The Study of Biological Wastewater Treatment through Biofilm Development on Synthetic Material vs. Membranes

A Major Qualifying Project Submitted to Faculty of Worcester Polytechnic Institute
In Partial Fulfillment of the Requirements for the Degree of Bachelor of Science

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Abstract

Water is one of the most important natural resources on our planet. Unfortunately, clean water availability is becoming scarcer each year given the current increase in waste disposal. Since recycling is the best way to salvage this dwindling natural resource, regulations concerning wastewater treatment have become more rigorous in recent years. Biofilms are being used more frequently to aid in the cleansing of wastewater through oxidation of organic particles and nitrification of ammonium.

This study focused on the biological treatment of wastewater through the use of biofilms. Six biofilms were grown in a reactor tank; four were on nylon mesh frames and two were on plastic membranes, one aerated and one anaerobic. The reactor tank consisted of 48.5 liters of diluted wastewater which was circulated and oxygenated to imitate a river. To determine the components of the diluted wastewater and to monitor the efficiency of biofilms in wastewater treatment, daily samples of the wastewater were tested using the TOC method, the Ammonium Nessler test, and UV-Visible Spectrometry. The development of the biofilm was monitored through opacity measurements.

Throughout the experiment, more biofilm growth was observed on the nylon mesh frames than on the plastic membrane frames. Furthermore, colonization on the aerated membrane surpassed that of the anaerobic membrane. It has been concluded that the use of biofilms is an effective step in wastewater treatment.

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Table of Contents

Table of Figures

Terminology

Aeration	Process by which air is circulated through, mixed with, or dissolved in a liquid or substance.
Biofilm	Bacteria that adheres to a surface.
Domestic Use	Household use of water for drinking, cooking, washing, watering a household garden and animals, but does not include the watering of crops or livestock for commercial purposes.
Effluent	Any liquid discharge as a result of domestic, commercial, industrial, or agriculture activities.
Membrane	A thin, pliable sheet like structure acting as a boundary, lining or partition in an organism.
Planktonic	The collection of small or microscopic organisms, including algae and protozoans, that float or drift in great numbers in fresh or salt water.
Sessile	Fixed in one place; immobile.
Waste	Includes any solid materials that is dissolved, suspended or transported in water, including sediment.
Wastewater	Wastewater is liquid waste discharged by domestic residences, commercial properties, industries, agriculture, which often contains some contaminants that result from the mixing of wastewater from different sources.

Chapter 1: Introduction

We are completely surrounded by water at every moment of every day. While covering 70.9% of the earth's surface and making up 70% of the average human being's body, water is, without a doubt, one of the most crucial natural resources on our planet (CIA- The World Factbook, 2012; The Basics of Water, 2009).

In recent years, the awareness of fresh water availability has increased significantly due to scientific predictions which state that in less than 20 years, two-thirds of the world's population might face water shortages (United Nations, 2009; The Nature Conservatory, 2012). Following the current increasing trend of waste disposal will be the main cause to this undesirable future. To prevent future water shortages, many countries have laws that require treatment of effluent from domestic and industrial sites so that water can be reused. In order to maintain the levels of clean water as high as possible and remove materials that are potentially harmful to human health, it is important for wastewater to be treated prior to being reused or discharged back into the environment. There are several ways of treating wastewater and different levels to which it is cleaned; this determines its future use.

Although it is necessary for water treatment systems to be engineered due to the amount of pollution contaminating wastewater, natural ways of cleaning wastewater through the use of microorganisms can also be incorporated into the process. For example, biofilm treatment systems employ the use of bacteria, fungi, algae, and protozoa to remove organic and inorganic materials from the surrounding liquid. The complexity of biofilm activity and behavior requires research contributions from many disciplines, such as biochemistry, engineering, mathematics and microbiology (MSU Center for Biofilm Engineering, 2008). Biochemistry and microbiology specialties are used to research and explore biofilm characteristics, such as the life cycle, growth and reproduction habits, the extracellular polymeric matrix, and environments in which biofilms grow sustainably. Engineering and mathematics specialties are used to devise methods under which biofilms can improve their surroundings, rather than have detrimental effects. Some situations in which biofilms may cause harm to their surroundings are in medical fields where biofilms often cause infections, and industrial fields where biofilms often cause corrosion of tools and equipment.

This study focused on the growth and effectiveness of biofilms in a reactor of diluted wastewater, intended to simulate a river. The experiment was conducted in the Laboratoire Réactions et Génie des Procédés (LRGP) at L'Ecole Nationale Supérieure des Industries Chimiques (ENSIC) in Nancy, France. Biofilms were grown on six frames submerged in the reactor tank; four of the frames consisted of a mesh made from nylon string in which the biofilm would adhere and grow, while the other two frames consisted of aerated and anaerobic plastic, respectively. For the first four weeks of experimentation, wastewater, taken from a treatment plant in Nancy, France, was replenished twice a week to feed the biofilms. For the final four weeks, the water level in the reactor tank was maintained using tap water, and no wastewater was added. The wastewater used in this project was primarily from domestic waste. Air was also pumped into the water, on either side of the reactor tank, to oxygenate and circulate the diluted wastewater. Each frame was scanned daily to measure biofilm thickness and observe the growth cycle. Biofilm growth on the aerated plastic was compared with that on the anaerobic plastic.

Methods used to determine the efficiency of the biofilm were the ammonium test, total organic carbon (TOC) and ultraviolet-visible spectroscopy. Daily samples of the wastewater were analyzed using these methods. The biofilm's ability to nitrify ammonium, maintain a constant chemical oxygen demand, and oxidize organic compounds in the wastewater allowed for the conclusion that biofilms are, in fact, an efficient step in the treatment of domestic wastewater.

Chapter 2: Literature Review

2.1 Wastewater and Its Treatment

Wastewater treatment is the only existent process in which you cannot control the inputs, but have to create a defined output. Treating wastewater is vital. Less than 3% of the world's total water supply is available as fresh water, and salt water is costly to desalinate. Due to this fact, water that was previously used for cleaning, bathing, and drinking will eventually end up back in a river or lake for it to be consumed once again (Ellis, 2004).

Nature has the ability to manage and clean certain amounts of waste and pollution in water through its use of microorganisms and algae. In the past few decades, however, the amount of waste that is disposed each year has increased, consequently affecting our environment in negative ways. Nature's inability to sufficiently treat the increasing amount of waste in bodies of water is one of the reasons why wastewater treatment plants have become a focal path in securing our future water supply. Wastewater treatment plants are essential to manage and clean the effluent from industrial, agricultural, and domestic sources. Treated wastewater is discharged to lakes and rivers and is eventually reused, so monitoring the chemical and bacteriological composition is crucial in guaranteeing public health (Ellis, 2004).

The treatment of wastewater is also imperative to the protection of many aquatic species. Wastewater can have low dissolved oxygen conditions, as well as excess nutrients such as nitrogen and phosphorous. Under these conditions the ecosystem is prone to develop a syndrome, in response to human activities, known as eutrophication. Eutrophication refers to excessive plant growth (mostly algae), which leads to a change in habitat from the lack of oxygen present in bodies of water.

Figure 1 provides a description of the process of eutrophication (Cloern, 2007).

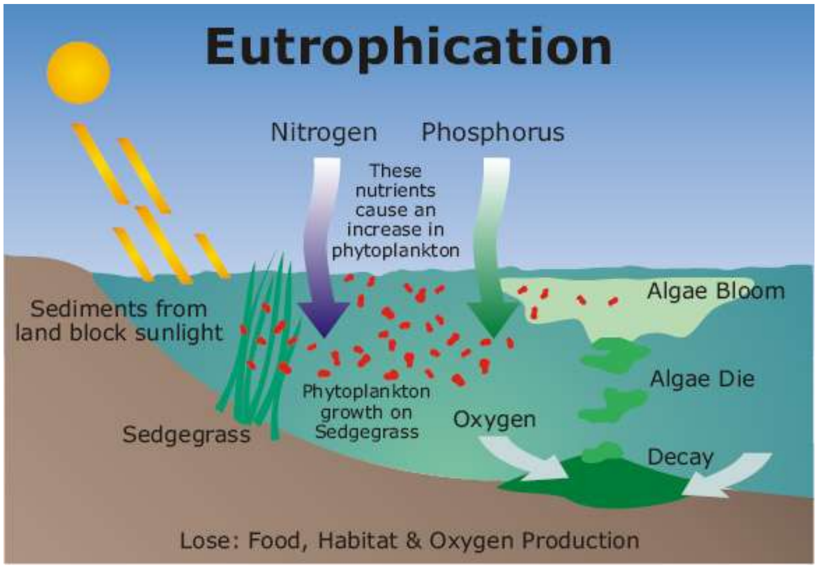

Figure 1: Process and Effects of Eutrophication (Cloern, 2007)

2.1.1 Wastewater Components

Wastewater is composed of many organic and inorganic materials, chemicals, and nutrients, among others. The composition of wastewater varies everyday. Components in the wastewater can differ depending on location, season, and time of day. There is no way to control the radical changes in wastewater, given that they depend on many factors. The composition of wastewater is roughly 99.9% water and 0.1% total organic and inorganic matter (Olutiola, 2010).

Microorganisms

Many types of microorganisms can be preset in wastewater. The most common are: bacteria, viruses, protozoa and helminthes. Most of these microorganisms can be considered harmless, given that they are present in the human body, but there are some that might cause disease. In order to get rid of disease causing microorganisms, the wastewater must go through rigorous processes such as filtering and chlorination (Jimenez et al., 2008).

Organic Material

“The organic composition of wastewater is approximately 50% proteins, 40% carbohydrates, 10% fats and oils, and trace amounts of priority pollutants and surfactants”

(Ellis, 2004). The organic material present in wastewater includes detergents, pesticides, fats and oils. These substances can be toxic and must be eliminated from wastewater.

Within the organic material, biodegradable organic material also resides. Biodegradable organic material refers to all waste that will naturally degrade with time. This material is comprised of wood, plant matter, paper, and human and animal waste. Even though it does not present a direct risk to the environment, it is a way in which microorganisms are transferred. Many microorganisms are introduced to the water in this manner, and since water composition needs to be monitored to prevent diseases, organic matter needs to be removed (Henze, 2002).

Basic Nutrients

Basic nutrients present in wastewater are most commonly nitrogen, phosphorous and ammonia. These nutrients can cause depletion of oxygen, i.e., eutrophication when they are present in excess. As seen in Figure 1, the nutrients are capable of causing significant damage to the environment, due to the changes they impart on the ecosystem (Henze, 2002).

Metals and Inorganic Material

Most of the metals and inorganic materials found in wastewater are part of industrial waste. Some of these metals and inorganic materials present in water are mercury, lead, cadmium, nickel and hydrogen sulphide. The amounts of these components that are present in wastewater greatly surpass the trace amounts that humans and animals need for health purposes. Wastewater is eventually reused due to its scarcity, so these components need to be eliminated. They can cause corrosion, and be toxic to the environment as well as humans (Henze, 2002).

Table 1, shows the components present in wastewater and their effect on the environment.

Component	Of special interest	Environmental effect
Micro-organisms	Pathogenic bacteria, virus and worms eggs	Risik when bathing and eating shellfish
Biodegradable organic materials	Oxygen depletion in rivers, lakes and fjords	Changes in aquatic life (less diverse)
Other organic materials	Detergents, pesticides, fat, oil and grease, colouring, solvents, phenol, cyanide	Toxic effect, aesthetic inconveniences, bio accumulation
Nutrients	Nitrogen, phosphorus, ammonia	Eutrophication, oxygen depletion, toxic effect
Metals	Hg, Pb, Cd, Cr, Cu, Ni	Toxic effect, bio accumulation
Other inorganic materials	Acids, for example hydrogen sulphide, bases	Corrosion, toxic effect
Thermal effects	Hot water	Changing living conditions for flora and fauna
Odour (and taste)	Hydrogen sulphide	Aesthetic inconveniences, toxic effect
Radioactivity	Toxic effect, accumulation	

Table 1: Components in Wastewater (Henze, 2002)

2.1.2 Overview of Wastewater Treatment

There are many ways in which wastewater is treated, primarily because many factors need to be taken into consideration prior to the treatment of sewage. As previously mentioned, the location and time of the year can drastically affect the composition of wastewater. For example, the area in which the water is treated and where the water will be discharged affects its cleaning process. If the water is to be discharged in a river, the chemical and bacteriological content needs to be less than if it were to be used for irrigation of fields. Wastewater can be treated for different purposes, some of which are for agricultural and potable use. The treatment of water for potable standards is very rigorous, while the treated water for agricultural use can have traces of organic matter and solid waste.

Wastewater treatment is generally divided in three or four main stages, which represent the degree to which the water is treated. These stages are: preliminary treatment, primary treatment, secondary treatment, and tertiary or advanced treatment. Within these stages there are different steps and methods that can be employed to treat the water (Prescod, 1992).

Preliminary Treatment

Preliminary treatment is used to eliminate large, solid objects that are often present in wastewater. Although this not an essential step in the cleaning process, treatment plants typically employ it as a precaution to prevent any damage these objects may cause to machinery in subsequent stages. The most common methods utilized in this stage are screening and grit removal.

Screening is the first step in the preliminary treatment of the wastewater. The water flows through a series of screens which filters out large, medium, and small objects from the liquid. Usually three different mesh sizes are used. The mesh size of the largest grid is about 5.0 centimeters, and it is used to remove large objects, such as branches and cloth. The mesh sizes of the second and third grids range between 0.5 to 3.0 centimeters, and 1.5 to 3.0 millimeters, respectively (Hogan, 2010). The grids are cleaned regularly, either manually or sometimes by using an automated process.

The next step in the preliminary process is known as grit removal. This process consists of separating sand, gravel, and any food particles present in the wastewater. There are different types of grit chambers, some of which are aerated; others just have a specific flow pattern. The turbulence, either from flow or air, settles the heavy components in the water, while light organic particles are suspended. The settled grit is then removed by a pumping system that takes it out of the chamber (EPA, 2004). Using this grit removing process will decrease the chances of damaging any type of mechanical system that may be used in the following steps (Vesilind, 2003).

None of the steps in preliminary treatment involve chemical or biological improvement of the wastewater. This is one of the reasons why some treatment plants, especially small ones, do not include this stage in their process, but include screening as the first step in their primary treatment instead (Hogan, 2010).

Primary Treatment

The primary treatment of wastewater is the stage in which the majority of organic and inorganic material, as well as contaminants in the water, are removed. Solids are removed by sedimentation, while oils and grease are removed by skimming (Hogan, 2010). "Approximately 25% to 50% of the incoming biochemical oxygen demand (BOD), 50% to 70% of the total suspended solids (SS), and 65% of the oil and grease are removed during primary treatment" (Prescod, 1992).

In this stage the wastewater is subjected to a process known as coagulation-flocculation, which leads to the settling of the solids. Since the particles in the wastewater are fairly small, a coagulant is added to the water to simplify the removal process. The purpose of this chemical is to attract the particles that are present to make them clump together; this simplifies their removal.

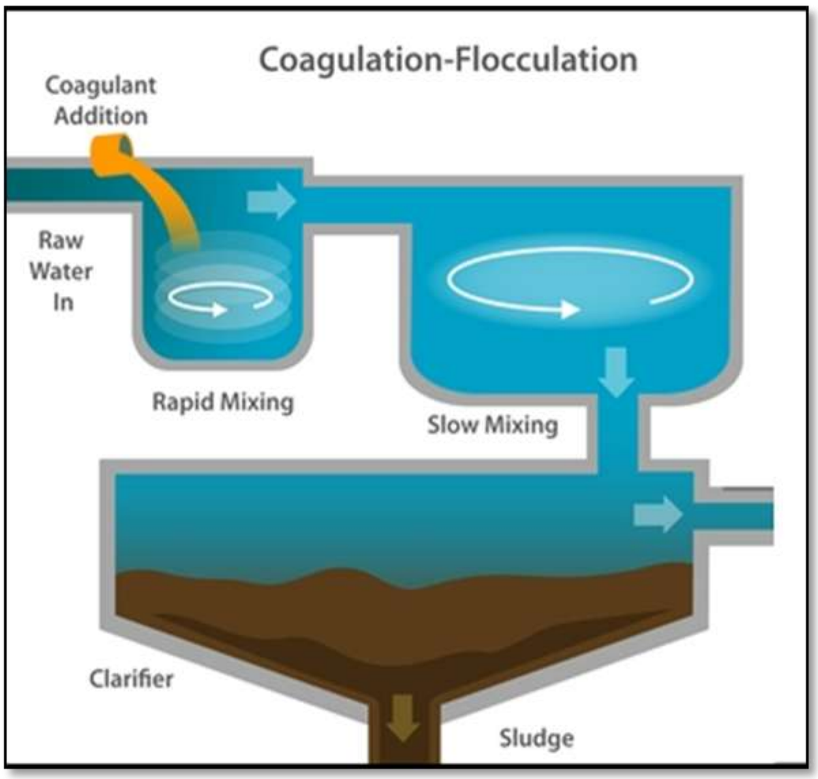

Figure 2: Steps Followed During the Primary Treatment (The Water Treatments, 2008)

Figure 2 shows the process that the wastewater follows in the primary treatment stage. First, the coagulant is added. Then, the water is rapidly mixed for a short period of time to ensure dispersion of the coagulant. After rapid mixing, the speed of the mixer is lowered to allow the particles clump. Once flocs are formed, they either settle to the bottom of the tank, or rise to the top. The bottom flocs are removed as sludge, while the top ones are removed as scum, using a continuously moving scrapper (Vesilind, 2003).

Secondary Treatment

Secondary treatment of wastewater employs biological processes and the use of microorganisms to rid the water of any organic compounds that may still be present. This stage simulates what actually happens in nature, when microorganisms break down organic wastes. There are three main approaches that can be used in this stage: fixed film, suspended film and lagoon systems (Mancl, 2009).

Fixed Film

In a fixed film system, microorganisms grow on rocks, sand, or plastic, to create a film. They grow on these surfaces by feeding off the organic matter and nutrients in the wastewater that flows over them. There are three main fixed film systems that are commonly used today: trickling filters, rotating biological contactors, and sand filters (Mancl, 2009).

Suspended Film

Suspended film systems consist of suspending the microorganisms in the wastewater. While in the water, they absorb the organic waste and nutrients around them, which allows them to grow and reproduce to form micro-colonies. These micro-colonies settle as sludge, which is then removed and either reused in the process by being re-suspended, or treated in a sludge treatment process. Some examples of suspended film are: activated sludge, extended aeration, and sequential batch reactor systems.

Lagoon Systems

Lagoon systems are settling ponds in which the wastewater is retained for an extended period of time. During this time, microorganisms degrade the organic compounds present in the water.

The systems used in secondary treatment completely rely on nature’s ability to clean the wastewater through natural resources. The use of sunlight, algae, oxygen, and microorganisms is essential (Mancl, 2009). “The result of effective primary plus secondary treatment is removal of about 90% of the organic matter, virtually all pathogens, and most solids. Between 10% and 20% of the nitrogen is also automatically removed because the decomposer bacteria require this much for their own growth" (The Sound Book, 2010). After these three treatment stages, the water is sufficiently clean for use in non-potable applications. These include: agricultural uses, non-potable household uses, and irrigation of parks.

Advanced Treatment

Advanced wastewater treatment refers to any steps that are utilized to raise the quality of water obtained from primary and secondary treatments. Problems such as color, odor, and taste are dealt with in this stage. The water is disinfected using either one or a combination of techniques such as chlorination, UV disinfection, and ozonation. Nutrients like nitrogen and phosphorous are also sometimes removed (Hogan, 2010).

Chlorination is the step in which chlorine is added to the water as a disinfectant to eradicate any bacteria left behind from the previous steps. UV disinfection consists of exposing the treated water to radiation by UV light. The UV light penetrates through an organism's cell wall and disrupts its genetic material, impeding any reproduction. Ozonation is the use of ozone gas (O_3) to disinfect and improve the color and odor in the water (Prescod, 1992).

An increasing number of wastewater treatment plants are incorporating this last stage in their processes. The water exiting at this stage in the treatment can be used for potable applications, which means the water can come in contact with humans.

Figure 3 depicts all of the stages used in wastewater treatment processes, prior to releasing the water to the environment.

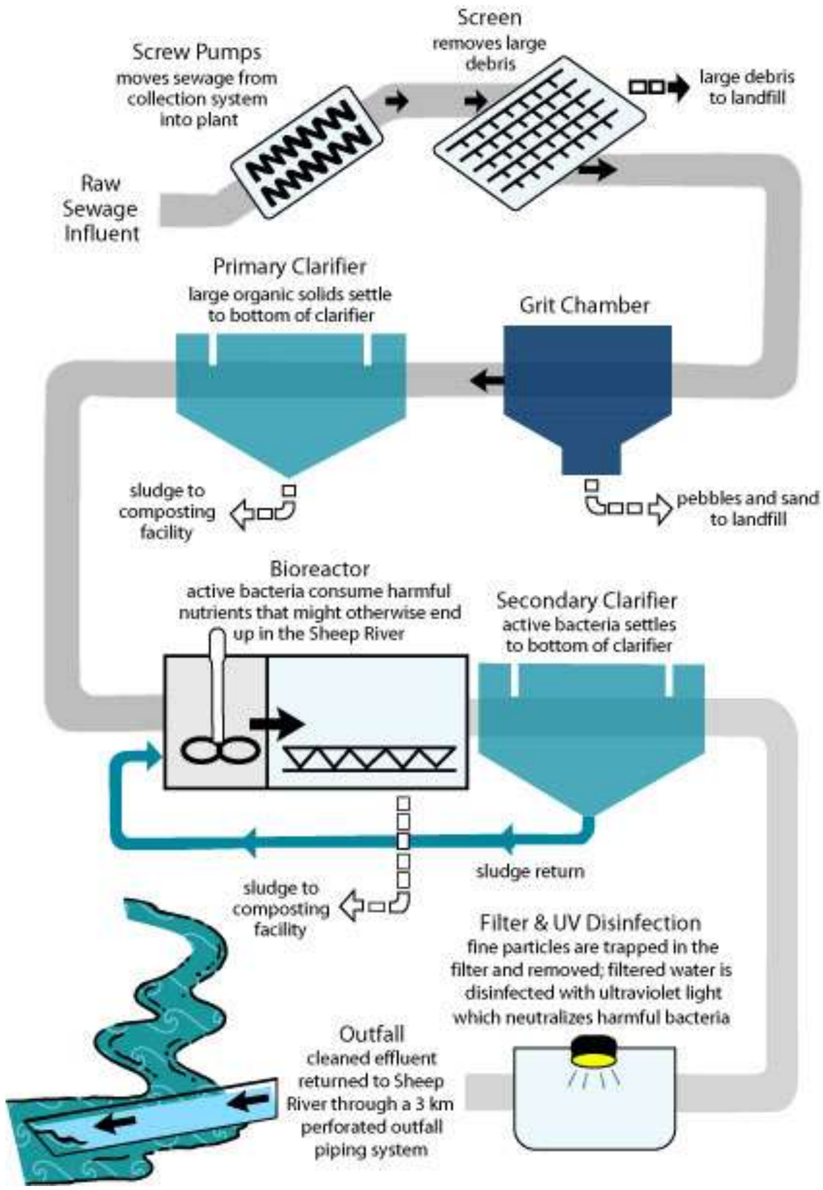

Figure 3: Stages in the Wastewater treatment Process (Sewage Treatment, 2009)

2.2 Biofilms

A biofilm is a community of microorganisms that develops in moist environments. The organic and inorganic materials that comprise a biofilm can range from decaying products in wastewater, to the millions of species of microorganisms in a lake (Deibel et al., 2003). A biofilm population can be made up of as little as one bacterial species, and is not limited to any particular amount of species. Dental plaque, for example, is one type of biofilm that is comprised of over 500 bacterial species (MSU Center for Biofilm Engineering, 2008).

The varying species in biofilms, such as bacteria, fungi, algae, yeasts, and protozoa, form clusters by producing an “extracellular polymeric substance,” also known as “EPS.” The cells are held together by these sugary molecular strands (EPS) and develop complex three-dimensional, resilient communities. These clusters of microorganisms excrete a glue-like substance, which allows them to adhere to a surface of almost any type (MSU Center for Biofilm Engineering, 2008). Where a combination of moisture and nutrients exists on a surface, it is likely to find a biofilm there. Biofilms have the ability to grow on natural materials above and below ground, on metals, plastics, medical implant materials, and plant and body tissue. Over time, a biofilm in the appropriate environment will grow and become strongly attached to the surface it lives on (Deibel et al., 2003).

Biofilms vary in size, depending on their surrounding conditions. A healthy biofilm can grow to be many inches thick in an environment with the proper nutrients and a steady temperature. To synthetically develop a sustainable biofilm, constant replenishing of nutrients is necessary (MSU Center for Biofilm Engineering, 2008).

