



Wastewater Treatment and Analysis of Fixed Film Systems

A Major Qualifying Project

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Abstract

Biofilms are used in wastewater treatment plants to degrade organic particles. This project investigates the effect of shear forces and surface structure on biofilm formation and detachment in rotating biological contactors. Biofilm formation in a photocatalytic reactor, and on stationary titanium dioxide deposited slides is also studied. Biofilm growth is monitored through measurements of opacity, while its efficiency is measured through ammonium, chemical oxygen demand, total organic carbon, and ion chromatography tests, as well as ultraviolet-visible and fluorescence spectroscopy.

Table of Contents

Table of Contents

Cover Page	1
Abstract.....	2
Table of Contents.....	2
Authorship	6
Acknowledgements	6
1 Executive Summary	7
2 Background	11
2.1 Wastewater Treatment	11
2.1.1 Importance of Wastewater Treatment.....	12
2.1.2 Wastewater Quality Measurement	12
2.1.3 Overview of Wastewater Treatment.....	13
2.1.4 Preliminary and Primary Treatment	15
2.1.5 Secondary Treatment	15
2.1.5.1 Fixed Film Systems	16
2.1.5.1.1 Rotating Biological Contactors.....	16
2.1.5.1.2 Trickling Filters.....	17
2.1.5.1.3 Biological Aerated Filters	17
2.1.5.2 Suspended Film Systems	18
2.1.5.2.1 Activated Sludge System	18
2.1.5.2.2 Aerated Lagoon	19
2.1.6 Sewage Sludge Treatment.....	20
2.1.7 Tertiary Treatment	20
2.2 Biofilm	21
2.2.1 Biofilm Composition	21
2.2.2 Biofilm Growth	22
2.2.3 Biofilm Research	24
2.2.3.1 Antagonistic Interactions	26
2.2.3.2 Surface Structure and Shear Force	27
2.2.4 Common Biofilm Components	28
2.2.4.1 Pseudomonas aeruginosa	28
2.2.4.2 Thiosphaera pantotropha	29
2.2.4.3 Metazoa	30
2.2.4.4 Rotifers and Nematodes	30
2.3 Testing Techniques	31
2.3.1 Fluorescence Spectroscopy	31

2.3.2 Ultraviolet-Visible Adsorption Spectroscopy	32
2.3.3 Ammonium Test	33
2.3.4 COD/BOD	33
2.3.5 Total Organic Carbon (TOC) Test.....	34
2.3.6 Ion Chromatography	35
2.3.7 Compound Light Microscope	36
2.3.8 Scanning Electron Microscope	36
2.4 Photocatalysis.....	37
2.4.1 Titanium Dioxide	38
3 Methodology.....	40
3.1 Experiment A, rotating biological contactors	40
3.2 Experiment B, photocatalytic reactor	42
3.3 Experiment C, stationary titanium dioxide deposited slides	43
3.3 Greyscale Fortran Program	46
4 Results and Discussion.....	47
4.1 Results of experiment A, rotating biological contactors	47
4.1.1 Fluorescence Spectroscopy	47
4.1.2 Ultraviolet-Visible Adsorption Spectroscopy	49
4.1.3 Ammonium Test	52
4.1.4 Chemical Oxygen Demand Testing	54
4.1.5 Ion Chromatography	55
4.1.6 Opacity	58
4.1.7 Biofilm Dry Mass/Opacity Correlation.....	61
4.1.8 Compound Light Microscopy	62
4.2 Results of experiment B, photocatalytic reactor	63
4.2.1 Fluorescence Spectroscopy	63
4.2.2 Ultraviolet-Visible Adsorption Spectroscopy	64
4.2.3 Ammonium Test	66
4.2.4 Total Organic Carbon Testing.....	68
4.2.5 Ion Chromatography	69
4.2.6 Opacity	71
4.2.7 Microscopy (Scanning Electron and Compound Light).....	72
5 Conclusions	74
5.1 Effect of shear forces and surface structure	74
5.2 Effect of photocatalysis, titanium dioxide and UV light.....	75
6 Appendices.....	77
6.1 Figures.....	77
6.1.1 Experiment A, rotating biological contactors.....	77

6.1.1.1 Disk Shape.....	77
6.1.1.1.1 Flat	77
6.1.1.1.2 Grooved Concentric Circles	77
6.1.1.1.3 Grooved Webbed	78
6.1.1.2 Compound Light Microscopy	78
6.1.1.2.1 Disk 3, 10X	78
6.1.1.2.2 Disk 8, 10X	79
6.1.2 Experiment B, photocatalytic reactor	80
6.1.2.1 Compound Light Microscopy	80
6.1.2.1.1 Slide 1, 10X, PC500	80
6.1.2.1.2 Slide 2, 10X, PC500	80
6.1.2.1.3 Slide 3, 10X, P25	81
6.1.2.1.4 Slide 4, 10X, P25	81
6.1.2.1.5 Slide 5, 10X	82
6.1.2.1.6 Slide 6, 10X	82
6.1.2.2 Scanning Electron Microscopy	82
6.1.2.2.1 Ion Composition, Slide 1, PC500	83
6.1.2.2.2 Ion Composition, Slide 2, PC500	83
6.1.2.2.3 Ion Composition, Slide 3, P25	84
6.1.2.2.4 Ion Composition, Slide 4, P25	84
6.1.2.2.5 Image, Slide 1, PC500	85
6.1.2.2.6 Image, Slide 2, PC500	85
6.1.2.2.7 Image, Slide 3, P25	86
6.1.2.2.8 Image, Slide 4, P25	86
6.2 Procedures	87
6.2.1 Housekeeping for Rotating Biological Contactors	87
6.2.2 Synthetic Substrate Preparation.....	91
6.2.3 Biofilm Scanning	91
6.2.4 Preparation of Titanium Dioxide Deposited Glass Slides	93
6.2.5 Ammonium Measurement.....	94
6.2.6 Chemical Oxygen Demand Measurement	95
6.2.7 Ultraviolet-Visible Adsorption and Fluorescence Spectroscopy.....	96
6.2.8 Total Organic Carbon and Ion Chromatography Measurement	97
6.2.9 Biofilm Dry Mass.....	97
6.2.10 Microscopy.....	98
7 Bibliography	99

Authorship

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1 Executive Summary

Wastewater treatment plants have been around for centuries, working to efficiently clean and reduce pollutants found in wastewater. Wastewater, which contains substances such as oils, detergents and food scraps, as well as organic and inorganic compounds that may potentially pose health problems for humans, must be purified before it can be reintroduced to the environment. Through a four step process the wastewater is filtered, organic molecules are degraded, ammonium is nitrified and other harmful chemicals are removed. As water continues to become scarcer in many areas of the world, it becomes increasingly more necessary to efficiently clean available wastewater.

The secondary step of wastewater purification is the most pertinent to this report, as it directly deals with the use of bacteria and microorganisms to degrade the organic and inorganic compounds. There are many approaches to secondary treatment, although activated sludge is the most commonly used (54). This technique which uses free floating bacterial flocs to degrade the organic compounds, is widely available, however, they take up a large amount of space resulting in extremely large wastewater treatment plants.

The biofilm, which may be composed of bacteria and any combination of microorganisms, has the ability to form in most any aqueous environments that can provide the microbes with the essential nutrients to survive. These colonies adhere to each other and to a fixed surface, which initiates the excess production of extracellular polymeric substance, EPS. The gene responsible for the production of EPS is immediately up regulated and is then excreted to enclose the biofilm. This provides the cells with an external barrier against biocides, harmful environmental conditions, and other microorganisms, making the biofilm more robust and efficient (5).

The first experiment; dealing with the rotating biological contactors (experiment A), was set up to focus on two variables and their overall effect on a growing biofilm. The first property tested was shear force due to rotational speed. Two rotating biological contactors were assembled, rotating 5 circular disks at two different speeds, 4 rpm and 40 rpm. Both of the contactors were given equal amount of nutrients, were identically cleaned and prepared and each had samples taken daily for testing. The goal was to see which contactor had more efficient biofilm growth, as well as which biofilm was the most efficient in regards to nitrification and oxidation.

The other property that was being tested was that of the surface structure. In each of the contactors, the five circular disks were arranged in identical order, with a flat surface disk, two grooved concentric circle disks, a grooved webbed disk, and finally another flat surface disk. It was hypothesized that the most heavily grooved disk (webbed) would provide the best surface for the biofilm due to the larger surface area provided through the grooving.

The samples collected from freshly prepared substrate and substrate from the two contactors was tested by ultraviolet-visible spectroscopy, fluorescent spectroscopy, ion chromatography, chemical oxygen demand (COD), and Nessler's reagent. These test for the properties of nitrate concentration, soluble COD concentration, ammonium concentration, and the compound composition of water samples, specifically phosphate, nitrite, and nitrate. The opacity was recorded using an Epson scanner and analyzed. The use of a light microscope was also used in order to observe the density of the biofilm on each of the 10 disks.

A reactor was also used in a separate experiment, experiment B, testing the photocatalytic properties of titanium dioxide and its ability to prevent biofilm formation in the presence of ultraviolet light. This reactor was composed of six glass slides, of which two had titanium dioxide P25, two had titanium dioxide PC500 deposited on them and the other two were bare.

The reactor was split into two parts, each of which had one of the three glass slides in identical order; one part was exposed to UV light at 365nm while the other part was shielded from the UV light. Clarified wastewater was run over the six glass plates.

Samples were also collected from this reactor and were tested by ultraviolet-visible spectroscopy, fluorescent spectroscopy, total organic carbon (TOC), Nessler's reagent, and ion chromatography, testing for concentration of nitrate, nitrite, ammonium, phosphate, and organic compounds. These slides were also imaged using the Epson scanner, and studied through light and electron microscopy to determine if biofilm was developing as well as any other ion formation.

The third experiment, experiment C, which consisted of stationary titanium dioxide deposited slides upon which biofilm was grown. There were 36 slides, 18 of which were coated in titanium dioxide, 18 of which were left uncoated. These slides were placed at the bottom of wells on two 24 well plates, with each plate getting 9 titanium dioxide coated slides, and 9 uncoated slides. On six of the slides (three titanium dioxide and three uncoated) biofilm was grown using wastewater from right before the secondary stage of wastewater treatment. On another six of the slides (three titanium dioxide and three uncoated) biofilm was grown using wastewater from after the settling tanks after the secondary stage of wastewater treatment. On the other six slides tap water was used to grow the biofilm. One of these 24 well plates was exposed to UV light at 350nm, while the other plate was exposed to indoor lighting. After biofilm growth, these slides were imaged through light microscopy and stained with crystal violet and safranin to determine biofilm attachment.

Photocatalysis using titanium dioxide deposits shows much promise as a technology that can be used for wastewater treatment to breakdown organic compounds that are non-

biodegradable. The build-up of biofilm upon the photocatalytic media would reduce efficiency. This study was inconclusive, as to whether or not there would be a noticeable growth of biofilm on any of the glass slides, due to its short time span.

2 Background

2.1 Wastewater Treatment

Wastewater contains solids, metals, biodegradable organic compounds, pollutants (such as organic and inorganic compounds that are carcinogenic, mutagenic, or teratogenic, nutrients, such as ammonium and phosphorous, and microorganisms, such as bacteria, protozoa, helminthes, rotifers, and viruses (53). Sewage wastewater specifically is high in nutrients, microorganisms, and biodegradable organic compounds. In developed countries, wastewater is treated either on site or through a municipal wastewater treatment facility, where the wastewater is routed to the facility from homes and businesses through a series of pipes (54). In some undeveloped countries, there are no wastewater treatment facilities, and this presents a number of problems such as health concerns and eutrophication. The goal of wastewater treatment is to free the wastewater of contaminants; to a degree of purity where it can be reintroduced into the water cycle without many negative repercussions. The regulations for the quality of the treated water varies throughout different locals, with guidelines being set by the EPA and each state's respective Department of Environmental Protection. Reclaimed wastewater is a second type of treated wastewater. It is wastewater that has gone through some but not all of the rigorous treatment processes that fully treated wastewater has, but has non-potable uses such as irrigation, as toilet water, and for fire suppression or cooling towers (52). Unlike fully treated wastewater, which is usually discharged into surface streams (56); these uses introduce the reclaimed water further from areas where the eutrophication resulting from the excessive nutrients is a problem.

