks, the concentration varied from 1-24 CFU/100ml.

2.2 *Legionella* background information

2.2.1 Characteristics and morphology.

Legionella bacteria are thin, rod-shaped, aerobic, pleomorphic, flagellated, non-spore forming, and gram-negative bacilli of the genus *Legionella*. They are a known pathogen to humans. There are 39 species and 60 distinct antigens. *Legionella* in the environment is facultative intracellular micro-organism, which is a parasite and needs a host such as amoeba. This host acts as a protecting shell for *Legionella* and also a source of nutrient. (Greub and Raoult, 2003) To grow, *Legionella* needs L-Cysteine hydrochloride, which is not freely available in the environment. It is widely debated whether *Legionella* can survive without the host in the environment, but it is certain that it cannot multiply without it. On reaching a mature stage, it may become filamentous when grown in-vitro. (Winn et al., 1993). The *Legionella* bacterium is not encapsulated and its cell wall has an outer cell membrane. This cell membrane has a high amount of lipopolysaccharide. (Brenner et al. 1979). It has a diameter of 0.3-0.9 μm and a length of about 4 μm.

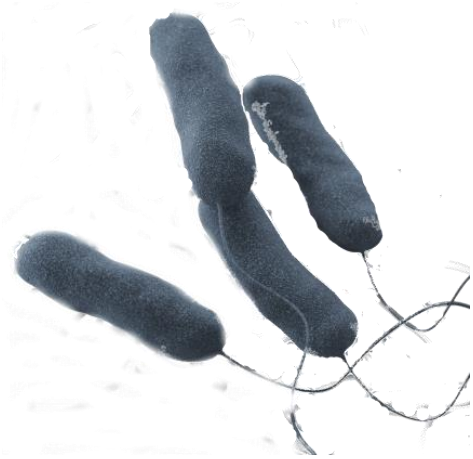


Figure 2.2 *Legionella* Bacteria

Source: Centers for Disease Control and Prevention. (2018, April 30). Retrieved 2018 from https://www.cdc.gov/legionella/images/materials-illustration.jpg

Table 2.1 Chlorine resistance of waterborne pathogens relative to *Escherichia coli*.

Genus or Species	**CT $_{99.9\%}$**	**Reference**
Escherichia coli	0.09 (reference)	Taylor et al. 2000
Legionella pneumophila		
Medium-grown	7.5 (83-fold)	Kuchta et al. 1985
Water-adapted	52.5 (580-fold)	Kuchta et al. 1985
Mycobacterium avium		
Medium-grown	51 (567- fold)	Taylor et al. 2000
Pseudomonas aeruginosa	1.92 (21 fold)	Grobe et al. 2001
Methylobacterium spp.	1.5 (16.7 fold)	Furuhata et al. 1989
Acinetobacter baumanii	59 (658- fold)	Karumathil et al. 2014
Aeromonas hydrophila	2.6 (29- fold)	Sisti et al (1998)

2.2.2 *Legionella* as a pathogen.

Legionella in the environment is a facultative intracellular parasite that needs a host such as amoeba. This host acts as a protecting shell for *Legionella* as well. *Legionella* feeds off the amoeba as it needs L-cysteine hydrochloride for growth which is not freely available in the environment. It is debated widely whether the survivability of *Legionella* is possible in the environment without the host, but it is certain that it cannot

multiply without it. It may become filamentous on reaching a mature stage when grown in vitro.

The *Legionella* bacterium is not encapsulated and its cell wall has an outer cell membrane, which has high lipopolysaccharide content (Brenner et al. 1979).

2.2.3 Transmission

Legionella is a common yet understudied pathogen. It is transmitted mostly via aerosols and come through humidifiers, showers, cooling towers. It has also been found in creeks and ponds and the soil from their banks. It has been seen that bacteria survive for months in environmental conditions. Although there is significant proof that there is *Legionella* in aquatic systems, the mode of transmission s via inhaling aerosols.

In a study done (Zacheus and Martikainen, 1994), there were *Legionella* [resent in BOCO of hot water distribution systems.

The existence of *Legionella* in water systems is a direct product of the presence of amoeba. It is well established that amoeba acts as a host for *Legionella*. It has been hypothesized that control of amoeba could lead to direct control of *Legionella* being transmitted.

2.2.4 Presence of *Legionella* in aquifers

Legionella is ubiquitous in water systems. L. *pneumophila* are responsible for the majority of the water-borne diseases (drinking water and non-recreational) in the United States. (McBurnett et al., 2018). The survival of *Legionella* depends on their association

with biofilms and endoparasitization of amoeba and ciliate hosts (Abu Kwaik et al., 1998). *Legionella* has been detected in 60% of the samples (3/5) collected from recharge basin samples collected from sampling sites in California. (McBurnett et al., 2018)

In a state like Arizona, which is one of the leading states when it comes to practicing groundwater recharge, the potential threat of *Legionella* being present in the water is very real. It has been seen that *Legionella* is present in not only reclaimed water, but also surface water. It has a high proclivity for growing in reclaimed water (McBurnett et al., 2018).

Legionella has been shown to have grown in chlorinated effluents as well with a concentration of 1.0×10^3 CFU/ml (Palmer et al. 1995) and treated Reclaimed water of 1.0×10^2 CFU/ml (Jjemba et al. 2010).

2.3 Mechanisms of transport of microbes through soil and aquifers

In a span of 30 years, there have been over five hundred thousand cases of water contamination, 14% of which have been attributed to bacterial infection. Detection of Pathogenic microbes in groundwater has been found via the multiple studies that have been done by multiple agencies like USGS. Interest in understanding the factors and the mechanism at play for deriving a suitable theory that illustrates a theory that supports the transport of microbes (Hornberger, et al. 1992). It has been seen that (Corapcioglu and Haridas, 1984) there is strong evidence that human waste is directly related to groundwater contamination. Contaminants, which in this case are microbes, enter the soil and travel long distances in groundwater if the conditions are suitable. It has been noted by multiple researchers that the sources are of fecal origin or due to agricultural waste not disposed of properly.

Most of the researches done to identify the mechanism of microbial transport have been performed from repacked column breakthrough curves (BTCs) and retention profiles (RPs), batch experiments and complementary micro-modeling studies. (Wang et al., 2012).

The major driving force for microorganism transport is via the bulk flow although there are multiple other mechanisms that occur. Microbes are at times motile in nature. This motility may attribute to transport of microbes as well as seen in literature. Microbial growth via the course of the experiment in the system can also affect the transport, although this is not taken into consideration while considering the experiments in bench scales. Primarily because the run time of one experiment is typically less as

compared to the incubation time of the microbe in that specific environment. Microbial dispersion on the other hand is another viable mechanism of transport.

In the broader scheme of things, mechanisms can be broken into physical, chemical, biological and geotechnical processes.

Chemical processes can be broken down into further sub categories such as convection, advection and hydrodynamic dispersion (Abu-Abshour et al., 1994). The transfer of microbes by the flow of fluid (water) horizontally/ vertically would be referred to as advection. In short, transport of the microbes by bulk motion of liquid is considered in this case the velocity of the carrying fluid (water) plays a very important role in such a mechanism. The process of dispersion can be described as a process by which agglomerated particles/ microbes are separated from each other. Dispersion can be further broken down into two subparts, namely diffusion and mechanical mixing. In a saturated column dispersion happens because ground water flows through different pores at different rates and various flow paths vary in length. Diffusion often defined by Fick's law is the mechanism of spreading of microbes as a result of difference in concentration gradient. Fick's law can be expressed by the following expression:

$$F = -D\frac{\mathrm{dC}}{\mathrm{dx}} \qquad \textit{eq.}\ 2.1$$

Where D is the diffusion coefficient (L^2/T) F is the mass flux of solute per unit area per unit time and C is the concentration of solute (M/L^3).

For a scenario where the concentration changes with time, the diffusion equation would be expressed via Fick's second law.

$$\frac{\mathrm{dC}}{\mathrm{dx}} = D\frac{\mathrm{d}^2C}{\mathrm{d}x^2} \qquad \textit{eq.}\ 2.2$$

Although diffusion is pretty common in transport of solutes in a saturated environment it does not play as important a role in microbial transport. It has been seen mechanical mixing is the preferred mode of transport when microbes are concerned. Mechanical mixing can occur due to fluid velocity distribution in pores, variability in pore velocity convergence/ divergence of pore channels, fractures and macropores.

The physical processes include filtration, adsorption/ desorption and sedimentation. Filtration is the most prominent out of all these. Filtration can be sub divided into separate sub categories. They include surface/ cake, vacuum filtration. This happens when microbes flow through the pores and are too large to penetrate, thus accumulating on the surface of the soil. This is common when the ratio between soil grain diameter and microbe is lesser than 10. The other mechanism, straining, happens when the ratio is between 10 and 20. (Abu- Abshour, et al., 1994). Physio-chemical filtration happens when the ratio increases past 20 and can even go up to 1000. This primarily happens when there is interaction between microbes and media. The collision between the media particles and the microbes determine the extent to which this process would affect the transport. The collecting efficiency of a media particle also plays an important role in the extent to which this would be effective. The collector efficiency or the ability of the media particles to adhere to the microbes can be explained by the following formula.

$$\eta = \frac{rate\ at\ which\ microbes\ strike\ the\ collector}{v_0 C_0 (\frac{\pi d^2}{4})} \qquad eq.\ 2.3$$

Where η is the efficiency of the collector, v_0 & C_0 are the water velocity and the concentration of the microbes, d is the grain diameter.

Adsorption is another physical process that governs transport of microbes. It relies on physical and chemical nature of the absorbate (microbes) and obsorbent (soil media), pH of the solution is also important, the characterization of flow and the degree of saturation. (Haridas and Corapcioglu, 1984). Salt content in the soil aids adsorption. It is directly proportional to the adsorption. This happens due to the formation of double layers. Another thing that affects the adsorption is the moisture content of the soil. It has been seen that the higher the soil moisture the lesser the attenuation. (Abu- Abshour et al. 1994) Studies have shown that the adsorption kinetics were better described by a Freundlich isotherm as compared to a Langmuir isotherm (Bitton et al. 1980).

Gravitational deposition of the microbes can happen if there is a difference in the bulk density of the soil and microbes. (Haridas and Corapcioglu, 1984) In general bacteria tend to be buoyant and tend not to settle. However it has been seen (Gerba et al. 1984) that there could be a scope for sedimentation to happen and some bacteria could be removed/ transported via this method.

Any particle ranging between the size range of 100 nm to 100 μm, can be theoretically classified as colloids. The colloid/ classical filtration theory (CFT) is often used to describe the transport of colloid or bacteria through saturated porous media. The theory summarizes that bacteria that are colloids will undergo less deposition during transport as compared to the larger ones. It would have lesser chance of interaction with the media. It has been shown that less removal translates to faster transport. (Abbaszadegan et al., 2011)

The governing equation for transport is a modified version of Richard's equation. The equation being used is,

$$\frac{dC}{dt} = \alpha_L\left(\frac{q}{n}\right)\frac{\partial^2 C}{\partial x^2} - \left(\frac{q}{n}\right)\frac{\partial C}{\partial x} - k_c C + k_y S \qquad eq.\ 2.4$$

$$\frac{dS}{dt} = k_c C - k_y S \qquad eq.\ 2.5$$

C being the concentration of the microbes, α_L is the dispersivity (cm), q the Darcian velocity, n the fractional porosity, k_c and k_y deposition and entrainment coefficients, S the concentration of bacteria associated with the solid phase but expressed per volume of pore water (cells mL^{-1}).

The macroscopic mass conservation equation for species in a porous medium in space can be denoted by:

$$R_a + \frac{\partial(\theta C)}{\partial t} = -\nabla \cdot J + R_d + R_g \qquad eq.\ 2.6$$

$$R_{df} = -k_d \theta C\ ; \qquad R_{ds} = -k_d \rho \sigma \qquad eq.\ 2.7$$

$$R_{gf} = \mu \theta C\ ; \qquad R_{df} = \mu \rho \sigma \qquad eq.\ 2.8$$

Where R_a is the rate of deposition, R_d is the rate of decay and R_g is the rate of growth, k_d is the decay rate constant and μ the growth rate constant. In the above equation, θ is the volume occupied by the flowing suspension per unit volume. σ is defined as the volume of captured bacteria in unit volume of bulk soil ρ density of bacteria.

R_{ds} and R_{df} is the decay rate in solid and liquid media.

R_{gf} and R_{gs} is the growth rate in fluid and solid media.

Combining the equation 2.6, 2.7 and 2.8 we get the following equation for macroscopic mass conservation equation for species in a porous medium.

$$\frac{\partial(\theta C)}{\partial t} = -\nabla \cdot J + (\mu - k_d)\,\theta C - R_a \qquad eq.\ 2.9$$

Where the flux of bacteria, $J = -D\theta\nabla C + (v_f + v_m + v_g)\,\theta C - R_a$ *eq.* 2.10

D is the coefficient of hydrodynamic dispersion, is the sum of Brownian diffusion (non-primary).

Effective diffusivity due to the tumbling of bacteria and mechanical dispersion (primary), v_f, v_m, v_g are the fluid, chemotropical and gravitational settling velocity. R_a being the combined mass transfer coefficient.

Now it must be kept in mind that equation 2.x does not have the growth and decay. It is ignored because the growth time of microbes in question under the conditions in which the experiment is done is more than the growth/decay time as is discussed below.

In addition to the above mechanisms of transport, the biological process affecting transport are growth, inactivation/death and is also influenced by pH of the media, the temperature of the surroundings, nutrient availability and competition with other microbes for survivability. (Tufenkji, 2007)

In must be noted that dynamics of the growth/ decay of microbes in specific conditions is poorly quantifiable and is often unpredictable. (Tufenkji, 2007) The intrinsic characteristics of the microbes also affect the transport of the microbes. Chemotactic migration happens in cases where motile microbes migrate via a chemical gradient. Microbes tend to move away from zones of high chemical concentration. (Abu-Abshour et al. 1994).

2.4 Factors which influence transport of microbes through soils and aquifers

Microbial Transport is affected by a variety of factors adhesion, filtration, physiological state of the cells, porous medium characteristics, flowrate of the water, predation and intrinsic mobility of the bacterial cell. On top of that, the composition of the ground water, the path taken during the flow, subsurface chemistry and the type of media through which bacteria travel, affect the transport of the microbes. The movement can be affected by the presence of by the presence of macropores in the packed media, which might lead to preferential flow (Abu-Abshour et al. 1994).

The transport of microbes is affected by microbial filtration, physiological state of the bacterial cell, microbial adhesion with the media particles, pH of the matrix solution, ionic strength of the solution, cellular appendages and hydrogeological factors.

2.4.1 Microbial Filtration

Transport of microbes is affected by the media that make up the packed column in an experiment. The mechanisms at play in this case is physiological straining details of which has been discussed in section 2.3. Filtration has shown a direct correlation with bacterial size (Gannon et al. 1991a). For sandy soil there wouldn't be a huge difference as there would be a significant difference between the size of the bacterial cell and the media particles. However, in a media where there would be a significant quantity of silt, the transport is seen to be significantly slower. In all viruses have shown lesser retention as compared to bacteria.

Microbial filtration can lead to a phenomenon called microspore exclusion, where bacteria are excluded from the microporous domain of structured porous media. This, in turn leads to a difference in microbial activity within the micropores that exclude microbes and the immediately adjacent colonized site, which affect the overall transport of the matrix.

2.4.2 Physiological State of the microbial cell

Many factors can lead to changes in the morphology of bacteria. The size of the bacteria plays an important role in transport and determining the potential of bacteria to be transported.

The physiological state is one of the key factors that determine the size of bacteria. In case of an abundance of nutrients, bacterial cells produce exopolymers that can form an outer shell/ coating on the surface bacterial cell. The exopolymers may also enhance attachment hence increasing size on the backdrop of an increase in diameter in the first place. This may lead to pore clogging, which can severely limit the transport. Under starvation conditions, the bacteria decrease in size and shed their exopolymer capsule (Young 2006). This reduces their overall diameter and adhering capability and increases transport. It has been seen bacteria have a higher transport potential as compared to cells with exopolymers (Wang et al., 2013).

2.4.3 Microbial adhesion to soil particles

The adhesion with soil particles and vadose zone materials require an initial interaction between the bacterial cell and the media is required. Thus might happen to either of the three methods: diffusion, active transport or advective transport. Brownian diffusion is the mechanism that would be the most prominent mechanism, for diffusion. It has been discussed earlier that the diffusion rate for microbes, bacteria is very less hence this will not be the primary mode of transport.

If the cell is mobile, then the cell will travel through active movements. The most prominent method would be with bulk flow advective transport.