2.2.1 The Life of Biofilms

Behaviors and survival strategies of bacterial colonies significantly surpass the capabilities of individual bacteria. For example, microbial biofilms are naturally tolerant of antibiotic doses up to 1,000 times greater than doses that kill free-swimming, planktonic bacteria (MSU Center for Biofilm Engineering, 2008). The picture in Figure 4, illustrates the five stages in biofilm development (Cogen et al., 2004):

1. Initial attachment
2. Irreversible attachment
3. Maturation 1
4. Maturation 2
5. Dispersal

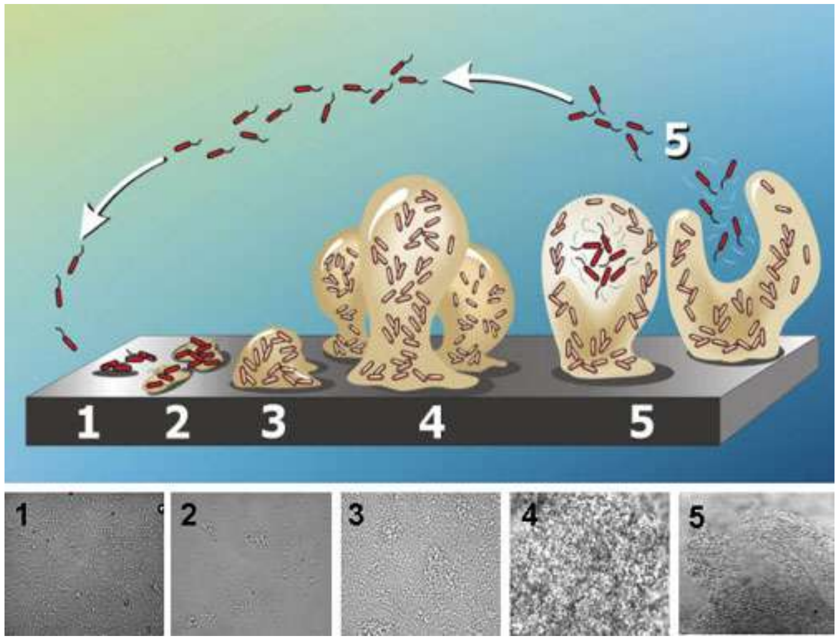

Figure 4: Stages of Biofilm Development (Cogen et al., 2004)

Initial Attachment

Initial attachment occurs when a material surface is exposed to an aqueous medium (e.g., water, blood) and is coated by polymers from that medium. This is known as the conditioning layer; it is organic, forms within minutes of exposure, and continues to grow for several hours. The cells that comprise the conditioning film attach quickly and efficiently to hydrophobic, nonpolar surfaces, such as plastics. In addition, initial attachment and colonization increase as surface roughness increases. This is due to the larger surface area on rougher surfaces (Donlan, 2002). Planktonic microorganisms instinctually attach themselves to this conditioning layer to become part of the developing biofilm (Cogen et al., 2004).

Irreversible Attachment

The second stage, irreversible attachment, begins once the microorganisms start producing the extracellular polymeric substance, EPS, which represents the "house of the biofilm cells" (Flemming et al., 2007). The polyhydroxyl groups in EPS anchor the bacteria in the biofilm to the surface through hydrogen bonding (Kjelleberg et al., 2007). At this time, the microbes can no longer move away from the surface. It has been determined that mature biofilms are anchored to their place until the final stage of growth (Kolari et al., 2001).

The Cell Matrix

The EPS is comprised of a wide variety of proteins, glycoproteins, glycolipids, and extracellular DNA. The biopolymers in the extracellular polymeric substance are highly hydrated and form a matrix that holds the biofilm together and retains water. This matrix interacts with the environment to provide nutrients for biofilm organisms. Micro-colonies are formed within the EPS during the maturation stages of development. The colonies are separated by pores and water channels, which function as pathways for nutrients and oxygen to flow through, and nourish the biofilm (Flemming et al., 2007).

Maturation

During the maturation stages, the biofilm's main goal is to grow in three dimensions. This is achieved by picking up debris in the surrounding environment, such as sand, and by recruiting new planktonic bacteria. The biofilm also grows in these stages through reproduction, which occurs regularly in the micro-colonies. Some biofilms can grow to be several inches thick, as seen in Figure 5, which is a photograph of a streambed in Yellowstone National Park in the United States. This particular biofilm is heavily populated by green algae (MSU Center for Biofilm Engineering, 2008).

Figure 5: Streambed in Yellowstone National Park (MSU Center for Biofilm Engineering, 2008)

Dispersal

The final stage of development is dispersal, or shedding of cells. This stage is sometimes referred to as "expansion". While many bacterial cells disperse from biofilms by passive processes, bacterial biofilms can also experience active dispersal events in which sessile, matrix-encased biofilm cells convert to free-swimming planktonic bacteria (Webb, 2007). Quorum sensing, commonly referred to as cell-to-cell signaling, is essential to biofilm dispersal (Donlan, 2002). Through quorum sensing, bacterial populations will activate dispersion only when they are able to sense that their population is numerous enough to make it advantageous. For example, one study observed a cellular division of labor through quorum sensing, where one group of cells stayed attached to the surface and made nutrients available to the second group, which reproduced and released daughter cells to the surrounding water (MSU Center for Biofilm Engineering, 2008).

Quorum Sensing

An illustration of quorum sensing can be seen in Figure 6.

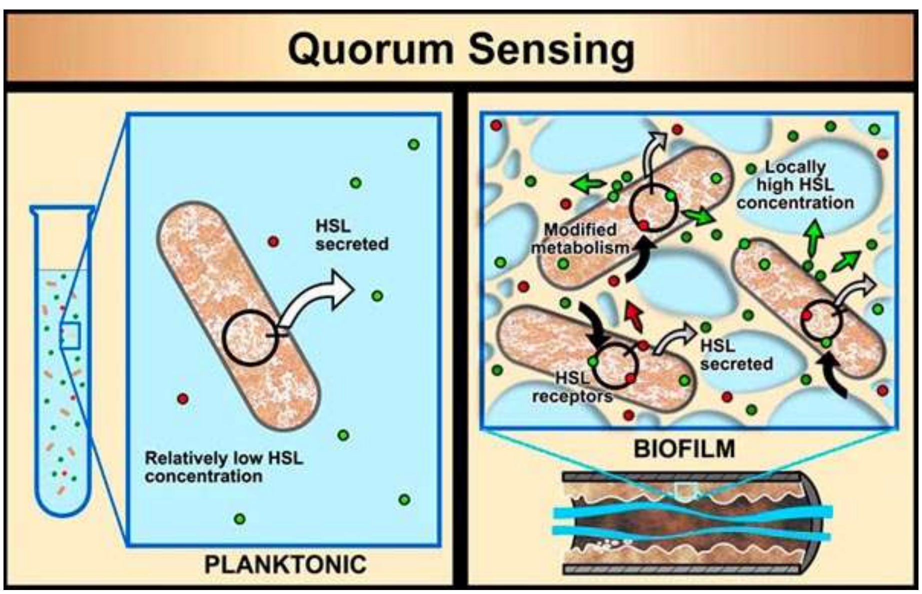

Figure 6: Quorum Sensing Depiction (Cunningham et al., 2011)

This image compares the cell-to-cell signaling abilities of planktonic bacteria to the bacteria in biofilms. Planktonic bacteria and biofilm bacteria both secrete chemical signals, or homoserine lactones (HSLs). The difference is that the population of bacteria in a biofilm is denser than that of planktonic bacteria, so the signals are more concentrated, and thus, more effective (Cunningham et al., 2011). Biofilm dispersal is not always intentional; passive dispersion, such as erosion and sloughing, can occur due to hydrodynamic or shear forces in the aqueous environment. Layers of biofilm may be broken off due to natural abrasion, nutrient deprivation, and/or hydrodynamic forces caused by the velocity of the liquid (Webb, 2007). Regardless of which way the dispersal occurs, a layer of cells (the conditioning layer) remains on the surface for the growth process to continue, or start over. It is important to note that the higher the shear force, the denser the biofilm will be. This means that a biofilm created under high shear conditions will be more compact and less porous than one created under low shear conditions (Celmer et al., 2008). Furthermore, a less porous biofilm will be less susceptible to natural erosion.

2.3 Biofilm Applications

The awareness of biofilms has increased enormously in recent years due to the impact of biofilms on natural and industrial systems, as well as human health. Biofilms cost the U.S. billions of dollars every year in energy losses, equipment damage, product contamination, and medical infections (MSU Center for Biofilm Engineering, 2008). The situations in which biofilms exist can range from contamination of catheters, to adhesion to rocks in steams, to corrosion of pipes in the oil industry. Because both disease-causing and non-disease-causing bacteria are incorporated into biofilms, food and medical industries have to be kept biofilm-free (Deibel et al., 2003). The formations of biofilms cause contamination or corrosion of equipment because of their physical properties, which make them resistant to anti-microbial agents (Cogen et al., 2004).

2.3.1 Biomedical Device Industry

An example of contamination caused from biofilms is found in the biomedical device industry. It is not uncommon for biofilms to grow on medical devices and implants within the human body. These organisms typically originate from the skin of a patient or health care worker, or tap water to which the device is exposed. Such medical devices include urinary catheters, central venous catheters, prosthetic heart valves, and artificial hip prosthesis (Kokare et al., 2008).

The organisms most commonly isolated from catheter biofilms are *S. epidermidis, S. aureus, C. albicans, P. aeruginosa*, and *K. pneumonia*. They gain access to the catheter by migrating externally from the skin along the exterior catheter surface or internally from the catheter port. It has been observed that colonization and biofilm formation can occur within 3 days of inserting the catheter, but biofilm formation on internal surfaces of the catheters is more likely to be present for those that remain in place for longer periods of time (Donlan, 2001).

According to the National Institutes of Health, more than 65% of all microbial infections are caused by biofilms (Proal, 2008). Specific to urinary catheters, biofilms that develop will infect the patient and result in a urinary tract infection. This is more likely to happen in an open system, where the catheter drains into an open collection center, than a closed system, where the catheter empties into a plastic bag. Time is also a variable; essentially all patients who have a urinary catheter for more than 30 days get infected with a UTI (Kokare et al., 2008).

2.3.2 Water Systems

Although biofilms sometimes have harmful effects, they also offer huge potential for certain applications, such as bioremediating hazardous waste sites, biofiltering municipal and industrial water and wastewater, and forming biobarriers to protect soil and groundwater from contamination (MSU Center for Biofilm Engineering, 2008). When used in engineered systems of wastewater treatment, biofilms are often beneficial.

Some of these systems include trickling filter systems, modified lagoons, and specialized systems for nutrient or waste removal. Biofilm based treatment systems are advantageous because the microbial communities are resistant to changing environmental conditions, which makes them resilient to variation in toxicity concentrations (Clark Ehlers et al., 2012).

Heavy metals such as copper, lead, and zinc are typical pollutants in freshwater and wastewater. A study was performed by Teitzel et al. to examine the effects of these heavy metals on biofilm and planktonic *P. aeruginosa*. A rotating-disk biofilm reactor was used in this experiment, and it was determined that biofilms can be up to 600 times more resistant to heavy metal stress than free-swimming cells. By binding metal ions to the EPS matrix, a biofilm is capable of eliminating heavy metals from the surrounding liquid (Teitzel et al., 2003).

2.4 Analytical Testing Techniques

Various techniques were applied in order to test the efficiency of biofilms in wastewater. These were used to monitor the development of the biofilm, as well as its ability to cleanse the wastewater. The nylon mesh and plastic membrane frames were scanned daily to observe the biofilms' growth. Additionally, water samples were taken daily to test for changes occurring in the water.

2.4.1 Total Organic Carbon (TOC) Test

The purpose of the total organic carbon (TOC) test was to determine the concentration of organic carbon in the reactor tank each day of the experiment. By testing for the existence of organic carbon in the liquid, a conclusion can be drawn on the biofilms' ability to function properly. In theory, a biofilm should oxidize the organic components in wastewater, which consist of organ carbon, causing the TOC results to decrease over time.

The TOC machine measures the organic carbon in the water by mixing the water sample with a strong acid. This acid reacts with the inorganic carbon, which is converted into CO_2 and is evaporated from the liquid. The organic matter is left behind. Combustion takes place in order to convert the organic carbon into water and CO_2. The CO_2 is then transferred to an infrared analyzer, where the adsorption wavelength of CO_2 is read, and the concentration of organic carbon can be obtained. Results are given in milligrams of organic carbon per liter of water (mg/L) (LAR, 2008).

2.4.2 Ammonium Test

The ammonium test determines the concentration of ammonium present in the wastewater sample. The method used for this test is known as Nesslerization. The Nessler reagent is an aqueous solution made of sodium hydroxide, potassium iodide, and mercuric iodide. This reagent is added to a diluted sample of wastewater after a mineral stabilizer and polyvinyl alcohol; the mineral stabilizer is added to reduce cloudiness in the samples. A spectrophotometer is then used to read the absorbance of ammonium present in the sample (Tissue, 2000). A calibration curve is prepared with a sample of pure distilled water, to which the reagent, stabilizer, and polyvinyl alcohol are also added.

The intensity of the color can be correlated with the ammonium concentrations present in the wastewater. A bright, yellow color implies a low concentration of ammonium, while dark, brown implies a high concentration. The concentration of ammonium in the wastewater can be directly correlated to biofilm health. High concentrations of ammonium suggest the biofilm is not growing properly, while low concentrations suggest the biofilms are healthy, and efficiently treating the wastewater.

In theory, the ammonium concentration of wastewater in which biofilms are growing should decrease. This is because a certain amount of the microorganisms that comprise biofilms have nitrifying properties. Nitrifying microorganisms oxidize ammonium in the wastewater, converting it to nitrite. These nitrites are then further oxidized to nitrates. Two different groups of bacteria work on each of these reactions. *Nitrosomonas*, the most common ammonium-oxidizing bacteria, work on the first reaction in which ammonium is converted to nitrite: $NH_3 + O_2 \rightarrow NO_2^- + 3H^+ + 2e^-$. *Nitrobacters* work on the second reaction in which nitrite is converted to nitrate: $NO_2^- + H_2O \rightarrow NO_3^- + 2H^+ + 2e^-$ (Environmental Protection Agency, 2002).

2.4.3 Ultraviolet-Visible Spectroscopy

The amount of nitrates and the chemical oxygen demand (COD) in a wastewater sample was determined through the use of ultraviolet-visible spectroscopy. In this qualitative testing technique, a beam of light passed through the given sample and measured the reflected wavelength. The range of wavelengths used for the wastewater samples was 190nm to 600nm. The beam of light (in the UV regions), excited the particles in the sample for each wavelength. After the sample was subjected to every wavelength in the determined range, a graph was displayed. The graph showed peaks at certain wavelengths, indicating the presence of certain compounds with which the wavelength was associated.

For wastewater, there was one absorbency wavelength found in UV-Vis spectroscopy that was important: 254nm. The absorbance at 254nm was correlated with the COD in the sample. The COD measured all the oxidizable matter present in the wastewater. Theoretically, as the biofilm treats the wastewater, the COD will decrease because there is less oxidizable matter in the wastewater, thus less need for oxygen.

On the contrary, nitrate concentrations, in theory, should increase over time. This is because the biofilms are oxidizing ammonium, therefore, creating nitrate. To find the nitrate concentration in the sample, the second derivative of the absorbencies was used. Within the range of 220 to 240nm, the highest value of the second derivatives corresponding to the specified wavelength range was isolated. These maximum values are directly proportional to the nitrate concentration. Using the following equation, the nitrate concentration in each sample was found (Pons, 2011):

$$Nitrate\ Concentration\ (mg/L) = {}^{UV''}/_{0.0006}$$

Chapter 3: Methodology

The following experiments were designed to determine the effect of biofilms on wastewater. These experiments were conducted in the Laboratoire Réactions et Génie des Procédés (LRGP) at the Ecole Nationale Supérieure des Industries Chimiques (ENSIC) in Nancy, France.

3.1 Diluted Wastewater

Biofilms grow naturally and abundantly in rivers, so the goal of the experiment was to simulate a river as closely as possible. A reactor tank was prepared prior to starting the experiments. First, the volume of the tank was calculated to be 53.8 liters, however; only 48.5 liters were used in order to leave space for maneuvering the experimental materials. A mixture of diluted wastewater was then made by filling a reactor tank with 2 liters of concentrated wastewater, and the remainder with tap water. This is similar to a river because when wastewater is released into a river, it is diluted by the large amount of water flowing in the river.

The wastewater used in this experiment was collected in 10 liter plastic jugs from a wastewater treatment plant in Nancy, France. Although the wastewater was collected before being treated, it was already slightly diluted due to rain water. It came from a domestic sewer, which means that the water from neighborhoods in Nancy drains into a sewer that leads to the wastewater treatment plant. It therefore only contained domestic waste, such as that from sinks and bathrooms. The wastewater was collected in separate batches from the treatment plant. When the lab was in need of more wastewater, an employee was sent to get more.

A filtered sample of the water from the tank was taken every day in a plastic, 25 mL labeled bottle. The samples were tested for ammonium content, and they were also tested using the TOC (total organic carbon) and UV-Visible Spectroscopy techniques. Since the tests were only conducted every two weeks, the samples were kept in a refrigerator inside the laboratory. Procedures for the Ammonium and UV-Visible Spectroscopy tests can be found in Appendices C and D, respectively. For the TOC tests, glass bottles were numbered and filled with samples in order of the date they were taken. The TOC tests were conducted by a lab technician, and results were sent via email.

Since a river was being simulated as closely as possible, the proper environment needed to be created for a biofilm to grow; microorganisms need certain nutrients to survive. Oxygen is one important nutrient required to grow a sustainable biofilm. To oxygenate the water in the tank, two “bubble strips” (similar to those used in fish tanks) were placed in the water on either side of the tank. Along with oxygenating the water, this method enabled the water to mix and circulate as it would in a river.

Biofilms also need to be in contact with other bacteria and microbes in order to grow and increase the sizes of their micro-colonies. In order to “feed” the biofilms, two liters of fresh, concentrated wastewater were added to the tank every Tuesday and Friday for the first four weeks of the experiment. The procedure to change the water was:

1. Take a sample of the water from the tank and label it with the date and “before”
2. Calculate the liquid volume in the tank and determine how much was lost to evaporation and samples being taken
3. Record the volume with the appropriate date
4. Calculate the amount that needs to be removed from the tank using the equation: 2L – amount lost = amount that needs to be removed
5. Remove the necessary amount from the tank using a beaker and a graduated cylinder
6. Measure 2L of concentrated wastewater in a graduated cylinder and add it to the tank
7. Recalculate the volume in the tank to make sure it equals 48.5L
8. Record the volume with the appropriate date
9. Take another sample of water from the tank and label it with the date and “after”

For the second four weeks of the experiment, once the biofilms had substantially grown, the original volume in the tank was maintained using tap water. The volume in the tank was calculated every Tuesday and Friday, and the amount that was lost due to evaporation and samples was replaced with tap water. Samples were still taken before and after the tap water was added.

The calculations for the volume and the "volume records chart" can be found in Appendix A.

3.2 Nylon Mesh Experiment

Two specific experiments were designed to determine the effect of biofilms on wastewater. The biofilms were grown on six frames submerged in the reactor tank; four of the frames consisted of mesh made from nylon string in which the biofilm adhered and grew, while the other two frames consisted of aerated and anaerobic plastic, respectively.

For the "nylon mesh experiment," nylon string was threaded through a plastic frame to form a criss-cross pattern within the frame (see Figure 7). The criss-crossing technique created a mesh in which the biofilm adhered and grew. The utilized frames were 12cm by 12cm by 0.5cm in dimension.

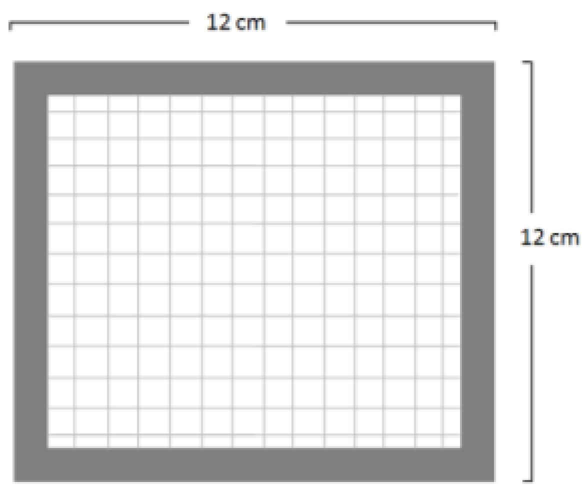

Figure 7: Diagram of Nylon Mesh Frame

Four of these frames were used to make comparisons on biofilm growth, and to see their effect on the wastewater. The nylon mesh frames were hung from two thin, metal sheets that were screwed in parallel, nine centimeters apart from each other. They were located at the top of the tank above the water (see Figure 8). In order to keep parameters constant, each frame was placed in the same order every day. Metal wire was used to hook the frames into place, while keeping them completely submerged in the diluted wastewater (see Figure 9). The metal hooks were designed to be easily removed for scanning purposes, without damaging the biofilm growth cycle. During the 4th week of the project, new hooks were made because the previous ones had rusted to the point of breakage.

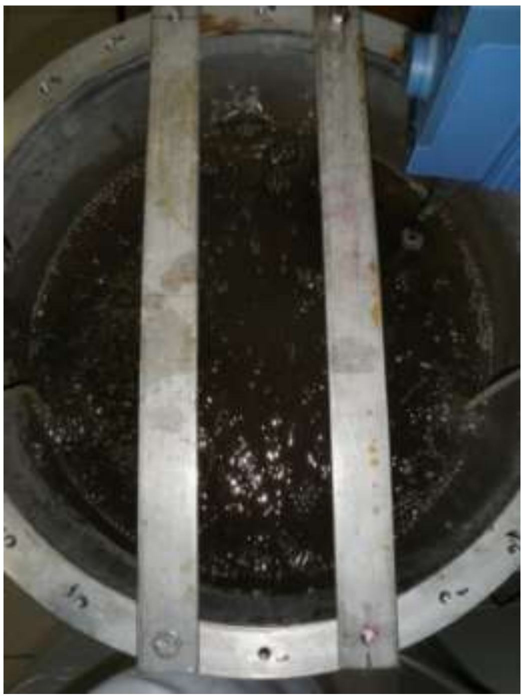

Figure 8: View from Top of Tank

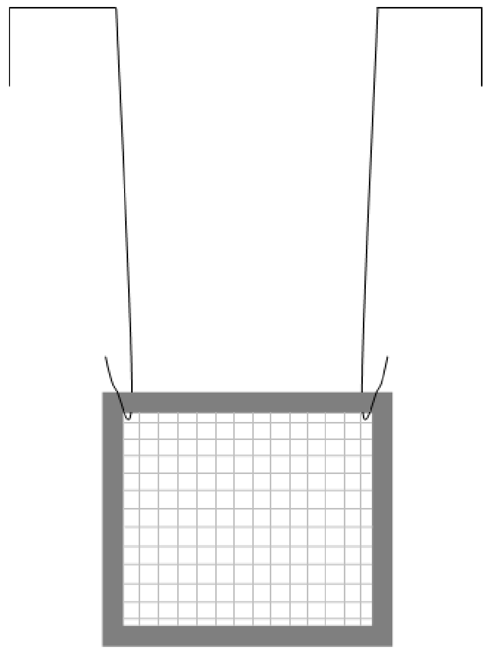

Figure 9: Diagram of Mesh Frames with Wire Hooks

3.3 Plastic Membrane Experiment

For this experiment, two membranes were used to determine the growth of biofilms on aerated vs. anaerobic plastic. Each membrane was constructed identically, except that one had air flowing through it, and the other was closed off.

The equipment was set up by using a metal frame comprised of two pieces that were screwed together. The first piece had glass glued to its frame. The second piece had plastic stretched across it before being screwed to the first piece (see Figure 10). A space of 0.9 centimeters separated the plastic from the glass. Because the membrane was completely submerged underwater, leakage was an issue. In the case that water entered, an opening on the bottom of the frame, sealed with a screw, allowed for drainage of the area between the plastic and glass. The membrane frame had dimensions of 15.5cm by 15.5cm by 2.4cm.

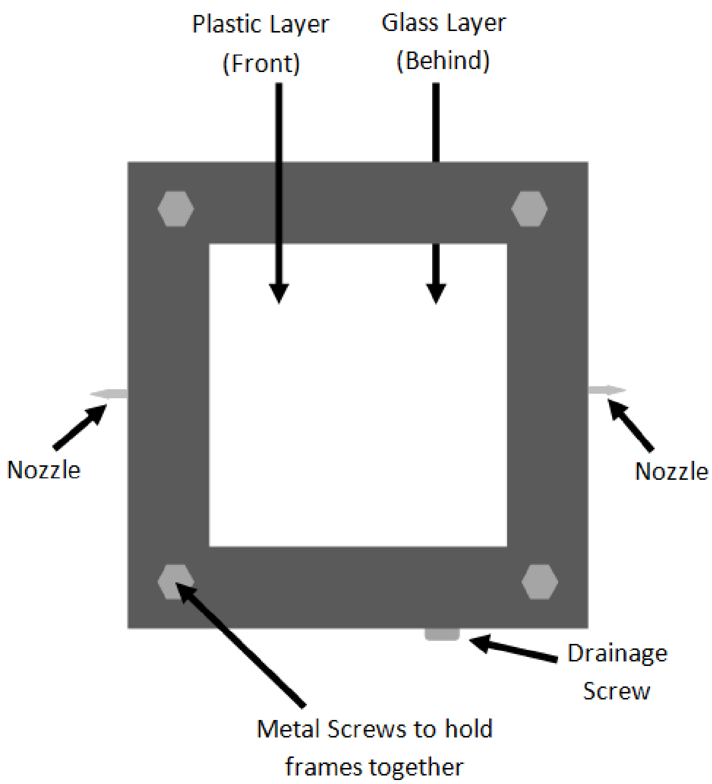

Figure 10: Diagram of the Plastic Membrane Frame

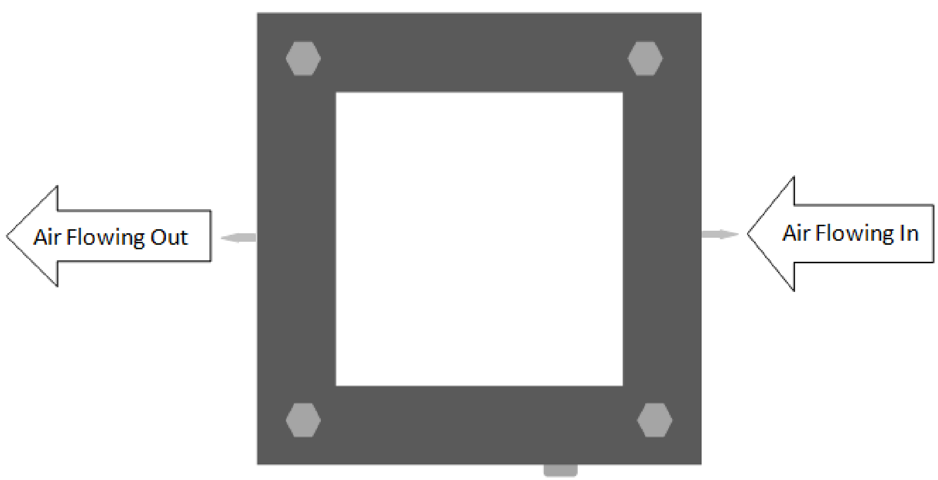

Figure 11: Diagram of the Aerated Plastic Membrane Frame

A nozzle was located on either side of the metal frame. For the experiment without air, small rubber piping was used to seal off the nozzles, so that no water could leak into the space between the plastic and glass. For the experiment with air, an air pump connected to small rubber piping was attached to one nozzle, while the other had piping that allowed the air to escape into the environment (see Figure 11). Figure 12 is an illustration of the reactor with the six frames submerged inside.