2.1.1 Importance of Wastewater Treatment.

Without proper wastewater treatment the pollutants in the water present problems when reintroduced to the water cycle. Eutrophication is an excessive buildup of nutrients that results in excessive plant growth. This growth cannot be supported by the ecosystem, so the plants die. When this happens the bacteria that decompose the plants deoxygenate in the water, creating a hypoxic environment, causing large aquatic species to die, and which the same bacteria also decompose (51). Another issue is undegraded chemicals that make their way into the water table, such as drugs, or organic compounds that are carcinogenic, mutagenic or teratogenic, which have varying effects on all species reliant on that water supply. Waterborne diseases are also prevalent anywhere without proper wastewater treatment (55).

2.1.2 Wastewater Quality Measurements.

There are many tests that treated wastewater undergoes to be deemed acceptable for usage. These can be broken down into chemical tests and physical tests. Some common physical tests are Total Dissolved Solids (TDS), Total Suspended solids (TSS), Fluorescence Spectroscopy, Ultraviolet-Visible Spectroscopy and Turbidity measurements. Common chemical measurements for water quality include Biological Oxygen Demand (BOD), Volatile Suspended Solids (VSS), Total Organic Carbon (TOC), Chemical Oxygen Demand (COD), Ion Chromatography, pH tests, and ammonium measurements using Nessler's Reagent.

Each one of these tests check for some amount of pollutant in the wastewater. BOD for example, is a measurement of how much oxygen is used by aerobic bacteria when breaking down the organic compounds that remain in the wastewater (3). TDS, TSS, and VSS are all quantitative measurements of how much suspended or dissolved solids there are in the water. TDS represents the overall level of organic and inorganic matter in the water, it is a measure of

how clean the water is overall It a measure of the weight of solids that would pass through a 1.2um filter with the water and generally consists of calcium, phosphates, nitrates, sodium, potassium, and chloride (5). TSS, is a measure of larger particles than those in TDS, but these particles are suspended in solution but could be filtered out with a 1.2um pore-sized filter (5). VSS consists of solids that can be removed by firing at 550C (5), Turbidity, Fluorescence Spectroscopy, and UV Spectroscopy qualitatively measure suspended solids, organic compounds, and nitrate, respectively in water. TOC and COD are both used to either directly or indirectly, respectively, measure the amount of organic compounds in water; TOC is a measurement only of the organic compounds while COD measures all of the oxidizable matter. Ion Chromatography measures all of the charged particles in the water quantitatively. Nessler's reagent is used to measure the ammonium in the water quantitatively. The pH of pure water is neutral, so bringing it as close as possible to that pH, is best for plants, and taste.

2.1.3 Overview of Wastewater Treatment.

The four stages of wastewater treatment can be combined reducing the number of treatment steps, and they are in facilities all over the world. The treated water is discharged into surface water. If treated properly, the water should not cause eutrophication, or disease. An overview of the stages of treatment:

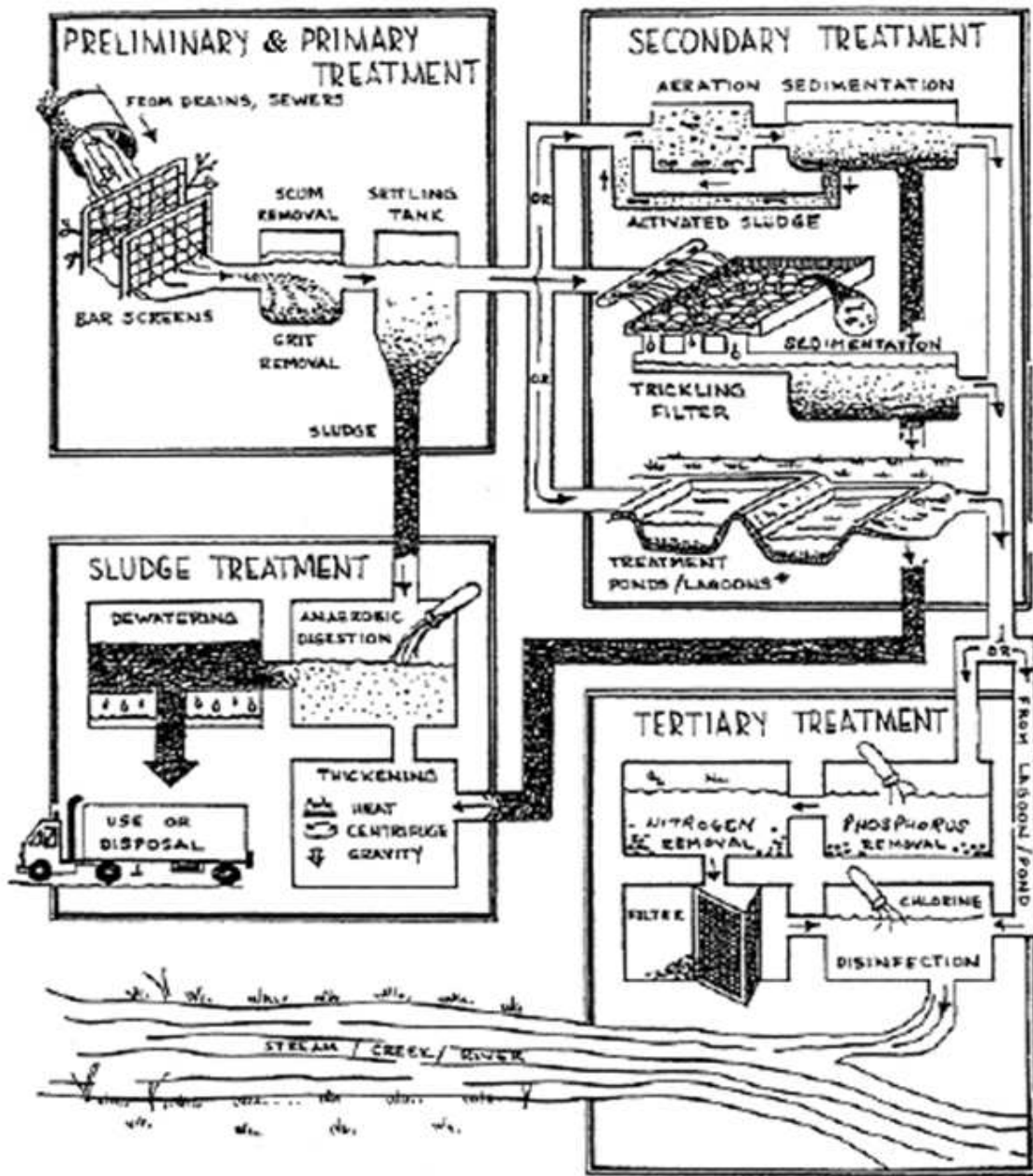


Image from : (Wastewater Treatment)

2.1.4 Preliminary and Primary Treatment.

All wastewater treatment processes have the same goal in mind. There are four main stages of treatment. This is sometimes reduced in systems which combine two or more stages of treatment into one stage. The first of these stages is referred to as Preliminary Treatment (25). In this stage of treatment large solids are removed from the wastewater. This can consist of gravel, sticks, large food particles, rags, etc. These materials are removed using bar screens, grit chambers, or macerators (25) and then the debris are taken to landfills and disposed of there (24).

The next phase of wastewater treatment is the primary phase. First the sewage is settled, in settling tanks, for example clarifiers or septic tanks (25) so that a large amount of the suspended solid waste material can be removed, over the course of two hours in the settling tanks, around 50% of the suspended solids are removed in a Winnipeg facility. Oils that make their way into the wastewater are skimmed off of the top of these clarifiers and sent to landfills (24). The solids are called Primary Sludge and are sent for further processing downstream. The liquids called partially clarified liquor (21) are sent for secondary treatment.

2.1.5 Secondary Treatment.

In the secondary phase of wastewater treatment, the water flows over the settlers and into another tank or tanks where it is mixed with oxygen or air and digested using a biological treatment process (24). The most popular options for biological treatment are fixed film systems, either rotating biological contactor, trickling filter, biological aerated filter or suspended film systems, such as activated sludge and lagoon systems (25). All of these utilize rich ecosystems of bacteria, protozoa, helminthes, rotifers, and viruses to digest the waste. The treatment plant speeds up the degradation of the sewage by creating optimal conditions for the

micro-organisms that decompose the sewage to do so. Both fixed film and suspended film systems refer to systems where the creation of biofilms is encouraged to protect the digesting bacteria.

Each of these systems has its benefits and downsides, and the decision as to which biological treatment process is best for a given municipality or on-site treatment facility depends on the amount of land that is available, the amount of water to be treated, the soil type, the proximity to a body of water, and the size of that water, amount and variability of wastewater to be treated, and expense.

2.1.5.1 Fixed Film Systems

2.1.4.1.1 Rotating Biological Contactors

Rotating Biological Contactors (RBCs) consist of closely spaced, slowly rotating, plastic disks on a shaft which is moved by a motor. Micro-organisms grow on the disks, forming a biofilm, and oxidize the organics and ammonium (7). During the rotation about 40% of the surface area of the disks is submerged (39). The aeration required for aerobic bacterial growth is provided by the rotation, and a large population of bacteria is available for the degradation of contaminants. The biofilm size is controlled by the shear forces of rotation. This is advantageous, because it prevents overgrowth of the biofilm which could limit oxygen to the aerobic bacteria. Sheared off excess biomass is removed through a settler or clarifier. RBCs are simpler to operate (32) than activated sludge systems, and have longer contact times between the bacteria and the pollutants than trickling filters (8). The start-up cost for RBCs is high, but the maintenance and energy costs are low (7).

2.1.5.1.2 Trickling Filters

Another fixed-film system is a trickling filter. A trickling filter is composed of a fixed bed of rocks, gravel, slag, sphagnum, peat moss, plastic media or another highly permeable media (28). An optimal filter medium would be one with high surface area per unit volume and a large amount of void space, since clogging with biofilm can cause ponding (28). The trickling filter works through the formation of a biofilm on the media which degrades the organic compounds and ammonium in the wastewater. The wastewater is fed to the top of the trickling filter using a pump and collected at the bottom in a sump (40). Oxygen is made accessible to the bacteria through the use of a fan or solely through natural convection. As the biofilm continues to grow, an anaerobic section of the filter is formed, since the oxygen cannot diffuse through the thickening biofilm well (50). Parts of the biofilm slough off as it grows in size, and this slough is clarified or settled out after being collected at the sump (14). Trickling filters are advantageous for small to medium communities that do not have a lot of land to dedicate to wastewater treatment.

2.1.5.1.3 Biological Aerated Filters

Biological Aerated Filter (BAF) is another form of fixed-film sewage treatment. BAFs generally contain a small sized filter media, composed of clay, schist or plastic (31). On this media (22) microorganisms grow just as in the RBC or trickling filter systems. Both the oxygen source and the sewage enter the BAF from the bottom of the container. As the sewage makes its way up the container the organic compounds and ammonium are degraded. When it reaches the top, it has successfully been treated, with very low ammonium levels (44). This process of secondary treatment does not require the use of another clarifier like the activated sludge, RBC, or trickling filter systems, because the solids are collected in the filter and the filter is

backwashed every few days, and the backwash is allowed to settle out in the primary settler, and then returned to the BAF for processing. Regardless of the hydraulic retention times, the BAF provides stable nitrification (37). The BAF is good solution in communities where the footprint of the treatment plant must be small, and is has the potential for wide variations in loads (31). Water must be backwashed every few days so that the pressure drop does not become unacceptably large, but this system does eliminate the need for a secondary clarification process.