Once the initial interaction happens, the attachment happens either via electrostatic interactions, Van Der Waals forces, hydrophobic interactions.

2.4.3.1 Electrostatic interactions

This kind of interaction happens between charged particles. The microbes have a negative surface charge which is a product of lipoteichoic acids and lipopolysaccharides. Soil particles including natural organic matter are all negatively charged. Hence the major interactions are repulsion due to similar charge.

2.4.3.2 van der Waals forces

Although electrically neutral particles don’t have a net charge or a permanent dipole moment, the particles have a dynamic charge distribution. These charge

distribution may become favorable for attenuation and the forces that are between the particles are called the van der Waals forces.

2.4.3.3 Hydrophobic interactions

This is the tendency of non-polar groups to associate in an aqueous environment. As a result of hydrophobicity, the cells accumulate at the solid-water interface.

2.4.4 Effect of pH on Transport

pH of the solution doesn't have a major effect on transport on bacteria. It as to be taken into account through viruses can be affected greatly by the pH. The primary restraining when it comes to bacteria are not adsorption, as it the case for viruses. The bacteria have an appreciable chemically diverse surface charge would not be noticed. In case of viruses have a more homogenous charge distribution on the surface, a change in pH beyond the isoelectric point would change the surface changes to positive from the inherent negative charges. This would affect adsorption hence transport of viruses would be affected. (Maier et al., 2009)

2.4.5 Effect of ionic strength of solution matrix on transport

Ionic strength is determined by the total concentration of cations and anions present. The transport is primarily influenced via two mechanisms,

1. Altering the size of the double layer.
2. Influencing soil structures.

The overall ionic strength of the solution is important to the transport. The porous medium is often thought to be a diffuse double layer because of the distribution of charges. The stern layer sticks tightly to the surface whereas the diffuse double layer interacts with the remaining charges and is much loosely attached. (Maier et al., 2009)

2.4.6 Effect of cellular appendages of microbes on transport

Bacteria cells have many appendages like flagella, pili and fimbriae. Flagella especially are responsible for motility. On the other hand, pili and fimbriae are involved in attachment. (Maier et al., 2009)

In general appendages do not make s very big difference in the transport since motility happens in a smaller scale and in course of micrometer. It must be kept in mind that for bacterial motility to make a major difference in transport, there is a need for an extensive continuous water film. It has been noted under non-advective conditions, the bacterial motility could lead to some change. (Maier et al., 2009)

2.4.7 Effect of hydrogeological factors on transport

Soil textures, structure of soil, porosity, water content and tortuosity are a few very important factors that need to be considered when analyzing transport.

Advection, the primary mechanism of transport of microbes in soil (saturated/unsaturated) is essential for the analysis and the soil structures/ porosity and tortuosity is important. (Maier et al., 2009)

Dispersion a phenomenon involving diffusion (minimal for microbes) and mechanical mixing is also governed by path tortuosity. The velocity of water in the system especially through pores is explained by Darcy's law and Darcy velocity. The Darcy velocity is determined by the height of the column (z) and the difference in hydraulic head (ΔH) The dash velocity is determined by equating hydraulic head difference or inlet and outlet. The hydraulic conducting constant (K) and the length of the column

The Darcy flux is given by

$$q = -K\frac{\partial H}{\partial z} \qquad eq.\ 2.11$$

While the pore velocity is a function of the flux water content.

Table 2.2 Factors Affecting the Survival of Bacteria in Soil

No.	Factor	Comment
1	Microorganism and their physiological State	
2	**Physical and Chemical nature of receiving water.**	
	pH	Shorter survival times in acidic soils (pH 3-5) than in alkaline soil.
	Soil water content	Longer survival time in wet soils and during times of high rainfall.
	Organic matter content	Increased survival and possible growth when sufficient amount of organic matter is present.
	Texture and particle size distribution	Finer soils especially clay minerals and humic substances increase water retention by soil which increases survival time.
	Temperature	Longer survival at lower temperature.
	Availability of nutrients	Increases survival time.
	Adsorption properties	Microorganisms appear to survive better in sorbed state.
3	**Atmospheric conditions**	
	Sunlight	Shorter survival time at the soil surface.
	Water(vapor and precipitation)	Longer survival time in wet soils and during times of high rainfall.
	Temperature	Longer survival at lower temperature.
4	**Biological interactions**	
	Competition from indigenous microflora	In sterile soil, survival is increased.
	Antibiotics	Many microorganisms cannot survive in presence of antibiotics
	Toxic substances	Many microorganisms cannot survive in presence of toxic substances
5	**Application method**	
	Technique	
	Frequency of application	
	organism density in waste water material	

Table 2.3 Factors Affecting Movement of Microorganisms in Soil

1	**Soil physical characteristics**
	Texture Particle size distribution Clay type and content Organic matter type and content pH Pore size distribution Bulk density
2	**Soil environment and chemical factors**
	Temperature Soil water content Soil water flux
3	**Chemical and microbial factors**
	Ionic strength of soil solution pH of infiltrating water Nature of organic matter in waste effluent solution (concentration and size) Type of microorganism Presence of larger organisms
4	**Application method**
	Soil drying between applications Time of application (winter, spring)

2.5 Modeling microbial transport

Modeling for microbial transport is necessary for many reasons, especially if there is an accidental release into the environment or an incident of unplanned release of untreated sewage. In order to investigate the role of the different factors that governs microbial transport, tests are conducted in bench/pilot scale packed porous media columns, when studies are done ex-situ (in laboratory). In-situ tests are performed, but only after extensive ex-situ analysis. (Maier et al., 2009)

Tracer tests are often done to measure the potential of microbial transport. Tracers are generally chemically inert and a good representative of the abiotic processes that affect transport.

Mathematical Model are used to not only predict the extent of transport but also predict the arrival of microbes at certain positions. The most common method of analysis is via an advection-dispersion equation as mentioned in section 2.3 (equation 2.4).

The transport models should ideally take into account, the method of transference through the media. Typically microbial transport models have a term incorporated in them that reflect its survival (decay/growth). The growth and decay term are often ignored (Hornberger et al, 1992) when formulating the equation for analysis. One major reason behind this is the time for which the experiments last don't exceed the growth cycle of the microbes. The in-situ surroundings also have very less nutrient media to facilitate growth.

It has been seen via literature review (Haridas and Corapcioglu, 1985) that some microbes have high rate of survivability in the environment. In the environment microbes

typically would not perish over a short time, hence the in-situ conditions being similar to the actual ones don't accommodate a decay term in the primary equation for calculating transport.

In principle, models are either:

1. Advection- Dispersion models.
2. Filtration models.

In an **advection dispersion model**, it is assumed that the contaminant is in solution and it has an average velocity of flow throughout the domain as if a transport via a matrix is taking place. Values for a flux and velocity of flow can be calculated empirically using concepts of Darcy flux and the Darcy velocity. Equations of which can be found in section 2.4.7. The calculations can also be approximated using a conservative tracer test. (Maier et al., 2009)

Empirical calculations or approximations such as the above mentioned ones may not be accurate while predicting microbial transport. The mechanics involved seem to be a bit varied as compared to chemicals. The microbes are suspended in solution whereas chemicals as dissolved. The equations generally have a growth and decay term incorporated in them. Heterogeneities in soil can be included in the equations by adding extra terms for it. Details of the equations can be found in section 2.3. (Maier et al., 2009)

In **filtration models**, contaminant is particulate in nature. Their removal is dependent primarily on physical straining. In a filtration model, particles of the media (soil particles) come into contact with the microbes. The microbes are effectively strained through the pores between the soil particles. (Maier et al., 2009)

3. COMPARATIVE TRANSPORT OF BACTERIA (*E. coli* AND *Legionella*) IN TWO-DIMENSIONAL POROUS MEDIA TANK

3.1 Abstract

The study was to analyze the extent of bacterial transport in a two-dimensional tank under completely saturated conditions. The experiments were done in a 2-D tank packed with 3,700 in^3 of fine grained, homogenous, chemically inert sand under saturated conditions. The tank used for transport was made sterile by backwashing with 0.6% chlorine solution. The transport of bacteria was measured using samples collected from ports at vertical distances of 5, 15 and 25 inches from the surface of the sand on both sides for the 2-D tank. An influent concentration of 10^5 *CFU/*mL was set as a standard for both microbes and the percolation rate was set at 11.37 inches/day using a peristaltic pump at the bottom outlet. At depths of 5, 15 and 25 inches, *E. coli* breakthroughs were recorded at 5, 17 and 28 hours for the ports on the right side and 7, 17 and 29 hours respectively. At respective distances *Legionella* the breakthroughs were recorded at 8, 22 and 35 hours for the ports on the right side and 9, 24, 36 hours for the ports on the left side, respectively which is homologous to its pleomorphic nature.

3.2 Introduction

Contaminant transport in ground water is a concern for communities relying on ground water and replenishing it by artificial recharge. It is estimated over 150 million Americans directly depend on ground water for their water supply (Dylan et al., 2014). The microbiological quality of groundwater is often times found to be better and more stable compared to surface water (Katayama, 2008, Feighery et al., 2012). This is a good reason why a vast majority of the population both in developed and developing countries depends on ground water as their primary source of water. In a passive surveillance of 11,000 private water supplies in England, (3520/11,000) 32% sites tested positive for *E. coli* (Richardson et al., 2009). 10% of the samples collected from 144 wells in the Netherlands were found to be contaminated by *E. coli* or enterococci (Schets et al., 2005). In the United States samples collected from 117 utilities across 35 states (445 samples) were assayed for presence of bacteria, bacteriophages and enteric viruses. 9.9% (44/445) were found to be positive for Total coliform, 8.7% (31/355) were found to be contaminated with Enterococci and 1.8% (1/57) of the samples was contaminated with *Clostridium*. 15.1% (67/445) of the samples were found to have at least one bacteria while none of the samples had all three test bacteria. (Abbaszadegan et al., 2003) Studies were conducted with samples from 1174 wells being analyzed. (347/1174) 29% of the wells were found to be contaminated by (USGS 2006).

Legionella is ubiquitous in water systems. *L. pneumophila* are responsible for majority of the water borne diseases (drinking water and non-recreational) in the United States. (Brunkard et al., 2011). The survival of *Legionella* depends on their association

with biofilms and endoparasitization of amoeba and ciliate hosts (Abu Kwaik et al., 1998). *Legionella* has been detected in 60% of the samples (3/5) collected from recharge basin samples collected from sampling sites in California. (Palmer et al., 1993b)

In a state like Arizona, which is one of the leading states when it comes to practicing ground water recharge, the potential threat of *Legionella* being present in the water is very real. It has been seen that *Legionella* is present in not only reclaimed water, but also surface water. (Palmer et al., 1995). It has a high proclivity for growing in reclaimed water (Jjemba et al., 2010).

Microbial transport in environments such as soil aquifer has extensively studied different groups of microbial surrogates. However, relevance of surrogate transport data for predicting risk of pathogens transport has been questioned.

The *Legionella* is a unique bacterium with high lipopolysaccharide content in cell membrane (Brenner et al., 1979). Pleomorphic nature of *Legionella* cells has been documented during it life cycle in environments. In environment, it can adapt into a facultative intracellular stage that needs a host such as amoeba. This host acts as a protecting shell for *Legionella* as well. *Legionella* feeds off the amoeba as it needs L-cysteine hydrochloride for growth which is not freely available in the environment. (Greub et al., 2003). The survivability of *Legionella* in the environment in the absence of the host amoeba is widely debated. Under normal conditions, *Legionella* is a Gram-negative bacillus that measure 2 to 20 μm depending upon the age of the culture, (WHO 2007) which can transform through different shapes and sizes under different

environmental conditions. This plasticity of *Legionella* cell warrants in-depth study of it transport through different types of aquifers.

The objective of this study was to investigate, compare and contrast the transport of an *E. coli* (bacterial indicator) and *Legionella* (human opportunistic pathogen) through two-dimensional packed porous media column under saturated conditions.

3.3 Materials and method

3.3.1 Preparation and packing of soil column of two-dimensional tank

The primary aim of this experiment was to measure the transport of *E. coli* and *Legionella* in a saturated 2D tank which was packed with porous media. The tank in use was built by the Engineering Technical Service shop at ASU and is made from stainless-steel along with acrylic glass enclosing it and steel coupling and braces protecting the structure from fracture. It was identified that the pressure points were at the 4 corners and the braces were attached accordingly.

The tank is 72 inches (183cm) tall, 24 inches (61cm) wide and 4 inches (10cm) deep. It has intermittent sampling ports as can be seen in the picture below. For this study sampling ports at 5 inches, 15 inches and 25 inches were used as they were identified to have given the best portrayal of the overall transport of bacteria and tracers throughout the tank. Approximately 3750 in^3 of dry Quikrete© Mesh Fine Silica Sand was packed into the tank and compacted. The bottom of the tank along with the top of the sand column was lined with gravel to stop the sand from getting dislodged. The tank was disinfected with a 0.6% bleach solution. This ensured that most, if not all microbial life in the column was removed and the tank was sterile. The tank was subsequently flushed 4

times after the bleaching was done, the last one with chlorine neutral water to ensure there was no free chlorine. The tank was saturated from the bottom and filled up at a slow rate of 3.6*ml*/min to ensure all the air entrapped amongst the void can be eliminated. It was made sure that a head of 1.5-2 in water was allowed above the gravel. The tank as can be seen in the picture has an outlet on the bottom right corner. This is connected to a peristaltic pump. This was done to ensure that a constant infiltration rate of 11 in/day was kept going. On the longitudinal side of the tank there is an outlet on either side. The one of the right side was used to pump in the sample during gravity flow and the left one for overflow to maintain a steady flow of water on the top. This was done to enable a steady concentration matrix and to avoid preferential flow as much as possible. The bottom of the tank below the gravel has been lined with a pipe with 3 openings which was connected to the outlet at the bottom right corner. This was done to ensure uniform flow through the column.