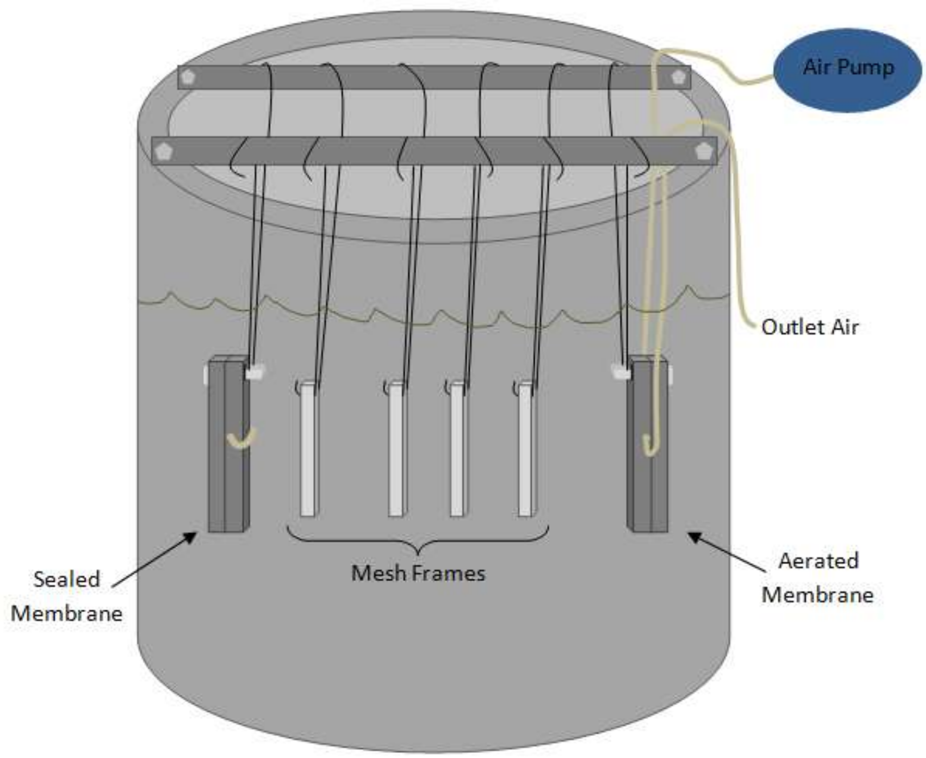

Figure 12: Diagram of Reactor

3.4 Monitoring Biofilm Development

The growth of the biofilms was monitored through daily scanning. The six frames were scanned using an Epson Perfection 4490 Photo Flat Bed Scanner and the Epson Scan Program. The parameters of the scanner were set to opaque, 8-bits gray image, and a resolution of 800 dpi. These parameters were the same for both the mesh and the membrane experiments.

The scanning for the mesh experiment was executed using *Paramètrage 2*, where the image size was set to 118.7mm x 120.5mm. Each frame was placed on an additional sheet of glass on top of the scanner with marks that labeled where the frame should be placed. A preview of the image was taken, and the scan was saved using the format, "wire_yymmdd _disknumber.jpg". The frames were scanned from the same side each day so as to not change any parameters.

The scanning for the membrane experiment was conducted using *Paramètrage 4*. Under this parameter the image size changed to 105.5mm x 107.4mm. The membrane frame was placed on the glass sheet with the plastic side face down. Long screws on the frame prevented the scanner from closing completely, so white computer paper was used to cover the other side of the membrane frame. These images were saved using the format, "memb_air_ yymmdd_disknumber.jpg" or "memb_noair_yymmdd_disknumber.jpg" to differentiate between the images of the frames with and without air flowing.

The complete scanning procedure can be found in Appendix B. Once the pictures were saved in the computer, they were cropped so that only the inner area of each frame could be seen. Although planktonic biofilm growth was noticed in the wastewater, as well as on the tank walls, only the growth within the six frames was monitored. The images were then evaluated with the help from Dr. M.-N. Pons, the on-site project sponsor. Using the Fortran Program, the mean gray level of the area in each picture was obtained. With the gray levels from day 1 (containing no biofilm growth) and the days following, the opacity of each frame for each day was found:

$$Opacity = initial\ gray\ level\ (day\ 1) - actual\ gray\ level\ (day\ in\ evaluation)$$

Using the opacity, the biofilm thickness was plotted over time. This was used to determine the time it took for the biofilm to enter each cycle of growth (initial attachment, maturation, and dispersal). A scanned image for each frame per week can be found in Appendix E.

Chapter 4: Results and Analysis

4.1 Biofilm Opacity

Opacity is determined by the amount of light that travels through an object; in this case, the biofilms growing on the nylon mesh and membrane frames were the objects. When there is barely any biofilm growth, the measured opacity is close to zero. On the other hand, the opacity is high when the biofilm growth is thick and dense. The opacity was measured by running the scanned frame images through the Fortran Program. This program calculated the mean gray level of the scanned area, as well as the standard deviation. Using Microsoft Excel, the opacity for each frame was calculated by subtracting the value of the actual mean gray level from the value of the initial scan, before being submerged in the tank.

4.1.1 Opacity on Nylon Mesh Frames

The biofilms that were grown on the four nylon mesh frames in this experiment followed a similar trend (see Figure 13). The growth cycle took place on days 1-18, which was 4 weeks. From days 1 to 15 they attached to the nylon mesh, matured, and grew. From day 15 to 18, dispersal took place. This was the period in which the biofilms detached from the nylon mesh within the frame and became planktonic bacteria. Then they either died, or found a new micro-colony in the environment to attach to.

As seen in Figure 13, frames 1 and 4 had a higher opacity than frames 2 and 3. The reason for more growth on these frames is most likely due to their placement in the tank. Frames 2 and 3 were closer, and thus more exposed, to the bubble strips located on either side of the reactor tank. Since the reactor tank had little shear force compared to that of a river, the force from the air through the bubble strips may have prohibited biofilms from attaching successfully on these two frames. Biofilms that grow under low shear force are less compact and more porous than biofilms formed under high shear conditions. Furthermore, a porous biofilm will be more susceptible to natural erosion and accidental detachment.

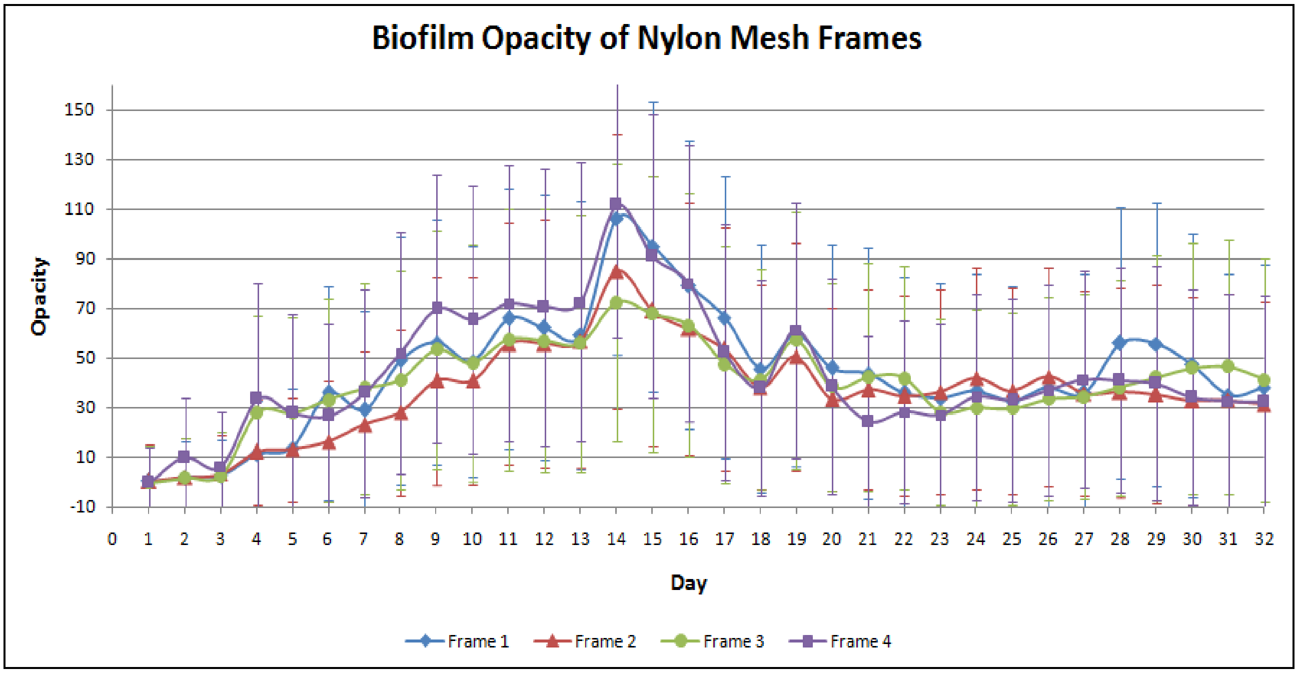

Figure 13: Biofilm Opacity on Nylon Mesh Frames

Attachment began again on day 18, after the previous growth cycle, which was a Friday when wastewater was added to the tank. On day 19 (Monday) the opacity was visibly higher than on Friday, illustrating the re-growth cycle which had begun over the weekend. Although biofilm development had been occurring in a healthy manner up to this point, undesirable trends were observed in the ammonium and nitrate concentrations within the tank. To offset these trends, tap water was added to the tank twice a week instead of wastewater, starting on day 20. The microbes and nutrients in the wastewater that had been "feeding" the biofilms and assisting in their growth from days 1-18 were not present in the tap water. Because these nutrients were not being replenished, the re-growth cycle on days 18-19 was unsuccessful.

From day 20 onwards, few significant changes took place in the opacity of the nylon mesh frames. During these last 3 weeks, Figure 13 illustrates that almost no detachment took place and the opacity plateau-ed. Throughout this time period, the biofilms' sustainability was successful; they relied on the bacteria and nutrients already in the reactor tank to survive. Since wastewater was not being added, growth was minimal because the lack of new microbes prohibited the biofilms from expanding their colonies. Frame 1, however, was an exception; an increase in the opacity is visible on days 27-29. Although some growth was present on this frame in the last 2 weeks of the experiment, detachment occurred shortly after.

Even though the growth trends for the four mesh frames were consistent with one another, there is still error associated with each sample in Figure 13. This error can be mainly attributed to accidental detachment, which occurred from everyday handling of the frames. Moving the frames in and out of the tank, removing their hooks, and placing them on the scanner were several ways in which accidental detachment occurred.

Another source of error, depicted by the error bars, can be attributed to the glare created by water droplets trapped in the squares of the mesh frames.

4.1.2 Opacity on Plastic Membrane Frames

The opacities of the plastic membrane frames vastly differ from those of the nylon mesh frames. In Figure 15, Day 1 represents the opacity of the frames before being submerged in the tank. During the first week of the experiment, the opacity values were negative for both the aerated and anaerobic membrane frames. These negative values were attributed to the presence of water on the plastic of the aerated membrane, as well as condensation on the inner layer of plastic on the anaerobic membrane. Since the opacity was calculated by subtracting the gray level of the membrane frames from the initial value, the glare and condensation created an illusion that the scan was less gray than it was on Day 1.

The glare from the water on the aerated membrane made the opacity seem lighter than the scan of the frame before being submerged in the tank. Similarly, the condensation in the anaerobic membrane made the scanned image even lighter than that of the aerated membrane. Figure 14 compares the aerated and anaerobic membranes on days 1 and 4.

“Air- Day 1” is an image of the aerated plastic membrane before being submerged in the reactor tank. “Air- Day 4” is the same membrane after being submerged in the reactor tank for four days. The glare from the wet plastic can clearly be seen on the bottom of the picture “Air- Day 4.” Inside the glare, little black specks can also be noticed; this is the initial attachment of microbes on the membrane. Since the computer program took the average gray level of the entire area within the frame, the glare caused the mean gray level to be lighter on day 4 than on day 1, even though biofilm had begun to form.

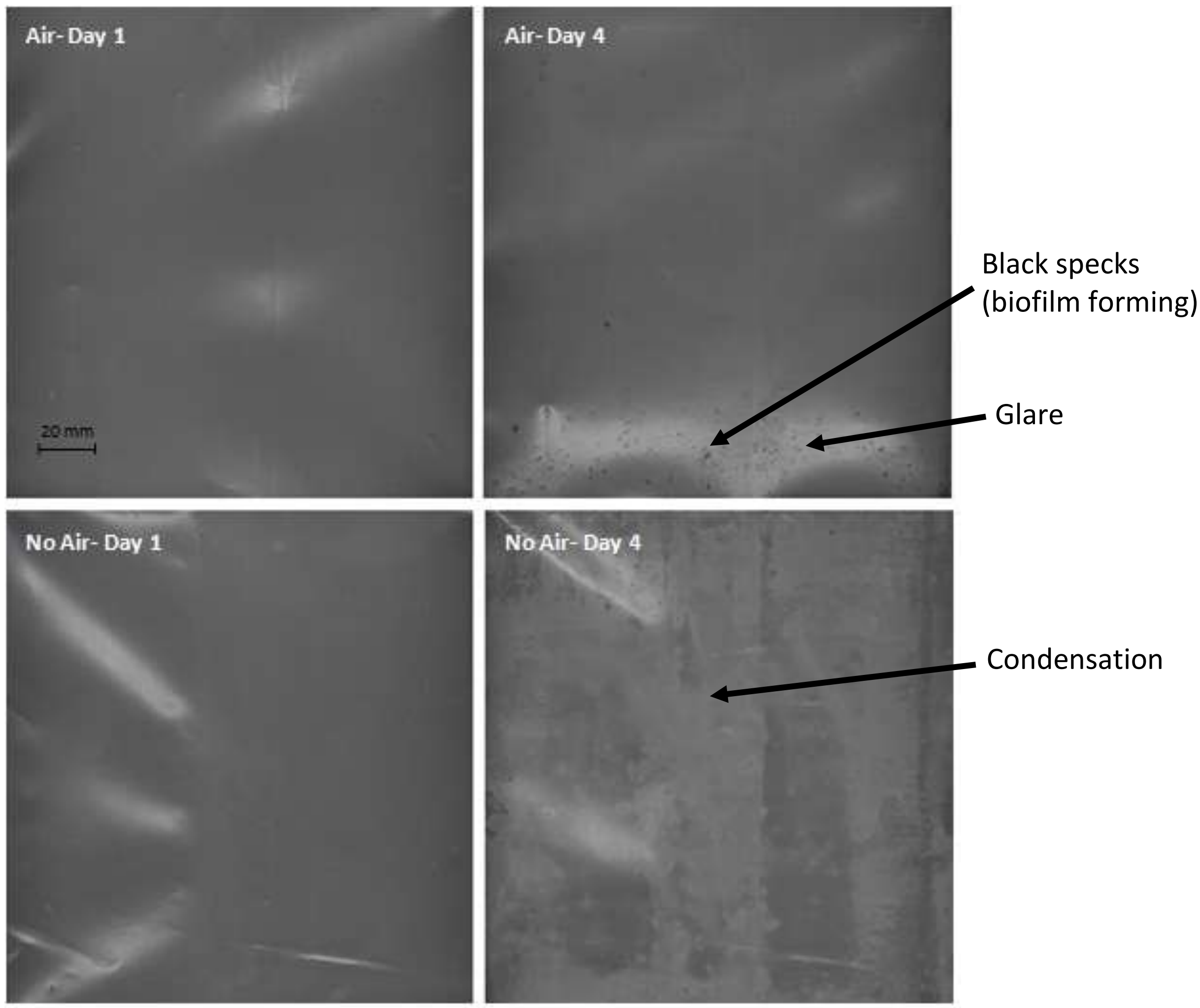

Figure 14: Comparison of Membranes on Day 1 and 4

The bottom two images make the same comparison as the top two; however, these are images of the anaerobic membrane. The condensation on the inside of the plastic membrane can easily be seen in “No Air- Day 4.” Similarly to the aerated membrane, this error gave undesirable results from the opacity testing technique.

Figure 15 displays the opacity of both membranes over time. It is necessary to note that the opacity testing technique was not entirely accurate in determining biofilm thickness on the membrane frames.

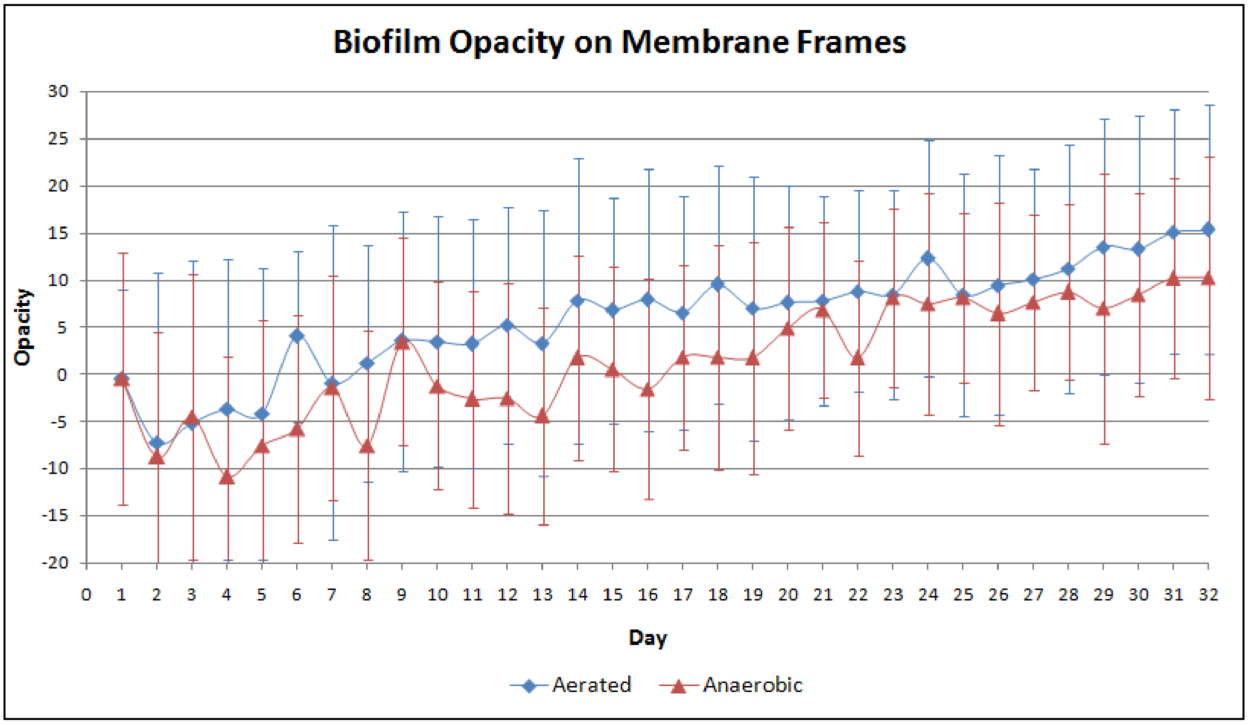

Figure 15: Biofilm Opacity on Plastic Membrane Frames

On day 9, the opacity of the aerated membrane reached a point above zero and growth of the biofilm steadily increased. On the other hand, even though the opacity of the anaerobic membrane also reached a point above zero on day 9, it decreased again until day 13. From day 17 onward, the opacity in both frames rose above zero and steadily increased; however, it is apparent that more biofilm attached to the aerated membrane than to the anaerobic membrane. This was attributed to the oxygen permeating the plastic layer, giving the film this nutrient from the surface.

Since the plastic on the membranes was extremely smooth, less attachment and growth took place this material than on the nylon mesh frames. This is because accidental detachment occurred more frequently due to water droplets dragging traces of biofilm off as the frames were handled.

It has been noticed that growth on the plastic membranes took considerably longer than on the nylon mesh frames. While the opacity of the nylon mesh frames had reached up to 110 by day 14, the highest opacity reached by the plastic membrane frames was only 15 during the last week. More time to run the experiment would have enabled better understanding of biofilm development on the aerated vs. anaerobic membranes.

4.2 Total Organic Carbon Test

The total organic carbon testing technique measured the concentration of organic carbon in a wastewater sample. The TOC results are depicted in Figure 16. Organic compounds, consisting of organic carbon, are a major component to wastewater; this why an increase in the concentration can be seen for each red bar, which is when more wastewater was added to the reactor tank. In theory, the concentration of organic carbon should decrease over time because the biofilm oxidizes organic components, making the water safe for human use.

In Figures 16, 18, 19, and 21, the red bars represent samples taken after two liters of wastewater were added to the reactor tank, while keeping the total volume constant. The blue bars on the graph represent samples taken after tap water was added to the reactor tank; this was done twice a week starting on day 20 to maintain the volume in the tank. The green bars represent the samples that were taken daily, including before wastewater or tap water was added to the tank.

In Figure 16, the first red bar represents the first sample taken the day that the tank was filled with diluted wastewater and the experiment began. On this day, there was no biofilm growth on the six frames submerged in the tank. Sufficient growth was needed for the microbes to oxidize the organic carbon in order to decrease the concentration. For this reason, trends can start to be noticed on day 7. Up until this point, the biofilm had not grown enough to sufficiently treat the wastewater.

Since there is no human error to report, the increasing TOC on days 2-3 can only be attributed to noise in the TOC machine or water mixing in the reactor tank. TOC is a qualitative test, so while the concentration of organic carbon in the sample can be determined, it does not guarantee that it is the exact concentration in the reactor tank.

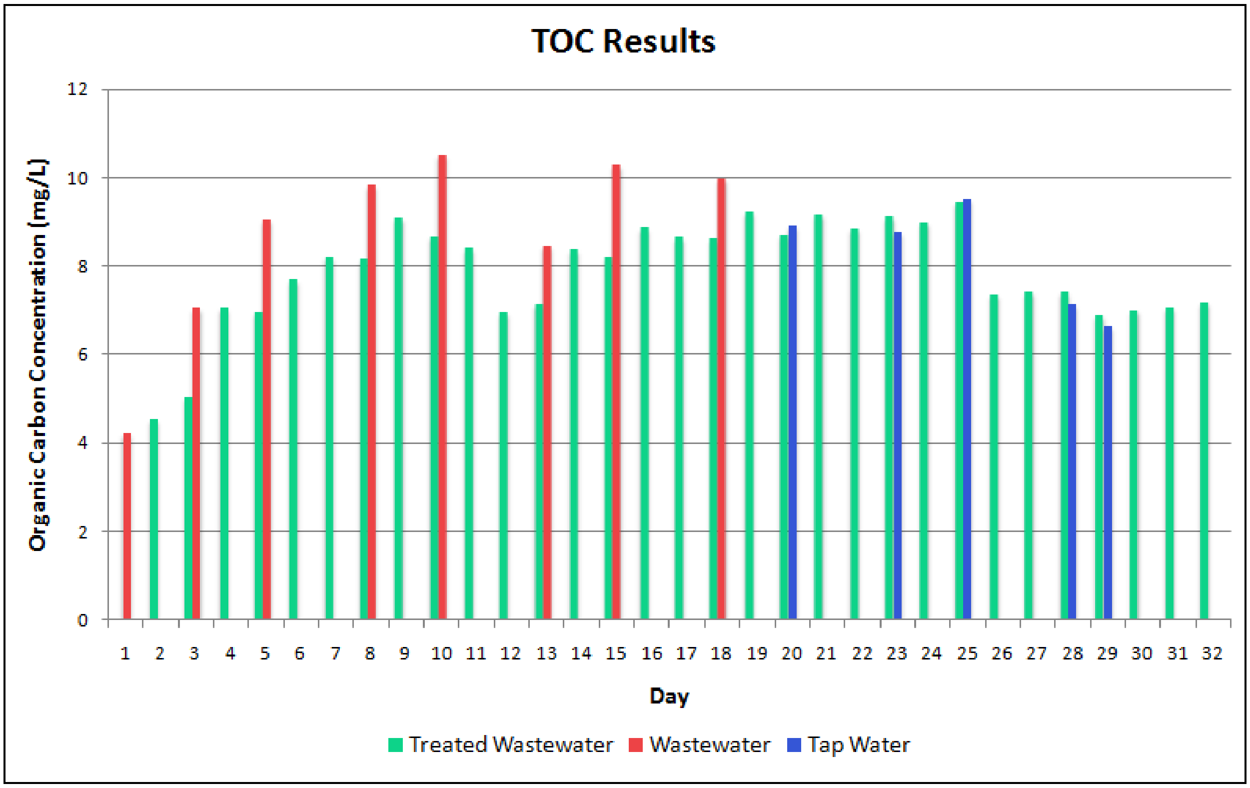

Figure 16: Total Organic Carbon Test Results

A sufficient decrease in the organic carbon concentration can be seen between the red and green bars, respectively, on days 8-9, 10-11, 13-14, 15-16, and 18-19. The biofilms react quickly to the organic matter that was added to the tank, and a large amount was oxidized by the next day, when a new sample was taken.