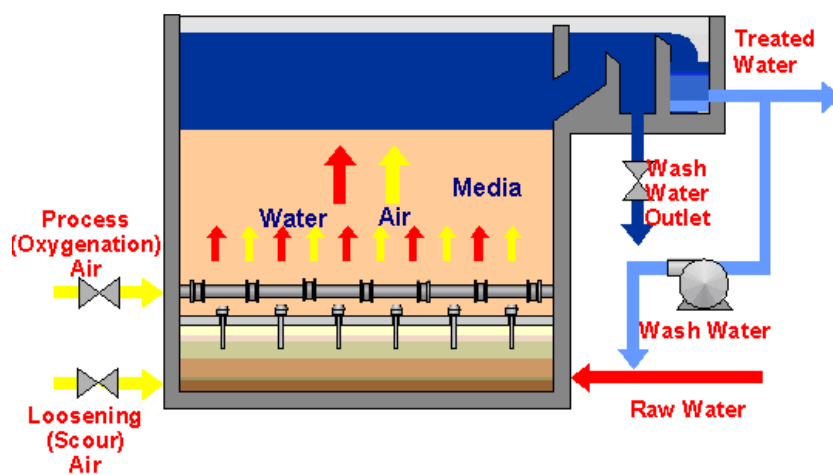


Image from (44)

2.1.5.2 Suspended Film Systems.

2.1.5.2.1 Activated Sludge System.

Activated sludge systems are the most frequently used systems (44). These systems require large amounts of surface area and reactor volumes (31), react slowly to high load variations, and have a tendency towards bulking and foaming. Bulking and foaming are caused when the population of filamentous bacteria that are normally present in the sludge increases beyond a certain point (Sludge Volume Index $>150\text{ml/g}$). This causes issues with settling, and

also the creation of stabilized gas bubbles, respectively (12). To control these problems the filamentous bacterial population must be kept under control.

In an activated sludge system, the micro-organisms are present in flocs (12). These flocs consist of bacteria, amoebae, spirotrichs, peritrichs, rotifers, and other organisms that are suspended in the water. These flocs develop spontaneously with the aeration of the water (12). This aeration also mixes the flocs and the water and provides oxygen for the aerobic breakdown of organics and ammonium. These flocs are a complex ecosystem which feeds on the sewage and reduces its biological content. These bacteria reduce the organic content of the water. The sludge must be separated from the treated water; this is done through the use of a clarifier or settler. Once the sludge has been settled out, some of it is sent back to the head of the reactor, to seed the incoming primary sludge, while the rest is sent downstream for further processing. After the water has been treated by the biological organisms to a sufficient degree it is sent for tertiary treatment.

2.1.5.2.2 Aerated Lagoon.

Another kind of suspended system is aerated lagoons, which have floating surface aerators. They work by the same principle as activated sludge systems in that the aeration transfers air into the water for aerobic bacteria to thrive, and also causes mixing. The retention times in aerated lagoons are much longer than for activated sludge systems because there is far less mixing. These systems take a lot longer than the other options and require a lot of land, but require much less manpower or energy to operate. They also have far less sludge disposal needed (13). These systems are good for small communities as well.

2.1.6 Sewage Sludge Treatment.

The sewage sludge originating from the primary settlers and secondary treatment phases must be treated before disposal. First, the sludge must be degraded under aerobic or anaerobic conditions to break down more of the organic compounds. Many plants use anaerobic digestion in this phase because it does not require costly aeration and because it produces Biogas, a mixture of methane and carbon dioxide, which many plants use to generate electricity. (24) The sludge is broken down using thermophilic (55C) or mesophilic (36C) bacteria. Then the waste sludge must be dewatered using centrifugation or heat (25), the water which is high in ammonium content is returned to the beginning of the secondary treatment reactor, while the dried solids are treated with caustic chemicals or heat to kill the bacteria in them and are then used as fertilizer in many locals due to their high nutrient content (24, 25).

2.1.7 Tertiary Treatment.

The last phase in wastewater treatment is called tertiary treatment and is used to raise the water quality. This is done through filtration, further biological treatment, and/or disinfecting the wastewater using chlorine or UV light or ozone (25). Chlorine is very effective for removing microbial pathogens, but needs special care considerations (11), and forms Trihalomethanes (THMS), carcinogenic compounds, in the presence of humic and fulvic acids (30), chlorination also allows for the water to remain disinfected while in the pipes. As a result, chloramines are used more frequently now because they are more stable and less liable to break down into THMS. Other disinfectant technologies are ozonation and UV light. Both of these have no ability to keep the water disinfected while in the pipes, so a secondary disinfectant must be used. Filtration can also be used since some parasites are not as susceptible to the toxicity of chloramines or chlorine. Other end stage treatments include an anaerobic phase of digestion

which denitrifies the water turning the nitrates generated from the breakdown of ammonium into nitrogen gas, or phosphorous coagulation and precipitation, a chemical process to remove excess phosphorous from the water (42).

2.2 Biofilm.

Bacteria have been around for billions of years thriving in various forms found throughout the world. Originally, bacteria had been studied in planktonic form, observing the characteristic of individual organisms floating in aqueous environments. It wasn't until the 1970's (and advanced microscopic technology) when scientists discovered that there are alternate forms, more specifically, biofilm. Not only did they determine that bacterial colonies existed in this form, scientists were now able to proclaim that it was, in fact, the predominate form. After this discovery, scientists have been utilizing bacteria's ability to grow biofilm in the fields of medical care and wastewater treatment.

2.2.1 Biofilm Composition.

Simply stated, biofilm is a population of bacteria, algae, yeast, fungi, protozoa or a combination of all of these that adhere in an aqueous environment to each other and to a solid surface. These clusters of organisms excrete a sticky, slimy substance that allows it to proliferate and remain intact to the fixed surface. This substance has been termed extracellular polymeric substances or exopolymersaccharides (EPS) and as the colonies develop, they release this matrix of polysaccharides, proteins and phospholipids (45). Biofilms have the ability of growing on any solid surface. They are not limited to living surfaces, such as in the tissues within the human body, but also nonliving entities including the rocks at the bottom of a riverbed, metals, plastics or even glass.

Although biofilm can form on essentially any solid surface in an aqueous environment,

there are several conditions that are necessary to produce a healthy, sustainable biofilm. Biofilms are composed primarily of living bacteria and providing a steady temperature and the proper nutrients is extremely vital for their development (45). The constant replenishing of nutrient, primarily carbon and nitrogen will provide the biofilm with the best opportunity to grow and thrive. This is especially important in synthetically developed biofilm for use in either a laboratory or in industry.

2.2.2 Biofilm Growth.

Bacterial colonies that grow as biofilm are genetically favorable over free-floating bacteria. As part of a colony, these bacterial organisms have an easier time avoiding harmful substances and protecting themselves against agents that may otherwise destroy the bacterial cell. The extracellular polymeric substance works to protect bacteria against the environmental toxins, creating a biological shield that blocks harmful substances from being absorbed into the pores of the growing cells (2). For this reason, biofilms are able to grow quickly and efficiently.

The formation of biofilm can be categorized into five stages, the conditioning layer, bacterial attachment, formation/EPS production, biofilm maturation and finally, detachment (3). Before any colonization can occur, free-floating bacteria, other microorganisms or debris must first come into direct contact with a fixed, solid surface. These cells form the conditioning layer of the biofilm. This interaction causes the newly attached bacteria's behavior to change, allowing the colonized cells to immediately begin producing EPS, which then allows other bacterial cells to adhere. The interaction between the bacteria and the fixed surface is mediated by hydrophobic interactions (23). Whether the biofilm is forming within a living system, allowing proteins, leukocytes and fibrin to condition the biofilm foundation, or if it is the collection of debris found in the aqueous environment, the newly attached bacterial cells are now ready to proliferate and

reproduce.

Bacterial attachment sparks the initiation for the upregulation of the EPS producing genes, allowing the colony to grow exponentially. This begins the maturation state and cells are reproducing regularly and maturing on the surface of the biofilm. This process has also been described as ‘irreversible attachment’, meaning that the microbes can no longer move away from the surface. These microbes are now intact and it has been determined that mature biofilms are cemented to their place until the final stage of growth (23). At this point, the film can now grow thicker as well as expand over a larger surface area. Naturally, some biofilms will remain just a few cells thick, such as dental plaque or on a surface such as a kitchen counter, whereas others can grow to be a several millimeters thick, similar to the biofilm found on top of rocks in a riverbed. In either case, the biofilm grow in accordance to the environmental, biochemical or hydrodynamic conditions, as well as the availability of nutrients.

The growth and maturation of the biofilm is regulated by hormone like signals, diffusible extrasignalling molecules composed of homoserine lactones and oligopeptides (23), produced by the biofilm cells in response to the environmental conditions. These signals allow the colony to ‘communicate’ and monitor their own population growth and toxin production, among others. The following image is a depiction for the development of biofilm growth process.

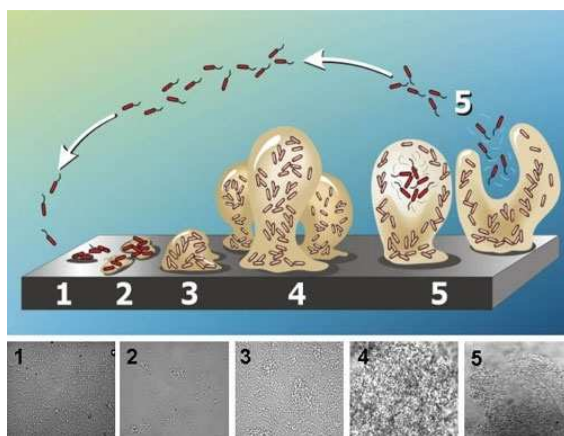


Image from (27)

It typically takes the colonies about 1-2 weeks in order reach a mature state. After maturation, the final stage, detachment, occurs. At this point, usually as a result of environmental forces, the top several layers of the biofilm is ripped from the surface and released back into the aqueous environment. Hydrodynamic and shear forces are usually the culprit for this process. As the cells extend upward, there is a much stronger hydrodynamic force as the aqueous solution flows over the surface (6). When the force becomes too strong, the bonds break and large fragments are broken off. An adherent layer of cells is maintained on the bottom surface however, and the growth process begins once again. In areas, or environmental conditions where these forces are not strong enough to cause significant amounts of detachment, the biofilm will undergo 'shedding', a process in which daughter cells are released from the biofilm. Fewer cells are lost at one time, however, the principle is the same in which the biofilm loses its top layers and is left with the conditioning layer on the fixed surface. The process will then repeat itself and more layers of biofilm will grow at the location of the shedding.

2.2.3 Biofilm Research

Significant research has been performed in order to determine which parameters (bacterial strains, surface structure and nutrient availability) provide for the most efficient growth of biofilm. In a study performed by Andersson *et al*, 13 strains of different bacterium were studied and assessed for the properties of initial adherence and production of extracellular polymeric substances. Four different culture media were used, wastewater, acetate medium, glucose medium and diluted nutrient broth in order to determine the nutrients that would best aid in biofilm production. The conditions in the laboratory were all held constant (2).

With the biofilm thickness analyzed by crystal violet microtiter plate assay, it was

determined that the acetate medium contained the necessary nutrients and carbon sources to encourage strong biofilm growth. These biofilm were able to thicker films and cover a larger surface area without significant disturbance. Wastewater and glucose medium also provided a good baseline for biofilm growth. Diluted nutrient broth, however, resulted in very weak and generally inexistent growth. It was determined that there were not enough nutrients to sustain all the cells in the colonies, significantly impacting their ability to grow to an appropriate thickness and proliferate successfully. During this experiment it was also determined that the culture time did not affect growth for the majority of the bacteria strains. All medium encouraged growth during the same time period, just the thickness and maturation of the biofilm was varied (2). The results from this initial experiment cemented the hypothesis that accessible nutrients are vital to the growth of biofilm. This becomes increasingly important in industrial use where biofilm is created in an artificial environment and must be continually monitored for healthy cells.

Diverse bacterial organisms have many different properties that allow them to grow healthier and stronger in variable conditions. In regards to initial adherence, Andersson *et al* was able to confirm properties of several common bacterial strands in regards to their adherence to polystyrene surfaces created in a laboratory setting. In particular the bacteria strain, *P. aeruginosa*, was found to be one of the most versatile strains forming uniform biofilm with moderate production of EPS on all surfaces that were being tested, not just the polystyrene surface. These results confirmed the hypothesis that different strains of organisms would be able to bind and efficiently proliferate on different medium. This experiment also showed that the formation of a biofilm is universal property among all of the tested strains of bacteria (2). This confirms the declaration that the biofilm is the predominate form of bacteria.