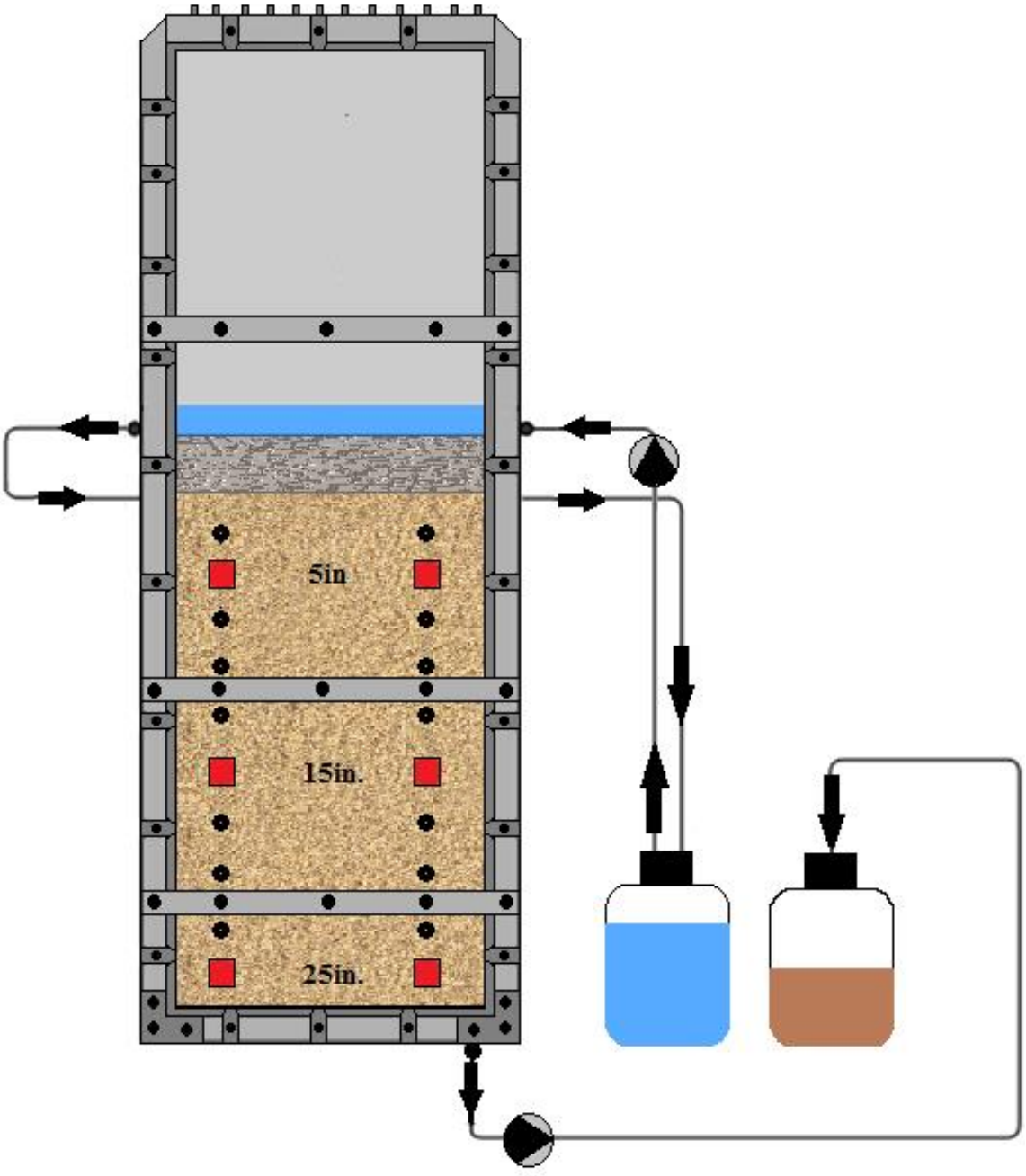


Figure 3.1: 2-D tank packed with Quikrete play sand media with sampling ports

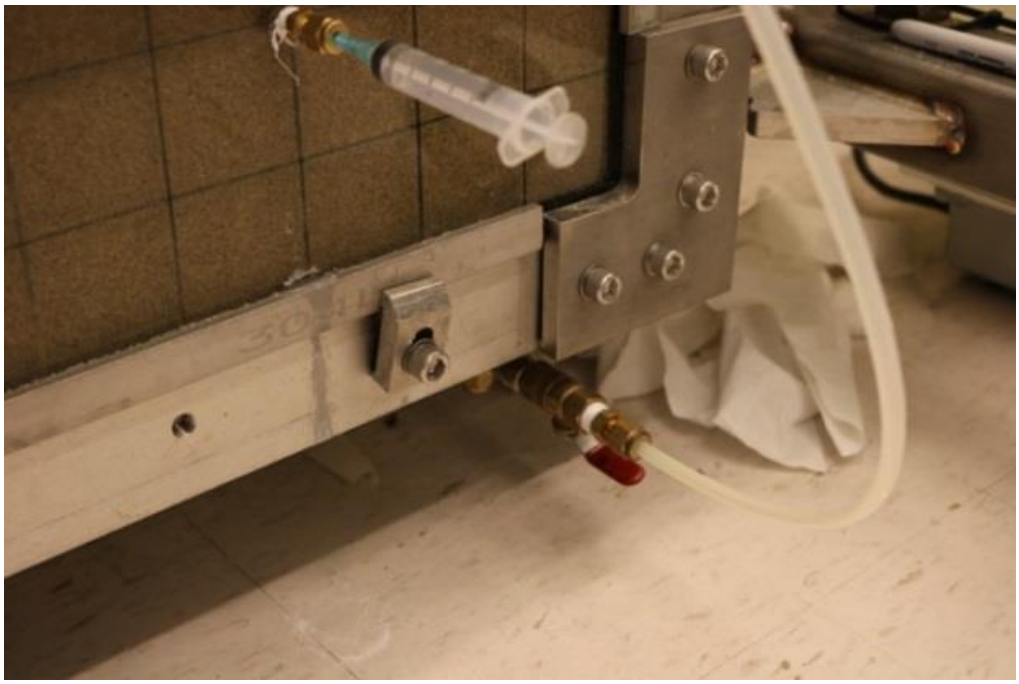


Figure 3.2 Inlet/Outlet point at bottom of tank

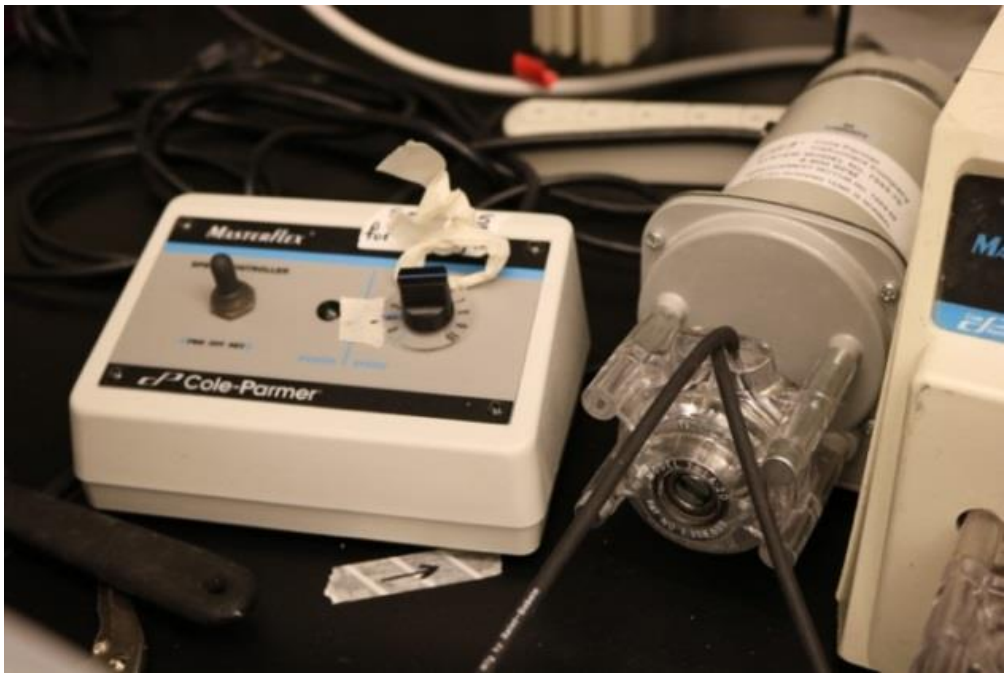


Figure 3.3 Peristaltic pump which controls tank flowrate

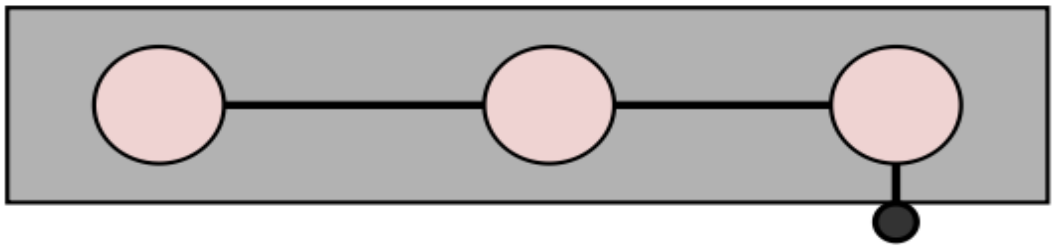


Figure 3.4 Schematic of outlet points along bottom of inside of tank

3.3.2 Media properties

Quikrete© (No. 1961) Mesh Fine Silica Sand was selected as the media to pack the column, datasheet has been attached herewith (Appendix B). The packed media is among the size range associated with the U.S. sieve number #30- #70 (0.6-0.2 mm) (Commercial Grade Sands: Product Nos. 1961, 1962, 1963). According to ANSI grit size, table B.1 found in Appendix B is contained mostly in the sieve size 0.01mm.It was seen post a sieve analysis (particles above 2.00 mm) comprised 8% of the total media. It must be noted that 83.75% of the media was restrained by sieve size greater than 0.01mm. The porosity of the homogenous layer of sand was measured to be 31% and allows for closely controlled sizing and particle size distribution. An enlarged view of the sand particle can be seen in the figure 3.6. Considering the factors that affect transport such as size of grain, chemical interactions, texture etc., the sand was chosen as ideal for the analysis of transport of microbes.

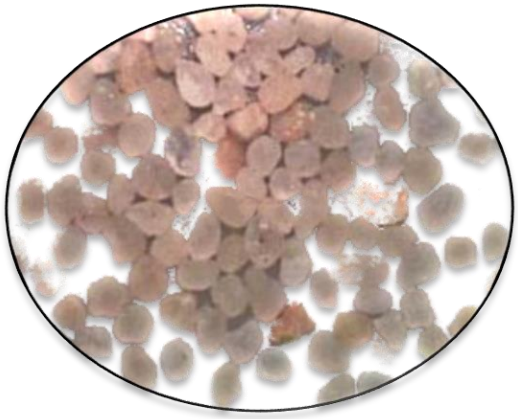


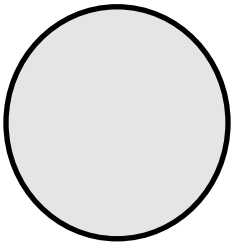
Figure 3.5 Quikrete© Silica Sand

Source: *Commercial Grade Sands: Product Nos. 1961, 1962, 1963* [Pamphlet]. (n.d.). Atlanta, GA: the QUIKRETE Companies©.

The packed media is chemically inert and has a boiling point which is relatively high. This can be attributed to the high inter atom bond strength. It is primarily composed of Silicon dioxide (SiO_2) and being chemically inert it is nonreactive. If the silica grains are compared with the size of *E. coli* and *Legionella* cells, there is a visible difference among them. The size of a silica grain is 250μm whereas for *E. coli* the size is somewhere between 0.5-2.0μm and for *Legionella* about 4μm. Table 3.1 gives an idea of the differences in sizes between the media and microbes. It must be kept in mind that ratio of soil grain diameter to the diameter of *E. coli* is about 125-500, whereas for *Legionella* it is about 60. This explains the vast size difference between the two.

Table 3.1 Micron Size Chart

Source: *The Best Air Purifier for Smoke Buying Guide. (2016, December 15). Retrieved 2017, from https://purifythis.com/best-air-purifier-for-smoke/*

	150 Microns	Average Human Hair
	25 Microns	Lint, Particles Visible to the Naked Eye
	10 Microns	Heavy Dust, Lint, Fertilizer, Pollen
	5-10 Microns	Average Dust, Plant Spores, Mold
	1-5 Microns	Bacteria, Light Dust, Animal Dander.
	0.3-1 Microns	Bacteria, Tobacco and Cooking Smoke, Metallic Fumes
.	0.001-0.01 Microns	Viruses

The porosity of the sand was determined by measuring out 1000 cubic centimeter of the media in a beaker (consolidated). Water was slowly added from the top. It was made sure that there was no air entrapped in the voids. The volume of water added was measured. It was recorded that 314 ml of water had been added to completely saturate the media. The porosity was calculated using the following formula.

$$porosity = \frac{volume\ of\ voids}{Total\ volume}$$

It was assumed that all the air voids had been replaced with water and the total volume of voids was equal to the volume of water added.

The test was repeated using 500 g of sand. The media was completely saturated and weighed again. The difference in weight was measured. The ratio of the difference between the weights and the total weight of the saturated media was calculated. The results were similar and the final porosity was calculated to 31% or 0.31.

3.3.3 Determining and setting flow rate through packed column

The outflow is controlled by a peristaltic pump set at a specific speed. This was done in order to make sure there was a constant infiltration rate throughout the experiment and a stable head is maintained on the top as it was essential to ensure that a uniform matrix is formed. The desired infiltration was something between 10 and 12 in/day. The flowrate coming directly from the tubing was measured to be 3.6mL/min. This measured flowrate was used to calculate the linear velocity of water and the total water that would infiltrate the media in one day. The tank was filled with about 3750in^3 (61451.49 cm^3) of Quikrete play sand. The porosity after compaction was noted to be 31% or 0.31. This would translate to 1162.5 in^3 (19049.9619 cm^3) of voids and 2587.5 in^3 (42401.5 cm^3) occupied by sand particles. The flow rate from just outside the pump was measured to be 3.6 mL/min, which converted to linear velocity of water through soil is 11.37 in/day. The following calculations entail the details:

$$\frac{0.0036\ L}{min} = \frac{3.6 \times 10^{-3} L}{min} \times \frac{60\ min}{hr} \times \frac{24 hr}{day} = \frac{5.184\ L\ fluid}{day}\ through\ column$$

This means that approximately 5.184 L of fluid would travel through the column in one day at the measured flowrate.

$$\frac{17.8\ L\ void\ volume}{\frac{5.184\ L}{day}\ fluid} = 3.43\ days$$

3.43 days would be the time it would take the water to fill out all the voids and move through the sand.

$$\frac{39\ in}{3.43\ days} \times \frac{day}{24\ hrs} = 0.47\frac{in}{hr}\ \ or\ \ 11.37\frac{in}{day}$$

The above calculations indicate that the infiltration rate will be 0.47 in/hr. or 11.37 in/day, which is in coherence with the national average of infiltration rates.

Once the infiltration had been adjusted to 11.37 in/day, the speed on the pump was locked and all pilot-scale studies were performed at the determined flowrate. In general modeling for ground water is done with a much slower infiltration. 11.37 inches per day may seem a bit high as compared to general modeling studies. However, when pollution in a saturated system is considered, infiltration is usually found to be higher. Multiple EPA reports suggest that contaminants can move rapidly through macropores and fractures, hence the higher infiltration rates.

3.3.4 Preparation of growth enrichment media.

3.3.4.1 Preparation of Brilliance media.

In the course of the experiment, brilliance agar from two different vendors was used. Brilliance agar media (Oxoid CM1046), a selective nutrient media for facilitating the growth of *E. coli*, was primarily used for assay. According to manual and documentation provided, 14.05 g (28.1 g/l) of powdered media was added to a conical flask containing 500 mL of distilled water. The suspension is brought to a gentle boil on a hot plate and

stirred using a magnetic stirrer. Stirring was done at 200 RPM. Once thoroughly mixed and boiled, the liquid media was cooled to 50°C and aseptically dispensed into petri dishes. 15ml of media was transferred to every petri dish. It must be kept in mind that it does not require autoclaving. The plates were left to cool at room temperature until it solidified. The plates were then bagged and stowed away at 4°C. The petri dishes are kept in an upside down position so that moisture does not collect on them. The analysis of samples was done by spread plating 0.1 ml of sample on every plate.

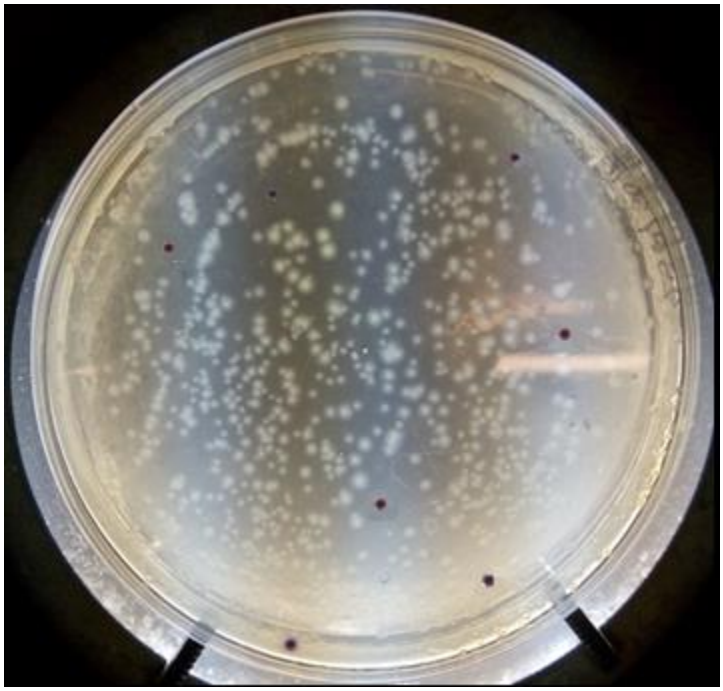


Figure 3.6: Brilliance media with sample plated containing *E .coli.*

Brilliant agar media (Sigma Aldrich 27815), a selective nutrient media for *E. coli* and Coliform bacteria was used for assay as well for triplicate plates. A comparative test was done and results were found to be satisfactory. According to manual and documentation provided by manufacturer, 10.35 g (20.7 g/l) of powdered media was

added to a conical flask containing 500 mL of distilled water. The suspension is brought to a gentle boil on a hot plate and stirred using a magnetic stirrer. Stirring was done at 200 RPM. Once thoroughly mixed and boiled, the liquid media was autoclaved for 15 minutes at 121 °C. The liquid media was cooled to 50°C and aseptically dispensed into petri dishes. 15ml of liquid media was transferred to every petri dish. The plates were left to cool at room temperature until it solidified. The plates were then bagged and stowed away at 4°C. The petri dishes are kept in an upside down position so that moisture does not collect on them. The analysis of samples was done by spread plating 0.1 ml of sample on every plate.