A decrease in concentration was also noticed between the green bars on days 7-8, 9-10, 11-12, 14-15, 16-18, 19-20, and 21-22. Here it is observed that the biofilms continue to oxidize the organic matter in the wastewater throughout several days, until new wastewater was added and the organic carbon concentration peaked up again.

There are several data points that do not follow the general trends. For example, on days 13 and 23, the green bars rise slightly above the bars from days 12 and 22, respectively, showing an increase in the organic carbon concentration between each of these two day periods. This also occurs on days 21 and 25, where the green bars rises above the blue and green bars from previous days. This is probably due to noise in the TOC machine, as no other explanation can be provided.

The blue bar on day 20 represents the first day that tap water was added to the tank. Although tap water should not increase the organic carbon concentration since there should not be any organic matter in tap water, there are still slight increases noticed between the green and blue points on days 20 and 25. The variation on day 25 is too small to give much credibility, but the increase on these days is probably because the samples were taken immediately after the tap water was added. When the water was poured into the reactor tank it may have stirred up the water (more than the bubble strips did regularly) causing organic matter sitting on the bottom of the tank to circulate.

To test this hypothesis, the diluted wastewater in the reactor tank was mixed with an electric mixer and ten samples were taken in one-minute intervals (starting with zero minutes). The results from this test can be seen in Figure 17, where it is apparent that mixing does have an effect on the samples taken from the tank.

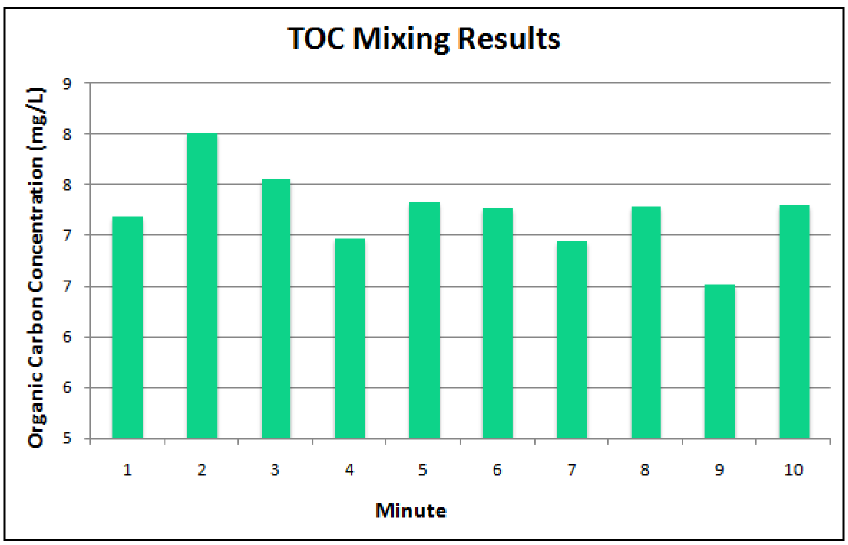

Figure 17: Organic Carbon Concentration of Mixing Test

The total organic carbon concentration varies between 6.5 and 8.0 mg/L, but the variation is random. The conclusion made from this mixing test is that it was important to be consistent throughout the experiment; when the wastewater or tap water was added to the tank, a designated amount of time should have been set to allow the water to settle before a sample was taken. This would have offset the error from the water mixing and more/less of certain components entering the sample.

4.3 Ammonium Concentration

The Ammonium Nessler Test was performed on daily wastewater samples taken from the reactor tank. This test gave a sense of the rate and efficiency at which the biofilms converted ammonium to nitrites and nitrates. Figure 18 is a representation of the ammonium content sampled each day.

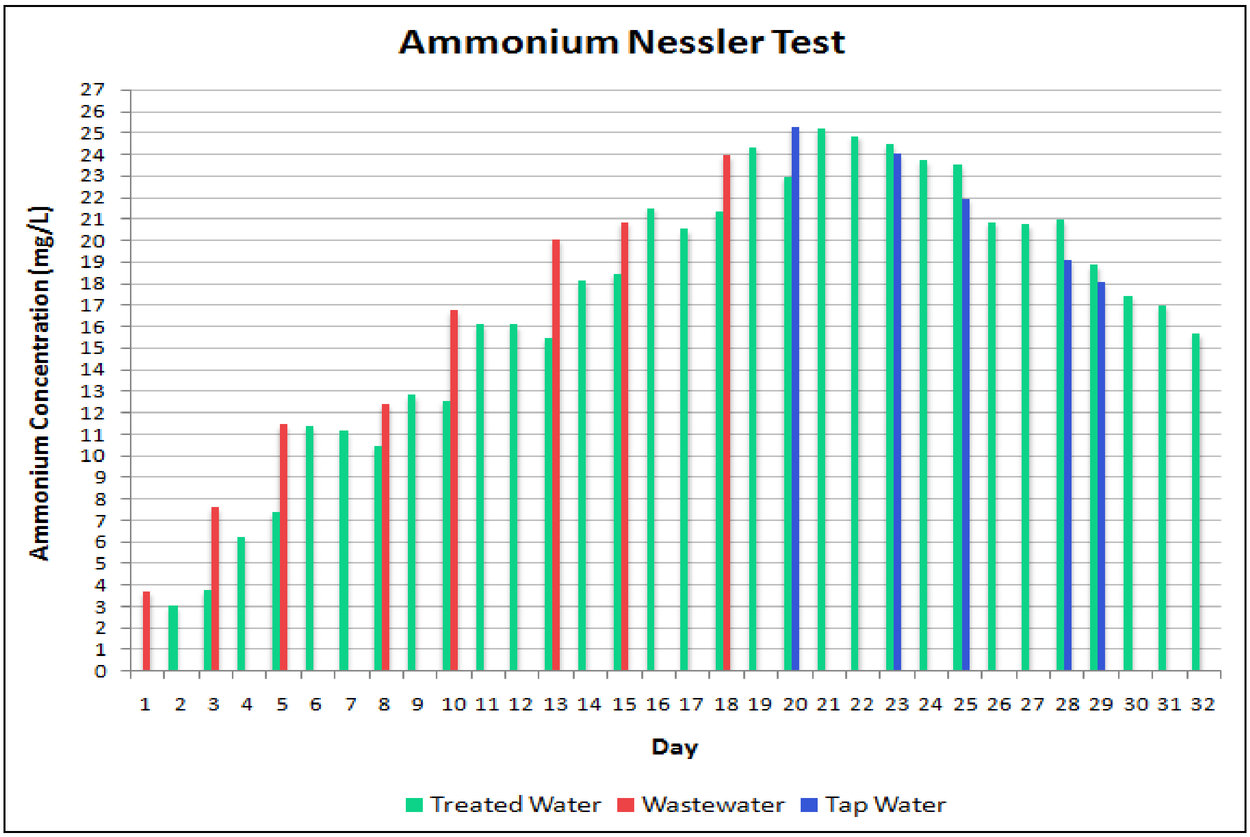

Figure 18: Ammonium Concentration in the Wastewater Reactor Tank

Since ammonium is a main component of wastewater, an upward trend can be noticed in the red bars, which were samples taken on days when two liters of wastewater were added to the tank. This caused the ammonium concentration to increase. The most significant trend that shows the effectiveness of the biofilms can be seen between the days in which addition of new liquid occurred (in the green bars). Even though the green bars show higher concentrations from week to week, a decrease starting on day 5 can be seen between the days in which wastewater was added. This is when the biofilm reached a mature level and could effectively oxidize the ammonium in the wastewater.

Although standard deviation was calculated and error bars were originally included on the graph, it only obstructed the graphs appearance and made it difficult to read. The overall amount of error was small enough to not be a major issue, so a graph with the error bars was not included. The amount of error in the data ranged from only ± 0.95% to ± 11%.

From days 5-8 and days 10-13, after wastewater was added, a significant decrease in the ammonium concentration can be seen. On other days, such as 15 and 18, the green bars show a higher concentration than expected. These slight errors are attributed to noise in the measurement of the sample, and are not high enough to be considered significant.

From day 20 onwards, when tap water was added, the decreasing trend is especially noticeable. This is because tap water does not contain ammonium, so the previous build-up from the wastewater began to decrease through the addition of tap water and the efficiency of the biofilms. On day 20, even though tap water was added, an increase in ammonium concentration is noticed. This was most likely due to the addition water which stirred the contents of the reactor tank more than usual, causing more ammonium to enter the sample at this point in time. In order to achieve exact results through the ammonium test, the reactor tank would have to be perfectly mixed when all the samples were taken.

4.4 Ultra Violet-Visible Spectroscopy

UV-Visible Spectroscopy was performed on the water samples to determine the nitrate concentration in each sample, as well as the chemical oxygen demand (COD). To find the nitrate concentrations, the second derivative of the UV absorbencies between 220 and 240nm was found. The highest value of the second derivative within the specified range gave a value that was directly proportional to the nitrate concentration in the sample. The COD of each sample corresponded to the peak at 254nm. The UV-Visible Spectroscopy technique is a qualitative test. Even though the values are accurate for the concentration within the given sample, it is difficult to get an exact concentration of the wastewater in the tank through this technique.

4.4.1 Nitrate Concentration

Nitrate concentrations, in theory, should rise as the biofilm treats the water because the bacteria in the biofilm oxidize ammonium in the wastewater, turning it into nitrate. This process is called nitrification. The formation of nitrates over time indicates an efficient biofilm, since ammonium in the wastewater is being converted. Figure 19 represents the nitrate concentration in the reactor tank over time. Since the volume in the tank was maintained constant throughout the duration of the experiment, the overall nitrate concentration decreased almost every time more wastewater or tap water was introduced to the reactor tank. This trend can be seen on days 3, 5, 8, 10, 15, 20, and 23.

Similar to the results from the ammonium test, the UV-Visible spectroscopy test illustrated that it took the biofilm approximately five days before it was capable of nitrifying a significant amount of ammonium to oxidize it to nitrate. Up until this point, the biofilm had not grown enough to sufficiently treat the wastewater.

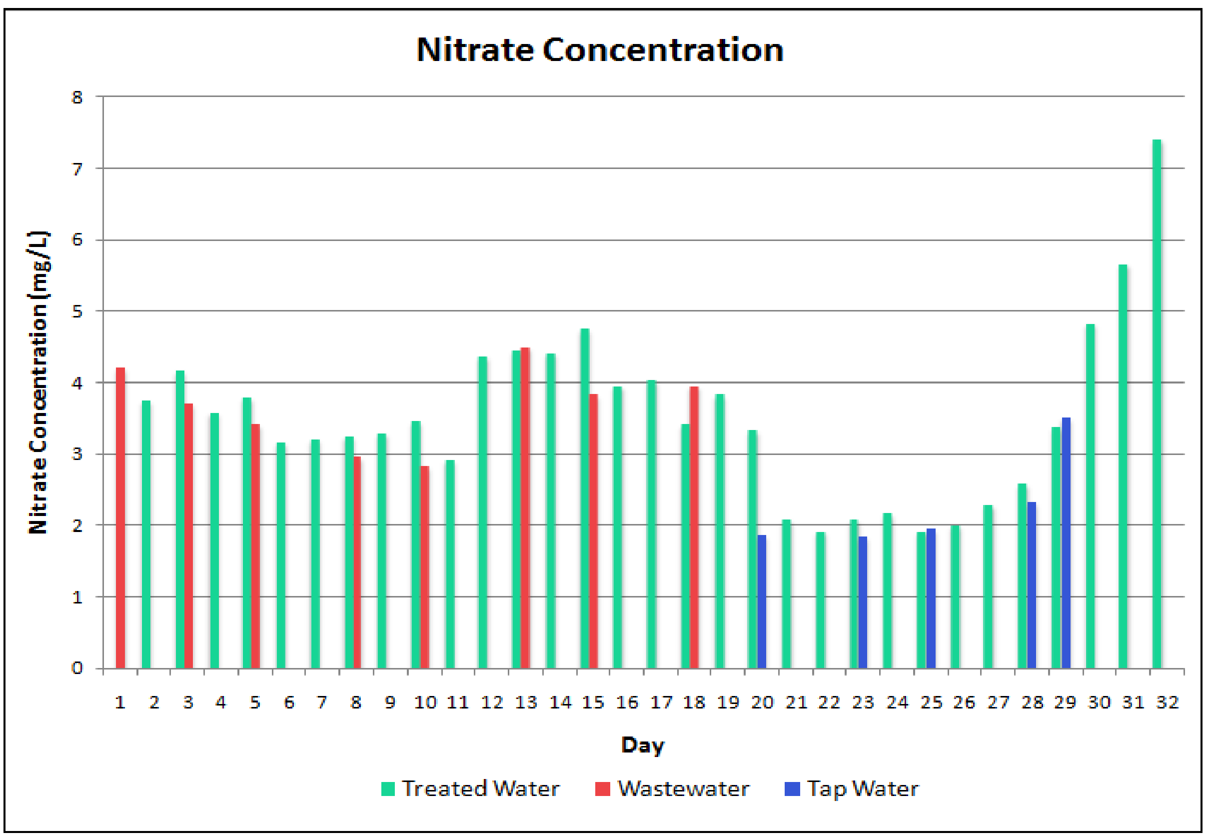

Figure 19: Nitrate Concentration in the Reactor Tank

The maturation stages of the biofilm life cycle occurred between days 6 and 15. This agrees with the trends of the nitrate concentration graph in Figure 19. The nitrate concentration increases when the biofilm is efficiently treating the wastewater by oxidizing ammonium to nitrate. This increase can be seen in the green bars between days 6 and 15. More specifically, the trend can be noticed on days 6-8, 9-10, 11-13, and 14-15; the days between when new wastewater is added to the tank. There is also an increase in the green bars on days 16-17, 22-23 and 26-28. Although dispersal and re-growth are occurring on these days, there are still planktonic microbes and biofilm on the tank walls which may have contributed to the oxidation of ammonium in the wastewater.

On days 29-32 the nitrate concentration increased immensely. This dramatic increase corresponds to the decrease in ammonium concentration on the same days. Although the biofilms had not entered a re-growth cycle during this time, the accelerated nitrification process was due to planktonic bacteria and biofilm on the walls of the reactor tank.

Decreasing trends on days 17-18 and 19-20 can be attributed to the fact that more ammonium was being added to the tank, but the microbes were not able to nitrify the ammonium because they were either in dispersal or re-growth mode. Similar decreasing trends can be seen on days 21-22 and 24-25. However, no ammonium was entering the tank after day 18 since only tap water was added. Given that there is no human error to report, the decrease in nitrate concentrations between these specific days is probably attributed to noise on the spectroscopy machine.

Data points that do not follow the trend occur on days 13 and 18, where the red bar is higher than the green bar, and days 25 and 29, where the blue bar is higher than the green bar. Adding wastewater or tap water to the tank should not increase the nitrate concentration because there should not be any nitrates in either liquid. The concentration increased on these days probably because the samples were taken immediately after the wastewater or tap water was added. When the liquid was poured into the reactor it may have stirred up the water (more than the bubble strips did regularly) causing more nitrate to enter the sample taken after the water was added to the tank.

To test this hypothesis, the diluted wastewater in the reactor tank was mixed and ten samples were taken in one minute intervals. Similarly to the TOC mixing test results, the nitrate concentration results randomly vary between 6.7 and 7.7 mg/L (see Figure 20).

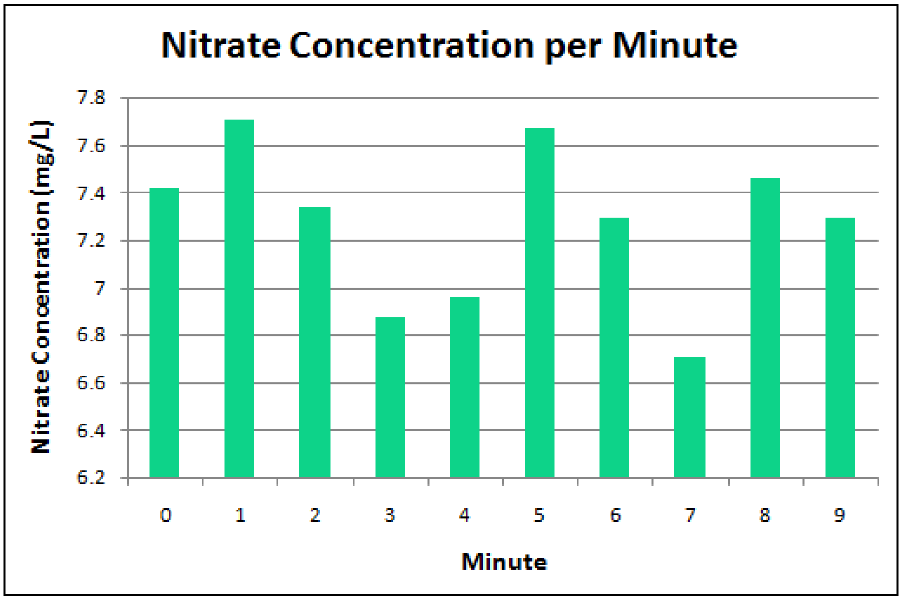

Figure 20: Nitrate Concentration of Mixing Test

Since the analytical testing techniques used in this experiment are qualitative, more care should have been to taken to ensure that each sample was taken in the same conditions without any variables affecting the results.

4.4.2 Chemical Oxygen Demand

The COD of each sample was found by plotting the peak at 254nm over time (see Figure 21). It is important to note that the scale on this graph is small; although it appears that there is a large drop in the absorbency on day 12, it only differs from day 10 by 0.25nm in the absorbance at 254nm. The reason for this drop is unknown, as there is no human error to report. One speculation is the dramatic decrease in the temperature of the environment around this day. Winds from Russia caused the average high temperature in Nancy, France to drop from 46°F to 32°F. Since the lab was located in the basement of the building, heat was minimal. This cold temperature lasted for about 2.5 weeks (days 12-24). The COD would drop during this period because cold temperatures prohibit bacteria from being active, so the demand for oxygen was reduced.

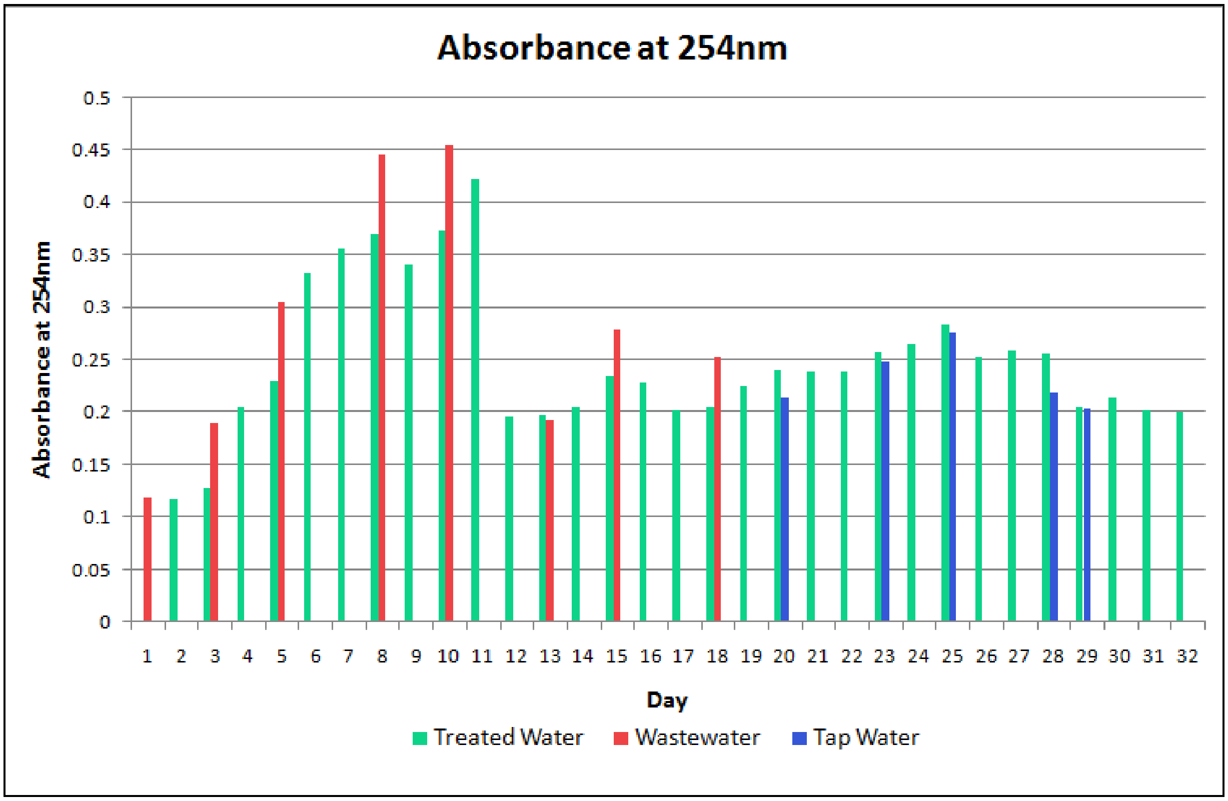

Figure 21: UV-Vis Absorbance at 254nm

Apart from the previous observation, an increasing trend is noticed from days 1-8. As the biofilms grew, the need for oxygen to oxidize ammonium in the wastewater (which was consistently being added to the tank) increased. It can also be noticed that adding wastewater to the tank makes the COD significantly higher than adding tap water. This is because tap water does not contain any oxidizable bacteria, so the need for oxygen is decreased.

A decreasing trend is observed on days 28-29, and then the low COD is sustained throughout the remainder of the experiment. At this point in time the wastewater had been continuously cleansed, so there were less oxidizable components; therefore, the need for oxygen is minimized.

Chapter 5: Conclusions and Recommendations

5.1 Biofilm Development

The opacity test was not the most adequate method for monitoring the biofilm thickness on the plastic membrane frames. The test was, however, useful in determining that the biofilms on the nylon mesh developed much easier than those on the plastic membranes. This was viewed in the opacity results, as well as the scanned images of each frame. Furthermore, biofilm development on the aerated plastic membrane surpassed that of the anaerobic plastic membrane by a substantial amount. This was attributed to the oxygen permeating the plastic layer, giving the film this nutrient from the surface.

The aerated membrane process was similar to that of a tree root in a river. Since oxygen passes through the root and into the biofilm, it is an ideal place for microorganisms to colonize. The texture of the plastic, however, was not ideal for the biofilm attachment process. This is why it took longer for the surface of the aerated membrane to become populated with biofilm; initial attachment occurred over the first 3 weeks and maturation continued through weeks 4-7. On the contrary, the texture of the nylon mesh was a much more suitable surface for attachment. Even though air was not permeating the surface of this material, the rough surface area allowed attachment to occur in as little as 4 days. The entire life cycle of the biofilms on the nylon mesh frame took approximately 4 weeks; maturation continued through weeks 2 and 3.

Accidental detachment occurred from everyday handling of the frames. Since the biofilms were developed in an environment with little shear stress, the colonies formed porous matrices which caused the biofilms to be more susceptible to accidental detachment. Colonization on the plastic, in particular, was prone to accidental detachment due to the smooth surface, which made it difficult for the microbes to formulate a strong initial attachment.

As hypothesized, the anaerobic membrane experiment did not follow the trend of the other two experiments. Although some colonization did occur on this membrane, it was irregular and inconsistent. Unlike the aerated membrane, oxygen was not permeating the plastic of the anaerobic membrane. Microbes on the anaerobic plastic were at a disadvantage because they did not receive additional oxygen from the membrane. The lack of this essential nutrient prohibited the biofilm from formulating a strong initial attachment, so everyday movement frequently caused accidental detachment.

Although a river was being mimicked in the reactor tank, biofilms would rarely detach from such slight movements in an actual river environment. The shear force in a river is much higher than that in the reactor tank; growing the biofilms in an environment with more shear stress would have lessened the occurrence of accidental detachment. Biofilm that detached from the frames became planktonic bacteria until it adhered to a new surface. Even though planktonic bacteria are capable of treating the wastewater, the development cannot be monitored or associated with the effectiveness of the treatment since the amount was unknown.

5.2 Wastewater Treatment

Methods used to evaluate the components in the wastewater over time were the Total Organic Carbon test (TOC), Ammonium test, and UV-Visible Spectrometry. From the UV-Visible Spectrometry technique, the concentration of nitrate in the sample was determined as well as the chemical oxygen demand (COD).

The concentrations of ammonium and nitrate in the samples go hand-in-hand. While the biofilm oxidizes ammonium in the wastewater, minimizing the concentration of ammonium, it forms nitrate through the chemical reaction that takes place. The ammonium test was most essential throughout this experiment; it gave insight that the wastewater concentration in the reactor tank was increasing too quickly with the addition of wastewater twice a week. At this point, tap water was added instead of wastewater and a decreasing trend in the ammonium concentration was observed as the biofilms oxidized the ammonium buildup from the previous weeks. Meanwhile, the nitrate concentration increased. These two tests were also useful in showing that planktonic microbes and biofilm on the tank walls definitely contributed to the oxidation of ammonium into nitrates.

Other analytical techniques were less significant, such as TOC and COD, but still demonstrated the ability biofilms have of cleaning wastewater. The chemical oxygen demand, obtained from the UV-Visible Spectroscopy method, concluded that as the biofilms clean wastewater, the need for oxygen is minimized because there are fewer bacteria to oxidize. The TOC technique demonstrated that biofilms also have the ability to oxidize organic compounds in wastewater, making it suitable for human use.