The most significant of the results however, was discovering the effect of dual-strain and

multi-strain biofilm and their ability to adhere and proliferate on a variety of fixed surfaces. It was observed that the strongest overall biofilm formation occurred when a mixture of all 13 bacteria were grown and cultured together. The synergistic effect that appears as species begin to grow together is important in formation. This allows the organisms to work together and while one strain of bacteria is poor at initial adherence, they may be vitally important in the EPS production.

Similarly, Andersson *et al* tested the effects of dual strain bacteria. In one particular case, they found that one particular strain of bacteria that were unable to form biofilm due to an inability to initially adhere to the fixed surface. By coupling that bacterium to a strain that was capable of adhering, they were able to observe the formation of very strong biofilm, again illustrating the synergistic effects of bacterial organisms. Although there is no way to determine which organisms will thrive when cultured together, it confirms the hypothesis that the organisms work together (2). What makes all this possible is the vital characteristic of the EPS, encasing the bacteria in a protective coating. When multiple strains are cultured together, the EPS becomes thicker, allowing the cells an even more efficient extra layer of protection from antibiotics and other harmful agents (6).

2.2.3.1 Antagonistic Interactions

Antagonistic interactions were also observed by Andersson *et al*. This was seen when two organisms that were originally capable of producing efficient single-strain biofilm were cultured together, it resulted in no biofilm formation. In particular, *B. cereus* SJV and *B. denitrificans* B79 completely stopped biofilm growth when mixed with *A. calcoaceticus* (2). It is impossible to determine which properties caused the halting of the biofilm formation but it is known that there must be certain properties that regulate the process.

Biofilm that is found in nature, either in riverbeds or in the human body are most often comprised of a variety of organisms. The guess work of matching compatible organisms is the next step towards manufacturing a biofilm that is most efficient for the degradation of organic particles found in wastewater treatment plants (2).

2.2.3.2 Surface Structure and Shear Forces

The basic structure of a biofilm can be broken down into two parts, the base film and the surface film. The base film, in which bacteria cells adhere to a solid surface in the aqueous environment, is generally found to be closely packed and continuous, where as the surface film is more discontinuous. Because the base of the biofilm sets the framework, different materials have been used to encourage the efficient growth and maintenance of the biofilm (6).

In regards to wastewater treatment, biofilm that have been cultivated on a porous medium have been found to aid in its growth. With the general goal of degrading organic materials, the ability for nutrients, water and waste to get filtered through the bacterial biofilm is necessary for efficiency. While this information has aided laboratory experiments in growing substantial biofilm, both in surface area and thickness, there are still other parameters that encourage efficiency for wastewater treatment (38).

Shear forces that the biofilm come into contact when growing have also been determined as a significant factor in the efficiency for nitrification rates. High shear forces improved the nitrification rates by reducing the overall thickness of the biofilm. Lower EPS accumulation observed in thinner biofilm allowed for higher nitrate removal from the wastewater (6). This information follows logically because the EPS works as a protective barrier. The more permeable the outer layer, the more ammonium can enter to become oxidized into nitrate.

These results were attained by experiments using a hollow-fiber membrane biofilm

reactor (MBfR) in wastewater treatment plants to determine the amount of ammonium removal from the water. Controlling the structure of the biofilm is the most vital factor affecting MBfR efficiency. Throughout this processes, it was found that the higher velocity the MBfR rotated in, the greater the decrease in boundary layer thickness which led to a higher substrate flux. As greater shear forces are acting on the biofilm, they become more compact than those created under lower shear conditions (6).

The EPS is important because it maintains the structural integrity of the biofilm matrix. The EPS is composed primarily of polysaccharides and proteins, along with lipids and nucleic acids and it has been suggested that it is the composition of the EPS that is more significant than the actual thickness of the layer (6). A shear force that is too strong will negatively impact the biofilm, causing it to overproduce EPS, thus lowering efficiency and water flow. It is necessary to find the appropriate ratio of proteins and polysaccharides to get a balanced and stable structure (17).

2.2.4 Common Biofilm Components.

Biofilm composition can be varied depending on the location of the biofilm, the availability of nutrients and the present environmental conditions. There are some common organisms that are found in biofilms, including two bacterial strains *Pseudomonas aeruginosa*, *Thiosphaera pantotropha*, as well as other microorganisms including metazoa, rotifers and nematodes.

2.2.4.1 *Pseudomonas aeruginosa*.

Pseudomonas aeruginosa are a type of bacteria that are commonly found as members of a biofilm. These bacteria are gram-negative, aerobic and rod shaped belonging to the family Pseudomonadaceae. Generally speaking, *Pseudomonas aeruginosa* can exist as a free living

bacterium, found in both water and soil environments, however, the typical *pseudomonas* bacterium will be found in a biofilm attached to a fixed surface. One reason for its ability to form biofilm is that the bacterium has simple nutritional requirements, satisfied in the laboratory with acetate as a source of carbon and ammonium sulfate as a source of nitrogen (47). Another aspect of this bacterium that makes it so versatile is its great tolerance for environmental conditions. These bacteria can survive temperatures in the range of 37 – 42 degrees Celsius, it is virtually resistant to high concentrations of salts, weak antiseptics and many commonly used antibiotics. This keeps the biofilm healthy, proliferating and maturing into efficient biofilm. In the laboratory it has been found that it takes *P. aeruginosa* 5-6 days to achieve its maximum thickness before detachment occurs, which is a relatively short amount of time to mature (2). These natural properties are what allow this bacterium to have such a great ecological contribution as an opportunistic pathogen.

2.2.4.2 Thiosphaera pantotropha.

Thiosphaera pantotropha is another type of bacterium that is capable of heterotrophic nitrification and aerobic denitrification, thus it is commonly found within laboratory biofilm (35). Many types of bacteria, including *T. pantotropha*, are able to nitrify if they are supplied with a source of energy and the proper nutrients. This allows the bacteria in the biofilm to successfully oxidize the ammonium within the wastewater as the growth rate of the biofilm increases. *T. pantotropha* was also shown to have high levels of nitrifications under a variety of growth conditions, suggesting that the bacteria are sustainable in several different environments (35).

2.2.4.3 Metazoa.

Metazoa is a multicellular organism, which has been known to consume unicellular or filamentous bacteria. Because of these characteristics, it has been found that ciliates and metazoa can flourish in biofilm, gaining nourishment from the bacterial cells that are both free-floating in the aqueous environment, or directly from the biofilm itself. Metazoa have the ability to alter their communities in an aqueous environment based upon the operating conditions present. This makes them a very good candidate for use in a biofilm because the environment of a wastewater treatment plant changes readily and rapidly. Also, changes in the inflow from artificial to municipal wastewater provoked a large increase in the percentage of metazoan. This organism, which feeds primarily on the bacteria of the biofilm, helps to remove their outer layer of cells and allowing the biofilm to proliferate (15).

2.2.4.4 Rotifers and Nematodes

Rotifers and Nematodes are generally found in biofilm as microorganisms who feed on bacterial cells. Rotifers exist in the biofilm by attaching themselves to the fixed biofilm. Rotifers can move through the techniques of jumping, swimming or inch worming, however, many rotifers can also fix themselves in one location for an extended period of time granted there are sufficient amounts of nutrients. The corona, which is attached to their mouth, acts similarly to a wheel and causes a current to sweep the bacteria into their pharynx, where the food is chewed (36). This process helps keep the bacterial population healthy by eating off the dead cells and allowing new cells to regrow in their places. Nematodes are also located in the biofilm eating the bacterial cells, as well as the other small protozoa and metazoa. The number of nematodes is much less numerous than the rotifers in the laboratory cultured biofilm.

2.3 Analytical Techniques to Characterize Biofilm Properties.

Several testing techniques are necessary in order to determine the efficiency of the biofilm. The opacity measurement, taken by a flat bed scanner with an 8-bit grey scale, will provide the most concrete data for the progression of biofilm growth over the duration of the experiment. Other tests, which are performed by taking samples of the synthetic substrate as well as the discharge in the RBC tanks, will provide the results for the efficiency of the biofilm.

2.3.1 Fluorescence Spectroscopy.

Electrons favor the lowest level of energy, generally referred to their ground state and it is in this ground state that they are the most stable. When a beam of light is sent through the molecule, the valence electrons are then elevated to their excited states as they gain more and more energy from the light. The energy in this system is found in fixed amounts of quanta, which then results in distinct absorption bands on the spectroscopy. Once the molecules are elevated to their excited states, they rapidly fall back down to their ground state, releasing the energy in the form of fluorescence (1).

The fluorescence spectrometer is a machine that contains a broadband light source, a monochromator, a sample cell, an appropriate grating, a diode array sensor and associated electronics. Light passes through the monochromator which begins the process of exciting the electrons within the molecules of the sample. The excitation light is focused onto the sample contained in a suitable cell and the fluorescent light is focused onto the diode array sensor. The monochromator is programmed to record the fluorescence spectrum of the solute at a fixed excitation wavelength, or as a range of wavelengths (16). The fluorescence spectrometer is very

efficient; however, emission and fluorescence spectra do not give concrete results for structure identification, they instead are primarily used to confirm substance identity.

2.3.2 Ultraviolet-Visible Adsorption Spectroscopy.

Ultraviolet and visible adsorption spectroscopy is used to determine the components of a sample by passing a beam of light through the sample and measuring the wavelength that is reflected. With the use of the Beer-Lambert Law it is also possible to determine the concentration of the analyte for which you are testing. The range of wavelengths that a UV-visible spectrophotometer can record is 200nm-600nm (43). For wastewater samples, the wavelength that is most important is that at 215nm, which correlates to the presence of nitrate. Nitrate, which is formed by the oxidation of ammonium as a result of the biofilm found in solution, is an important indicator on whether the biofilm is working efficiently.

The ultraviolet and visible adsorption spectroscopy works by sending two beams of light through the sample, usually a deuterium discharge lamp for UV measurements and a tungsten-halogen lamp for visible measurements (43). While the switching of the lamps occurs, there is generally a small peak that results on the reading (around 400nm) and can be overlooked during the analysis. The wavelengths of these beams of light are dispersed by a holographic grating in a single or double monochromator or spectrograph before it is sent to the sample. The wavelength is then determined by the monochromator slit width or by the array-element width in array-detector spectrometer (43).

When the reading of the sample is complete, it is then possible to quantitatively determine the components of your sample based upon the intensity of the wavelengths that were reflected off of the sample.

2.3.3 Ammonium Test

Another test that can be performed on the water samples is the ammonium test. This test is done to determine the concentration of ammonium bound nitrogen in the sample. This is done by adding Nessler's reagent to wastewater samples and using a spectrophotometer to get an accurate reading. The Nessler's reagent is able to detect very minute amounts of ammonium and has a color variation of bright yellow, for lower levels, to dark brown for extremely high concentrations (29). The Nessler's reagent is also extremely accurate by reacting with the ammonium, which creates the coloration for detection in the spectrophotometer. This allows an accurate reading of ammonium in the sample. A wastewater sample that has very low amounts of ammonium is regarded to have been in the presence of a very efficient biofilm. A high amount of ammonium results in a biofilm that is not properly growing or has poor nitrifying properties.

2.3.4 COD/BOD.

The Chemical Oxygen Demand test is a way to estimate the total amount of organic compounds in water. Wastewater treatment plants use this value in order to determine the level of water purity in their plants before expelling back into the water cycle. COD results are defined by milligram of oxygen consumed per litre of water.

The principle behind the COD testing is to measure the total amount of oxygen that is required to oxidize all organic compounds to carbon dioxide in solution (9). This process is done by combining a strong oxidizing agent, potassium dichromate and mercury sulphate, to an extremely strong acid, sulfuric acid and silver sulfite. The potassium dichromate is reduced during the chemical reaction, and its reduced state, Cr^{3+} is used as an indirect measurement of the organic compounds in the solution. Cr^{3+} has a distinct coloration, ranging from yellow to dark green-blue. With the use of a spectrophotometer a concentration of COD can be conducted.