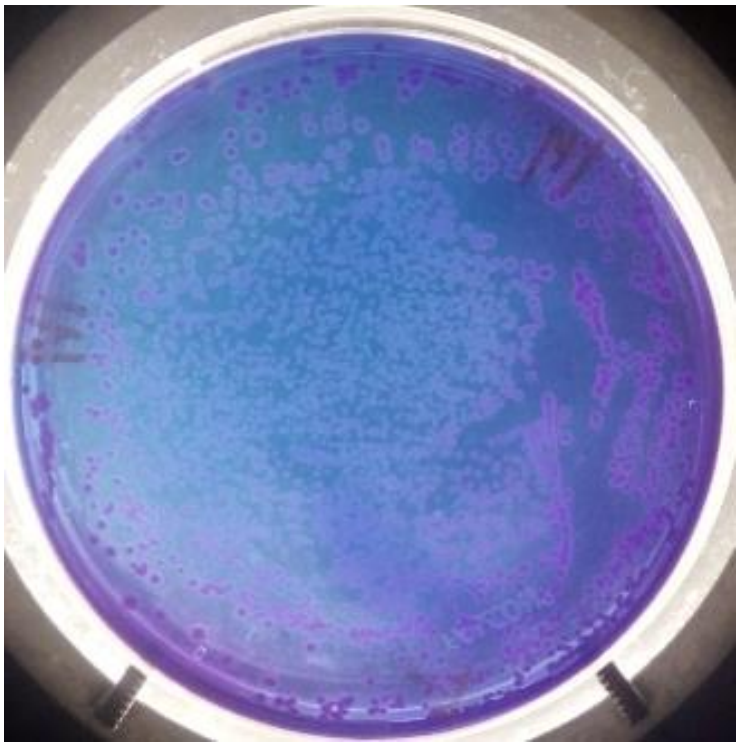


Figure 3.7: Brilliant media, sample plated containing *E. coli.*

3.3.4.2 Preparation of TSA media.

Tryptic Soy Agar (Sigma Aldrich 22091) was used for isolation of *E. coli*. TSA (Tryptic Soy Agar) is non- selective media in itself. Frozen stock was streaked on TSA plates. These TSA plates were then used as the monthly plates. Once streaking was done the sample was left at 37°C for 24 hours for the *E. coli* growth to attain log phase. After isolation was done, one colony forming unit was picked up using a sterile inoculation loop. This one colony forming unit was then transferred to Broth for liquid culture.

3.3.4.3 Preparation of TSB media

Bacto ™ TSB (Soybean-Casein Digest Medium) was used for the preparation of liquid microbial culture. 30 g of powder was suspended in a flask with 1000 mL of nano-pure water and boiled on a hot plate. Mixing was done using a magnetic stir bar set at 200 RPM. Once the media was mixed and boiled, it was sterilized by autoclaving at 121 °C. The TSB was then stowed away at 4°C. This handled carefully while stowing, and left under UV (Ultra violet) light for 30 minutes before use to maintain sterile media.

3.3.4.4 Preparation of BCYE media.

Buffered Charcoal Yeast Extract BBL™ BCYE Agar Base (Thermo Fisher Scientific 212327). Buffered Charcoal media with α-ketoglutarate and Ferric Pyrophosphate are essential for providing *Legionella* the required base for growth. L-cysteine hydrochloride is another essential nutritional requirement for the successful growth of *Legionella.* BCYE powdered media in its self is not selective for *Legionella*,

although BCYE media with L- cysteine HCl will ensure growth of *Legionella* from a frozen stock.

For making 500 *ml* of media, 19.15 g of powdered media was dissolved in 250 *ml* of nanopure water. (38.3g/ 1000 *ml*). 2.4g of KOH pellets were added to bring the pH to 6.9 (+/-) 0.05. 250 *ml* of nanopure water was further added to make the suspension to 500 *ml*. The liquid media is autoclaved and sterilized. Autoclave cycle was done at 121°C for 15 minutes. The media is taken out of autoclave and put in water bath at 45-48°C. Growth supplements are added to the media after it cools down to 45-48°C. The growth supplements include L-cysteine HCl (10% by volume); 4 *ml* is added to the liquid media. Polymyxin B, Vancomycin and Cycloheximide are antibiotics to inhibit the growth of other unwanted bacteria and fungi. Polymyxin B and Vancomycin are for inhibition of bacteria and Cycloheximide for fungi. The required dosages according to the CDC are 100 units/*ml* for Polymyxin B, 5µg/*ml* for Vancomycin and 80 µg/*ml* for Cycloheximide. The liquid media is then aseptically dispensed into petri dishes. 15ml of liquid media was transferred to every petri dish. The plates were left to cool at room temperature until it solidified. The plates were then bagged and stowed away at 4°C. The petri dishes are kept in an upside down position so that moisture does not collect on them. The analysis of samples was done by spread plating 0.1 ml of sample on every plate.

Figure 3.8: *Legionella* plated on BCYE media

3.3.4.5 Preparation of PBS buffer

Phosphate Buffer Saline (1X strength) solution was made to wash off the *Legionella* lawn off the plates and create a suspension. This suspension would have a high number of colonies present. To make 1L 10X strong Buffer 80g of NaCl, 2g of KCl, 14.4 g of Na_2HPO_4 and 2.4 g of K_2HPO_4 were added to 800 *ml* of DI/ nanopure water. The final was adjusted to 6.8. 200 *ml* DI water was added to make the full liter. Serial dilution was done to make the final solution of the required concentration.

3.3.5 Preparation of microbial stocks

E. coli and *Legionella* were chosen as the two microbes for analysis. *E. coli (*ATCC® 25922 ™) and *Legionella* (ATCC® 33153 ™) were accrued from ATCC for this test. It is known that *Legionella* in environment needs amoeba as a host for its nutrients and cannot multiply without a surrogate, primarily because of the lack of L-cysteine hydrochloride in the free environment. However under laboratory conditions, it can grow freely if provided with necessary nutrients. The primary aim of this study was analyze the transport of *E. coli* and *Legionella.* This study is more prominent for states that do artificial recharge. Arizona is one of the states that do prominent artificial recharge. Artificial recharge is a process where water is artificially fed into the ground with the help of artificial forces like pumping.

3.3.5.1 *E. coli* culture

E. coli (ATCC® 25922™) strain was acquired from the ATCC. The antigen properties of the strain are listed to be Serotype O6 and Biotype 1. It is widely used as an indicator organism (Minogue et al., 2014). The frozen stock (kept at -80 °C) was carefully taken out and using aseptic techniques. The frozen stock was left to thaw. A sterile inoculation loop was used to streak on a TSA plate for isolating colonies. This also served as the monthly plate. This was left inside the incubator at 37 °C overnight. *E. coli* generally reaches log-phase overnight. An isolated colony was picked up and inoculated in 15 ml of TSB. This was left in the incubator at 37 °C for 24 hours. In theory, the stock stays good for 7 days, but 1ml of stock was transferred to 9 ml of TSB every day and to

keep the stock fresh. This was done every day till the liquid culture was used in the experiment for analysis. A suspension having about $3*10^8$ CFU/ml was hence created. The number were primarily based of OD-600 readings and then confirmed by plate count assay. Serial dilutions were made and 10^6, 10^7, 10^8 dilutions were plated and counted.

3.3.5.2 *Legionella* culture

Legionella (ATCC® 33153™) strain was acquired from the ATCC. This strain is representative of *Legionella pneumophila* from Knoxville [NCTC 11286]. Direct fluorescent antibody (DFA) test is done to identify the *Legionella* species. This specific strain falls under the category of serogroup 1 with fluorescent antibody. The frozen stock (kept at -80 °C) was carefully taken out and using aseptic techniques. The frozen stock was left to thaw. 0.1 ml of the thawed stock was taken and spread plating was done on a BCYE agar media plate to create a uniform lawn of bacteria. This was left in the incubator at 37 °C with 5% CO_2 for 3-4 days. Generally the incubation time for *Legionella* is 72-96 hours. The uniform lawn is washed off by 1X strength PBS buffer with 10% glycine. This ensured that all the bacteria from the plates were recovered into the liquid media that was created. A suspension having a concentration of $5*10^9$ CFU/ml was recovered. This was verified using plate count assay. Serial dilutions were made and 10^7, 10^8, 10^9 dilutions were plated and counted.

3.3.6 Pilot-scale investigations of saturated spiked column

A column study was done to understand and investigate the transport and behavior of the bacteria (*E. coli* and *Legionella*) and dye under laboratory conditions. The experiments were performed in saturated conditions (wet packing). The column was saturated with Tap water. While filling the tank a slow flow was used to flush out all air bubbles. The tank was subsequently bleached. 0.6% Beach solution was used. The bleaching was repeated twice. The free chlorine in the tank was neutralized using Sodium Thiosulfate ($Na_2S_2O_3$). The source of water was water circulated by the City of Tempe, Table 3.4 lists influent water quality parameters. Table 3.5 lists the theoretical detection limit for the microorganisms, which does not include the detection of dead cells.

Table 3.2 Column Feed Water Quality, City of Tempe

(2016 City of Tempe Water Quality Report)

Constituent	**Units**	**Range**	**Typical Values**
Chlorine Residual	mg/L	0.0-2.0	0.64
Hardness	mg/L	220-420	244
Alkalinity	mg/L	130-370	172
pH	-	6.9-7.7	7.3
Turbidity	NTU	-	<0.07
TDS	mg/L	360-1200	658
Coliform	-	ND-1.5%	ND
NO_3	mg/L	-	6.4
Temperature	°F	70-80	74
Conductivity	MHOS/cm		
Chloride	mg/L	60-420	215

Table 3.3 Microbial Detection Limits by Analysis Method

Microorganism	**Detection Limit**	**Analytical Method**
E. coli bacteria	1 cell/mL	Spread Plate Method
Legionella bacteria	1 cell/mL	Spread Plate Method

3.3.6.1 Spiked dose *E. coli* experiments, gravity flow

The experiment to analyze transport of *E. coli* was done under gravity flow. Via the gravity flow it was possible to simulate water flow in the ground. The flow of water is controlled via a peristaltic pump set a predetermined flowrate. This was done to have a steady infiltration rate. The Inlet was fed with a peristaltic pump. The carboy that housed the influent containing *E. coli* had neutral water in it. It was seen the residual chlorine found in tap water was detrimental to maintaining a stable *E. coli* colony in the influent. A stable colony count of 10^5 CFU/ *ml* was maintained. About 7ml of *E. coli* culture was put in the influent carboy, based on the following calculation.

$$C_1V_1 = C_2V_2$$

$$\frac{10^5\ CFU}{mL} \times 20{,}000\ mL = (3 \times 10^8 CFU/mL) * V$$

$$3 \times 10^8 \frac{CFU}{mL} \times V = 2 \times 10^9\ CFU/mL$$

$$\frac{2 \times 10^9 CFU}{3 \times 10^8 CFU/mL} = 6.67\ mL\ stock$$

The required colony count was 10^5 CFU/ *ml* in the influent which is the C_1 in the above equation. C_2 is the concentration of the broth; V_1 being the volume of Carboy and V_2 is the volume of broth required.

3.3.6.2 Spiked dose *Legionella* Experiments, Gravity Flow

The experiment to analyze transport of *Legionella* was done under gravity flow like the previous experiment with *E. coli*. Via the gravity flow it was possible to simulate water flow in the ground. The flow of water is controlled via a peristaltic pump set a predetermined flowrate. This was done to have a steady infiltration rate. The Inlet was fed with a peristaltic pump. The carboy that housed the influent containing *Legionella* had neutral water in it. It was seen the residual chlorine found in tap water was detrimental to maintaining a stable *Legionella* colony in the influent. It is known that *Legionella* is sensitive to chlorine when living as an isolate. A stable colony count of 10^5 CFU/ *ml* was maintained. About 1 ml of *E. coli* culture was put in the influent carboy, based on the following calculation.

$$C_1V_1 = C_2V_2$$

$$\frac{10^5\ CFU}{mL} \times 20{,}000\ mL = (5 \times 10^9 CFU/mL) * V$$

$$5 \times 10^9 \frac{CFU}{mL} \times V = 2 \times 10^9\ CFU/mL$$

$$\frac{2 \times 10^9 CFU}{5 \times 10^9 CFU/mL} = 0.40\ mL\ stock$$

The required colony count was 10^5 CFU/ *ml* in the influent which is the C_1 in the above equation. C_2 is the concentration of the broth; V_1 being the volume of Carboy and V_2 is the volume of broth required.

3.3.7 Sample collection and assay

3.3.7.1 Sample Collection from two-dimensional tank

Samples were collected from the 5 in, 15 in and 25 in sampling ports, along with the influent (from the carboy). The distances of the sampling ports are aligned with the distances from the layer from which the sand starts. Like for instance the sampling port labeled as the 15 in port is 15 inches vertically down from the point where the sand level starts. The tank has the facility to measure transport on the left and the right. The tank has ports on both sides. The right port is designated by the letter 'R' next to the vertical height of the port. The same is done for the left port. Hence a sample taken from the right port at 15in depth would read 15R and a sample taken from the left would read 15L. It must be noted that the sampling ports are at the same vertical distance and samples are collected at the same time. A 18G needle was placed directly in the middle of the port septa, and 5-10 mL were flushed out before the sample was collected. After flushing, 10 mL of sample was collected. Following collection, the sampling port was plugged using a syringe. It must be kept in mind artificial sealants were not used as it might have tampered with the samples collected.

Figure 3.9 Right Side Sampling Ports

3.3.7.2 *E. coli* analysis by spread plate technique.

To detect *E. coli*, samples were collected from the sampling ports. The samples were separately vortexed to ensure homogeneous distribution of the microbe. The Brilliance media, either type were used. The samples were taken and 0.1*ml* was transferred to the petri dish. A spreader was first dipped in Ethanol and flamed. This ensured sterility. The volume transferred was uniformly spread throughout the petri dish.

The petri dish was kept in an incubator at 37°C for 24 hours. The *E. coli* on the Brilliance media (OXOID CM1046) shows as purple bacterial colonies. The *E. coli* on the Brilliant media (SIGMA ALDRICH 27815) appears as pink bacterial colonies. The colony forming units were counted and reported as CFU (Colony Forming Unit)/ *ml*. The count is finally multiplied with 10 to get the final plate count accounting for a 10 fold dilution when 0.1 *ml* is transferred to the plate.

3.3.7.3 *Legionella* analysis by spread plate technique

To detect *Legionella,* samples were collected from the sampling ports. The samples were separately vortexed to ensure homogeneous distribution of the microbe. The BCYE media (with antibiotics Polymyxin B, Vancomycin, Cycloheximide and L-cysteine HCl), either type were used. The samples were taken and 0.1 *ml* was transferred to the petri dish. A spreader was first dipped in Ethanol and flamed. This ensured sterility. The volume transferred was uniformly spread throughout the petri dish. The petri dish was kept in an incubator at 37°C for 72-96 hours. The *Legionella* on the BCYE media (Thermo Fisher Scientific 212327) shows as white bacterial colonies. The colony forming units were counted and reported as CFU (Colony Forming Unit)/ *ml*. The count is finally multiplied with 10 to get the final plate count accounting for a 10 fold dilution when 0.1 *ml* is transferred to the plate.

3.3.8 Data analysis

Microsoft Excel 2017, Origin Pro and R programming softwares were used to perform data analysis and prepare graphical representation of data from the column studies.

3.4. Results

3.4.1 *E. coli* transport experiments

E. coli transport was studied by beginning collection of sample at the 5 inch ports 4 hours after the start of the experiment. Sampling continued for 9 hours. A 2-hour difference was observed for bacterial breakthrough at the right and the left port at this distance: The breakthrough for the right port was measured around 5 hours whereas the same for left port was 7 hours after the start of sampling. Sampling for 15 inches was started 16 hours elapsed time and continued until 20 hours. Like the 5 inch port, it was seen that there was a 2 hour difference in the arrival time for *E. coli* between the right and the left ports; the breakthrough was 17 hours and 19 hours for the right and left ports, respectively. The delay between ports was consistent with that seen at 5 inches.

Sampling was started at 25 inches from 28 hours after start of experiment. Sampling was continued till 32 hours. Like the 5 and 15 inch ports, it was seen that there was a difference in the arrival time for *E. coli* between the right and the left port at the same height. It must be noted that the difference in time was reduced to 1 hour as compared to the 2 hour difference in the above ports. The breakthrough for the right port was measured around 30 hours whereas the same for left port was 31 hours.

All experiments were repeated 5 times. It was seen there were changes in the breakthrough concentrations at the respective ports when the experiments were repeated. This could be an indication of an earlier breakthrough although samples from the previous hour before the breakthrough yielded negative results. Therefore the breakthrough is believed to vary by less than an hour. The concentration at all the ports follow a similar trend, there is an increase in concentration after breakthrough. After a certain time the concentration starts decreasing. This is consistent with the hypothesis that the microbes travel in a discontinuous matrix and there are intermittent gaps between subsequent plumes.