Testing the opacity of the biofilms was used to correlate the biofilm development with the cleanliness of the wastewater. The opacity results for the biofilms on the plastic membrane frames were inconclusive; however, the opacity results of the nylon mesh frames were more significant. This allowed for the conclusion that biofilms are most effective at oxidizing ammonium and organic compounds in wastewater during the maturation stages of development.

It is important to note that biological treatment is typically just a step in wastewater treatment procedures. Several other methods are also employed to treat the recycled water that our world is so desperately in need of; however, it has been concluded through extensive experimentation and analysis that biofilms do effectively clean wastewater.

5.3 Recommendations

Although it has successfully been determined that biofilms are efficient in wastewater treatment processes, certain measures could have been taken to increase the accuracy of the results obtained throughout the experiment. These recommendations are listed below:

- Create more shear force in the tank to prevent the occurrence of accidental detachment. This can be achieved with constant mixing of the wastewater in the tank with an electric mixer.
- Wait a designated amount of time before taking a sample after adding wastewater or tap water to the tank. This will allow the particles to settle so that samples can be taken in a consistent manner.

- Run each sample in the UV-Visible Spectrometer at least 2 or 3 times so the standard deviation can be calculated to determine the exact amount of error from noise on the machine.
- Run the experiment for a longer period of time to see more development on the plastic membranes.

Overall, from this study, it has been concluded that the application of biofilms is an effective step in the biological treatment of wastewater. Even though the development of the biofilm takes time, once attachment and maturation has occurred, the films are proficient in increasing water quality by oxidizing ammonium and organic compounds in wastewater. It is important that alternate steps in wastewater treatment are also applied in order to obtain the highest possible water quality. This will ensure the future water demand does not exceed its availability.

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Appendix A: Volume

Total Volume of Cylindrical Tank

$$Volume = \pi r^2 h$$

$$Height = 44.5\ cm$$

$$Diameter = 44.5\ cm \quad so, \quad Radius = 22.25\ cm$$

$$Volume = \pi * 22.25cm^2 * 44.5cm = 69210.17\ cm^3$$

$$69210.17\ cm^3 * \frac{(1\ m)^3}{(100cm)^3} = 0.06921017\ m^3 * \frac{1000\ L}{1m^3} = 69.21\ Liters$$

Actual Volume

The tank could not be filled to the top because space was needed to maneuver the biofilm equipment, so the tank was filled to a height of 31.2 centimeters. The volume was recalculated:

$$Volume = \pi r^2 h$$

$$Volume = \pi * 22.25cm^2 * 31.2cm = 48524.88\ cm^3 = 0.0485\ m^3$$

$$Volume = 48.5\ Liters$$

Original Concentration of Wastewater

$$Volume\ of\ Wastewater = 2\ Liters$$

$$Volume\ of\ Tap\ Water = 48.5 - 2 = 46.5\ Lters$$

$$Concentration\ of\ Wastewater \rightarrow \frac{2\ L\ wastewater}{48.5\ L\ total} = \frac{x}{100\%}$$

$$Concentration\ of\ Wastewater = 4.12\%$$

Maintaining the Volume

Week 1-4: Two new liters of wastewater were added in the tank twice a week in order to feed the biofilms. This was done through a series of steps. First, the volume in the tank was calculated. Then, the amount of water that was lost due to evaporation and sampling was determined using the equation: 48.5 L – current volume = volume lost. The volume to be removed from the tank was determined using the equation: 2 L – volume lost = volume to be removed. Finally, 2 liters of wastewater were added to the tank, and the volume was recalculated to equal the original volume of 48.5 liters.

Week 4-8: Regular tap water was added to the tank to maintain the original volume. The volume of the tank was calculated, then the amount of water lost to evaporation and sampling was replaced by tap water.

The chart below shows the volumes of the tank (before and after adding water) on dates that the water was changed:

	Before (Liters)	After (Liters)
Friday, January 13^{th}	47.44	48.5
Tuesday, January 17^{th}	47.125	48.5
Friday, January 20^{th}	47.436	48.5
Tuesday, January 24^{th}	47.59	48.5
Friday, January 27^{th}	47.436	48.5
Tuesday, January 31^{st}	47.59	48.5
Friday, February 3^{rd}	47.75	48.5
Tuesday, February 7^{th}	46.91	48.5
Friday, February 10^{th}	47.28	48.5
Tuesday, February 14^{th}	47.28	48.5
Friday, February 17^{th}	47.28	48.5
Tuesday, February 21^{st}	47.59	48.5

Note: Friday, February 3^{rd} was the last day that wastewater was added in the tank. Tuesday, February 7^{th} was the date when tap water started being added.

Appendix B: Procedures to Monitor Biofilm Development

Scanning Procedure

1. Turn scanner and computer *ON*
2. Log into account *Zeiss* (password = zeisszeiss)
3. Open program: EPSONScan
4. Select *Paramétrage 2* (for nylon mesh frames) or *Paramétrage 4* (for membrane frames)
5. Place the frame on the glass sheet on the scanner using marked lines
6. Click on *Aperçu* (Preview)
7. Adjust the frame if necessary
8. Click on *Numériser* (Scan)
9. Name the file in appropriate format
10. Click *OK* to begin the scan
11. Repeat steps 5 to 11 until all frames have been scanned
12. Shut computer and scanner *OFF*

Notes:
- The first scan should be blank (this is used as a baseline for the gray level tests)
- Turn the lights in the room off during scanning

Image Editing Procedure

1. Create a folder called *Work* and copy the *wire* and *membrane* images
2. Open *Paint Pro Shop 5*
3. Open a scanned image in the window to edit
4. Select the *Crop* tool
5. Select the inner square area of the imagine where there is no frame or shades visible (only the material on which biofilm has grown)
6. Copy and Paste the cropped image in the same window
7. Save it as a *.tiff* document to replace the previous image
8. Cropped images are then analyzed to obtain the gray levels and opacities

Appendix C: Ammonium Nessler Test

Procedure

1. Collect the amount of test tubes needed for the samples being tested, plus 1 extra for a zero test
2. Number test tubes in order of their date, starting with zero, and place them in a test tube rack
3. Turn *ON* the *Hamilton Digital Diluter*
4. Adjust percentages on the diluter to *90%* on the distilled water side and *50%* on sample side
5. Clean the suction pipe once by pressing the down arrow on the Digital Diluter to fill the syringe with distilled water
6. Empty the distilled water from the suction pipe into the glass container provided by pressing the up arrow on the Digital Diluter
7. Insert the suction pipe inside plastic bottle containing the sample
8. Press the down arrow on the Digital Diluter to fill syringes (one with the sample and the other with distilled water)
9. Insert the suction pipe into the numbered test tube corresponding to the sample and press the up arrow to dispense the sample
10. Repeat steps 6 to 8 once more with the same sample to fill the entire test tube
11. Repeat steps 6 to 8 (twice) for every sample being tested
12. Adjust percentages on the diluter to 99% on the distilled water side and 0% on the sample side
13. Clean the suction pipe again by pressing the down arrow to fill the syringe with distilled water
14. Empty the distilled water from the suction pipe into the glass container provided by pressing the up arrow
15. To fill the "0" test tube, press the down arrow on the diluter to fill the syringe with distilled water

16. Empty the distilled water into the “0” test tube by pressing the up arrow
17. Once all the numbered test tubes are filled, shut off the Hamilton Digital Diluter
18. Add 2 drops of mineral stabilizer to each diluted sample
19. Add 2 drops of Polyvinyl alcohol to each diluted sample
20. Add 400 micro liters of the Nessler Reagent to each diluted sample
21. Cap all of the samples and turn them upside down to mix contents
22. Turn on the Hach Spectrometer
23. On the menu, select *Signal Unique* then set the wavelength to 425 nm
24. Place the blank sample into the spectrometer
25. Press *Zero* (the spectrometer will read 0 for the absorbance)
26. Place the first sample in the spectrometer and press *Lire* (read)
27. Record the number displayed on the screen
28. Rotate the sample in the spectrometer about 90 degrees
29. Press *read* again and record the absorbance
30. Repeat step 22 and 23 so that 3 absorbencies are recorded for each sample
31. Repeat steps 20 to 24 until all samples are finished
32. Insert the absorbance values into an excel spreadsheet
33. Take the average of the three absorbencies for each sample
34. Use the following equation to determine the concentration of ammonium in the wastewater in mg/L:

$$Ammonium\ Concentration = Average\ Absorbance * 3.446 * 10$$

Data

	Date	Day	1	2	3	AVG	NH_4	STDEV	CV
	11-01	**1**	0.105	0.109	0.108	0.107	3.699	0.0021	1.94%
	12-01	**2**	0.087	0.088	0.087	0.087	3.010	0.0006	0.66%
B	13-01	**3**	0.111	0.121	0.097	0.110	3.779	0.0121	10.99%
A	13-01	**3**	0.226	0.218	0.219	0.221	7.616	0.0044	1.97%
	16-01	**4**	0.182	0.183	0.179	0.181	6.249	0.0021	1.15%
B	17-01	**5**	0.211	0.222	0.209	0.214	7.374	0.0070	3.27%
A	17-01	**5**	0.335	0.332	0.329	0.332	11.441	0.0030	0.90%
	18-01	**6**	0.329	0.317	0.343	0.330	11.360	0.0130	3.95%
	19-01	**7**	0.316	0.326	0.328	0.323	11.142	0.0064	1.99%
B	20-01	**8**	0.307	0.306	0.298	0.304	10.464	0.0049	1.62%
A	20-01	**8**	0.369	0.355	0.355	0.360	12.394	0.0081	2.25%
	23-01	**9**	0.366	0.368	0.381	0.372	12.808	0.0081	2.19%
B	24-01	**10**	0.373	0.364	0.356	0.364	12.555	0.0085	2.33%
A	24-01	**10**	0.479	0.488	0.492	0.486	16.759	0.0067	1.37%
	25-01	**11**	0.468	0.469	0.466	0.468	16.116	0.0015	0.33%
	26-01	**12**	0.467	0.461	0.471	0.466	16.070	0.0050	1.08%
B	27-01	**13**	0.453	0.448	0.446	0.449	15.473	0.0036	0.80%
A	27-01	**13**	0.576	0.571	0.598	0.582	20.044	0.0144	2.47%
	30-01	**14**	0.522	0.520	0.535	0.526	18.114	0.0081	1.55%
B	31-01	**15**	0.528	0.548	0.530	0.535	18.448	0.0110	2.06%
A	31-01	**15**	0.605	0.605	0.606	0.605	20.860	0.0006	0.10%
	1-02	**16**	0.617	0.631	0.625	0.624	21.515	0.0070	1.13%
	2-02	**17**	0.597	0.593	0.596	0.595	20.515	0.0021	0.35%
B	3-02	**18**	0.634	0.615	0.607	0.619	21.319	0.0139	2.24%
A	3-02	**18**	0.702	0.697	0.687	0.695	23.961	0.0076	1.10%
	6-02	**19**	0.694	0.721	0.702	0.706	24.317	0.0139	1.97%
B	7-02	**20**	0.669	0.666	0.664	0.666	22.962	0.0025	0.38%
A	7-02	**20**	0.745	0.732	0.725	0.734	25.294	0.0101	1.38%
	8-02	**21**	0.725	0.730	0.737	0.731	25.179	0.0060	0.82%
	9-02	**22**	0.736	0.713	0.715	0.721	24.857	0.0127	1.77%
B	10-02	**23**	0.715	0.706	0.708	0.710	24.455	0.0047	0.67%
A	10-02	**23**	0.704	0.697	0.690	0.697	24.019	0.0070	1.00%
	13-02	**24**	0.704	0.671	0.689	0.688	23.708	0.0165	2.40%
B	14-02	**25**	0.670	0.679	0.698	0.682	23.513	0.0143	2.09%
A	14-02	**25**	0.640	0.631	0.640	0.637	21.951	0.0052	0.82%
	15-02	**26**	0.612	0.601	0.603	0.605	20.860	0.0059	0.97%
	16-02	**27**	0.597	0.593	0.614	0.601	20.722	0.0112	1.85%
B	17-02	**28**	0.614	0.606	0.604	0.608	20.952	0.0053	0.87%
A	17-02	**28**	0.558	0.539	0.568	0.555	19.125	0.0147	2.65%
B	21-02	**29**	0.533	0.553	0.555	0.547	18.850	0.0122	2.22%
A	21-02	**29**	0.533	0.510	0.531	0.525	18.080	0.0127	2.43%
	22-02	**30**	0.509	0.506	0.504	0.506	17.448	0.0025	0.50%
	23-02	**31**	0.485	0.486	0.508	0.493	16.989	0.0130	2.64%
	24-02	**32**	0.447	0.459	0.457	0.454	15.656	0.0064	1.42%

Appendix D: UV-Visible Spectra

Procedure

1. Turn PC *ON*
2. Login to ECCMA9 (password = eccma)
3. Turn Antlhélie Light spectrophotometer on (switch on the back of the machine)
4. Wait for the machine to run the autotest
5. Check whether the cuvette holder is empty and press “Val”
6. When *Imprimer* is displayed on the spectrophotometer screen, move right arrow to *Abbandoner* and press “Val”
7. Once the autotest is finished, *Absorbance* is indicated on the spectrophotometer screen, move with the down arrow and go to sub-menu *Configuration*.
8. Move with right arrow to *Liason RS232* and press “Val”
9. On the PC, start the program *LabPowerJ*
10. In *LabPowerJ*, go to the *Méthode* sub-menu
11. Click on *Nouvelle méthode* and then on *Balayage de spectra*
12. Click on *Editer* at the bottom of the main window to edit the parameters
13. Make *Début* (start) = 190 nm and *Fin* (end) = 600nm
14. Fill the cuvette with deionized water and start the baseline acquisition by clicking on *OK*
15. Once the baseline is obtained, start the spectrum collection for a sample by filling the cuvette with the sample and inserting it into the cuvette holder
16. Click on the yellow *M* on the tool bar to start the run
17. To save the spectrum as an Excel file, *export* from the sub-menu *Fichier*, and save in desired folder
18. Repeat steps 15 and 16 until all samples have been measured

Data: 254nm

	1	2	3	3	4	5	5	6	7	8	8	9	10	10	11	12	13	13
	Wed	Thursday	Friday	Friday	Monday	Tuesday	Tuesday	Wed	Thursday	Friday	Friday	Monday	Tuesday	Tuesday	Wed	Thu	Fri	Fri
	11-Jan	12-Jan	13-Jan B	13-Jan A	16-Jan	17-Jan B	17-Jan A	18-Jan	19-Jan	20-Jan B	20-Jan A	23-Jan	24-Jan B	24-Jan A	25-Jan	26-Jan	27-Jan A	27-Jan B
190	1.4069	1.4474	1.4251	1.6163	1.6167	1.6728	1.8507	1.8653	1.9336	1.9253	2.0903	1.867	1.9213	2.1586	2.0272	1.6185	1.6136	1.6666
191	1.4113	1.4645	1.422	1.6551	1.6289	1.6938	1.8947	1.9	1.9537	1.9666	2.1476	1.901	1.9582	2.2069	2.0299	1.6044	1.6108	1.6635
192	1.3931	1.4491	1.4007	1.6469	1.6207	1.6812	1.9002	1.8921	1.9439	1.9538	2.1453	1.8883	1.9528	2.2245	2.0144	1.5597	1.5691	1.6285
193	1.3687	1.4253	1.3783	1.6293	1.602	1.6634	1.8934	1.8779	1.9222	1.9253	2.1362	1.8613	1.929	2.2206	1.9863	1.5081	1.5196	1.5779
194	1.3384	1.3937	1.3554	1.6034	1.5729	1.6412	1.8747	1.8582	1.8887	1.8819	2.1219	1.8204	1.8869	2.1949	1.9454	1.4525	1.4639	1.5183
195	1.3116	1.3667	1.3288	1.567	1.5438	1.6043	1.8228	1.8248	1.8451	1.8405	2.0619	1.7724	1.8338	2.1388	1.897	1.3954	1.3973	1.4544
196	1.2835	1.338	1.3022	1.5323	1.5105	1.5666	1.7781	1.786	1.8062	1.7991	2.006	1.7229	1.7811	2.0816	1.8436	1.3423	1.3398	1.3939
197	1.254	1.3076	1.2755	1.4992	1.4733	1.526	1.7401	1.742	1.7714	1.7576	1.9538	1.6722	1.7287	2.0235	1.7855	1.2919	1.2879	1.3372
198	1.2244	1.2724	1.2462	1.4672	1.4374	1.4888	1.698	1.6946	1.7207	1.7149	1.9006	1.6216	1.6798	1.9677	1.7285	1.2447	1.2415	1.289
199	1.1956	1.2399	1.2171	1.4324	1.4032	1.4517	1.6547	1.6468	1.6729	1.6704	1.8506	1.571	1.6306	1.914	1.6729	1.2005	1.1979	1.2393
200	1.1675	1.2096	1.1883	1.3955	1.3705	1.4166	1.6105	1.5989	1.6279	1.6249	1.8031	1.5209	1.5813	1.8618	1.6187	1.1589	1.157	1.1909
250	0.1251	0.1243	0.1341	0.199	0.2127	0.237	0.3148	0.3429	0.3663	0.3807	0.4579	0.3489	0.3824	0.4677	0.4319	0.1987	0.2	0.196
251	0.1234	0.1224	0.1326	0.197	0.2107	0.2351	0.3125	0.3402	0.3636	0.378	0.4541	0.3462	0.38	0.4644	0.4291	0.1982	0.1996	0.195
252	0.1217	0.1208	0.131	0.195	0.2088	0.2333	0.3103	0.3376	0.361	0.3754	0.4505	0.3439	0.3777	0.4611	0.4264	0.1977	0.199	0.1941
253	0.1194	0.119	0.1295	0.1921	0.2065	0.2307	0.3067	0.3343	0.3574	0.3719	0.4477	0.3416	0.3748	0.4569	0.4233	0.197	0.198	0.1933
254	0.118	0.1176	0.1284	0.19	0.2048	0.229	0.304	0.3321	0.3551	0.3696	0.4443	0.3396	0.3724	0.4542	0.4211	0.196	0.1971	0.1921
255	0.1171	0.1167	0.1275	0.1882	0.2034	0.2276	0.3016	0.3301	0.3532	0.3676	0.441	0.3376	0.3704	0.4522	0.4191	0.195	0.1963	0.1911
256	0.1167	0.1165	0.1268	0.1864	0.2021	0.2263	0.2995	0.328	0.3513	0.3655	0.4385	0.3355	0.3685	0.4503	0.417	0.1941	0.1954	0.1904
257	0.1163	0.116	0.1261	0.1847	0.2015	0.2253	0.2985	0.3265	0.3496	0.3639	0.4367	0.334	0.367	0.448	0.4154	0.1932	0.1953	0.1898
258	0.1155	0.1153	0.1253	0.1831	0.2007	0.2244	0.2974	0.3248	0.3479	0.3623	0.4347	0.3325	0.3655	0.4457	0.4139	0.1925	0.1948	0.1893
259	0.1141	0.1144	0.1244	0.1814	0.1994	0.2235	0.2959	0.3225	0.346	0.3605	0.4322	0.3307	0.3637	0.4435	0.4124	0.1921	0.1939	0.1889
260	0.1132	0.1133	0.1235	0.18	0.1982	0.2222	0.295	0.321	0.3442	0.3589	0.4303	0.3291	0.3622	0.4419	0.4107	0.192	0.1927	0.1884

	14	15	15	16	17	18	18	19	20	20	21	22	23	23	24	25	25	26
	Mon	Tues	Tues	Wed	Thu	Fri	Fri	Mon	Tues	Tues	Wed	Thu	Fri	Fri	Mon	Tues	Tues	Wed
	30-Jan	31-Jan B	31-Jan A	1-Feb	2-Feb	3-Feb B	3-Feb A	6-Feb	7-Feb B	7-Feb A	8-Feb	9-Feb	10-Feb B	10-Feb A	13-Feb	14-Feb B	14-Feb A	15-Feb
190	1.676	1.698	1.8448	1.752	1.6683	1.6911	1.8485	1.7415	1.7917	1.8265	1.8984	1.8939	1.9221	1.8774	1.9163	1.9677	1.9429	1.8702
191	1.6683	1.716	1.8782	1.7556	1.6778	1.688	1.8775	1.7444	1.7959	1.8103	1.9115	1.8784	1.9015	1.851	1.9061	1.9471	1.9395	1.8385
192	1.6282	1.6724	1.8563	1.7192	1.6377	1.6429	1.8808	1.7287	1.7531	1.7489	1.8553	1.8061	1.8299	1.7806	1.8592	1.8746	1.8664	1.7521
193	1.5758	1.619	1.8138	1.6633	1.5805	1.5842	1.8252	1.6727	1.6845	1.6726	1.7833	1.726	1.7481	1.7013	1.7789	1.7871	1.7825	1.6658
194	1.5163	1.5598	1.7589	1.5975	1.5137	1.5175	1.7762	1.5976	1.6024	1.5891	1.7026	1.6411	1.6612	1.6181	1.6858	1.6941	1.6939	1.5797
195	1.4536	1.4971	1.6953	1.5317	1.4434	1.4464	1.7093	1.5294	1.5189	1.5019	1.6142	1.5516	1.5709	1.5341	1.5962	1.6026	1.6039	1.4942
196	1.3926	1.4353	1.6276	1.4669	1.3776	1.3771	1.6393	1.4555	1.4398	1.424	1.5303	1.4703	1.4882	1.4525	1.5101	1.517	1.5157	1.419
197	1.3342	1.3758	1.5591	1.4037	1.3145	1.311	1.5671	1.381	1.3654	1.3503	1.4508	1.393	1.4101	1.3745	1.4283	1.4366	1.4325	1.3489
198	1.2824	1.3232	1.4946	1.3446	1.2546	1.253	1.4923	1.3134	1.3013	1.2791	1.3794	1.3188	1.3383	1.3048	1.3559	1.3656	1.362	1.2842
199	1.2334	1.2734	1.4358	1.2905	1.2015	1.1977	1.4257	1.2513	1.2412	1.2148	1.3137	1.2529	1.2735	1.2419	1.2887	1.3009	1.298	1.2268
200	1.1869	1.2262	1.3812	1.2397	1.1523	1.1452	1.3642	1.1934	1.1851	1.1553	1.2517	1.1923	1.213	1.1836	1.2256	1.2404	1.2385	1.1746
250	0.208	0.238	0.2841	0.2333	0.2048	0.2088	0.2596	0.2311	0.246	0.2203	0.2461	0.2453	0.2643	0.2553	0.2728	0.2908	0.2826	0.2594
251	0.2073	0.2371	0.283	0.2321	0.2039	0.2079	0.2579	0.2298	0.2445	0.2187	0.2443	0.2427	0.2619	0.2532	0.2707	0.2884	0.2806	0.2568
252	0.2068	0.2362	0.2819	0.2309	0.2029	0.2071	0.256	0.2285	0.2429	0.2172	0.2425	0.2409	0.26	0.2513	0.2687	0.2862	0.2786	0.2549
253	0.2065	0.2353	0.2805	0.2295	0.2015	0.2063	0.2538	0.227	0.2413	0.2156	0.2404	0.2396	0.2586	0.2494	0.2666	0.2841	0.2764	0.2535
254	0.2053	0.2339	0.2789	0.2279	0.2009	0.2051	0.2517	0.2253	0.2399	0.2143	0.2385	0.2378	0.2569	0.2479	0.2653	0.2826	0.2749	0.2519
255	0.2043	0.2328	0.2774	0.2266	0.2	0.204	0.2498	0.2238	0.2386	0.213	0.237	0.2362	0.2552	0.2462	0.2639	0.2811	0.2732	0.2503
256	0.2034	0.2319	0.2759	0.2254	0.1986	0.2029	0.2481	0.2224	0.2374	0.2119	0.2359	0.2349	0.2536	0.2446	0.2624	0.2796	0.2715	0.2487
257	0.2024	0.2313	0.2753	0.2248	0.198	0.2019	0.2463	0.2218	0.2357	0.2104	0.2347	0.2335	0.2521	0.2433	0.261	0.2795	0.2696	0.2469
258	0.2014	0.2305	0.2743	0.2238	0.1975	0.2012	0.2451	0.2211	0.2345	0.2098	0.2339	0.2327	0.251	0.2422	0.2602	0.2784	0.2684	0.2459
259	0.2004	0.2291	0.2731	0.2226	0.1969	0.2006	0.2444	0.2204	0.2336	0.2097	0.2332	0.2321	0.2501	0.2414	0.2596	0.2766	0.2676	0.2453
260	0.1996	0.2285	0.2725	0.2218	0.1954	0.1996	0.2432	0.2194	0.2328	0.209	0.2325	0.2309	0.2489	0.2409	0.258	0.2756	0.266	0.2443