Although this test has been used for several years in wastewater treatment plants, it is not the most accurate test available to determine oxygen consumption. The COD test is unable to differentiate between the oxygen levels in the sample and all other organic matter present that can be chemically oxidized (9). For this reason it is only a rough estimate for determining how fast the bacteria are using up the oxygen. The Biochemical Oxygen Demand (BOD) test, however, is more accurate in accomplishing this goal. The BOD measures the total consumption of oxygen in water samples (26).

There are two methods commonly used to determine the BOD, the dilution and the manometric methods. These methods are similar in principle. Each method measures the initial amount of dissolved oxygen in a sample, and then takes another measurement 5 days later to determine the total amount of dissolved oxygen. The difference between the initial reading and the final reading is the total BOD. Although this process is highly accurate and easy to perform, it is hardly done in wastewater treatment plants due to its time scale. As stated, one run takes 5 days to complete, which is impractical for industrial use (26). The concentration of biofilm and activated sludge fluctuates regularly and once a reading for the BOD test as been achieved preformed, it is irrelevant to the concentrations that are currently present in the plant. For this reason COD testing is preferred.

2.3.5 Total Organic Carbon (TOC) Test.

The total organic carbon (TOC) test is used to determine the total amount organic compounds in wastewater, resulting in a convenient way to determine whether or not the active biofilms are working to successfully oxidize the contaminants. The general principles of all TOC machines are the same. A liquid sample is injected into the machine and it is combined with a strong acid. This causes all the inorganic carbon to be converted into CO₂ gas, which lifts out of

the liquid. What is left is the organic carbon. In order to determine the total organic carbon, combustion takes place in order to convert the organic carbon into water and CO₂ (48). This CO₂ is carried then into the infrared analyzer by carrier gas. Here, the adsorption wavelength of CO₂ can be read and the concentration of carbon will be expressed in mg/L (49). This test is similar to the COD and BOD tests; however, it is more accurate because it is only focusing on the organic carbon. COD is unable to differentiate between all oxidizable compounds, where as BOD focusing just on the consumed oxygen.

2.3.6 Ion Chromatography.

Ion chromatography was used to determine the molecular compounds found within the water samples. In particular nitrate, nitrite and phosphate were analyzed. Chloride and sulfate were also detected, but in regards to our project they were not used.

Ion chromatography uses the principle of liquid chromatography to separate anions with respect to their charge. Before samples are injected into the machine, it is necessary to filter them through a 0.45um filter. This is done to remove any particulates or other debris that may be left in the sample after filtration through a coffee filter. The pores on the coffee filter are larger; therefore it needs to be filtered again to refine the sample. The water sample (mobile phase) is then passed through a cation resin column (solid phase). This will cause the anions that have strong interactions with the resin to pass through the column with the slowest speed, where as those with few interactions will pass through relatively quickly. This then separates the different components and they will appear on the spectra at different times.

2.3.7 Compound Light Microscope

The compound light microscope is used to observe images too small to be seen by the naked eye, allowing scientists to study specimens more carefully. In regards to this project, the light microscope was used to detect the microorganisms that were located on the contactors disks and the glass plates, as well as the free-floating biofilm that detached itself within the contactor.

The light microscope works by shining a light through a series of lenses. The projector lens located near the eyepiece, the condenser lens, located beneath the sample and the optical lens can be adjusted to achieve the level of magnification desired. With this design, most compound microscopes magnifying samples up to 2000x the original size. The compound light microscope is only able to see specimens that can be detected by visible light, such as the rotifers, metazoa and nematodes found in biofilm; however organisms, such as viruses, are too small to be detected in this way (10).

2.3.8 Scanning Electron Microscope

The Scanning Electron Microscope (SEM) is a microscopy technique used to see very detailed pictures of a given sample. Rather than the conventional method of using lenses and lights to see an image, the SEM uses electrons. The SEM relays a 3-Dimensional image. This process allows for imaging at much higher magnification, allowing scientists to more effectively determine the makeup, and appearance of a specimen.

As stated, the SEM works by sending a beam of high powered electrons to the sample. This requires the sample to have a positive charge in order to attract the electrons to it. For this reason, samples are coated in a very thin layer of gold. This will provide enough charge on the sample to allow an image to be seen.

The inner workings of the SEM are very complex. First, the sample is placed into the microscope in a vacuum chamber. Once the microscope is turned on, the air is completely removed from the chamber. The electron gun, located at the top of the microscope, emits the beam of electrons back down throughout the chamber. Condensing and objective lenses are positioned throughout the microscope to focus the electrons into a centralized location near the sample at the bottom of the chamber. The scanning coils are also located there. These coils are responsible for moving the beam of electrons over the sample. As this occurs, the electrons focused from the beam hit other electrons already present on the specimen; these secondary electrons are removed from the surface and can then be detected by the microscope. The results are sent to the amplifier and a final image is relayed based upon the electrons emitted from each section of the sample (18).

This microscope can be used to image samples at a magnification from 15x to 200,000x the actual size, with a resolution of just 5 nanometers. This makes the scanning electron microscope an invaluable tool in the laboratory (18).

2.4 Photocatalysis.

Photocatalysis is the process by which light is absorbed onto a surface. This generates electron-hole pairs by moving an electron from the valence band to the conduction band. This electron-hole pairs are highly reactive. In water, the hole reacts with water to create the highly reactive hydroxyl radical, while the electron also reacts to create other radicals (33).

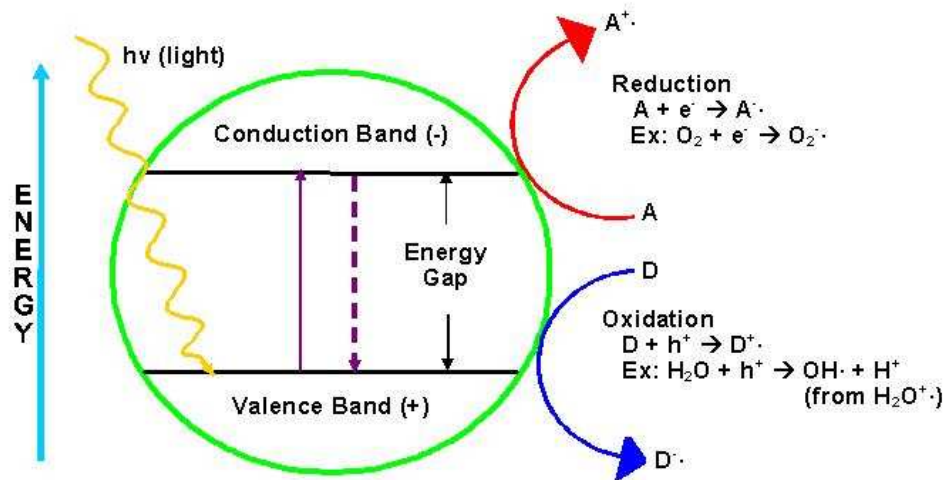


Image from: (33)

Semi-conductors are frequently used for photocatalysis due to the small amounts of energy required to transition electrons from the valence band to the conduction band. Titanium dioxide is the most frequently used due to its low cost, and slow k_{cat} when not exposed to UV light. In wastewater treatment, the use of Titanium dioxide is being explored due to its ability to completely oxidize the substrates to carbon dioxide, its ability to function as an immobilized photocatalysis, and its low cost. Titanium dioxide is known to have bacteriacidal properties, damaging first the outer membrane and peptidoglycan layer, then the cytoplasmic membrane (19).

2.4.1 Titanium Dioxide.

Titanium Dioxide (TiO₂) is often used as a method to reduce the formation of biofilm on solid surfaces located within a wastewater treatment plant. Titanium dioxide acts as a conductor in a photocatalysis reaction by releasing an influx of electrons into the system when UV light is presented. The surface of the TiO₂ then reacts with the water in the system to form hydroxyl radicals.

It has been hypothesized that it is these hydroxyl radicals that disrupt the growth of the bacteria by causing rapid cell death throughout the colony and that the hydroxyl radicals act as a biocide due to its high oxidation potential and its nonselective behavior. This allows the radicals to react with anything that has the ability to be oxidized. This is vital for wastewater treatment plants who wish to keep their tubing/piping free from biofilm formation where significant build-up could cause blockage.

Titanium is a common element used as a biocide due to its nontoxic characteristic. There are no proven detrimental side effects to using this TiO_2 in water treatment plants where the water will be reintroduced into the environment upon its cleaning.

3 Methodology

3.1 Experiment A, rotating biological contactors

The rotating biological contactor experiment was designed to test two different parameters, speed and surface structure, to determine the most efficient conditions to grow biofilm. To conduct the rotating biological contactor experiment, two tanks were set up, a slow rotating disk, rotating at about 4 rpm and a fast rotating disk spinning at 40 rpm. The shafts were rotating via Bioblock Scientific Eurostar digital motors. The two holding tanks were of the same size, with dimensions of 27.5 cm x 10.5 cm x 10.5 cm containing 3.03 L of volume. Fresh substrate was pumped in at a flow rate of 120 mL per hour for the slow reactor and 150 mL per hour for the fast reactor. Air was also flowing in at 100 L/hour using a Shego Prima pump and placing a tube directly into the holding tank via a small opening near the top. Both tanks had a drainage tube allowing the volume to remain constant in the tanks.

The disks in the reactors were arranged identically in both tanks. Each shaft held five disks, numbered accordingly, 1-5 originally in the slow contactor and 6-10 in the fast contactor. The disks were put onto the shaft in the same arrangement each day. Disk one was a flat surfaced glass plate, with a thickness of 0.25 cm and a diameter of 10cm. Disk two was a grooved surface with concentric circles around the area. It was thicker than the first with a width of 0.5 cm and also had a diameter of 10 cm. Disk three was identical to disk two. Disk four was also a grooved surface, however, the grooves made webbing over the surface, with a thickness of 0.5cm and a diameter of 10 cm. And finally, disk five was another smooth surface, identical to the first. As previously mentioned, this arrangement was identical in both of the RBCs.

Each morning the tanks were replenished, cleaned, and scanned. New synthetic substrate was prepared. The concentrated substrate was prepared by adding 1L of tap water, 5 g of

ammonium chloride, 0.25g of di-sodium hydrogen phosphate, 1 sugar cube and 100g of Viandox. Viandox was the solution that gave the synthetic properties of wastewater. It is used in cooking and consists of beef and yeast extract, soy sauce, citric and lactic acid, glutamate de sodium, salt, water and caramel for coloration. Once the stock solution was made, it was then diluted before being put into the RBCs. 50mL of stock substrate was added to 4L of water. The cleaning protocol was followed daily and it consisted of cleaning out the tubing that replenished the substrate due to the buildup of unwanted biofilm. This was done by aggravating the biofilm by pinching the tubing and running water through it. It was repeated several times to ensure it was all removed. The water in the tanks was also filtered every morning with a sieve, removing any biofilm detachment from the previous night and growing fungi present in the tank. Samples were taken from the holding tanks on a daily basis, collecting 50mL to be used for the testing. A sample of the freshly prepared synthetic substrate was also taken. The samples were filtered through coffee filters before collected and stored in the refrigerator. The contactor samples were tested using the Ammonium Test, COD test, Chromatography, Fluorescence spectroscopy and UV-Vis Spectroscopy.

Scanning the biofilm was done using an Epson Perfection 4490 Photo Flat Bed Scanner, as well as the Epson Scan program. The parameters of the scans were set to 8-bit grey image with a resolution of 800dpi. The area that was scanned had dimensions of 118.2mm for the height and 119.7mm for the width. Each disk was scanned in numerical order and named appropriately, following the format of run16_ (year/month/date) _disk number. A blank was scanned as disk number 001 and the rest followed accordingly.

Half way through the experiment the water in the tanks were emptied, filtered and replaced into the opposite tank. The rotating disks from the fast tank were switched into the slow

tank and vice versa in order to determine their ability to grow in different environmental conditions. The actual disks were kept in the same order and scanned in the same fashion.