The velocity of *E. coli* showed some variation with height but the average velocity was noted to be 0.83 in/ hr. on the right side and 0.81 in/hr. on the left side. The flow pattern as can be seen via the results is via a matrix. The difference in breakthrough in the right and left port can be a function of the way the microbes/ dye has been introduced. It could also mean variability in the packing of the tank.

Table 3.4: Breakthrough of *E. coli* across all ports.

Sampling Port	**Breakthrough (Right) (hr.)**	**Breakthrough (Left) (hr.)**
5 in (12.7 cm)	5:00	7:00
15 in (38.1 cm)	17:00	19:00
25 in (63.5 cm)	30:00	31:00

3.4.2 *Legionella* transport experiments

After *E. coli* was used to study microbial transport, *Legionella* was used as the second microbe so that a comparative study could be done. *Legionella* was introduced in the system under similar conditions as that of *E. coli*. A *Legionella* liquid culture (*Legionella* was washed off BCYE plates using PBS buffer having 10% glycine) having a concentration of 2 x 10^9 CFU/mL, was used to generate a reservoir concentration of 2 x 10^5 CFU/mL. *Legionella* was sampled for breakthrough based on experimental results from *E. coli* and literature. The samples were collected from ports at 5, 15, 25 inches.

Sampling at 5 inches began at 5 hours after start of experiment. Sampling continued until 11 hours. The breakthrough for the right port was measured around 8 hours, whereas, it was 9 hours for the left. The delay is consistent with the pleomorphic nature of *Legionella.*

Relative to the lag between the right and left ports at any level, it was seen that there was a 1 hour difference between the right and the left port at the same height. This was consistent with the arrival time delay seen in the experiments with *E. coli,* although with *E. coli* there was a 2 hour difference.

Sampling was started at 15 inches from 17 hours after start of experiment. This was done since breakthrough for *E. coli* was seen to be around 17 hours. Sampling was continued till 26 hours. It was seen that there was a 2 hours difference between the right and the left port at the same height. This was consistent with the *E. coli* data. The breakthrough for the right port was measured around 22 hours whereas the same for left

port was 24 hours. The delay compared to *E. coli* is consistent with the pleomorphic nature of *Legionella.*

Sampling was started at 25 inches from 28 hours after start of experiment. This was done since breakthrough for *E. coli* was seen to be around 28 hours. Sampling was continued till 38 hours. It was seen that there was a 1 hour difference between the right and the left port at the same height. This was consistent with the *E. coli* data. The breakthrough for the right port was measured around 35 hours whereas the same for left port was 36 hours. The delay compared to *E. coli* is consistent with the pleomorphic nature of *Legionella.*

The concentration at all the ports follow a similar trend as *E. coli*, there is an increase in concentration after breakthrough. It must be noted that the delay between the right and left ports were 1 hour as opposed to 2 hours for *E. coli* in the sampling port at 5 inches. The delay between the ports at 15 inches was consistent with that of *E. coli* with both showing a 2 hour difference. The port 25 inches also showed similar delay trends as *E. coli*. It has been seen the breakthrough at 25 inches was after 0.44 pore volumes had passed.

The velocity of *Legionella* showed some variation with height but the average velocity was noted to be 0.71 in/ hr. on the right side and 0.69 in/hr. on the left side.

Table 3.5: Breakthrough of *Legionella* across all ports.

Sampling Port	**Breakthrough (Right) (hr.)**	**Breakthrough (Left) (hr.)**
5 in (12.7 cm)	8:00	9:00
15 in (38.1 cm)	22:00	24:00
25 in (63.5 cm)	35:00	36:00

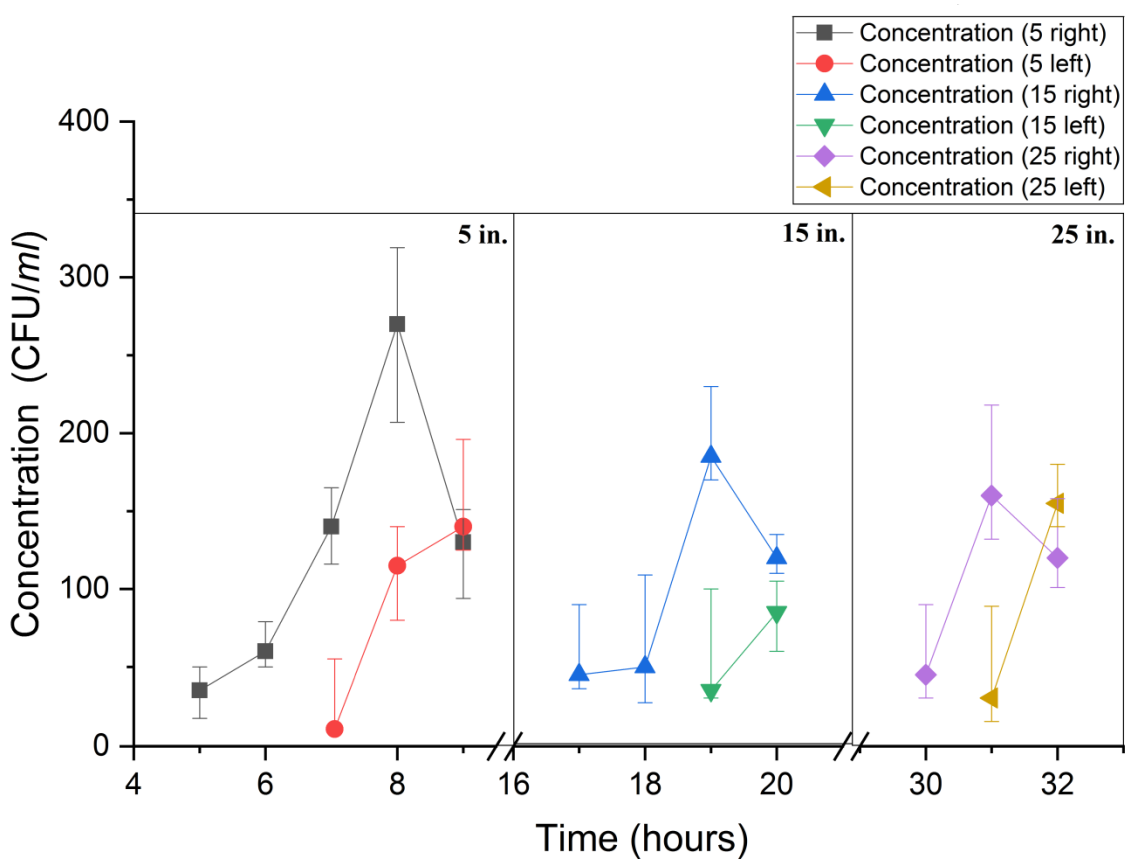


Figure 3.10: Breakthrough and concentration curve of *E. coli* as a function of time.

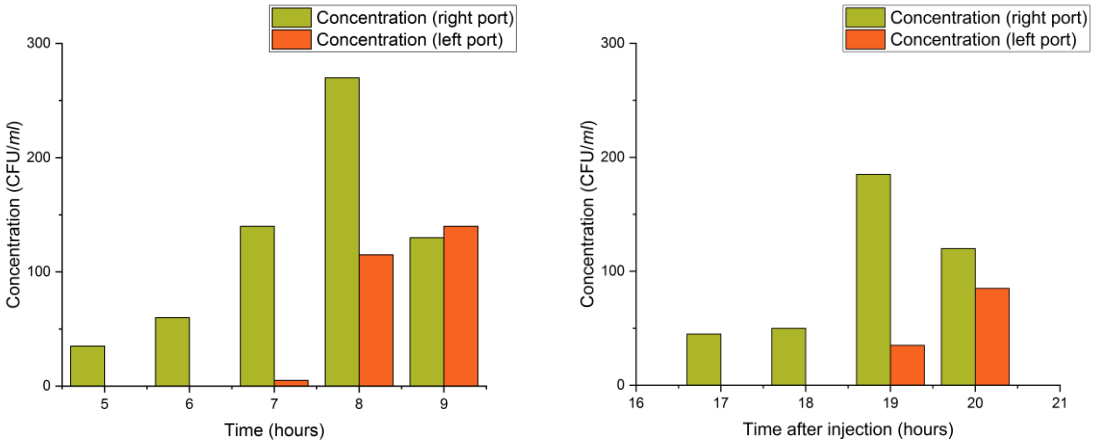


Figure 3.11: Breakthrough and concentration curve of *E. coli* as a function of time for 5 (Left) and 15 inches (Right).

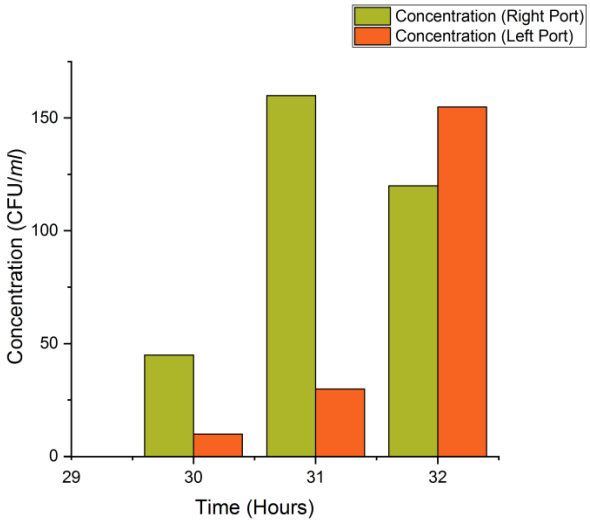


Figure 3.12: Breakthrough and concentration curve of *E. coli* as a function of time for port at 25 inches deep.

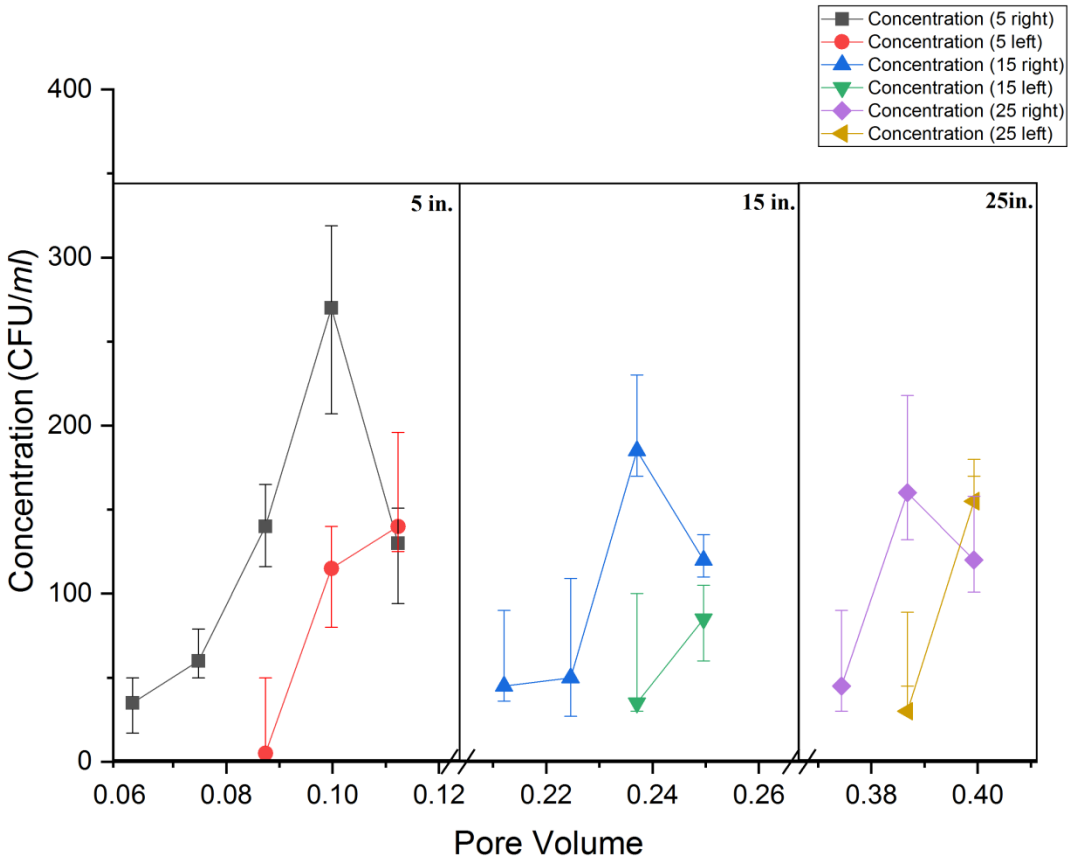


Figure 3.13: Breakthrough and concentration curve of *E. coli* as a function of pore volume.

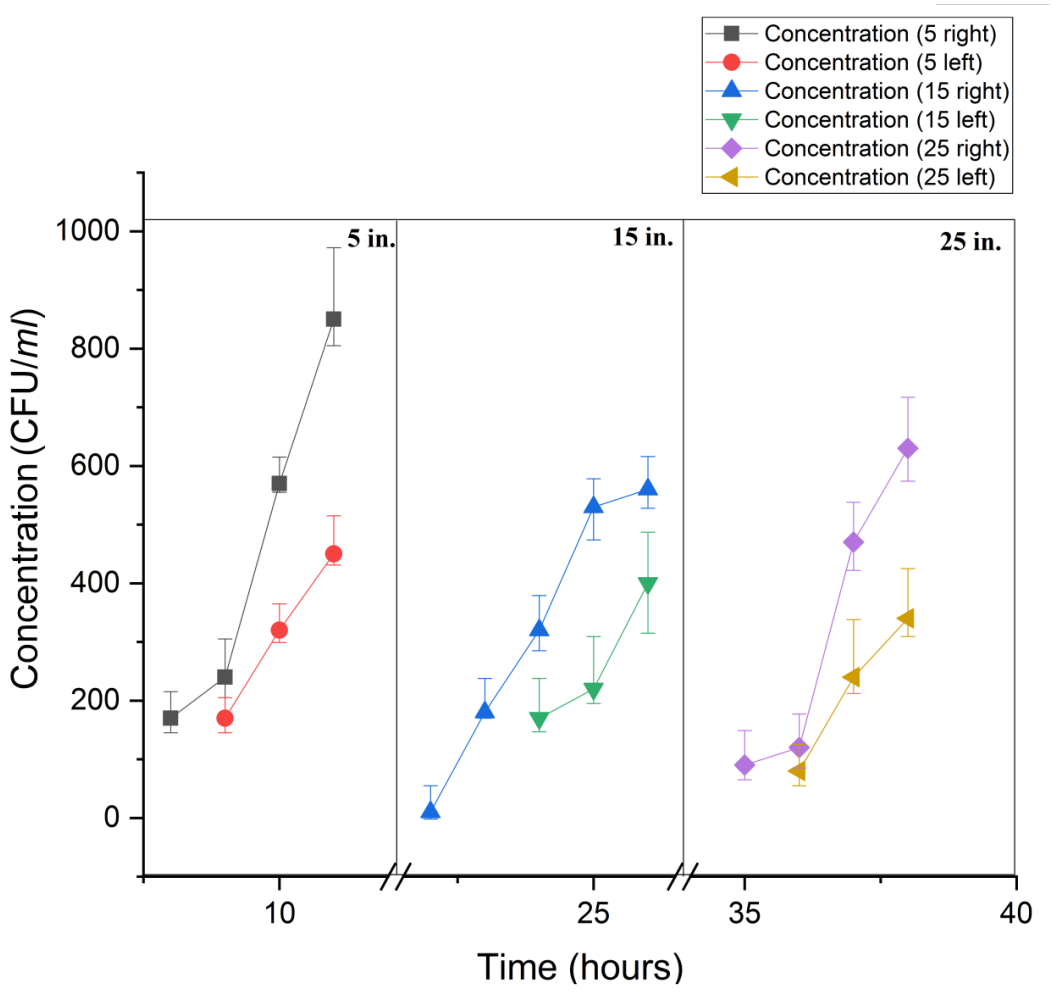


Figure 3.14: Breakthrough and concentration curve of *Legionella* as a function of time.

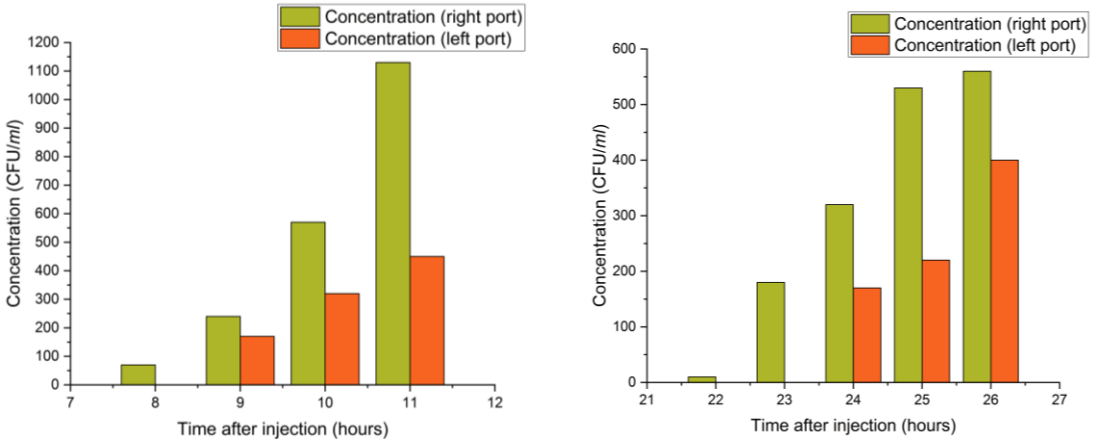


Figure 3.15: Breakthrough and concentration curve of *Legionella* as a function of time for 5 (left) and 15 inches.