	27	28	28	29	29	30	31	32
	Thurs	Fri	Fri	Tues	Tues	Wed	Thurs	Fri
	16-Feb	17-Feb B	17-Feb A	21-Feb B	21-Feb A	22-Feb	23-Feb	24-Feb
190	1.8798	1.9255	1.8111	1.8807	1.9017	1.9609	1.9283	2.08
191	1.8393	1.8858	1.7838	1.8612	1.871	1.9734	1.9601	2.1113
192	1.7713	1.8196	1.7587	1.801	1.8062	1.9385	1.9663	2.1236
193	1.6832	1.7388	1.6414	1.7287	1.7302	1.8764	1.9342	2.0952
194	1.5904	1.6535	1.5594	1.6535	1.6517	1.8071	1.8902	2.0528
195	1.5003	1.5692	1.4748	1.5803	1.5754	1.7377	1.8427	2.0021
196	1.4254	1.4871	1.4039	1.5145	1.5104	1.6749	1.7942	1.9625
197	1.3557	1.4096	1.3386	1.4538	1.4517	1.6173	1.7463	1.9293
198	1.2891	1.3408	1.278	1.4	1.4004	1.5673	1.7004	1.9042
199	1.2316	1.2751	1.2233	1.3534	1.3554	1.5271	1.6664	1.8843
200	1.1789	1.2146	1.173	1.3116	1.3144	1.4916	1.6363	1.8662
250	0.2654	0.2646	0.2256	0.2116	0.2089	0.2209	0.2094	0.2089
251	0.263	0.2621	0.2239	0.2092	0.2076	0.2193	0.207	0.2066
252	0.2609	0.2597	0.222	0.2072	0.2057	0.217	0.2048	0.2037
253	0.2591	0.2575	0.2201	0.2055	0.2036	0.2142	0.2024	0.2004
254	0.2579	0.256	0.2191	0.204	0.2026	0.2135	0.2013	0.1993
255	0.2561	0.2546	0.2177	0.2028	0.2012	0.2128	0.2003	0.1986
256	0.2541	0.2532	0.2161	0.2017	0.1993	0.2119	0.1992	0.1979
257	0.2527	0.2515	0.2147	0.1999	0.1979	0.2103	0.1975	0.1963
258	0.2517	0.2505	0.2139	0.1989	0.197	0.2088	0.1959	0.1949
259	0.2508	0.2497	0.2133	0.1983	0.1963	0.2075	0.1948	0.1938
260	0.2499	0.2485	0.2124	0.1974	0.1954	0.2067	0.1943	0.1931

Data: Derivates

	Wed			Thursday			Friday			Friday			Monday			Tuesday			Tuesday		
	11-Jan	1st	2nd	12-Jan	1st	2nd	13-Jan B	1st	2nd	13-Jan A	1st	2nd	16-Jan	1st	2nd	17-Jan B	1st	2nd	17-Jan A	1st	2nd
220	0.4897	-0.034	0.0008	0.5055	-0.0345	0.0007	0.5084	-0.035	0.0008	0.6245	-0.0361	0.00085	0.6387	-0.0359	0.00105	0.6723	-0.0365	0.00075	0.8106	-0.0377	0.00035
221	0.4557	-0.0332	0.00165	0.471	-0.0338	0.00145	0.4734	-0.0342	0.00155	0.5884	-0.0353	0.00155	0.6028	-0.0349	0.00145	0.6358	-0.0358	0.00155	0.7729	-0.0373	0.00107
222	0.4233	-0.0307	0.00252	0.4379	-0.0316	0.00225	0.44	-0.0319	0.00232	0.554	-0.033	0.00223	0.569	-0.033	0.00175	0.6008	-0.0334	0.00225	0.7359	-0.0355	0.00197
223	0.3943	-0.0282	0.0021	0.4078	-0.0293	0.0019	0.4096	-0.0296	0.0021	0.5224	-0.0308	0.00195	0.5368	-0.0314	0.00188	0.569	-0.0313	0.0016	0.7018	-0.0334	0.00177
224	0.367	-0.0265	0.00175	0.3793	-0.0278	0.00177	0.3809	-0.0277	0.00185	0.4924	-0.0291	0.00185	0.5063	-0.0292	0.00208	0.5383	-0.0302	0.00158	0.6691	-0.032	0.00165
225	0.3413	-0.0246	0.00193	0.3522	-0.0257	0.00215	0.3542	-0.0258	0.00192	0.4642	-0.0271	0.00195	0.4783	-0.0272	0.0019	0.5086	-0.0281	0.00227	0.6378	-0.0301	0.0019
226	0.3177	-0.0226	0.00215	0.3278	-0.0235	0.00217	0.3292	-0.0239	0.00235	0.4382	-0.0252	0.0019	0.4519	-0.0254	0.00195	0.4821	-0.0257	0.00213	0.6089	-0.0282	0.0017
227	0.296	-0.0204	0.00235	0.3052	-0.0214	0.00208	0.3065	-0.0212	0.0025	0.4138	-0.0233	0.00212	0.4274	-0.0233	0.00215	0.4573	-0.0238	0.0018	0.5814	-0.0267	0.00185
228	0.277	-0.0179	0.00193	0.285	-0.0193	0.00178	0.2869	-0.0188	0.0019	0.3916	-0.021	0.00205	0.4053	-0.0212	0.00177	0.4344	-0.022	0.00165	0.5555	-0.0245	0.00205
229	0.2601	-0.0165	0.00115	0.2665	-0.0179	0.0014	0.2688	-0.0173	0.00155	0.3719	-0.0192	0.00138	0.3851	-0.0197	0.00123	0.4132	-0.0205	0.00152	0.5324	-0.0226	0.00145
230	0.244	-0.0157	0.0013	0.2493	-0.0166	0.00158	0.2522	-0.0157	0.00172	0.3532	-0.0182	0.00137	0.3658	-0.0187	0.00147	0.3933	-0.019	0.00167	0.5103	-0.0216	0.00118
231	0.2288	-0.0139	0.00172	0.2334	-0.0147	0.00173	0.2373	-0.0139	0.00165	0.3355	-0.0165	0.00175	0.3477	-0.0168	0.0018	0.3752	-0.0172	0.00162	0.4892	-0.0202	0.00138
232	0.2162	-0.0122	0.00122	0.2199	-0.0131	0.00122	0.2244	-0.0125	0.00118	0.3203	-0.0147	0.00138	0.3322	-0.0151	0.0012	0.3589	-0.0158	0.0013	0.4698	-0.0188	0.00125
233	0.2044	-0.0115	0.00123	0.2072	-0.0122	0.00118	0.2124	-0.0116	0.0012	0.3061	-0.0137	0.00113	0.3175	-0.0144	0.0011	0.3437	-0.0146	0.00138	0.4515	-0.0178	0.00142
234	0.1933	-0.0097	0.00163	0.1954	-0.0107	0.00162	0.2013	-0.01	0.00143	0.2929	-0.0124	0.00127	0.3034	-0.0129	0.00163	0.3297	-0.013	0.0016	0.4343	-0.016	0.0018
235	0.1849	-0.0082	0.00097	0.1857	-0.009	0.00152	0.1923	-0.0087	0.00102	0.2812	-0.0112	0.00115	0.2917	-0.0111	0.0014	0.3177	-0.0114	0.00137	0.4195	-0.0141	0.00155
236	0.1769	-0.0078	0.0007	0.1774	-0.0077	0.00115	0.1839	-0.008	0.00095	0.2706	-0.0102	0.00123	0.2811	-0.0101	0.00127	0.3069	-0.0103	0.00125	0.406	-0.0129	0.0015
237	0.1693	-0.0068	0.00108	0.1703	-0.0067	0.00085	0.1763	-0.0068	0.00113	0.2609	-0.0087	0.00127	0.2715	-0.0086	0.00133	0.2972	-0.0089	0.00125	0.3937	-0.0112	0.00162
238	0.1633	-0.0056	0.00085	0.164	-0.006	0.00063	0.1703	-0.0057	0.00075	0.2532	-0.0076	0.00068	0.2639	-0.0074	0.00075	0.2891	-0.0078	0.00095	0.3837	-0.0097	0.00113
239	0.158	-0.0051	0.00038	0.1583	-0.0054	0.0004	0.1648	-0.0053	0.00032	0.2457	-0.0073	0.0002	0.2566	-0.0071	0.00028	0.2817	-0.007	0.00058	0.3744	-0.0089	0.00057
240	0.1531	-0.0049		0.1531	-0.0052		0.1597	-0.0051		0.2385	-0.0072		0.2497	-0.0069		0.2751	-0.0066		0.3659	-0.0085	

	Wed			Thursday			Friday			Friday			Monday			Tuesday			Tuesday		
	18-Jan	1st	2nd	19-Jan	1st	2nd	20-Jan B	1st	2nd	20-Jan A	1st	2nd	23-Jan	1st	2nd	24-Jan B	1st	2nd	24-Jan A	1st	2nd
220	0.8223	-0.0367	0.0005	0.8475	-0.0361	0.0005	0.8469	-0.0365	0.0007	0.9699	-0.0367	0.00035	0.7699	-0.0339	0.00075	0.8109	-0.0342	0.00025	0.9643	-0.0354	0.0005
221	0.7856	-0.0362	0.0013	0.8114	-0.0356	0.001	0.8104	-0.0358	0.00142	0.9332	-0.0363	0.00097	0.736	-0.0331	0.0015	0.7767	-0.034	0.00105	0.9289	-0.0349	0.00102
222	0.7499	-0.0341	0.0022	0.7763	-0.0341	0.00158	0.7753	-0.0337	0.00218	0.8972	-0.0348	0.0017	0.7036	-0.0309	0.00212	0.743	-0.0321	0.00205	0.8945	-0.0334	0.0016
223	0.7174	-0.0318	0.0018	0.7432	-0.0324	0.0015	0.7431	-0.0315	0.00193	0.8637	-0.033	0.00148	0.6742	-0.0289	0.00142	0.7125	-0.0299	0.00183	0.8622	-0.0317	0.00148
224	0.6863	-0.0305	0.0015	0.7114	-0.0311	0.00155	0.7124	-0.0298	0.0016	0.8313	-0.0318	0.00148	0.6458	-0.0281	0.00132	0.6833	-0.0284	0.00163	0.8311	-0.0304	0.0015
225	0.6564	-0.0288	0.0017	0.681	-0.0293	0.0018	0.6835	-0.0282	0.00155	0.8001	-0.03	0.00178	0.6181	-0.0263	0.00197	0.6556	-0.0266	0.00192	0.8014	-0.0287	0.00165
226	0.6287	-0.0271	0.00145	0.6527	-0.0275	0.00177	0.6559	-0.0267	0.00185	0.7713	-0.0282	0.00138	0.5933	-0.0241	0.00175	0.6301	-0.0246	0.00207	0.7737	-0.0271	0.00145
227	0.6022	-0.0259	0.0016	0.626	-0.0258	0.00193	0.6301	-0.0246	0.00195	0.7436	-0.0272	0.00157	0.5699	-0.0227	0.0015	0.6064	-0.0225	0.00205	0.7472	-0.0258	0.00158
228	0.5769	-0.0239	0.0019	0.6011	-0.0236	0.0019	0.6068	-0.0228	0.00138	0.7168	-0.0251	0.00208	0.5478	-0.0211	0.0016	0.5852	-0.0205	0.00145	0.7221	-0.0239	0.0017
229	0.5544	-0.0221	0.00133	0.5787	-0.022	0.0012	0.5845	-0.0218	0.00105	0.6934	-0.0231	0.00115	0.5277	-0.0195	0.00137	0.5654	-0.0196	0.00077	0.6993	-0.0224	0.0012
230	0.5327	-0.0212	0.00117	0.5571	-0.0213	0.00117	0.5632	-0.0207	0.00145	0.6706	-0.0228	0.00082	0.5087	-0.0184	0.00137	0.5461	-0.019	0.00133	0.6773	-0.0215	0.00115
231	0.5119	-0.0198	0.00155	0.5362	-0.0197	0.0016	0.5431	-0.0189	0.00177	0.6478	-0.0215	0.00165	0.491	-0.0168	0.00158	0.5275	-0.0169	0.00203	0.6562	-0.0201	0.0014
232	0.4932	-0.0182	0.00138	0.5178	-0.018	0.00113	0.5254	-0.0172	0.00145	0.6277	-0.0195	0.00163	0.4751	-0.0152	0.00148	0.5123	-0.0149	0.0013	0.6371	-0.0188	0.00103
233	0.4756	-0.017	0.0014	0.5001	-0.0174	0.0012	0.5088	-0.016	0.00135	0.6088	-0.0182	0.0013	0.4606	-0.0139	0.00128	0.4977	-0.0143	0.00103	0.6187	-0.018	0.0011
234	0.4592	-0.0153	0.00155	0.483	-0.0157	0.00187	0.4934	-0.0144	0.00155	0.5913	-0.0169	0.00137	0.4474	-0.0126	0.00118	0.4837	-0.0128	0.00155	0.601	-0.0166	0.00165
235	0.4449	-0.0139	0.00118	0.4688	-0.0137	0.00155	0.4799	-0.0129	0.00135	0.575	-0.0155	0.00155	0.4353	-0.0115	0.00118	0.472	-0.0112	0.00127	0.5856	-0.0148	0.00155
236	0.4314	-0.013	0.00142	0.4557	-0.0125	0.00158	0.4676	-0.0118	0.00137	0.5604	-0.0138	0.00175	0.4244	-0.0103	0.00122	0.4613	-0.0103	0.0011	0.5715	-0.0134	0.00145
237	0.4189	-0.0111	0.0018	0.4437	-0.0105	0.0018	0.4564	-0.0102	0.0014	0.5474	-0.012	0.00157	0.4147	-0.0091	0.0011	0.4514	-0.009	0.0012	0.5587	-0.0118	0.00145
238	0.4093	-0.0094	0.00105	0.4347	-0.0089	0.00085	0.4473	-0.0089	0.00078	0.5365	-0.0107	0.00085	0.4063	-0.0081	0.00083	0.4433	-0.0079	0.00073	0.5478	-0.0105	0.001
239	0.4001	-0.009	0.00035	0.4258	-0.0088	0.00012	0.4385	-0.0086	0.00028	0.5261	-0.0103	0.00028	0.3985	-0.0074	0.00055	0.4356	-0.0076	0.00025	0.5376	-0.0098	0.00053
240	0.3914	-0.0087		0.4171	-0.0087		0.4301	-0.0084		0.516	-0.0101		0.3915	-0.007		0.4282	-0.0074		0.5281	-0.0095	

	Wed			Thursday			Friday			Friday			Monday			Tuesday			Tuesday		
	25-Jan	1st	2nd	26-Jan	1st	2nd	27-Jan A	1st	2nd	27-Jan B	1st	2nd	30-Jan	1st	2nd	31-Jan B	1st	2nd	31-Jan A	1st	2nd
220	0.8656	-0.0318	0.0007	0.4899	-0.0326	0.00085	0.4918	-0.0324	0.00045	0.5001	-0.0319	0.0004	0.503	-0.0309	0.00035	0.5416	-0.0314	0.00065	0.6354	-0.0318	0.00065
221	0.8338	-0.0311	0.0013	0.4573	-0.0318	0.00175	0.4594	-0.0319	0.00147	0.4682	-0.0315	0.00145	0.4721	-0.0305	0.00137	0.5102	-0.0308	0.00175	0.6036	-0.0312	0.0013
222	0.8034	-0.0292	0.00175	0.4264	-0.0291	0.0026	0.4279	-0.0294	0.00267	0.4371	-0.029	0.0027	0.4419	-0.0282	0.00265	0.4801	-0.0279	0.00285	0.5731	-0.0292	0.00195
223	0.7754	-0.0276	0.00112	0.3991	-0.0265	0.002	0.4005	-0.0266	0.0022	0.4102	-0.0261	0.00222	0.4158	-0.0253	0.00223	0.4544	-0.025	0.00207	0.5452	-0.0272	0.0016
224	0.7482	-0.027	0.0009	0.3733	-0.0251	0.001975	0.3747	-0.025	0.002	0.3849	-0.0246	0.00152	0.3914	-0.0237	0.00165	0.43	-0.0238	0.00138	0.5186	-0.026	0.00177
225	0.7215	-0.0258	0.00142	0.3489	-0.0226	0.002625	0.3504	-0.0226	0.00255	0.3611	-0.0231	0.00173	0.3684	-0.0219	0.0019	0.4069	-0.0223	0.00175	0.4932	-0.0237	0.0023
226	0.6966	-0.0241	0.0017	0.3281	-0.0199	0.0023	0.3295	-0.02	0.00227	0.3388	-0.0211	0.00208	0.3475	-0.0199	0.002	0.3854	-0.0202	0.00217	0.4712	-0.0214	0.00177
227	0.6733	-0.0224	0.0017	0.3092	-0.018	0.0022	0.3105	-0.0181	0.0022	0.3189	-0.0189	0.00243	0.3286	-0.0179	0.00225	0.3664	-0.0179	0.0022	0.4504	-0.0202	0.00165
228	0.6518	-0.0207	0.00142	0.2921	-0.0155	0.002375	0.2934	-0.0156	0.00225	0.301	-0.0162	0.00237	0.3116	-0.0154	0.00225	0.3495	-0.0159	0.00195	0.4309	-0.0181	0.002
229	0.6319	-0.0195	0.00092	0.2783	-0.0132	0.001625	0.2794	-0.0135	0.00147	0.2864	-0.0142	0.00155	0.2978	-0.0134	0.00132	0.3347	-0.0141	0.00155	0.4142	-0.0161	0.0015
230	0.6127	-0.0189	0.00128	0.2656	-0.0122	0.001425	0.2663	-0.0126	0.0016	0.2727	-0.0131	0.00145	0.2847	-0.0127	0.00127	0.3214	-0.0127	0.0013	0.3986	-0.0151	0.0014
231	0.5942	-0.017	0.00168	0.2539	-0.0104	0.001975	0.2542	-0.0103	0.00235	0.2601	-0.0112	0.00212	0.2723	-0.0109	0.002	0.3092	-0.0114	0.00145	0.384	-0.0133	0.0017
232	0.5787	-0.0155	0.00067	0.2448	-0.0082	0.0018	0.2456	-0.0079	0.00182	0.2502	-0.0089	0.00192	0.2629	-0.0088	0.00165	0.2985	-0.0098	0.00155	0.3719	-0.0117	0.00122
233	0.5632	-0.0157	0.00042	0.2374	-0.0068	0.0014	0.2384	-0.0067	0.00117	0.2423	-0.0074	0.00113	0.2548	-0.0076	0.0011	0.2895	-0.0083	0.00167	0.3606	-0.0109	0.00118
234	0.5474	-0.0147	0.0014	0.2312	-0.0055	0.001175	0.2322	-0.0056	0.00107	0.2354	-0.0067	0.0008	0.2477	-0.0065	0.00103	0.2818	-0.0065	0.00145	0.3501	-0.0094	0.00145
235	0.5339	-0.0129	0.00153	0.2265	-0.0045	0.0007	0.2273	-0.0046	0.00078	0.229	-0.0058	0.00093	0.2417	-0.0055	0.00087	0.2765	-0.0055	0.00053	0.3419	-0.008	0.00088
236	0.5217	-0.0116	0.0014	0.2223	-0.004	0.000275	0.2231	-0.004	0.00053	0.2238	-0.0048	0.0009	0.2366	-0.0048	0.0006	0.2709	-0.0054	0.0003	0.3341	-0.0076	0.00063
237	0.5107	-0.01	0.00135	0.2184	-0.0039	0.00045	0.2193	-0.0035	0.00055	0.2194	-0.004	0.0007	0.2321	-0.0043	0.00062	0.2656	-0.0049	0.00062	0.3267	-0.0067	0.00107
238	0.5016	-0.0089	0.00078	0.2145	-0.0032	0.0009	0.2161	-0.0029	0.00053	0.2158	-0.0034	0.00053	0.2279	-0.0036	0.00087	0.2612	-0.0042	0.00058	0.3206	-0.0055	0.0011
239	0.4929	-0.0085	0.0003	0.2121	-0.0021	0.000675	0.2135	-0.0024	0.0003	0.2126	-0.0029	0.00035	0.225	-0.0026	0.00063	0.2572	-0.0037	0.0004	0.3158	-0.0046	0.00058
240	0.4846	-0.0083		0.2103	-0.0018		0.2112	-0.0023		0.2099	-0.0027		0.2227	-0.0023		0.2538	-0.0034		0.3115	-0.0043	

	Wed			Thursday			Friday			Friday			Monday			Tuesday			Tuesday		
	1-Feb	1st	2nd	2-Feb	1st	2nd	3-Feb B	1st	2nd	3-Feb A	1st	2nd	6-Feb	1st	2nd	7-Feb B	1st	2nd	7-Feb A	1st	2nd
220	0.5436	-0.03	0.0009	0.4927	-0.0288	0.00075	0.4885	-0.0278	0.00075	0.605	-0.0303	0.0005	0.5213	-0.027	0.00015	0.5256	-0.027	0.00090003	0.5397	-0.0221	0.0003
221	0.5136	-0.0291	0.0018	0.4639	-0.028	0.0017	0.4607	-0.0271	0.0014	0.5747	-0.0298	0.0014	0.4943	-0.0268	0.00115	0.4986	-0.0261	0.00155003	0.5176	-0.0218	0.0006
222	0.4854	-0.0264	0.00237	0.4366	-0.0254	0.00242	0.4344	-0.025	0.002	0.5454	-0.0275	0.00238	0.4676	-0.0247	0.0023	0.4734	-0.0239	0.002	0.4961	-0.0209	0.00097
223	0.4608	-0.0244	0.0013	0.4131	-0.0232	0.00142	0.4107	-0.023	0.00165	0.5197	-0.025	0.00187	0.4449	-0.0222	0.0017	0.4508	-0.0221	0.00137499	0.4758	-0.0199	0.00093
224	0.4367	-0.0238	0.0011	0.3902	-0.0226	0.00133	0.3883	-0.0217	0.0015	0.4953	-0.0238	0.0013	0.4231	-0.0213	0.00125	0.4292	-0.0212	0.001275	0.4564	-0.019	0.00083
225	0.4132	-0.0221	0.00197	0.368	-0.0205	0.00208	0.3673	-0.0201	0.00172	0.4722	-0.0225	0.00135	0.4023	-0.0197	0.0017	0.4085	-0.0195	0.00175001	0.4377	-0.0182	0.00092
226	0.3924	-0.0199	0.00205	0.3491	-0.0184	0.0016	0.3482	-0.0183	0.00173	0.4504	-0.021	0.00142	0.3836	-0.0179	0.0017	0.3901	-0.0176	0.00167499	0.42	-0.0172	0.00092
227	0.3735	-0.0181	0.002	0.3312	-0.0174	0.00172	0.3308	-0.0166	0.00195	0.4301	-0.0196	0.00175	0.3665	-0.0164	0.00158	0.3732	-0.0162	0.001775	0.4033	-0.0164	0.0008
228	0.3563	-0.0158	0.00197	0.3144	-0.015	0.00225	0.315	-0.0143	0.00205	0.4112	-0.0176	0.00195	0.3509	-0.0148	0.0015	0.3577	-0.0141	0.00192501	0.3873	-0.0156	0.0008
229	0.3418	-0.0141	0.0013	0.3013	-0.0129	0.00138	0.3021	-0.0125	0.0013	0.395	-0.0157	0.0014	0.337	-0.0133	0.0012	0.345	-0.0123	0.001225	0.3721	-0.0148	0.00085
230	0.3281	-0.0133	0.0014	0.2887	-0.0122	0.0012	0.29	-0.0117	0.00102	0.3798	-0.0147	0.0011	0.3242	-0.0123	0.0013	0.333	-0.0116	0.0011	0.3578	-0.0139	0.0008
231	0.3153	-0.0113	0.00175	0.2769	-0.0104	0.0017	0.2786	-0.0105	0.00142	0.3655	-0.0135	0.00142	0.3123	-0.0108	0.00142	0.3217	-0.0102	0.00152499	0.3443	-0.0131	0.00087
232	0.3055	-0.0097	0.00092	0.2678	-0.0088	0.00117	0.2691	-0.0089	0.00133	0.3528	-0.0119	0.0015	0.3027	-0.0095	0.00083	0.3127	-0.0086	0.001125	0.3315	-0.0122	0.001
233	0.2958	-0.0095	0.00085	0.2593	-0.0081	0.00107	0.2608	-0.0078	0.00105	0.3417	-0.0105	0.0013	0.2933	-0.0091	0.00075	0.3045	-0.0079	0.00077501	0.32	-0.0112	0.00057
234	0.2866	-0.0081	0.00147	0.2516	-0.0067	0.00127	0.2535	-0.0068	0.00105	0.3318	-0.0093	0.00107	0.2845	-0.008	0.00112	0.2969	-0.007	0.00094999	0.3092	-0.011	0.0005
235	0.2797	-0.0065	0.00113	0.246	-0.0056	0.0006	0.2472	-0.0057	0.00098	0.3231	-0.0083	0.00072	0.2773	-0.0069	0.00095	0.2904	-0.006	0.00089999	0.298	-0.0102	0.00113
236	0.2736	-0.0058	0.00058	0.2405	-0.0054	0.00018	0.2421	-0.0049	0.00057	0.3151	-0.0079	0.0004	0.2708	-0.0061	0.0009	0.2849	-0.0053	0.00037501	0.2889	-0.0087	0.00088
237	0.2681	-0.0053	0.00055	0.2351	-0.0052	0.00057	0.2375	-0.0046	0.0005	0.3074	-0.0076	0.00075	0.2651	-0.0051	0.00095	0.2799	-0.0052	0.00025	0.2805	-0.0084	0.00047
238	0.2629	-0.0047	0.00065	0.2301	-0.0043	0.00098	0.233	-0.0038	0.0008	0.3	-0.0063	0.00133	0.2607	-0.0042	0.0006	0.2744	-0.0047	0.0008	0.2721	-0.0078	0.00075
239	0.2587	-0.0041	0.0004	0.2265	-0.0032	0.0007	0.2298	-0.0029	0.00057	0.2947	-0.0049	0.00092	0.2567	-0.0038	0.00025	0.2704	-0.0036	0.00072501	0.2649	-0.0069	0.0006
240	0.2548	-0.0039		0.2236	-0.0029		0.2271	-0.0027		0.2902	-0.0045		0.253	-0.0037		0.2671	-0.0033		0.2583	-0.0066	0.0003