3.2 Experiment B, photocatalytic reactor

The other experiment that was run dealt with the UV reactor. Four glass plates, with the dimensions of 29.0 cm x 5 cm by 0.1 cm, were coated with a titanium dioxide and DIH_2O solution that was brought to a pH of 3 with HNO_3 . The solution was sonicated for 15 minutes before use in a Branson 1210 Sonicator. There were two types of TiO_2 powder used, P25 and PC500, and two glass plates were coated with each. Approximately 6mL of solution was uniformly placed in the glass plate. They were given several hours to dry and were then fired in an oven. The oven program heated was set to heat the plate $100\text{ }^\circ\text{C}$ in 1 hour, remained at $100\text{ }^\circ\text{C}$ for 1 hour, then the temperature rose for an hour to reach 475C , remained at that temperature for 5 hours, and then cooled back to room temperature. For the PC500, plates numbered 1 and 2, 4 coats were added resulting in a total thickness of 53.5 mg and 41.8 mg, respectively. 4 coats of PC25 were also added on plates 3 and 4 for a total thickness of 33.3 mg and 40.2 mg, respectively. Two blank plates were also prepared, washing them carefully with acetone.

These glass plates were then arranged into the reactor in the order of PC500, P25, blank, PC500, P25 and another blank. 4 L of Fresh clarified water from the Nancy, France wastewater plant was washed over the plates using a Pump Drive PD5001 Heidolf at a rate of 80 rpm. Originally a Bioblock Scientific Masterflex was used at a rate of 100rpm. On the 14th of February, however, that pump broke and was switched out. The slower pump rate was taken into account. Because the TiO_2 works as a photocatalyst to prevent the growth of biofilm, a UV lamp was placed over half of the slides in order to accurately determine a difference in the amount of

biofilm growth on the slides. Cardboard was taped onto the coverplate and was used to prevent the light from reaching the top three slides arranged in the holding tank.

Each morning the slides were removed and scanned using the Epson flatbed scanner and the Epson scan program. They were replaced in the same order after scanning. The scanning of the glass plates were also performed using an Epson Perfection 4490 Photo Flat Bed Scanner, as well as the Epson Scan program. The parameters of the scans were set to 8-bit grey image with a resolution of 800dpi. The area that was scanned had dimensions of 47.7mm for the height and 70.1mm for the width, taken from the middle of the glass plate. The plates were arranged in the same way on the scanner in order to guarantee the most accurate results. Each disk was scanned in numerical order and followed the format of run17_ (year/month/date) _disk number. A blank was scanned as disk number 001 and the rest followed accordingly. The images were saved into the run17 folder.

The clarified water in the tanks was replaced every other day and samples were taken of the old water remaining in the tank and the new water that was added. These samples went through the Ammonium Testing, Chromatography, Fluorescence spectroscopy, UV-Vis Spectroscopy and TOC.

3.3 Experiment C, stationary titanium dioxide deposited slides

The third experiment that dealt with the stationary titanium dioxide deposited slides that were exposed and not exposed to UV light. Thirty-six round glass slides, with the an 8mm diameter (Fisher Sci), were coated with a titanium dioxide and DIH_2O solution that was brought to a pH of 3 with HNO_3 . The solution was sonicated for 15 minutes before use in a Branson 1210 Sonicator. Titanium (IV) dioxide powder, <5 micron, 99.9+% (Sigma), was deposited on 18 glass slides. Approximately 2mL of solution was uniformly placed on these glass slides. They

were given several hours to dry and were then fired in an oven. The oven program heated was set to heat the plate 100 °C in 1 hour, remained at 100 °C for 1 hour, then the temperature rose for an hour to reach 475C, remained at that temperature for 5 hours, and then cooled back to room temperature.

These glass slides were then arranged into the 24 well plate so that there were three adjacent columns in the 24 well plate that had titanium dioxide coated slides, and three adjacent columns on the plate that had blank or uncoated slides. For each of these three columns in the left most column, each of the wells were filled with about 5ml of wastewater from after the settling tanks following the secondary phase of wastewater treatment from the Upper Blackstone Water Pollution Abatement District. In the middle of the three columns, each of the wells were filled with about 5ml of wastewater from right before the secondary phase of wastewater treatment from the Upper Blackstone Water Pollution Abatement District. In the rightmost of these three columns, about 5ml of Worcester tap water was filled into each of the wells.

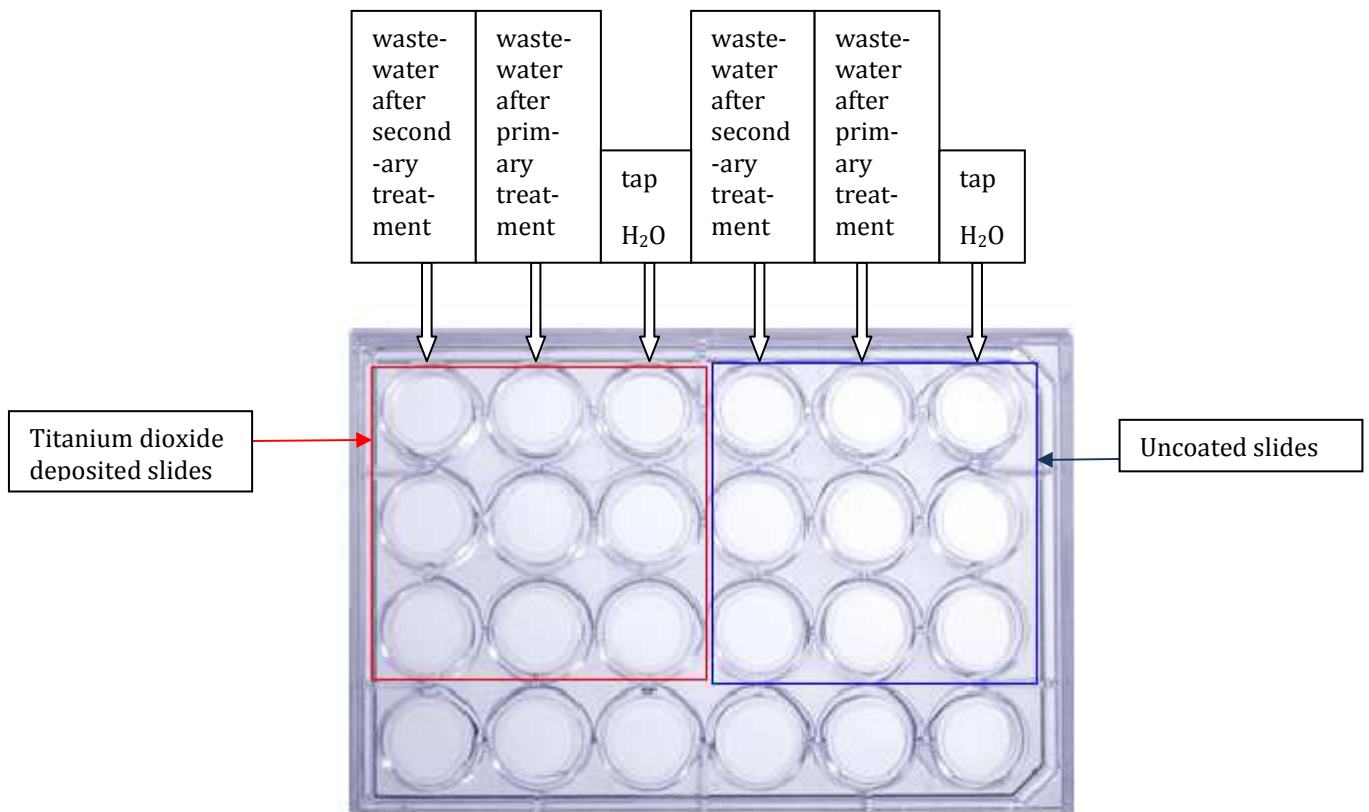
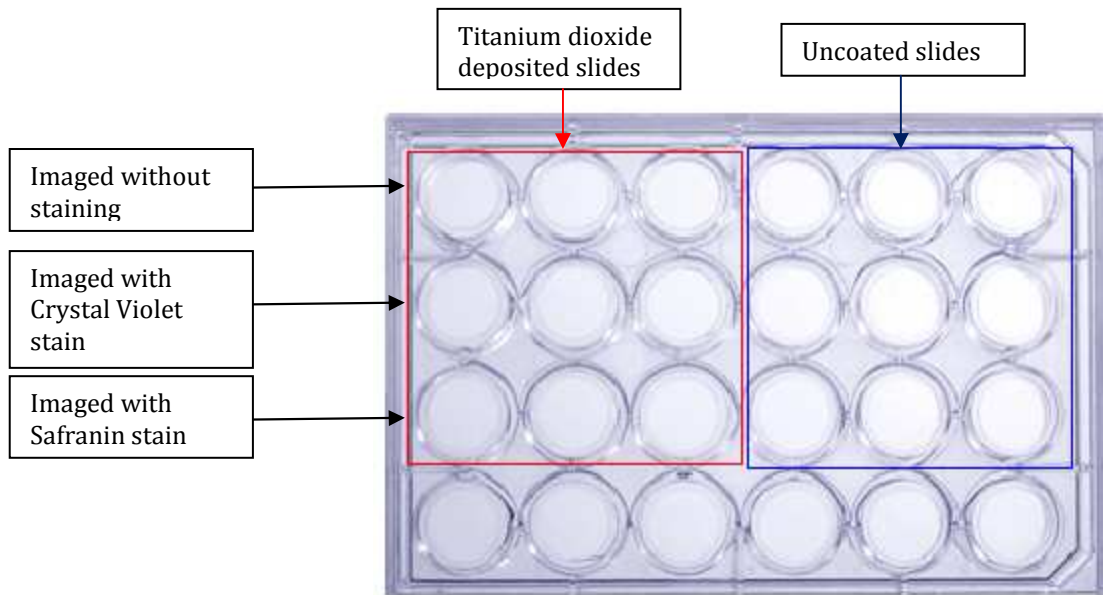


Image from: (58)

Two of these 24 well plates were prepared (58). One of these was placed under a UV light at 350nm, while the other was exposed to ambient light. Because the TiO₂ works as a photocatalyst to prevent the growth of biofilm, a UV lamp was placed over one of the 24 well plates in order to accurately determine a difference in the amount of biofilm growth on the slides.

After 24 hours of growth, a biofilm could be visualized using the optical microscope on the slides that were not exposed to the UV light and were uncoated. At this point all of the slides were imaged.



First the slides that were not stained were imaged at 60X and 40X magnification. Then the other slides were stained with one drop of Crystal Violet or Safranin and incubated at room temperature for 15 minutes. The slides were then rinsed thoroughly with MilliQ water to remove unattached cells and residual dye and imaged at both magnifications.

3.3 Greyscale Fortran Program

The Fortran program, written by Professor Pons, averages the darkness of the disks from 38% of the radius to 98% of the radius. The disks are only imaged starting at 38% of the radius because the disks sit on an axel above the water, and above 38% of the radius, the disk is not submerged in the water, so there is generally no biofilm growing at that point. It also generates the standard deviation of the darkness of the disks from 38% to 98% of the radius, to give a

picture of how variable the biofilm mass is on the disk. The higher the standard deviation, the less homogenous the biofilm thickness is on the surface of the disk.