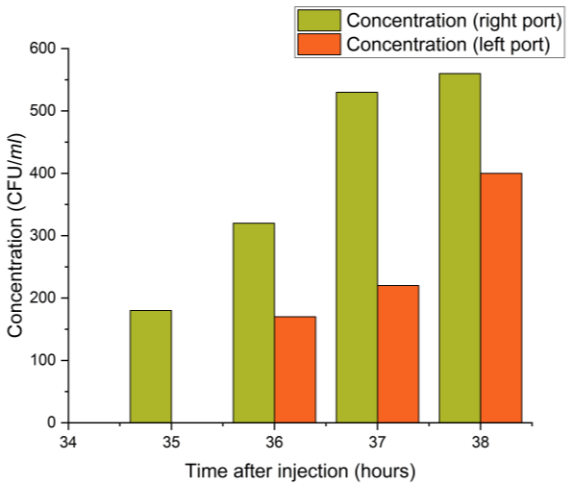


Figure 3.16: Breakthrough and concentration curve of *Legionella* as a function of time for 25 inches.

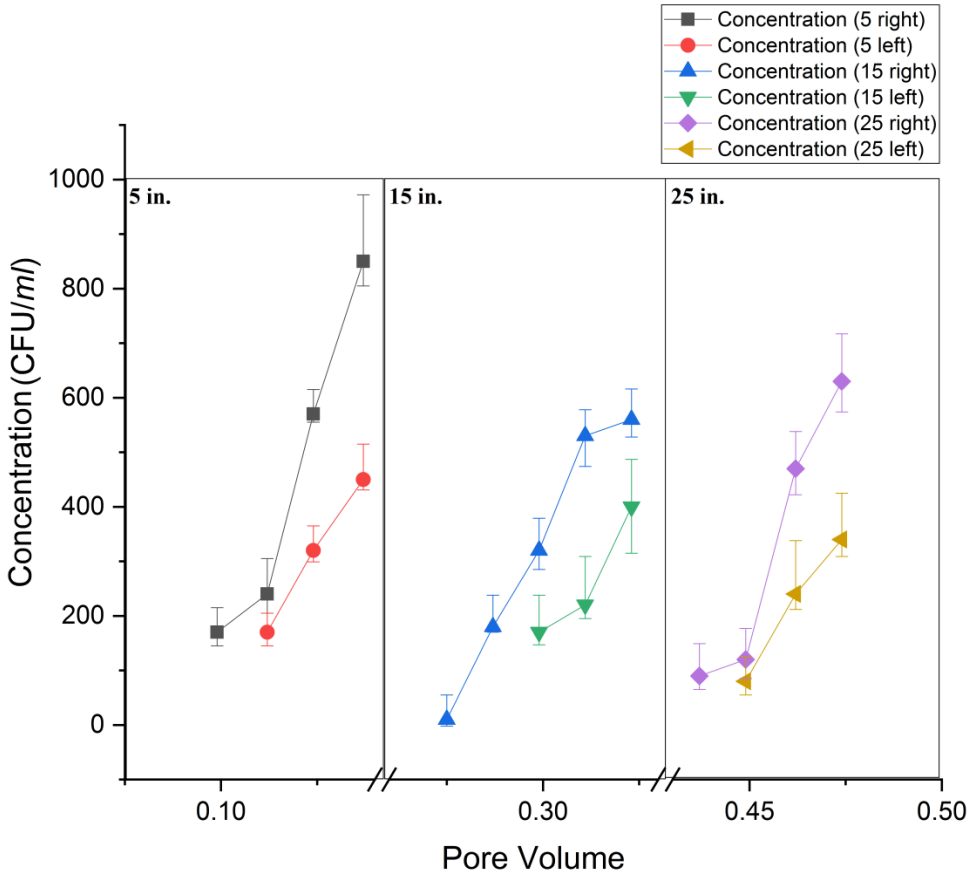


Figure 3.17: Breakthrough and concentration curve of *Legionella* as a function of pore volume.

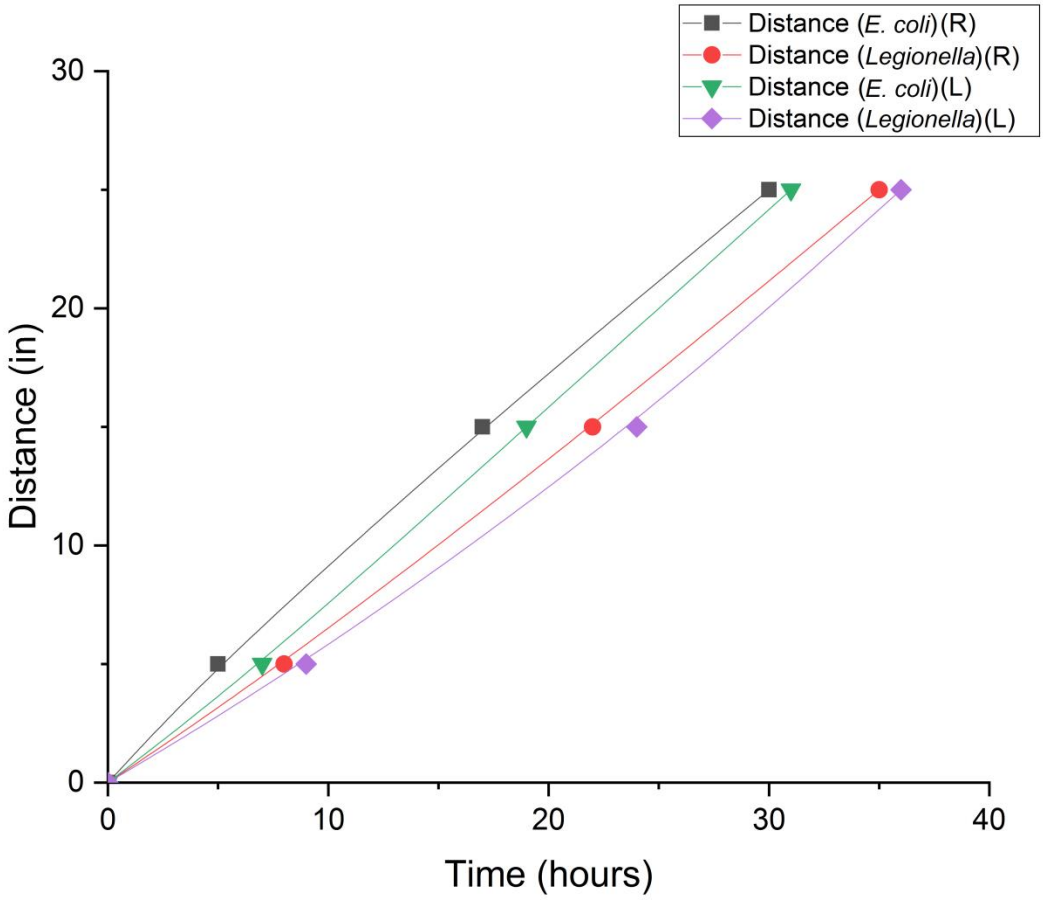


Figure 3.18: Distance traveled by *Legionella* and *E. coli* as a function of time. R stands for right ports and L stands for left ports.

3.5 Discussion

The average linear velocity of transport of *E. coli* was 0.83 in/hr (5 cm/day) and 0.81 in/hr (4.9 cm/day) on the right and left side, respectively. However, for *Legionella* it was 0.71 in/hr (4.3 cm/day) and 0.69 in/ hr (4.2 cm/day) on right and the left side, respectively. McBurnett et al., 2018 had reported difference in breakthrough between *E. coli* and *Legionella* under saturated conditions. As it can be seen there is variability in the velocity of transport velocities on the two sides of the tank. The microbes seem to travel

faster on the right side as compared to the left side. This can be due to a variety of reasons, starting with un-uniform packing on the right side as compared to the left side to horizontal transport and mechanical mixing (Fetter, 1988). Identification of the root cause behind this anomaly was beyond the scope of the experiment.

It was also noted that the test bacteria (both *E. coli* and *Legionella*) seemed to accelerate as they traveled further from the point of entry. Similar trend was noticed by McBurnett et al., 2018 during a column study. This could indicate un-uniform packing of media, although such a trend was not noticed in the tracer test (chapter 4). However it must be taken into account that the tracer test was based on the visual results of breakthrough, which might not reflect the true breakthrough of the tracer at the specific heights.

It was seen that there was a delay in the breakthrough of *Legionella* as compared to the breakthrough for *E. coli* at all three ports. The delay was consistent throughout the tank hence validating the trend. The delay in the breakthrough is in line with the hypothesis of *Legionella* being pleomorphic in nature. When under stress and deprived from nutrient, *Legionella* tends to become long and filamentous (Greub and Raoult, 2003). Size of the microbe is known to affect its transport under saturated conditions (Gupta et al., 2003).

It was seen that the average velocity of the test bacteria was more compared to the linear velocity of the water in the sand. This can be possible because the experiments were done in a completely saturated condition. Although advection (flow of contaminant/

microbe with the fluid) is the primary mechanism that governs transport, there are a myriad of other mechanisms at play. One possible reason could be mechanical mixing. (Fetter, 1988).

This study provided a good basis for the premise that microbial transport exists under recharge practices.

4. TRANSPORT OF FLORESCEIN TRACER IN TWO-DIMENSIONAL POROUS MEDIA TANK

4.1 Abstract

The study was to analyze the extent of tracer transport in a two-dimensional tank under completely saturated conditions. The experiments were done in a 2-D tank packed with 3,700 in^3 of fine grained, homogenous, chemically inert sand under saturated conditions. The tank used for transport was made sterile by backwashing with 0.6% chlorine solution. The transport of tracer was measured using samples collected from ports at vertical distances of 5, 15 and 25 inches (12.7, 38.1, 63.5 cm) from the surface of the sand on both sides for the 2-D tank. An influent concentration of 2g/L was set as a standard and the percolation rate was set at 11.37 inches/day using a peristaltic pump at the bottom outlet. At depths of 5, 15 and 25 inches, breakthroughs were recorded at 13.5, 41 and 67 hours for the ports on the right side and 15, 42.5 and 69 hours respectively.

4.2 Introduction

Contaminant transport in ground water is a concern for communities relying on ground water and replenishing it by artificial recharge. It is estimated over 150 million Americans directly depend on ground water for their water supply (Dylan et al., 2014). It has been well established that total dissolved solids (TDS) are a good measures of water quality. (Fetter, 1988). The major constituents of the water are inorganic compounds like magnesium, nitrogen and potassium in their salt forms like sulfates, halides and

bicarbonates or otherwise. Some compounds like those involving nitrogen can lead to eutrophication in lakes (Beeton, AM, 1965). Trace elements carcinogenic to human and aquatic life like arsenic, lead etc. (Fetter, 1988). It has been seen via studies that these contaminants have significant potential for transport in aquifers (Sathe et al., 2018). A good way to understand this would a tracer test. While there are more advanced methods like electrical resistivity tomography (ERT.) for quantification of tracer plumes and analyze their transport, a simpler visual tracer transport through a packed column can be as useful for understanding the flow gradient and flow patterns. It has been seen that accurate information about transport of chemicals are restricted by the lack of density of sampling locations because local heterogeneity and preferential flow (Kim and Lee, 2017).

The groundwater is generally contaminated by infiltrate released by contaminants through vadose zone subsequently spread following the particular flow directions and hydrodynamic conditions of ground water bodies. Localization and monitoring of contaminants is an important step to start remediating the contaminant. (Meyer, et al., 1994; Herrera and Pinder, 2005; Lekmine 2017).

The objective of this study was to analyze the transport of a tracer (Fluorescein-Na salt) in a 2-D tank packed with porous media under completely saturated conditions. This in turn would lend a good idea of the transport of like contaminants and the mechanisms hence involved.

4.3 Materials and method

4.3.1 Preparation and packing of soil column of two-dimensional tank

The primary aim of this experiment was to measure the transport of a chemically inert tracer (Fluorescein-Na salt) in a saturated 2D tank which was packed with porous media. The tank in use was built by the Engineering Technical Service shop at ASU and is made from stainless-steel along with acrylic glass enclosing it and steel coupling and braces protecting the structure from fracture. It was identified that the pressure points were at the 4 corners and the braces were attached accordingly.

The tank is 72 inches (183cm) tall, 24 inches (61cm) wide and 4 inches (10cm) deep. It has intermittent sampling ports as can be seen in the picture below. For this study sampling ports at 5 inches, 15 inches and 25 inches were used as they were identified to have given the best portrayal of the overall transport of bacteria (chapter 3) and tracers throughout the tank. Approximately 3750 in^3 of dry Quikrete© Mesh Fine Silica Sand was packed into the tank and compacted. The bottom of the tank along with the top of the sand column was lined with gravel to stop the sand from getting dislodged. The tank was disinfected with a 0.6% bleach solution. This ensured that most, if not all microbial life in the tank was removed and the tank was sterile. The tank was subsequently flushed 4 times after the bleaching was done, the last one with chlorine neutral water to ensure there was no free chlorine. The tank was saturated from the bottom and filled up at a slow rate of 3.6*ml*/min to ensure all the air entrapped amongst the void can be eliminated. It was made sure that a head of 1.5-2 in water was allowed above the gravel. The tank as can be seen in the picture has an outlet on the bottom right corner. This is connected to a

peristaltic pump. This was done to ensure that a constant infiltration rate of 11 in/day was kept going. On the longitudinal side of the tank there is an outlet on either side. The one of the right side was used to pump in the sample during gravity flow and the left one for overflow to maintain a steady flow of water on the top. This was done to enable a steady concentration matrix and to avoid preferential flow as much as possible. The bottom of the tank below the gravel has been lined with a pipe with 3 openings which was connected to the outlet at the bottom right corner. This was done to ensure uniform flow through the tank.