	Wed			Thursday			Friday			Friday			Monday			Tuesday		
	8-Feb	1st	2nd	9-Feb	1st	2nd	10-Feb B	1st	2nd	10-Feb A	1st	2nd	13-Feb	1st	2nd	14-Feb B	1st	2nd
220	0.5923	-0.0246	0.000700027	0.5731	-0.0227	0.00030002	0.5904	-0.023	0.00045	0.5759	-0.0224	0.0005	0.6044	-0.0228	0.0004	0.6253	-0.023	1E-04
221	0.5677	-0.0239	0.000925019	0.5504	-0.0224	0.00062501	0.5674	-0.0225	0.00073	0.5535	-0.0219	0.000775	0.5816	-0.0224	0.00072	0.6023	-0.0229	0.000475
222	0.5445	-0.0227	0.001049995	0.5283	-0.0214	0.000975	0.5453	-0.0215	0.00105	0.5321	-0.0209	0.0009	0.5596	-0.0214	0.00102	0.5795	-0.022	0.001
223	0.5222	-0.0218	0.000949994	0.5075	-0.0204	0.00082499	0.5243	-0.0204	0.00105	0.5118	-0.0201	0.000575	0.5389	-0.0204	0.0008	0.5582	-0.0209	0.00095
224	0.5009	-0.0209	0.000950009	0.4874	-0.0198	0.000875	0.5044	-0.0194	0.00092	0.4919	-0.0197	0.000625	0.5189	-0.0197	0.00078	0.5377	-0.0202	0.00085
225	0.4805	-0.0199	0.001000002	0.4679	-0.0187	0.00115	0.4854	-0.0186	0.00095	0.4724	-0.0188	0.001025	0.4994	-0.0188	0.001	0.5179	-0.0192	0.001075
226	0.4611	-0.0188	0.001024999	0.45	-0.0175	0.00097501	0.4672	-0.0176	0.00108	0.4542	-0.0176	0.0011	0.4813	-0.0177	0.00082	0.4993	-0.018	0.00115
227	0.4428	-0.0178	0.000875004	0.4329	-0.0167	0.000775	0.4503	-0.0165	0.00078	0.4371	-0.0167	0.0011	0.4639	-0.0171	0.00067	0.4819	-0.0169	0.000925
228	0.4254	-0.0171	0.0009	0.4165	-0.0159	0.00089999	0.4343	-0.016	0.00073	0.4209	-0.0155	0.0011	0.447	-0.0164	0.00095	0.4655	-0.0161	0.00085
229	0.4086	-0.016	0.00114999	0.401	-0.015	0.000975	0.4183	-0.015	0.00125	0.4062	-0.0144	0.000775	0.4311	-0.0152	0.00113	0.4496	-0.0152	0.00105
230	0.3933	-0.0148	0.001024999	0.3866	-0.014	0.00075001	0.4043	-0.0135	0.0009	0.392	-0.0139	0.0007	0.4165	-0.0141	0.00095	0.4351	-0.014	0.000875
231	0.379	-0.014	0.000750013	0.373	-0.0134	0.0007	0.3913	-0.0132	0.00045	0.3784	-0.0131	0.000925	0.4028	-0.0134	0.00072	0.4215	-0.0134	0.000675
232	0.3653	-0.0133	0.000724994	0.3597	-0.0126	0.00099999	0.3779	-0.0126	0.00085	0.3659	-0.0121	0.0009	0.3898	-0.0127	0.00058	0.4082	-0.0127	0.00085
233	0.3524	-0.0126	0.000849992	0.3478	-0.0115	0.0009	0.3661	-0.0115	0.00083	0.3543	-0.0112	0.00075	0.3774	-0.0122	0.0006	0.3961	-0.0117	0.00075
234	0.3402	-0.0116	0.00122501	0.3368	-0.0108	0.00077501	0.3549	-0.0109	0.00073	0.3434	-0.0106	0.000825	0.3654	-0.0115	0.00102	0.3847	-0.0112	0.00085
235	0.3292	-0.0101	0.001249999	0.3262	-0.0099	0.000925	0.3442	-0.01	0.00095	0.3332	-0.0096	0.000875	0.3544	-0.0102	0.00118	0.3737	-0.01	0.00115
236	0.32	-0.0091	0.000349998	0.317	-0.0089	0.00057499	0.3348	-0.009	0.00077	0.3242	-0.0088	0.000575	0.3451	-0.0091	0.00045	0.3646	-0.0089	0.00055
237	0.311	-0.0094	0.000250004	0.3083	-0.0088	0.00055	0.3261	-0.0085	0.0007	0.3156	-0.0085	0.000625	0.3361	-0.0093	0.00045	0.3559	-0.009	0.000375
238	0.3012	-0.0086	0.001249999	0.2995	-0.0078	0.0011	0.3178	-0.0077	0.0009	0.3073	-0.0075	0.00095	0.3266	-0.0082	0.0013	0.3467	-0.0081	0.00105
239	0.2938	-0.0069	0.001100004	0.2926	-0.0065	0.00082499	0.3108	-0.0067	0.00063	0.3005	-0.0065	0.000625	0.3196	-0.0067	0.00097	0.3396	-0.0068	0.000775
240	0.2874	-0.0064	0.000500008	0.2864	-0.0062	0.00034998	0.3044	-0.0064	0.0003	0.2942	-0.0063	0.00025	0.3133	-0.0063	0.00035	0.333	-0.0066	0.00025

	Tuesday			Wed			Thursday			Friday			Friday			Tuesday		
	14-Feb A	1st	2nd	15-Feb	1st	2nd	16-Feb	1st	2nd	17-Feb B	1st	2nd	17-Feb A	1st	2nd	21-Feb B	1st	2nd
220	0.6186	-0.0237	0.00055	0.5985	-0.0237	0.00035	0.6067	-0.0238	0.00035	0.6358	-0.0239	0.00035	0.5939	-0.0264	0.00045	0.7443	-0.0356	-0.00
221	0.5949	-0.0232	0.00057	0.5748	-0.0234	0.0006	0.5829	-0.0235	0.00078	0.6119	-0.0236	0.00045	0.5675	-0.0259	0.00062	0.7087	-0.0359	-0.000
222	0.5723	-0.0226	0.00068	0.5518	-0.0225	0.000925	0.5598	-0.0222	0.00113	0.5887	-0.023	0.00075	0.542	-0.0252	0.0008	0.6725	-0.0361	0.000400
223	0.5498	-0.0218	0.00103	0.5298	-0.0215	0.000975	0.5384	-0.0212	0.00075	0.5659	-0.022	0.00105	0.5172	-0.0244	0.00085	0.6365	-0.0351	0.0012
224	0.5287	-0.0205	0.00112	0.5088	-0.0205	0.001025	0.5174	-0.0208	0.00085	0.5446	-0.0209	0.00075	0.4933	-0.0235	0.00115	0.6023	-0.0335	0.001399
225	0.5088	-0.0196	0.00098	0.4887	-0.0194	0.0011	0.4969	-0.0195	0.00125	0.5241	-0.0206	0.0003	0.4703	-0.0221	0.00128	0.5694	-0.0323	0.001
226	0.4896	-0.0185	0.00105	0.4699	-0.0184	0.0009	0.4784	-0.0183	0.00082	0.5035	-0.0203	0.0004	0.4492	-0.0209	0.00083	0.5377	-0.0313	0.0009
227	0.4717	-0.0174	0.00102	0.452	-0.0176	0.000725	0.4604	-0.0178	0.00045	0.4835	-0.0197	0.0007	0.4285	-0.0204	0.00075	0.5069	-0.0303	0.001
228	0.4547	-0.0165	0.00105	0.4346	-0.0169	0.001	0.4427	-0.0174	0.00087	0.464	-0.0189	0.00092	0.4084	-0.0194	0.00125	0.477	-0.029	0.001
229	0.4387	-0.0153	0.00118	0.4182	-0.0157	0.0012	0.4257	-0.0161	0.00138	0.4457	-0.0179	0.00092	0.3897	-0.0179	0.0014	0.449	-0.0269	0.0020
230	0.424	-0.0141	0.0011	0.4033	-0.0145	0.001	0.4105	-0.0146	0.00135	0.4282	-0.017	0.00113	0.3726	-0.0166	0.00118	0.4233	-0.0249	0.001
231	0.4104	-0.0132	0.0009	0.3892	-0.0137	0.00075	0.3965	-0.0134	0.00087	0.4116	-0.0156	0.00105	0.3565	-0.0156	0.00113	0.3992	-0.023	0.001
232	0.3977	-0.0124	0.00073	0.376	-0.013	0.0006	0.3837	-0.0129	0.00043	0.3969	-0.015	0.00037	0.3415	-0.0143	0.00113	0.3774	-0.0212	0.001500
233	0.3857	-0.0117	0.0007	0.3632	-0.0124	0.000825	0.3708	-0.0125	0.00073	0.3817	-0.0149	0.0007	0.3278	-0.0133	0.00097	0.3568	-0.0199	0.0014
234	0.3743	-0.0109	0.001	0.3511	-0.0113	0.001175	0.3586	-0.0114	0.00107	0.3671	-0.0135	0.00135	0.3149	-0.0124	0.0011	0.3375	-0.0183	0.001999
235	0.3638	-0.0097	0.00113	0.3405	-0.0101	0.000975	0.348	-0.0104	0.00075	0.3546	-0.0122	0.00107	0.303	-0.0111	0.00127	0.3203	-0.016	0.001975
236	0.3549	-0.0087	0.00042	0.3309	-0.0094	0.00055	0.3378	-0.0099	0.00065	0.3427	-0.0114	0.00115	0.2927	-0.0099	0.00113	0.3056	-0.0143	0.001
237	0.3464	-0.0089	0.00028	0.3217	-0.009	0.0009	0.3282	-0.0091	0.00105	0.3318	-0.0099	0.00155	0.2833	-0.0088	0.00113	0.2917	-0.0137	0.001224
238	0.3372	-0.0081	0.00105	0.3129	-0.0076	0.00145	0.3196	-0.0078	0.00118	0.3229	-0.0083	0.00123	0.275	-0.0076	0.00115	0.2783	-0.0118	0.0019
239	0.3301	-0.0068	0.00087	0.3065	-0.0061	0.0009	0.3126	-0.0068	0.00065	0.3152	-0.0074	0.00055	0.2681	-0.0065	0.0007	0.268	-0.0098	0.001274
240	0.3237	-0.0064	0.00035	0.3007	-0.0058	0.0003	0.3061	-0.0065	0.00025	0.308	-0.0072	0.00025	0.2619	-0.0062	0.00035	0.2587	-0.0093	0.000499

	Tuesday			Wed			Thursday			Friday		
	21-Feb A	1st	2nd	22-Feb	1st	2nd	23-Feb	1st	2nd	24-Feb	1st	2nd
220	0.7411	-0.0361	1E-04	0.9022	-0.0445	-0.0001	1.0294	-0.0523	-0.0004	1.2408	-0.0656	0.0006
221	0.705	-0.036	0.000125	0.8577	-0.0446	-0.0002	0.9771	-0.0528	-0.0004	1.1752	-0.065	0.00058
222	0.6691	-0.0358	0.00045	0.8129	-0.0448	7.5E-05	0.9239	-0.0532	-0.0001	1.1108	-0.0645	-1E-08
223	0.6333	-0.0351	0.001025	0.768	-0.0445	0.0006	0.8707	-0.053	0.0003	1.0463	-0.065	-0.0005
224	0.5989	-0.0338	0.001325	0.7239	-0.0437	0.0012	0.8178	-0.0526	0.001	0.9808	-0.0655	0.00015
225	0.5657	-0.0324	0.001375	0.6807	-0.0421	0.00187	0.7655	-0.0511	0.0019	0.9152	-0.0647	0.00172
226	0.534	-0.031	0.001275	0.6397	-0.0399	0.002	0.7157	-0.0488	0.002	0.8514	-0.0621	0.00268
227	0.5036	-0.0299	0.00125	0.6009	-0.0381	0.00145	0.6679	-0.047	0.00173	0.791	-0.0593	0.00242
228	0.4742	-0.0285	0.001625	0.5635	-0.037	0.00143	0.6216	-0.0453	0.00203	0.7327	-0.0573	0.00242
229	0.4465	-0.0266	0.0019	0.5269	-0.0352	0.00203	0.5772	-0.043	0.00242	0.6765	-0.0545	0.0029
230	0.4209	-0.0248	0.001925	0.493	-0.0329	0.00227	0.5356	-0.0405	0.00272	0.6237	-0.0514	0.00325
231	0.397	-0.0228	0.001825	0.461	-0.0307	0.00267	0.4962	-0.0376	0.00333	0.5736	-0.048	0.0041
232	0.3753	-0.0211	0.00145	0.4316	-0.0276	0.0029	0.4605	-0.0338	0.0034	0.5277	-0.0433	0.00445
233	0.3548	-0.0199	0.001475	0.4058	-0.0249	0.00223	0.4285	-0.0308	0.003	0.4871	-0.0391	0.00378
234	0.3355	-0.0182	0.0019	0.3818	-0.0231	0.00195	0.399	-0.0279	0.0028	0.4495	-0.0357	0.00365
235	0.3185	-0.0161	0.001725	0.3595	-0.021	0.00215	0.3728	-0.0252	0.00243	0.4157	-0.0318	0.00345
236	0.3033	-0.0147	0.00125	0.3398	-0.0188	0.00212	0.3487	-0.023	0.00245	0.3859	-0.0288	0.00265
237	0.2891	-0.0136	0.001625	0.3218	-0.0168	0.0021	0.3268	-0.0202	0.00288	0.3581	-0.0265	0.00297
238	0.2761	-0.0114	0.0021	0.3063	-0.0147	0.00188	0.3082	-0.0173	0.00257	0.3329	-0.0229	0.00363
239	0.2662	-0.0094	0.001275	0.2925	-0.013	0.00123	0.2923	-0.0151	0.00148	0.3124	-0.0192	0.00243
240	0.2573	-0.0089	0.0005	0.2803	-0.0122	0.0008	0.278	-0.0143	0.0008	0.2944	-0.018	0.00125

Data: Nitrate Concentration

			UV"	WL	CONC
1	Wed	11-Jan	0.00252499	222	4.20832386
2	Thurs	12-Jan	0.00225	222	3.75000139
3	Fri	13-Jan B	0.00250001	227	4.16667511
3	Fri	13-Jan A	0.00222501	222	3.70835265
4	Mon	16-Jan	0.00214999	227	3.58331949
5	Tues	17-Jan B	0.002275	225	3.79166255
5	Tues	17-Jan A	0.00205001	228	3.41668725
6	Wed	18-Jan	0.00189999	228	3.16664577
7	Thurs	19-Jan	0.00192501	227	3.20834418
8	Fri	20-Jan B	0.00195	227	3.24999293
8	Fri	20-Jan A	0.00177501	225	2.95835237
9	Mon	23-Jan	0.001975	225	3.29166651
10	Tues	24-Jan B	0.00207499	226	3.45831116
10	Tues	24-Jan A	0.00170001	228	2.83335646
11	Wed	25-Jan	0.00175001	222	2.91667879
12	Thurs	26-Jan	0.002625	225	4.37499334
13	Fri	27-Jan B	0.002675	222	4.45832809
13	Fri	27-Jan A	0.00270001	222	4.50001409
14	Mon	30-Jan	0.00264999	222	4.41665451
15	Tues	31-Jan B	0.00285	222	4.7500059
15	Tues	31-Jan A	0.00230001	225	3.83334855
16	Wed	1-Feb	0.00237499	222	3.95831962
17	Thurs	2-Feb	0.00242499	222	4.04165437
18	Fri	3-Feb B	0.00205	228	3.41666241
18	Fri	3-Feb A	0.00237501	222	3.95834446
19	Mon	6-Feb	0.00229999	222	3.83332372
20	Tues	7-Feb B	0.002	222	3.33334009
20	Tues	7-Feb A	0.00112501	235	1.87501311
21	Wed	8-Feb	0.00125	235	2.08333135
22	Thurs	9-Feb	0.00115	225	1.91667428
23	Fri	10-Feb B	0.00124999	229	2.08331893
23	Fri	10-Feb A	0.0011	228	1.83333953
24	Mon	13-Feb	0.0013	238	2.16666609
25	Tues	14-Feb B	0.00115001	226	1.91668669
25	Tues	14-Feb A	0.001175	229	1.95833544
26	Wed	15-Feb	0.00120001	229	2.00000902
27	Thurs	16-Feb	0.001375	229	2.29167442
28	Fri	17-Feb B	0.00155	237	2.58333981

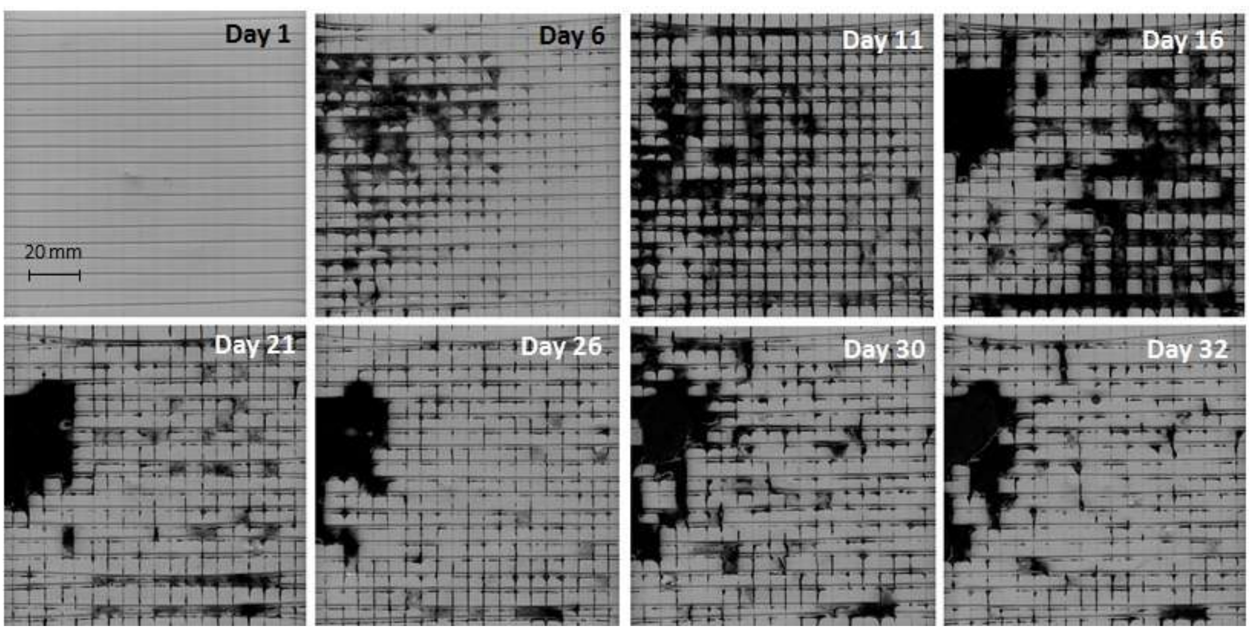
28	**Fri**	**17-Feb A**	0.0014	229	2.33333558
29	**Tues**	**21-Feb B**	0.002025	229	3.37500125
29	**Tues**	**21-Feb A**	0.00210001	238	3.50000958
30	**Wed**	**22-Feb**	0.00290001	232	4.83335306
31	**Thu**	**23-Feb**	0.0034	232	5.66666325
21	**Fri**	**24-Feb**	0.00444999	232	7.4166432

Appendix E: Images

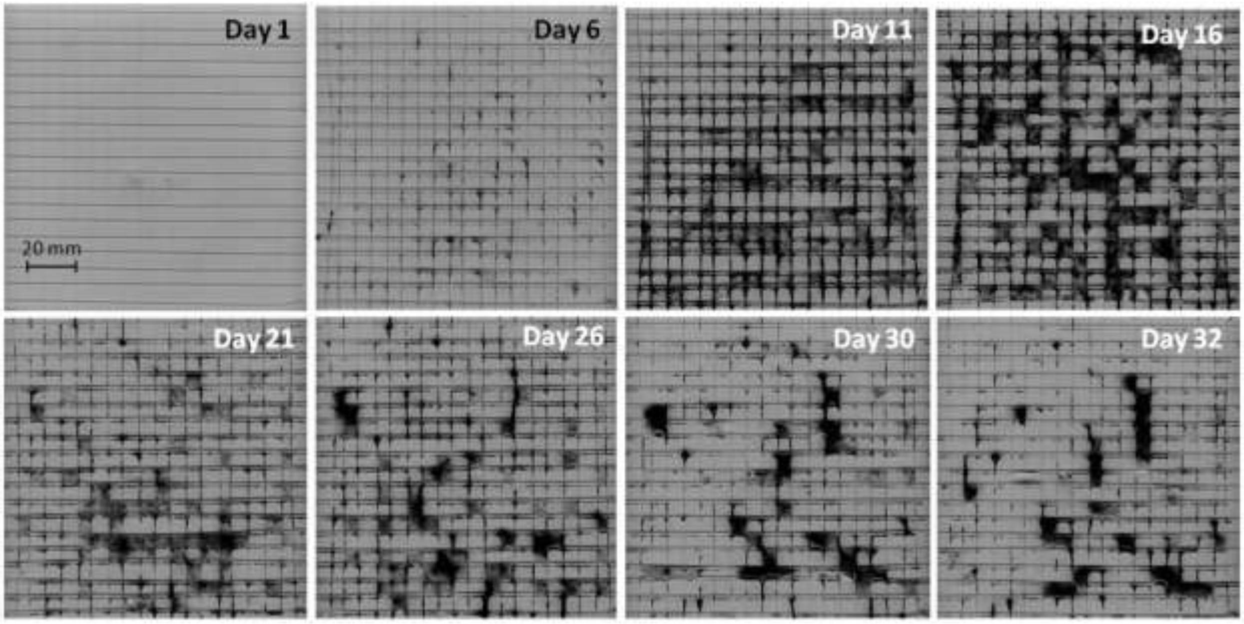
PICTURE KEY			
Day 1	January 11th	Week 1 (first day)	Wednesday
Day 6	January 18th	Week 2	Wednesday
Day 11	January 25th	Week 3	Wednesday
Day 16	February 1st	Week 4	Wednesday
Day 21	February 8th	Week 5	Wednesday
Day 26	February 15th	Week 6	Wednesday
Day 30	February 22nd	Week 7	Wednesday
Day 32	February 24th	Week 7 (last day)	Friday