4 Results and Discussion

4.1 Results of experiment A, rotating biological contactors

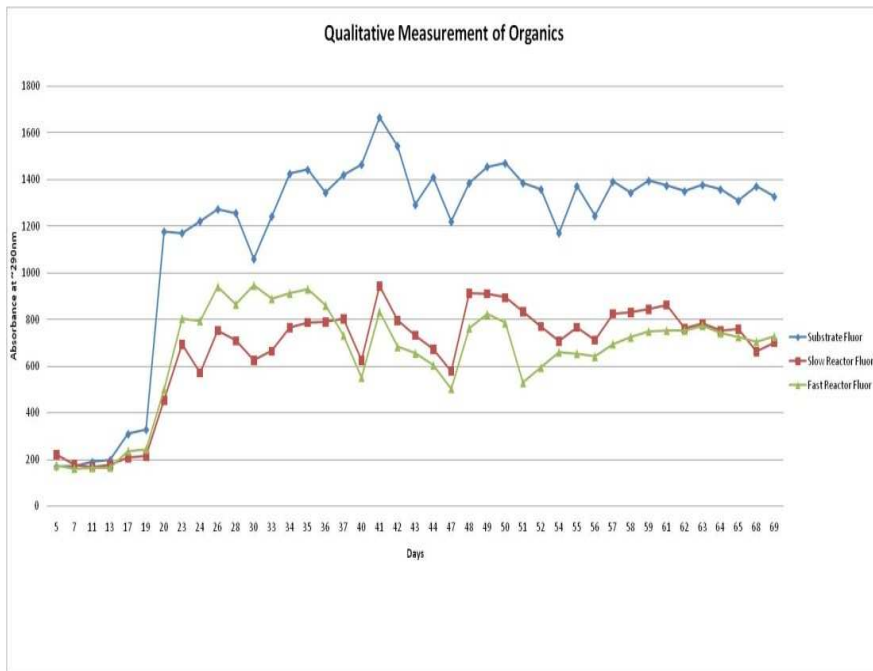
The following result sections summarize the tests performed on the two rotating biological contactors. The Fluorescence Spectroscopy, UV-Vis spectroscopy, COD, Ammonium and Ion Chromatography were all tested from the water samples collected daily. The opacity results were gathered from the direct scanning of the biofilm.

4.1.1 Fluorescence Spectroscopy

The fluorescence spectroscopy was used to determine the concentration of organic compounds located in each of the samples. Common aromatic structures that are present in the wastewater are tryptophan and organic nitrogen, both of which are visible in the peak at 285nm. The organic nitrogen gets converted to ammonium, which is then converted by the bacteria located in the biofilm to form the end product of Nitrate. In theory, the more efficient the biofilm the lesser concentration of organic nitrogen found within the sample.

The results gathered by this data were consistent throughout the duration of our experiment, as depicted in the following graph. The blank substrate, that is the sample of synthetic substrate freshly prepared before entering the rotating biological contactor, contained the highest concentration of organic nitrogen when compared to the samples taken each day from the two RBCs. As previously stated, the nitrogen in this sample was unable to become oxidized to nitrate due to the absence of biofilm. The organic compounds remained untouched and there

was a relatively constant concentration of nitrogen throughout the entirety of the three months. Slight fluctuations in concentration were attributed to noise and human preparation error.



The initial low concentrations of the organic nitrogen seen in days 1-15 of the previous graph were due to the fact that the initial onset of the contactors, real clarified water from the wastewater treatment plant in Nancy, France was used. Clarified water had relatively low amounts of organic nitrogen compared to that of the synthetically prepared substrate in which the Viandox was added. The peaks that were formed in those first 15 days are attributed to the amino acids and various proteins found within natural wastewater. On that 16th there is a large spike in the concentration of organic carbon, particularly in the substrate. The two contactors also showed an increase in concentration. At this point in the experiment there was substantial biofilm growth

on the rotating disks and the bacteria already begun oxidizing the nitrogen into ammonium and nitrate.

The two rotating biological contactors had relatively similar results, with both concentrations fluctuating at roughly the same places. The slow reactor developed biofilm at a slower rate than the fast reactor, however, it is seen that it is the slow reactor that was initially more efficient at oxidizing the organic nitrogen. There are many variables that can attribute to these findings; the bacterial strains are different in each of the contactors due to the fact that differing conditions favor different bacteria. While the biofilm in the fast contactor may seem denser through the grey-scale measurements, the conditions may not favor bacteria that are particularly good nitrifiers. Another reason for any differences could be that filamentous bacteria is free-floating in the substrate, or bacteria may be found clinging to the walls of the tank, neither of which were measured.

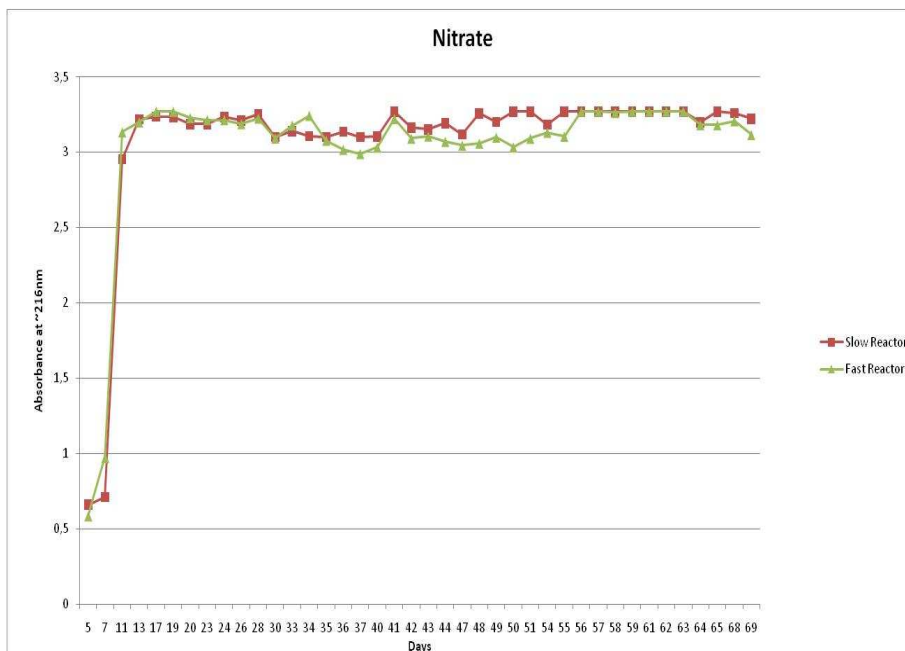
On day 34 and day 50 both contactors saw a very significant detachment of biofilm, yet the amount of nitrification remained relatively constant, or in some cases increased. This is due to the overall nature of the biofilm. As the outermost layer of cells detach from the biofilm, the lower level remains and continues to behave as healthy, mature biofilm. The microorganisms responsible for the nitrification are still intact and they maintain their function. In particular, on the recording after the first major detachment (day 34), the concentration of organic nitrogen decreased, meaning that the nitrification increased. Biofilm has a preferred thickness, which enables the most efficient breakdown of organic particles, and for this case it found to occur right after the old, dead cells were removed.

4.1.2 Ultraviolet-Visible Adsorption Spectroscopy

The UV-Vis spectroscopy was used to test two different aspects of the samples. The peak formation at 215nm showed a concentration of nitrate in the sample and the peak at 254nm showed the concentration of soluble COD. The UV-Vis spectroscopy is a qualitative test, and although the values are accurate, it is difficult to get a total concentration based on the results.

In regards to the nitrate concentration, only the fast and slow contactors were analyzed. The substrate sample taken daily has no nitrate peak simply because there are no bacteria present in the sample to nitrify the ammonium. These samples instead contain high levels of ammonium and organic nitrogen. The substrate samples, however; were considered when analyzing the soluble COD.

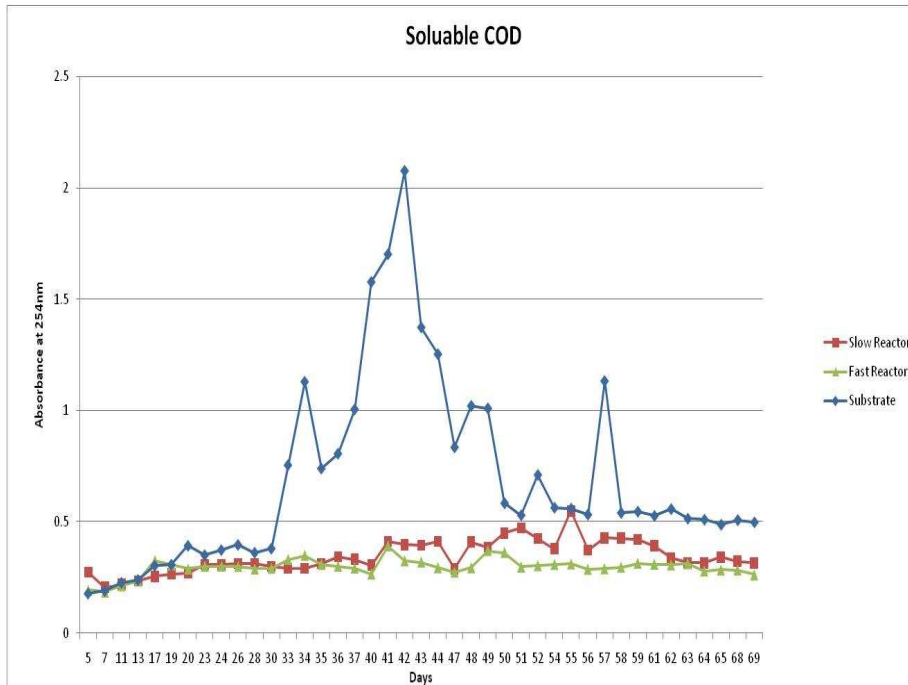
The following figure shows the graphical depiction of the concentration of nitrate. As represented by the curve, it took five days to develop enough substantial bacterial growth before they were capable of performing significant nitrification.



Upon maturation of the biofilm, the cells were very efficient and very consistent in their nitrification process. Between days 33-52 the slow reactor was slightly more efficient, having an overall greater concentration of nitrate. The biofilm growing in the contactors are composed of different organisms and therefore have different properties. The slow reactor in this respect would be expected to contain a higher number of organisms that are efficient at the nitrification process. At day 54, both contactors reached a plateau. The bacteria at this point are nitrifying more ammonium in the contactors than can be detected by the UV-Vis spectrophotometer.

A few human errors can be attributed to several of the large peaks. The larger peak found at day 30 can be attributed to the accidental preparation of substrate in which it was prepared twice as strong. This would cause an excess of ammonium in the tanks. The biofilm was able to nitrify the same percentage of the excess, illustrating the stability of the bacteria present. Other fluctuations in the graph are due to background noise and human error when preparing the synthetic substrate.

The UV-Vis spectroscopy with respect to the Soluble COD (peak at 254nm) shows a slightly different trend, as seen in the following graph.

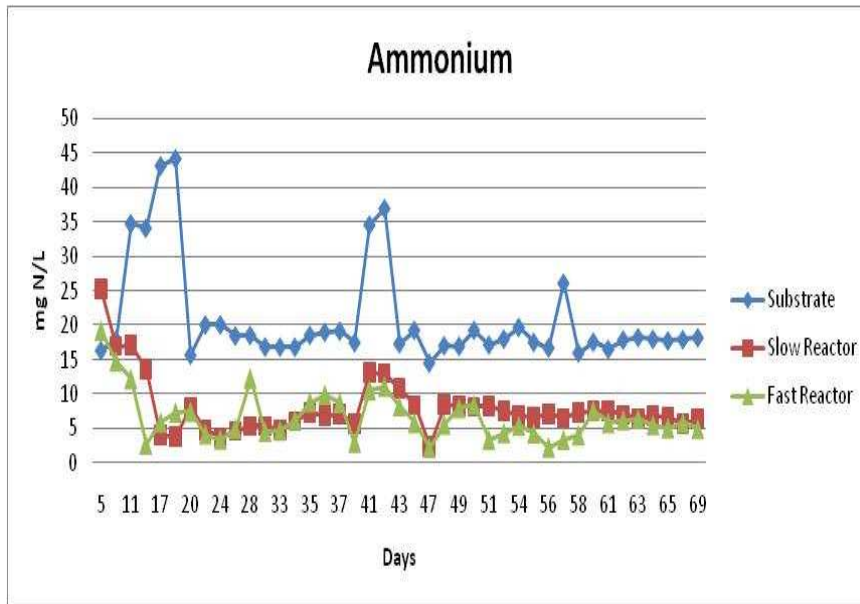


Soluble COD is reference so the total amount of carbon in the sample. Because carbon is located in the synthetic substrate, not an end product of the biofilm the substrate curve was included. The soluble COD value shown in the graph should be a constant value. Each wastewater treatment plant has its own concentration of soluble COD based upon the local diets and other compounds found in the local area. The above figure shows a relatively steady line for both of the contactors. The scale is large and the slight fluctuation is due to noise on the machine. The substrate curve, however, is less constant. Preparation of the synthetic substrate is a reason for these results. A higher concentration of the Viadox, which is added to the synthetic substrate, contains beef extract and therefore would show higher concentration of the soluble COD.