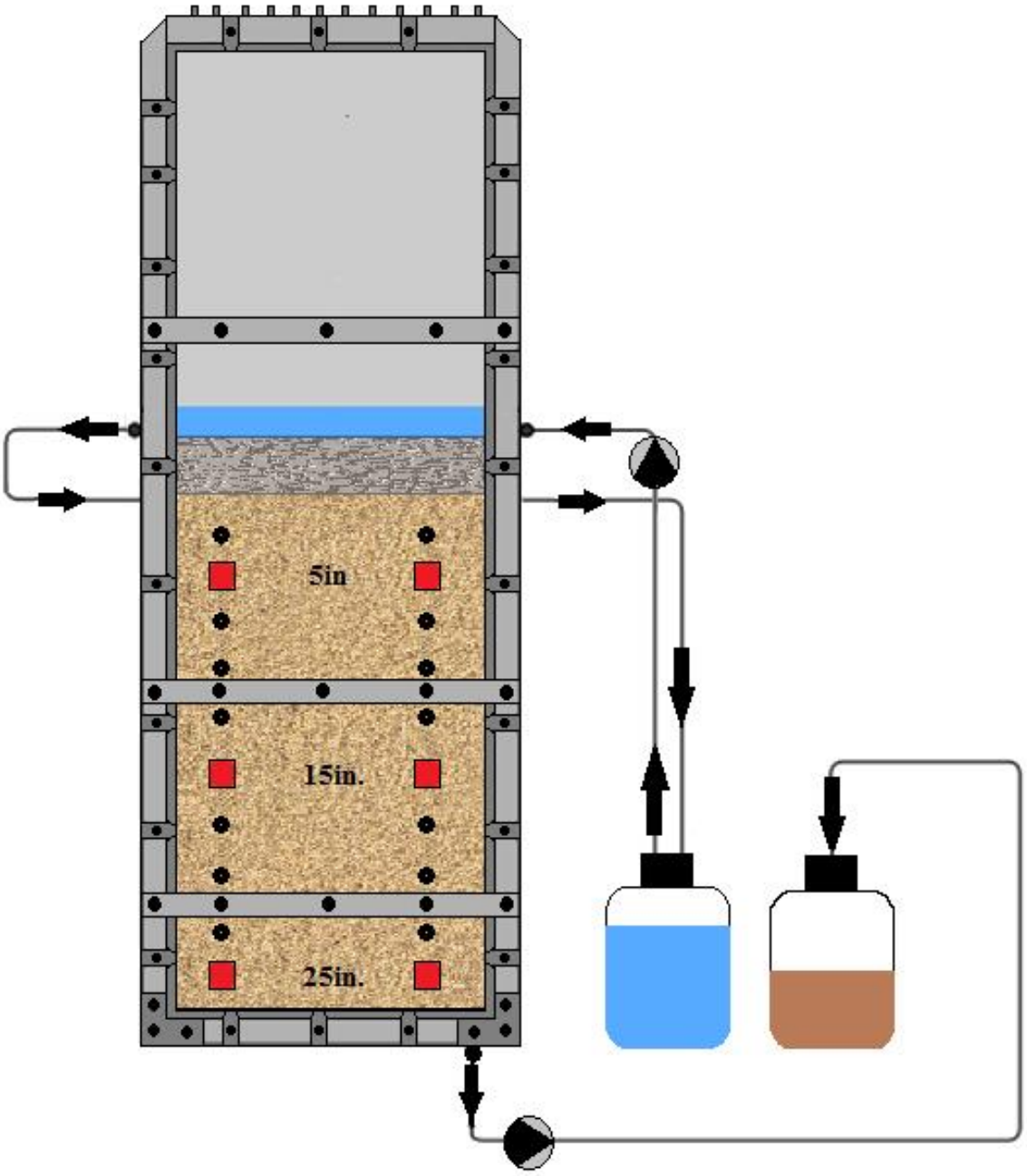


Figure 4.1: 2-D tank packed with Quikrete play sand media with sampling ports

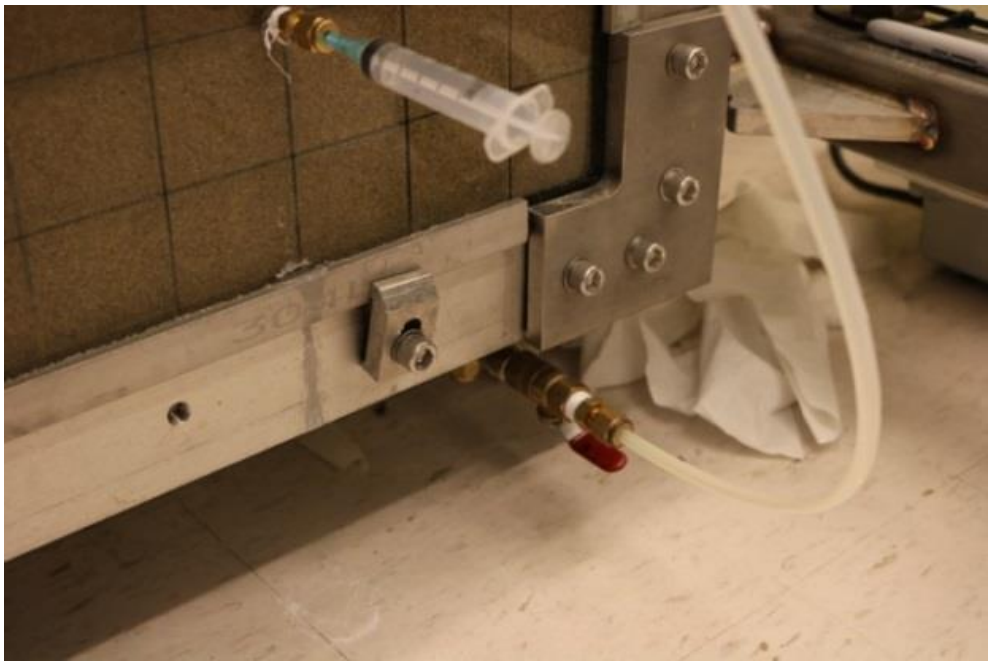


Figure 4.2 Inlet/Outlet point at bottom of tank

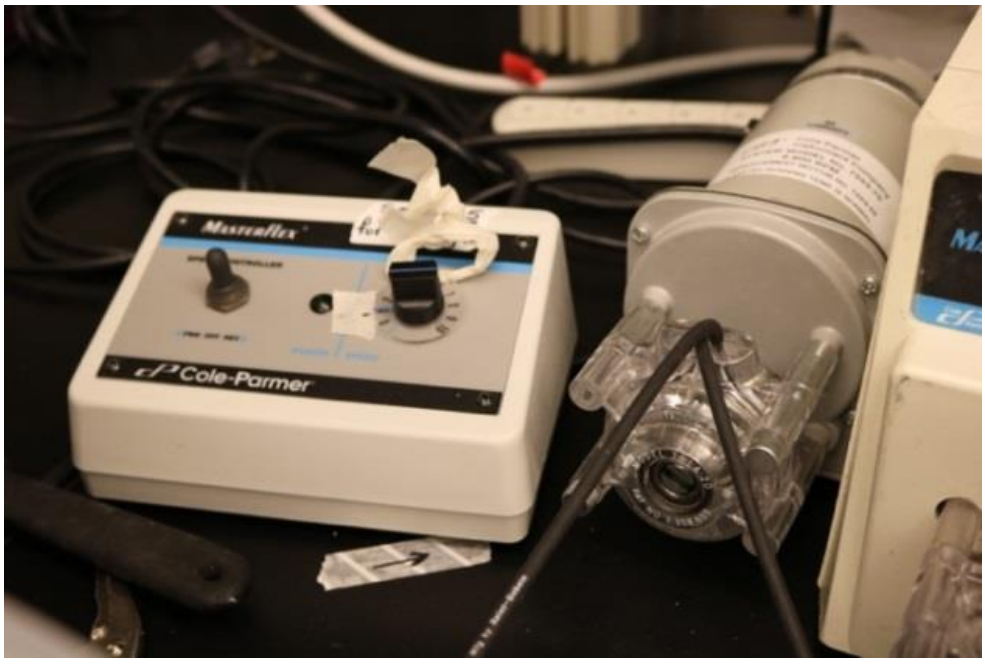


Figure 4.3 Peristaltic pump which controls tank flowrate

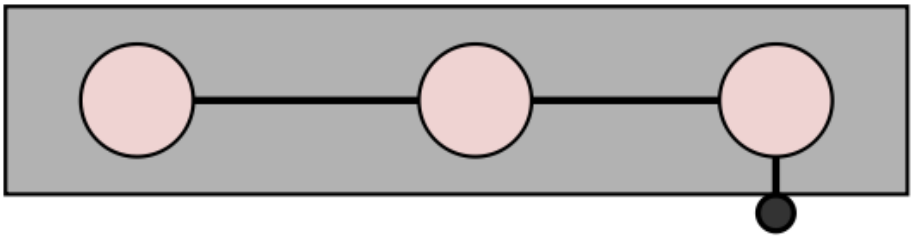


Figure 4.4 Schematic of outlet points along bottom of inside of tank

The two pumps during a gravity flow experiment were set at different rotational speeds. The inflow pump was set at 60 RPM whereas the pump controlling the outflow (Figure 3.3) was set at 15 RPM. The flow rate for the outflow pump was set at 3.5 ml/min. Both the pumps are capable to rotational speeds of a minimum of 1 RPM and a maximum of 200 RPM.

4.3.2 Media properties

Quikrete© (No. 1961) Mesh Fine Silica Sand was selected as the media to pack the column, datasheet has been attached herewith (Appendix B). The packed media is among the size range associated with the U.S. sieve number #30- #70 (0.6-0.2 mm) (Commercial Grade Sands: Product Nos. 1961, 1962, 1963). According to ANSI grit size, table B.1 found in Appendix B is contained mostly in the sieve size 0.01mm.It was seen post a sieve analysis (particles above 2.00 mm) comprised 8% of the total media. It must be noted that 83.75% of the media was restrained by sieve size greater than 0.01mm. The porosity of the homogenous layer of sand was measured to be 31% and allows for closely

controlled sizing and particle size distribution. An enlarged view of the sand particle can be seen in the figure 4.5. Considering the factors that affect transport such as size of grain, chemical interactions, texture etc., and the sand was chosen as ideal for the analysis of transport of microbes.

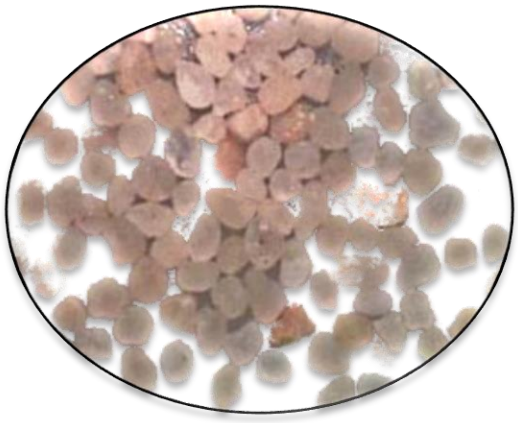


Figure 4.5 Quikrete© Silica Sand

Source: *Commercial Grade Sands: Product Nos. 1961, 1962, 1963* [Pamphlet]. (n.d.). Atlanta, GA: the QUIKRETE Companies©.

The packed media is chemically inert and has a boiling point which is relatively high. This can be attributed to the high inter atom bond strength. It is primarily composed of Silicon dioxide (SiO_2) and being chemically inert it is nonreactive. If the silica grains are compared with the size of *E. coli* and *Legionella* cells, there is a visible difference among them. The size of a silica grain is 250μm whereas for *E. coli* the size is somewhere between 0.5-2.0μm and for *Legionella* about 4μm. Table 4.1 gives an idea of the differences in sizes between the media and microbes. It must be kept in mind that ratio of

soil grain diameter to the diameter of *E. coli* is about 125-500, whereas for *Legionella* it is about 60. This explains the vast size difference between the two.

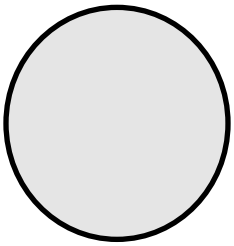
	150 Microns	Average Human Hair
	25 Microns	Lint, Particles Visible to the Naked Eye
	10 Microns	Heavy Dust, Lint, Fertilizer, Pollen
	5-10 Microns	Average Dust, Plant Spores, Mold
	1-5 Microns	Bacteria, Light Dust, Animal Dander.
	0.3-1 Microns	Bacteria, Tobacco and Cooking Smoke, Metallic Fumes
.	0.001-0.01 Microns	Tracers

Table 4.1 Micron Size Chart

Source: *The Best Air Purifier for Smoke Buying Guide. (2016, December 15). Retrieved 2017, from https://purifythis.com/best-air-purifier-for-smoke/*

The porosity of the sand was determined by measuring out 1000 cubic centimeter of the media in a beaker (consolidated). Water was slowly added from the top. It was made sure that there was no air entrapped in the voids. The volume of water added was measured. It

was recorded that 314 ml of water had been added to completely saturate the media. The porosity was calculated using the following formula.

$$porosity = \frac{volume\ of\ voids}{Total\ volume}$$

It was assumed that all the air voids had been replaced with water and the total volume of voids was equal to the volume of water added.

The test was repeated using 500 g of sand. The media was completely saturated and weighed again. The difference in weight was measured. The ratio of the difference between the weights and the total weight of the saturated media was calculated. The results were similar and the final porosity was calculated to 31% or 0.31.

4.3.3 Determining and setting flow rate through packed column

The outflow is controlled by a peristaltic pump set at a specific speed. This was done in order to make sure there was a constant infiltration rate throughout the experiment and a stable head is maintained on the top as it was essential to ensure that a uniform matrix is formed. The desired infiltration was something between 10 and 12 in/day. The flowrate coming directly from the tubing was measured to be 3.6mL/min. This measured flowrate was used to calculate the linear velocity of water and the total water that would infiltrate the media in one day. The tank was filled with about 3750in^3 (61451.49 cm^3) of Quikrete play sand. The porosity after compaction was noted to be 31% or 0.31. This would translate to 1162.5 in^3 (19049.9619 cm^3) of voids and 2587.5 in^3 (42401.5 cm^3) occupied by sand particles. The flow rate from just outside the pump was

measured to be 3.6 mL/min, which converted to linear velocity of water through soil is 11.37 in/day. The following calculations entail the details:

$$\frac{0.0036\ L}{min} = \frac{3.6 \times 10^{-3} L}{min} \times \frac{60\ min}{hr} \times \frac{24 hr}{day} = \frac{5.184\ L\ fluid}{day}\ through\ column$$

This means that approximately 5.184 L of fluid would travel through the tank in one day at the measured flowrate.

$$\frac{17.8\ L\ void\ volume}{\frac{5.184\ L}{day} fluid} = 3.43\ days$$

3.43 days would be the time it would take the water to fill out all the voids and move through the sand.

$$\frac{39\ in}{3.43\ days} \times \frac{day}{24\ hrs} = 0.47 \frac{in}{hr}\ \ or\ \ 11.37 \frac{in}{day}$$

The above calculations indicate that the infiltration rate will be 0.47 in/hr. or 11.37 in/day, which is in coherence with the national average of infiltration rates.

Once the infiltration had been adjusted to 11.37 in/day, the speed on the pump was locked and all pilot-scale studies were performed at the determined flowrate. In general modeling for ground water is done with a much slower infiltration. 11.37 inches per day may seem a bit high as compared to general modeling studies. However, when pollution in a saturated system is considered, infiltration is usually found to be higher. Multiple EPA reports suggest that contaminants can move rapidly through macropores and fractures, hence the higher infiltration rates.

4.3.4 Preparation of stock solution

A study was completed to investigate the transport of a chemical tracer, Fluorescein Sodium Salt ($C_{20}H_{10}O_5 \cdot 2\ Na$) (Fisher Scientific A833-100) also known as Uranine was used for the experiments, through the two dimensional tank packed with porous media at a predetermined flowrate. The influent had a dose of 2g/L of the tracer and 40g of tracer were added to the carboy having 20L of water. It was observed via previous experiments that at lower concentrations, the dye was detectable in the samples collected from the ports for analysis via spectrophotometry but was not visible on the media.

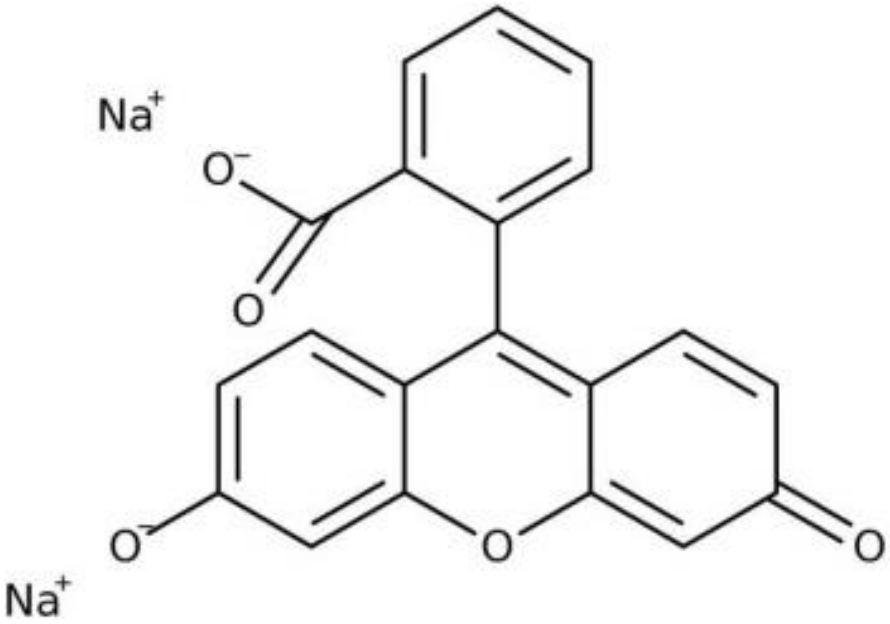


Figure 4.6: Fluorescein-Na salt

4.3.5 Pilot-Scale investigations of saturated spiked column

A transport study was performed to investigate the transport of an inert chemical tracer, Fluorescein Sodium Salt (Fisher Chemical A833-100) ($C_{20}H_{10}O_5 \cdot 2\ Na$), through the porous media inside the column at the set flowrate. The influent, a carboy with 20 L of water, was dosed with the dye to reach a concentration of 2 g/L. A lower dose although it was detectable by spectrophotometry, but wasn't visible with the naked eye. The primary aim of the study along with studying the transport of tracer was to identify the fluid flow patterns inside the media. The results (transport as a function of depth) if linear would enforce the idea that the column was uniformly packed and the gradient would give an idea if transport was via a matrix or if there were any preferential flow on either side.