Nylon Mesh Frames

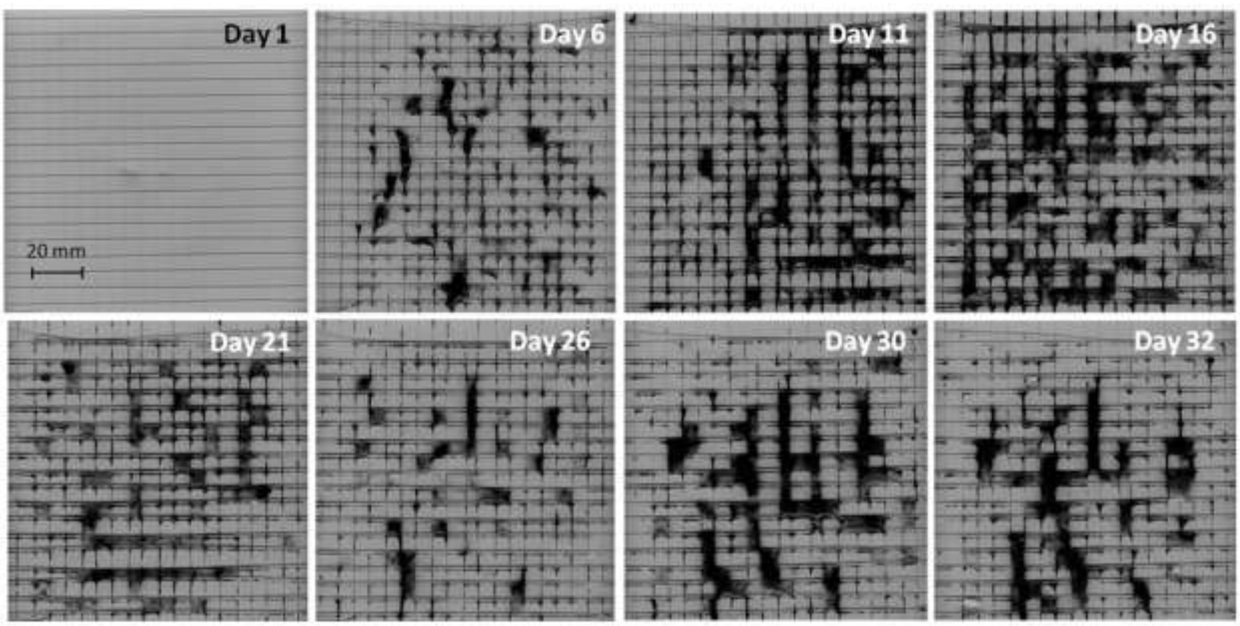
Frame 1



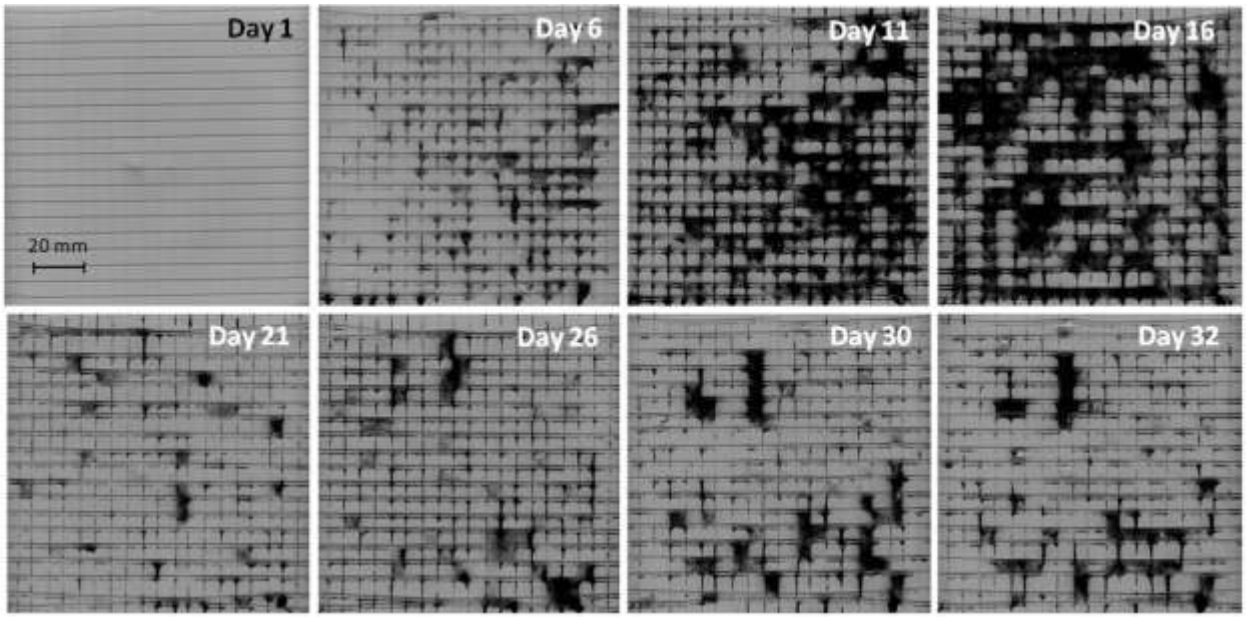
Frame 2



Frame 3

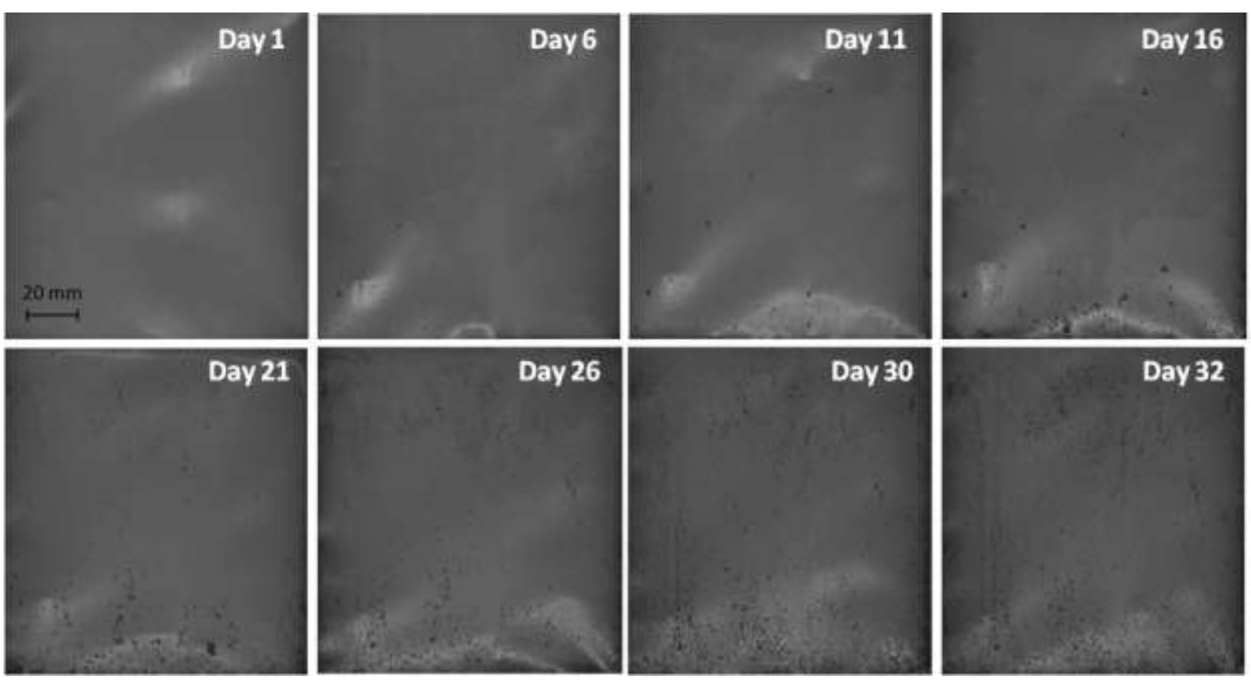


Frame 4

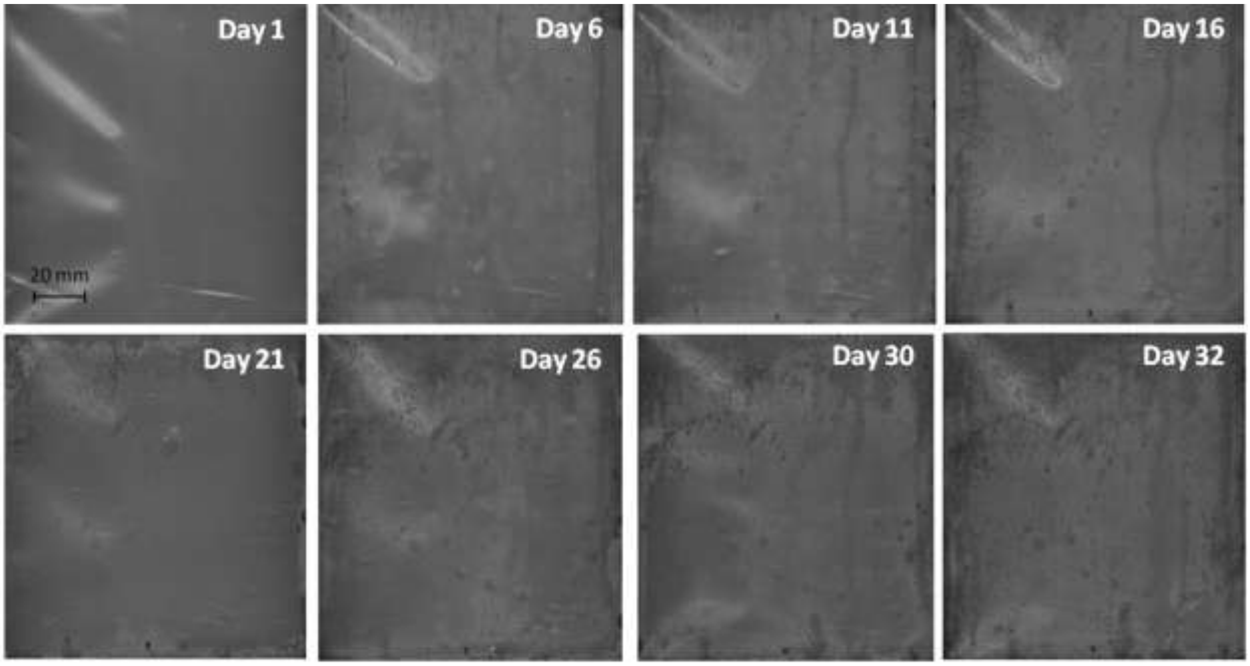


Plastic Membranes

Aerated Membrane



Anaerobic Membrane



Appendix F: Charts

Nylon Mesh Opacity

Day #	Date	Blank	initial	Mean	OPACITY	StDev	Frame #1	initial	Mean	OPACITY	StDev
1	10-Jan	wire_120110_001.ti	206	206	0	3.63E+00	wire_120110_002.tif	155	155	0	15
2	12-Jan	wire_120112_001.ti	206	206	0	4.65E+00	wire_120112_002.tif	155	153	2	15
3	13-Jan	wire_120113_001.ti	206	206	0	3.77E+00	wire_120113_002.tif	155	153	2	15
4	16-Jan	Wire_120116_001.ti	206	206	0	3.83E+00	Wire_120116_002.tif	155	144	11	22
5	17-Jan	Wire_120117_001.ti	206	206	0	3.93E+00	Wire_120117_002.tif	155	141	14	24
6	18-Jan	Wire_120118_001.ti	206	206	0	4.02E+00	Wire_120118_002.tif	155	119	36	43
7	19-Jan	Wire_120119_001.ti	206	206	0	4.01E+00	Wire_120119_002.tif	155	126	29	40
8	20-Jan	Wire_120120_001.ti	206	205	1	3.97E+00	Wire_120120_002.tif	155	106	49	50
9	23-Jan	Wire_120123_001.ti	206	205	1	4.04E+00	Wire_120123_002.tif	155	99	56	49
10	24-Jan	Wire_120124_001.ti	206	206	0	4.05E+00	Wire_120124_002.tif	155	106	49	47
11	25-Jan	Wire_120125_001.ti	206	206	0	3.95E+00	Wire_120125_002.tif	155	89	66	52
12	26-Jan	Wire_120126_001.ti	206	207	-1	3.95E+00	Wire_120126_002.tif	155	93	62	54
13	27-Jan	Wire_120127_001.ti	206	207	-1	3.97E+00	Wire_120127_002.tif	155	96	59	54
14	30-Jan	Wire_120130_001.ti	206	206	0	4.00E+00	Wire_120130_002.tif	155	49	106	55
15	31-Jan	Wire_120131_001.ti	206	207	-1	3.83E+00	Wire_120131_002.tif	155	60	95	58
16	1-Feb	Wire_120201_001.ti	206	206	0	3.88E+00	Wire_120201_002.tif	155	76	79	58
17	2-Feb	Wire_120202_001.ti	206	207	-1	3.99E+00	Wire_120202_002.tif	155	89	66	57
18	3-Feb	Wire_120203_001.ti	206	207	-1	3.85E+00	Wire_120203_002.tif	155	109	46	50
19	6-Feb	Wire_120206_001.ti	206	206	0	3.75E+00	Wire_120206_002.tif	155	95	60	53
20	7-Feb	Wire_120207_001.ti	206	206	0	3.70E+00	Wire_120207_002.tif	155	109	46	50
21	8-Feb	Wire_120208_001.ti	206	206	0	4.22E+00	Wire_120208_002.tif	155	111	44	51
22	9-Feb	Wire_120209_001.ti	206	206	0	4.14E+00	Wire_120209_002.tif	155	119	36	47
23	10-Feb	Wire_120210_001.ti	206	206	0	3.86E+00	Wire_120210_002.tif	155	121	34	47
24	13-Feb	Wire_120213_001.ti	206	206	0	3.84E+00	Wire_120213_002.tif	155	119	36	47
25	14-Feb	Wire_120214_001.ti	206	207	-1	4.05E+00	Wire_120214_002.tif	155	123	32	46
26	15-Feb	Wire_120215_001.ti	206	206	0	3.72E+00	Wire_120215_002.tif	155	117	38	48
27	16-Feb	Wire_120216_001.ti	206	206	0	3.73E+00	Wire_120216_002.tif	155	119	36	48
28	17-Feb	Wire_120217_001.ti	206	207	-1	3.71E+00	Wire_120217_002.tif	155	99	56	55
29	21-Feb	Wire_120221_001.ti	206	206	0	3.72E+00	Wire_120221_002.tif	155	100	55	57
30	22-Feb	Wire_120222_001.ti	206	206	0	3.82E+00	Wire_120222_002.tif	155	108	47	53
31	23-Feb	Wire_120223_001.ti	206	206	0	3.80E+00	Wire_120223_002.tif	155	120	35	49
32	24-Feb	Wire_120224_001.ti	206	206	0	3.95E+00	Wire_120224_002.tif	155	117	38	50

Frame #2	initial	Mean	OPACITY	StDev	Frame #3	initial	Mean	OPACITY	StDev
wire_120110_003.tif	155	155	0	15	wire_120110_004.tif	154	154	0	15
wire_120112_003.tif	155	153	2	16	wire_120112_004.tif	154	153	1	16
wire_120113_003.tif	155	152	3	16	wire_120113_004.tif	154	152	2	17
Wire_120116_003.ti	155	143	12	21	Wire_120116_004.ti	154	126	28	39
Wire_120117_003.ti	155	142	13	21	Wire_120117_004.ti	154	126	28	38
Wire_120118_003.ti	155	139	16	24	Wire_120118_004.ti	154	121	33	41
Wire_120119_003.ti	155	132	23	29	Wire_120119_004.ti	154	116	38	42
Wire_120120_003.ti	155	127	28	33	Wire_120120_004.ti	154	113	41	44
Wire_120123_003.ti	155	114	41	42	Wire_120123_004.ti	154	101	53	48
Wire_120124_003.ti	155	114	41	42	Wire_120124_004.ti	154	106	48	48
Wire_120125_003.ti	155	99	56	49	Wire_120125_004.ti	154	97	57	53
Wire_120126_003.ti	155	99	56	50	Wire_120126_004.ti	154	97	57	53
Wire_120127_003.ti	155	98	57	51	Wire_120127_004.ti	154	98	56	52
Wire_120130_003.ti	155	70	85	55	Wire_120130_004.ti	154	82	72	56
Wire_120131_003.ti	155	86	69	54	Wire_120131_004.ti	154	86	68	55
Wire_120201_003.ti	155	93	62	51	Wire_120201_004.ti	154	91	63	53
Wire_120202_003.ti	155	102	53	49	Wire_120202_004.ti	154	107	47	48
Wire_120203_003.ti	155	117	38	41	Wire_120203_004.ti	154	113	41	44
Wire_120206_003.ti	155	105	50	46	Wire_120206_004.ti	154	97	57	52
Wire_120207_003.ti	155	122	33	37	Wire_120207_004.ti	154	116	38	42
Wire_120208_003.ti	155	118	37	40	Wire_120208_004.ti	154	112	42	46
Wire_120209_003.ti	155	120	35	40	Wire_120209_004.ti	154	112	42	45
Wire_120210_003.ti	155	119	36	41	Wire_120210_004.ti	154	126	28	37
Wire_120213_003.ti	155	113	42	45	Wire_120213_004.ti	154	124	30	40
Wire_120214_003.ti	155	118	37	42	Wire_120214_004.ti	154	125	29	39
Wire_120215_003.ti	155	113	42	44	Wire_120215_004.ti	154	121	33	41
Wire_120216_003.ti	155	119	36	42	Wire_120216_004.ti	154	120	34	41
Wire_120217_003.ti	155	119	36	42	Wire_120217_004.ti	154	116	38	44
Wire_120221_003.ti	155	120	35	44	Wire_120221_004.ti	154	112	42	49
Wire_120222_003.ti	155	122	33	42	Wire_120222_004.ti	154	108	46	51
Wire_120223_003.ti	155	122	33	43	Wire_120223_004.ti	154	108	46	51
Wire_120224_003.ti	155	124	31	42	Wire_120224_004.ti	154	113	41	49

Frame #4	intial	Mean	OPACITY	StDev
wire_120110_005.tif	153	153	0	14
wire_120112_005.tif	153	143	10	24
wire_120113_005.tif	153	147	6	22
Wire_120116_005.tif	153	120	33	47
Wire_120117_005.tif	153	125	28	40
Wire_120118_005.tif	153	127	26	37
Wire_120119_005.tif	153	117	36	42
Wire_120120_005.tif	153	101	52	49
Wire_120123_005.tif	153	83	70	54
Wire_120124_005.tif	153	88	65	54
Wire_120125_005.tif	153	81	72	55
Wire_120126_005.tif	153	83	70	56
Wire_120127_005.tif	153	81	72	56
Wire_120130_005.tif	153	42	111	53
Wire_120131_005.tif	153	62	91	57
Wire_120201_005.tif	153	73	80	56
Wire_120202_005.tif	153	101	52	52
Wire_120203_005.tif	153	115	38	43
Wire_120206_005.tif	153	92	61	52
Wire_120207_005.tif	153	115	38	43
Wire_120208_005.tif	153	129	24	34
Wire_120209_005.tif	153	125	28	37
Wire_120210_005.tif	153	126	27	37
Wire_120213_005.tif	153	119	34	42
Wire_120214_005.tif	153	120	33	41
Wire_120215_005.tif	153	116	37	42
Wire_120216_005.tif	153	112	41	44
Wire_120217_005.tif	153	112	41	45
Wire_120221_005.tif	153	113	40	47
Wire_120222_005.tif	153	119	34	43
Wire_120223_005.tif	153	121	32	44
Wire_120224_005.tif	153	121	32	43

Membrane Opacity

Day #	Image	initial	Mean	OPACITY	StDev	Air Membrane	intial	Mean	OPACITY	StDev
1	Memb_120111_002.tif	240	240	0	3.68E+00	Memb_air_120111_003.ti	93	93	0	9.52E+00
2	memb_120112_001.tif	240	225	15	5.52E+00	memb_air_120112_003.t	93	100	-7	1.81E+01
3	Memb_120113_001.tif	240	225	15	3.73E+00	Memb_air_120113_003.ti	93	98	-5	1.73E+01
4	Memb_120116_007.tif	240	224	16	3.92E+00	Memb_air_120116_003.ti	93	97	-4	1.59E+01
5	Memb_120117_001.tif	240	224	16	3.84E+00	Memb_air_120117_003.ti	93	97	-4	1.54E+01
6	Memb_120118_001.tif	240	224	16	3.93E+00	Memb_air_120118_003.ti	93	89	4	9.04E+00
7	Memb_120119_001.tif	240	224	16	3.86E+00	Memb_air_120119_003.ti	93	94	-1	1.66E+01
8	Memb_120120_001.tif	240	224	16	3.86E+00	Memb_air_120120_003.ti	93	92	1	1.25E+01
9	Memb_120123_001.tif	240	224	16	3.96E+00	Memb_air_120123_003.ti	93	90	3	1.37E+01
10	Memb_120124_001.tif	240	224	16	4.02E+00	Memb_air_120124_003.ti	93	90	3	1.33E+01
11	Memb_120125_001.tif	240	224	16	3.95E+00	Memb_air_120125_003.ti	93	90	3	1.32E+01
12	Memb_120126_001.tif	240	226	15	3.84E+00	Memb_air_120126_003.ti	93	88	5	1.26E+01
13	Memb_120127_001.tif	240	226	14	3.71E+00	Memb_air_120127_003.ti	93	90	3	1.41E+01
14	Memb_120130_001.tif	240	225	15	3.84E+00	Memb_air_120130_003.ti	93	85	8	1.51E+01
15	Memb_120131_001.tif	240	225	15	3.68E+00	Memb_air_120131_003.ti	93	86	7	1.19E+01
16	Memb_120201_001.tif	240	225	15	3.69E+00	Memb_air_120201_003.ti	93	85	8	1.39E+01
17	Memb_120202_001.tif	240	225	15	3.65E+00	Memb_air_120202_003.ti	93	86	7	1.24E+01
18	Memb_120203_001.tif	240	225	15	3.65E+00	Memb_air_120203_003.ti	93	84	9	1.26E+01
19	Memb_120206_001.tif	240	224	16	3.51E+00	Memb_air_120206_003.ti	93	86	7	1.39E+01
20	Memb_120207_001.tif	240	225	15	3.56E+00	Memb_air_120207_003.ti	93	85	8	1.24E+01
21	Memb_120208_001.tif	240	225	15	4.10E+00	Memb_air_120208_003.ti	93	85	8	1.11E+01
22	Memb_120209_001.tif	240	225	15	3.89E+00	Memb_air_120209_003.ti	93	84	9	1.07E+01
23	Memb_120210_001.tif	240	225	15	3.74E+00	Memb_air_120210_003.ti	93	85	8	1.12E+01
24	Memb_120213_001.tif	240	225	16	3.66E+00	Memb_air_120213_003.ti	93	81	12	1.26E+01
25	Memb_120214_001.tif	240	225	15	3.64E+00	Memb_air_120214_003.ti	93	85	8	1.29E+01
26	Memb_120215_001.tif	240	225	15	3.67E+00	Memb_air_120215_003.ti	93	84	9	1.38E+01
27	Memb_120216_001.tif	240	225	15	3.75E+00	Memb_air_120216_003.ti	93	83	10	1.17E+01
28	Memb_120217_001.tif	240	225	15	3.72E+00	Memb_air_120217_003.ti	93	82	11	1.32E+01
29	Memb_120221_001.tif	240	225	15	3.62E+00	Memb_air_120221_003.ti	93	79	14	1.36E+01
30	Memb_120222_001.tif	240	225	15	3.65E+00	Memb_air_120222_003.ti	93	80	13	1.41E+01
31	Memb_120223_001.tif	240	225	15	3.75E+00	Memb_air_120223_003.ti	93	78	15	1.30E+01
32	Memb_120224_001.tif	240	225	15	3.84E+00	Memb_air_120224_003.ti	93	78	15	1.32E+01

No Air Membrane	initial	Mean	OPACITY	StDev
Memb_noair_120111_002.ti	94	94	0	1.33E+01
memb_noair_120112_002.t	94	103	-9	1.32E+01
Memb_noair_120113_002.ti	94	99	-5	1.51E+01
Memb_noair_120116_002.ti	94	105	-11	1.28E+01
Memb_noair_120117_002.ti	94	102	-8	1.33E+01
Memb_noair_120118_002.ti	94	100	-6	1.20E+01
Memb_noair_120119_002.ti	94	95	-1	1.18E+01
Memb_noair_120120_002.ti	94	102	-8	1.22E+01
Memb_noair_120123_002.ti	94	91	3	1.10E+01
Memb_noair_120124_002.ti	94	95	-1	1.11E+01
Memb_noair_120125_002.ti	94	97	-3	1.15E+01
Memb_noair_120126_002.ti	94	97	-3	1.22E+01
Memb_noair_120127_002.ti	94	98	-4	1.14E+01
Memb_noair_120130_002.ti	94	92	2	1.08E+01
Memb_noair_120131_002.ti	94	93	1	1.08E+01
Memb_noair_120201_002.ti	94	96	-2	1.17E+01
Memb_noair_120202_002.ti	94	92	2	9.76E+00
Memb_noair_120203_002.ti	94	92	2	1.19E+01
Memb_noair_120206_002.ti	94	92	2	1.23E+01
Memb_noair_120207_002.ti	94	89	5	1.08E+01
Memb_noair_120208_002.ti	94	87	7	9.30E+00
Memb_noair_120209_002.ti	94	92	2	1.04E+01
Memb_noair_120210_002.ti	94	86	8	9.52E+00
Memb_noair_120213_002.ti	94	87	7	1.17E+01
Memb_noair_120214_002.ti	94	86	8	9.02E+00
Memb_noair_120215_002.ti	94	88	6	1.18E+01
Memb_noair_120216_002.ti	94	86	8	9.37E+00
Memb_noair_120217_002.ti	94	85	9	9.33E+00
Memb_noair_120221_002.ti	94	87	7	1.44E+01
Memb_noair_120222_002.ti	94	86	8	1.08E+01
Memb_noair_120223_002.ti	94	84	10	1.06E+01
Memb_noair_120224_002.ti	94	84	10	1.29E+01

TOC Results

Date	Day	Treated	Addition
11-Jan	1		4.226
12-Jan	2	4.534	
13-Jan	3	5.042	7.049
16-Jan	4	7.058	
17-Jan	5	6.961	9.058
18-Jan	6	7.688	
19-Jan	7	8.206	
20-Jan	8	8.152	9.823
23-Jan	9	9.089	
24-Jan	10	8.673	10.51
25-Jan	11	8.407	
26-Jan	12	6.9500	
27-Jan	13	7.1430	8.4630
30-Jan	14	8.3700	
31-Jan	15	8.2060	10.2800
1-Feb	16	8.8600	
2-Feb	17	8.6480	
3-Feb	18	8.6220	9.9800
6-Feb	19	9.2210	
7-Feb	20	8.7060	8.9220
8-Feb	21	9.1670	
9-Feb	22	8.8490	
10-Feb	23	9.1200	8.7660
13-Feb	24	8.9960	
14-Feb	25	9.4450	9.5040
15-Feb	26	7.3590	
16-Feb	27	7.4210	
17-Feb	28	7.4110	7.134
21-Feb	29	6.8920	6.655
22-Feb	30	7.0000	
23-Feb	31	7.0620	
24-Feb	32	7.1820	

Date	Minute	Conc
24-Feb	0	7.1820
24-Feb	1	8.0140
24-Feb	2	7.5560
24-Feb	3	6.9710
24-Feb	4	7.3240
24-Feb	5	7.2670
24-Feb	6	6.9370
24-Feb	7	7.2850
24-Feb	8	6.5100
24-Feb	9	7.2950

Calendar

Week	DAY	Day of Week	Date	Samples taken	NOTES
1	1	Wed	11-Jan	1	JUG 1
	2	Thurs	12-Jan	1	
	3	Fri	13-Jan	2	JUG 1
2	4	Mon	16-Jan	1	
	5	Tues	17-Jan	2	JUG 2
	6	Wed	18-Jan	1	
	7	Thurs	19-Jan	1	
	8	Fri	20-Jan	2	JUG 2
3	9	Mon	23-Jan	1	
	10	Tues	24-Jan	2	JUG 2
	11	Wed	25-Jan	1	
	12	Thurs	26-Jan	1	
	13	Fri	27-Jan	2	JUG 2
4	14	Mon	30-Jan	1	
	15	Tues	31-Jan	2	mix
	16	Wed	1-Feb	1	
	17	Thurs	2-Feb	1	
	18	Fri	3-Feb	2	JUG 3
5	19	Mon	6-Feb	1	
	20	Tues	7-Feb	2	TAP WATER
	21	Wed	8-Feb	1	
	22	Thurs	9-Feb	1	
	23	Fri	10-Feb	2	
6	24	Mon	13-Feb	1	
	25	Tues	14-Feb	2	
	26	Wed	15-Feb	1	
	27	Thurs	16-Feb	1	
	28	Fri	17-Feb	2	
7	29	Tues	21-Feb	2	
	30	Wed	22-Feb	1	
	31	Thurs	23-Feb	1	
	32	Fri	24-Feb	2	