4.3.6 Data analysis

Microsoft Excel 2017 and Origin Pro were used to perform data analysis and prepare graphical representation of data from the column studies.

4.4. Results

Fluorescein Sodium Salt ($C_{20}H_{10}O_5 \cdot 2\ Na$) (Fisher Scientific A833-100) also known as Uranine was used for the experiments. A dye test was performed to better understand the fluid dynamics of the tank. A dosage of 2g/L was chosen as the concentration. It was seen that lower doses were detectable via photo chromatography, but the dye was not visible below this concentration. Visual results indicate breakthrough

at 5 inches was 13:30 hours and 15:00 hours elapsed time for the right and left ports, respectively. This equates to 0.15 and 0.17 pore volume (PV), respectively. For 15 inches the breakthrough (right) was around 41:00 hours and (left) 42:30 hours after the start of the experiment, which is 0.47 and 0.49 pore volume (PV). For 25 inches the breakthrough (right) was around 67:00 hours and (left) 69:00 hours after the start of the experiment, which is 0.77 and 0.79 pore volume (PV). There was slight preferential flow observed on the right side. This can be seen better illustrated in figure 4.7

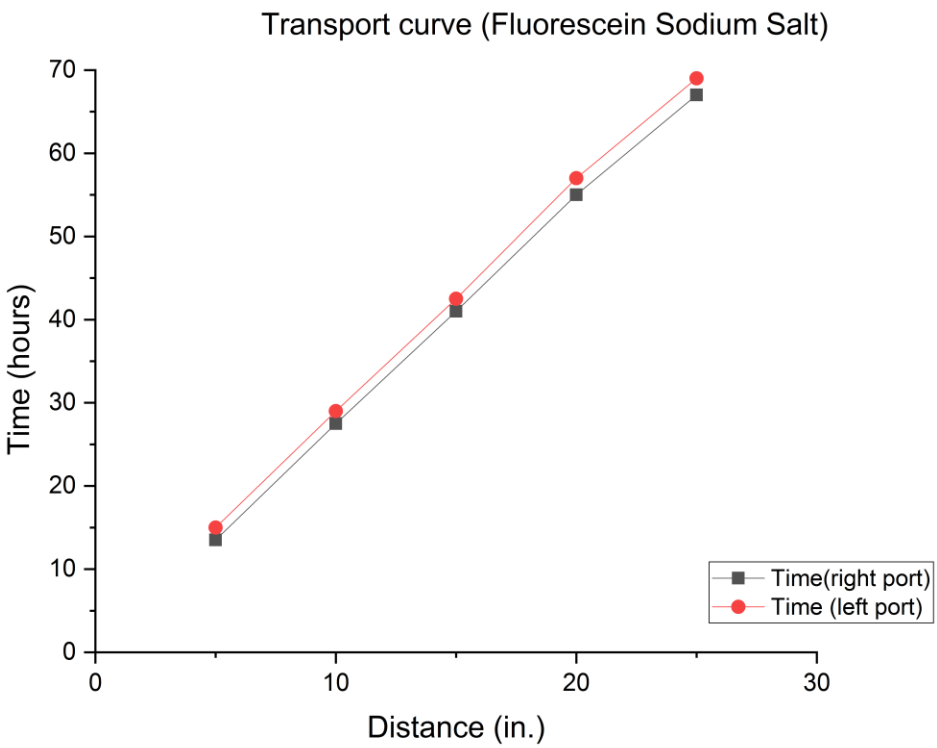


Figure 4.7: Transport of Fluorescein dye over time.

Table 4.2: Summary of visual breakthrough of Dye at different sampling ports (Visual)

Sampling Port	Breakthrough (R)	Breakthrough (L)
5 in	13:30	15:00
15 in	41:00	42:30
25 in	67:00	69:00

4.5 Discussion

The average linear velocity of transport of tracer was 0.37 in/hr (0.94 cm/day) and 0.36 in/hr (0.92 cm/day) on the right and left side, respectively. As it can be seen there is variability in the velocity of transport velocities on the two sides of the tank. The dye although ever so slightly, seems to travel faster on the right side as compared to the left side. This can be due to a variety of reasons, starting with un-uniform packing on the right side as compared to the left side to horizontal transport and mechanical mixing. Fetter (1984)

It was also noted that the tracer seemed to accelerate as they traveled further from the point of entry. This could indicate un-uniform packing of media, as a similar trend was noticed in the bacterial transport test. However it must be taken into account that the tracer test was based on the visual results of breakthrough, which might not reflect the true breakthrough of the tracer at the specific heights.

This study provided a good basis for the premise that transport of chemicals exists under recharge practices.

FUTURE INVESTIGATION

Additional experiments are needed with varying hydrogeological and chemical factors to improve overall understanding of microbial transport kinetics. Use of sophisticated softwares like HYDRUS 2D/3D can give a better representation of the flow dynamics via multiple simulations. HYDRUS uses a modified version of Richards's equation to simulate flow. Since it has been seen that the microbes have been subjected to significant removal, the porous media needs to be analyzed after the completion of an experiment in order to validate the state of the microbes since it has been seen that microbes especially *Legionella* changes form under stress. Analyzing the porous media also gives us the solid state concentration which is necessary for analysis of transport.

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APPENDIX A

COMMERICAL GRADE QUIKRETE© DATA SHEET

COMMERCIAL GRADE SANDS

PRODUCT NOS. 1961, 1962, 1963

DIVISION **32**

Aggregate
32 15 00

PRODUCT DESCRIPTION

QUIKRETE® Commercial grade sands are narrowly graded clean dry silica sands. They are available in three grades, fine (No. 1961), medium (No. 1962) and coarse (No. 1963).

PRODUCT USE

QUIKRETE® Commercial grade sands are silica sands which can be used alone or in combinations to provide a variety of gradations for any commercial application requiring high quality silica sands.

SIZES

• Available in 50 lb (22.7 Kg) and 100 lb (45.4 Kg) bags.

TECHNICAL DATA

QUIKRETE® Commercial grade sand is available in the following sizes:

Grade	Predominant Size Range US sieve number (mm)
Coarse No. 1963	#12 - #30 (1.7-0.6 mm)
Medium No. 1962	#20 - #50 (0.8-0.3 mm)
Fine No. 1961	#30 - #70 (0.6-0.2 mm)

PRECAUTIONS

• NOT RECOMMENDED FOR ABRASIVE BLASTING

WARRANTY

The QUIKRETE® Companies warrant this product to be of merchantable quality when used or applied in accordance with the instructions herein. The product is not warranted as suitable for any purpose or use other than the general purpose for which it is intended. Liability under this warranty is limited to the replacement of its product (as purchased) found to be defective, or at the shipping companies' option, to refund the purchase price. In the event of a claim under this warranty, notice must be given to The QUIKRETE® Companies in writing. This limited warranty is issued and accepted in lieu of all other express warranties and expressly excludes liability for consequential damages.

The QUIKRETE® Companies
One Securities Centre
3490 Piedmont Rd., NE, Suite 1300, Atlanta, GA 30305
(404) 634-9100 • Fax: (404) 842-1425

** Refer to www.quikrete.com for the most current technical data, MSDS, and guide specifications*

www.quikrete.com

Commercial Grade Sands: Product Nos. 1961, 1962, 1963 [Pamphlet]. (n.d.). Atlanta, GA: the QUIKRETE Companies©.

APPENDIX B

ANSI PARTICLE SIZE CONVERSION CHART

Table B.1 ANSI Particle Size Conversion Chart

Millimeters	Microns	Inches	ASTM	Tyler Sieve	*ANSI Table 2	*ANSI Table 3
5.60	5600	0.220	3 1/2	3 1/2	S-S	-
4.75	4750	0.187	4	4	4	-
4.00	4000	0.157	5	5	5	-
3.35	3350	0.132	6	6	6	-
2.80	2800	0.110	7	7	7	-
2.36	2360	0.093	8	8	8	-
2.00	2000	0.079	10	9	10	-
1.70	1700	0.067	12	10	12	-
1.40	1400	0.055	14	12	14	-
1.18	1180	0.046	16	14	16	16
1.00	1000	0.039	18	16	20	20
0.850	850	0.033	20	20	22	24
0.710	710	0.028	25	24	24	-
0.600	600	0.024	30	28	30	30
0.500	500	0.02	35	32	36	36
0.425	425	0.018	40	35	40	-
0.355	355	0.014	45	42	46	46
0.300	300	0.012	50	48	54	54
0.250	250	0.010	60	60	60	60
0.212	212	0.008	70	65	70	70
0.180	180	0.007	80	80	80	80
0.150	150	0.006	100	100	90	90
0.125	125	0.005	120	115	100	100
0.106	106	0.004	140	150	120	120
0.075	75	0.0030	200	200	150	150
0.063	63	0.0025	230	250	180	180
0.053	53	0.0021	270	270	220	220
0.045	45	0.0018	325	325	240	240

Micro Grits

Millimeters	Microns	Inches	ANSI Grit
0.0500	50.0	0.00200	240
0.0395	39.5	0.00156	280
0.0295	29.5	0.00116	320
0.0230	23.0	0.00091	360
0.0183	18.3	0.00072	400
0.0139	13.9	0.00055	500
0.0106	10.6	0.00042	600
0.0077	7.8	0.0003	800
0.0058	5.8	0.00023	1000
0.0038	3.8	0.00015	1200
0.0450	45	0.0018	F
0.0275	27.5	0.0011	FF
0.0160	16	0.00063	FFF
0.0110	11	0.00043	FFFF

*Columns Tyler Sieve, ANSI Table 2, and ANSI Table 3 correspond to grit size

Grit Sizes – ANSI. (2017). Retrieved 2017, from http://www.washingtonmills.com/guides/grit-sizes-ansi/particle-size-conversion-chart-ansi/

APPENDIX C

BACTERIA COLONY APPEARANCE MORPHOLOGY

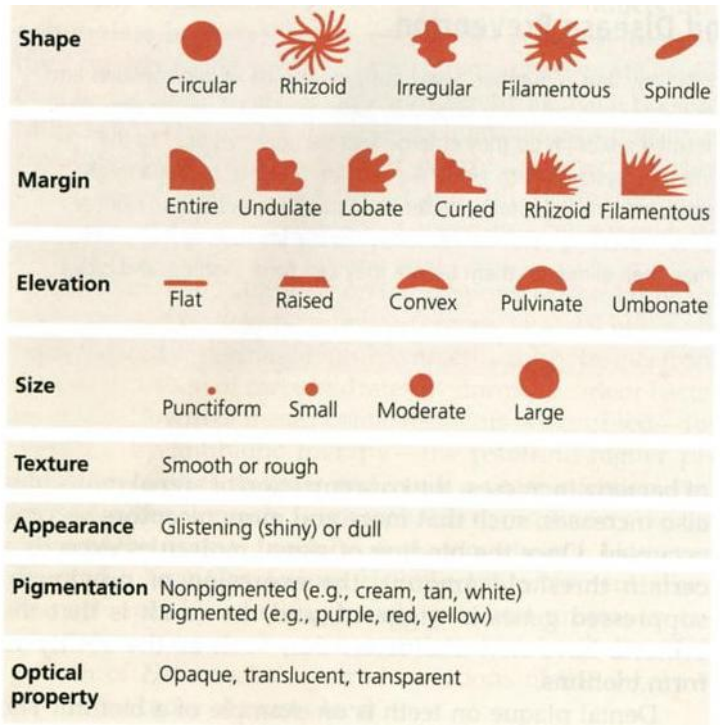

Figure C.1 Bacteria Colony Appearance Morphology Breakdown

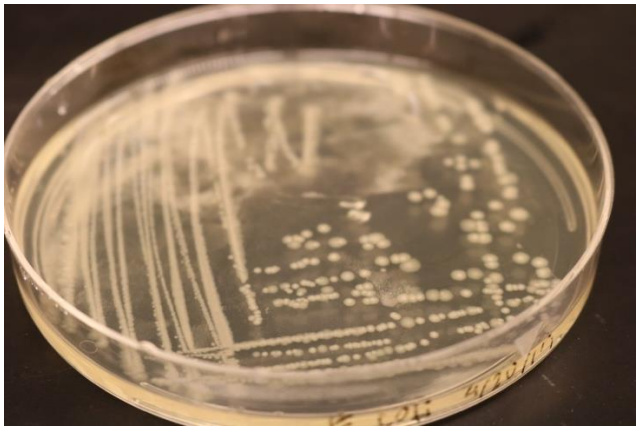

Figure C.2 *E. coli* Streak Plate on TSA Media

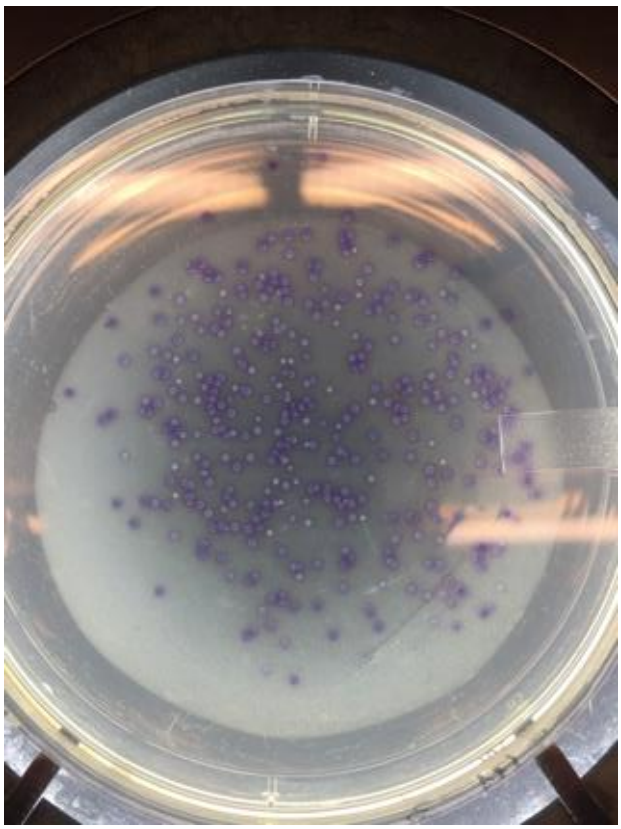

Figure C.3 *E. coli* Spread Plate on Brilliance Media

APPENDIX D

TANK CROSS-SECTION

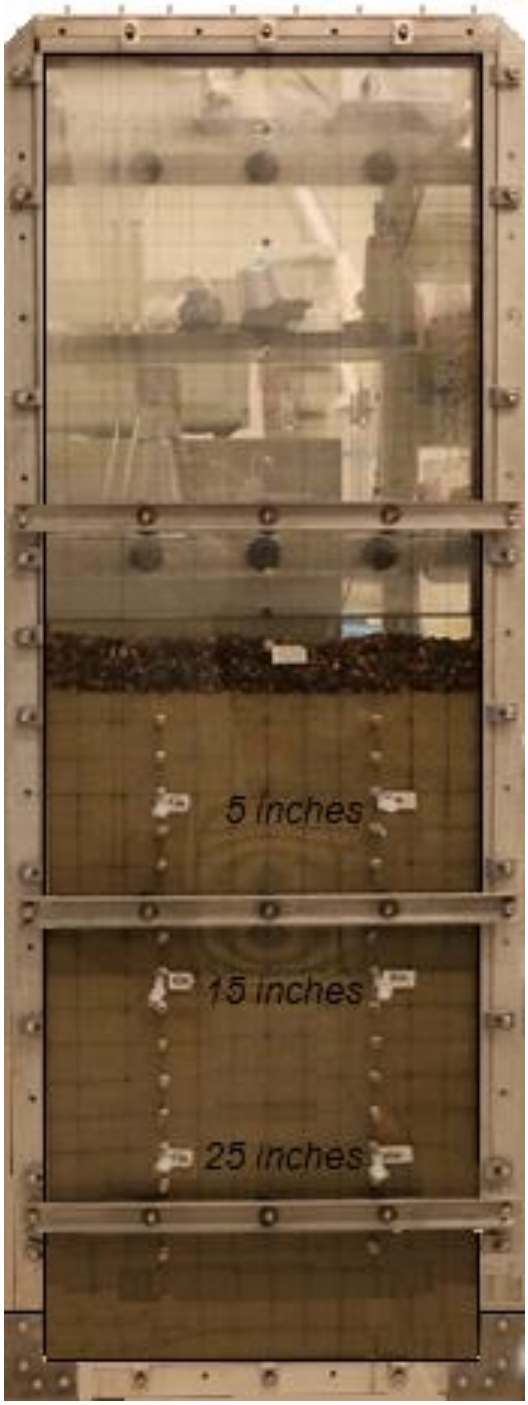

Figure D.1 Picture of the 2-D tank