4.1.3 Ammonium Testing

The ammonium tests performed on the samples taken from the RBCs were done to test the total amount of nitrogen fixed ammonium within the samples. A lower concentration of ammonium in this graph would relate to a higher efficiency of nitrification by the bacteria and

for this reason it was expected that the substrate would have a higher concentration than the samples for both of the RBCs. The following graph illustrates data from collected samples of both actual wastewater (days 1-15) and synthetically prepared wastewater (days 16-60).



The substrate curve, depicted in blue on the previous figure has a greater concentration of ammonium than the two reactors. As previously stated, this is due to the fact that the substrate samples do not come into contact with bacteria and therefore, is unable to oxidize the ammonium into nitrate. Ammonium is found in actual wastewater in very high quantities, which is represented by our graph. The first 15 days of the experiment were conducted using actual wastewater from the Nancy, France Wastewater Treatment Plant. This allowed our contactors to grow healthy bacteria before switching to synthetically prepared substrate. The concentration of ammonium in these samples is significantly higher than the samples taken after the switch to synthetic wastewater.

The other large peak, seen from days 39-40 resulted from a miscalculation and a two-fold increase in concentration of synthetic wastewater. The mistake was noticed two days later and

the concentration levels quickly returned back to normal. Other such peaks in the substrate curve can also be attributed to human error when preparing the new synthetic substrate daily. The spectrophotometer is very accurate and the Nessler's reagent used to test for the ammonium is extremely accurate and can detect very minute fluctuations of ammonium in the sample.

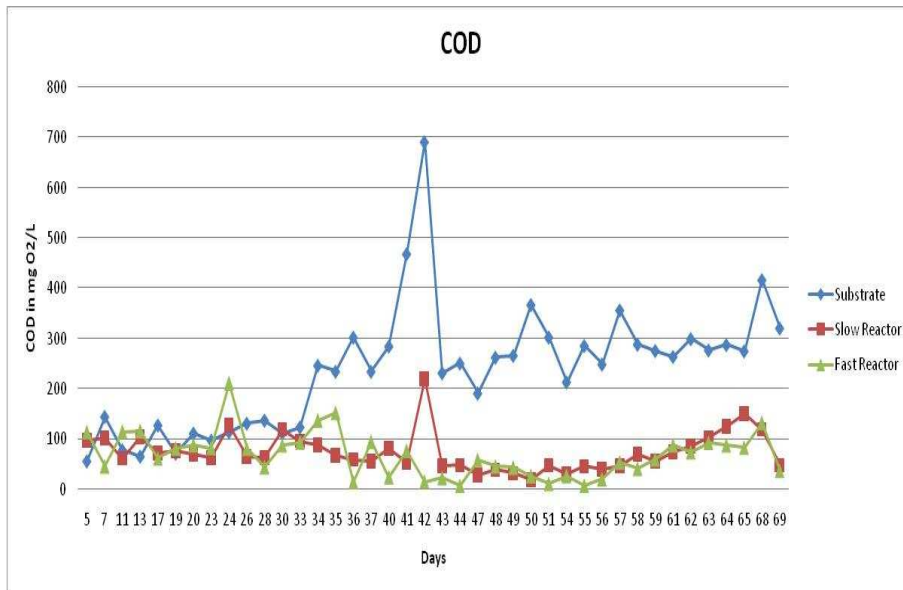
The two curves created by the fast and slow reactors are very similar and show stable results. The bacteria was nitrifying efficiently, even in the presence of actual wastewater as soon as the contactors began and mature biofilm formed. It took just about 13 days for the biofilm to mature and reach a steady level of nitrification. Again, on day 39, when the concentration of the substrate was doubled, it is shown that the biofilm was still able to nitrify a similar percentage of ammonium present. These results again show the effectiveness of our biofilm.

The major detachment of the biofilm on day 37 and day 50 also did not affect the biofilm's ability to nitrify the ammonium in solution. Although the total mass of biofilm greatly decreased, the majority of lost cells were already dead and not aiding function the biofilm. As the top layers were sloughed off, the healthy, mature layers underneath were capable of working with the same level of success. This base layer of healthy cells was also responsible for the quick regrowth of biofilm.

4.1.4 Chemical Oxygen Demand Testing

The Chemical Oxygen Demand (COD) test was performed in order to measure all oxidizable molecules in solution. This is inclusive of all molecules located in the sample, not just organic compounds. The concentration levels are measured in mg of O₂/L. The addition of a very strong oxidizer, in this case Potassium dichromate, into a very strong acid causes the chemical reaction, oxidizing compounds into the end products of carbon dioxide, ammonium and water. By this reaction the potassium dichromate is reduced to the chromic ion. This ion is

detectable through the use of a spectrophotometer, which absorbs strongly in the 600nm region and can be measured using a spectrophotometer. On the following graph, the larger peaks depict the more oxidizable compounds in the water.



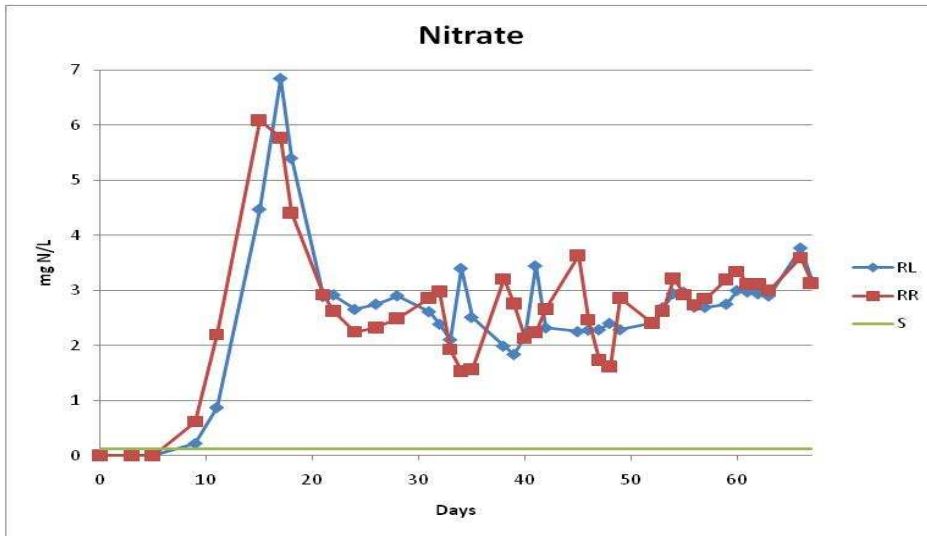
The substrate curve in the graph contains the highest concentration of COD, which is expected. The synthetic substrate was prepared with Viandox, a product of yeast and beef extract which contains many oxidizable compounds, and for this reason yields a much higher concentration of COD than the actual wastewater (days 1-15). The concentration of the synthetic substrate prepared between days 15-31 was prepared at lower concentrations and is the result of the lower curve. On days 32-60, the substrate was prepared at a greater concentration, and was prepared in the same manner for the duration of the experiment. The largest peak, located on days 39-40 again was due to a two-fold increase in substrate concentration. The excess Viandox in the substrate sample was responsible for the excess carbon.

The two contactors again show a similar curve on the previous COD graph. Both reactors were able to develop successful biofilm which were able to convert the oxidizable compounds into carbon dioxide and water on their own. This resulted in the potassium dichromate doing less work and resulting in an overall lower concentration of COD. In comparison of the substrate curve to both of the contactors, the larger the difference between the two depicted a greater efficiency in the biofilm. Similar to the other findings, the major detachment that occurred on our biofilm did not disrupt the biofilm's ability to convert the oxidizable compounds in solution.

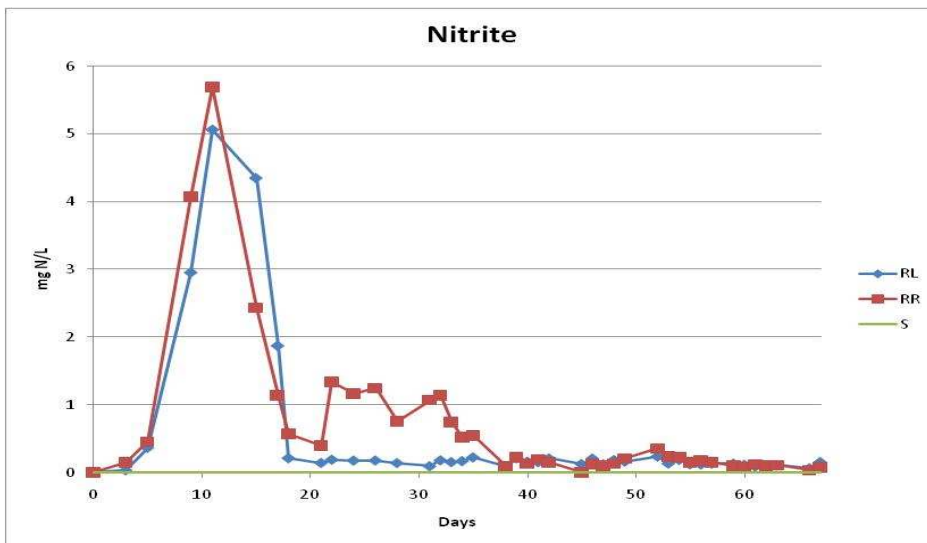
4.1.5 Ion Chromatography

The results of the ion chromatography are broken down depending on the compound found within the water sample. Included are nitrate, nitrite and phosphate. Sulfate and chloride were also present in the sample, however, these values did not change based upon the efficiency of the biofilm and will be discussed within this report.

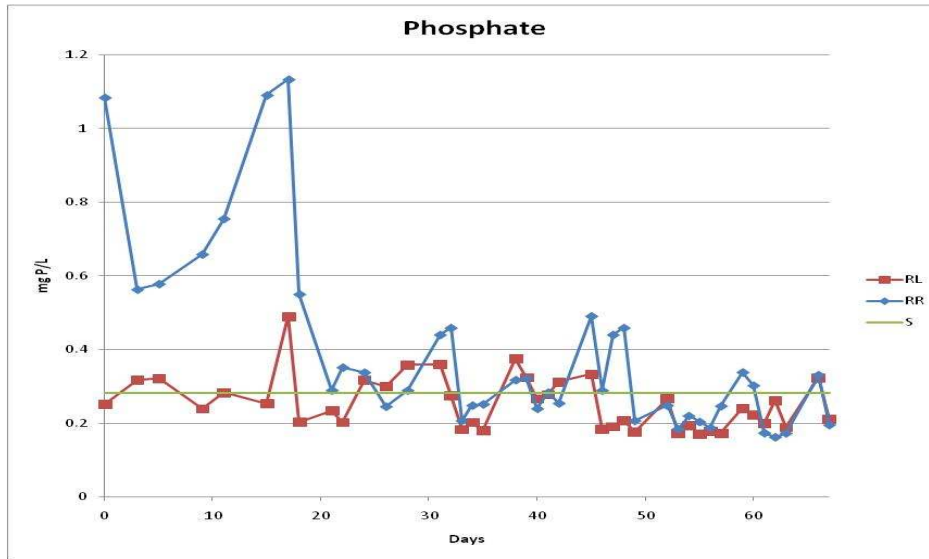
The nitrate results, received from the chromatography were consistent with the results that were received from the UV-Vis spectroscopy. The concentration of nitrate significantly increased after 10 days. This is due to the biofilm formation on the disks. Without proper maturation of the biofilm, the nitrification process cannot begin. The large peak found on day 18 was attributed to the miscalculation in the preparation of synthetic substrate and soon after the mistake was noticed, the values fell back to normal and remained constant for the duration of the experiment.



The nitrite concentrations found within the samples were relatively low, with an average of 0.5mg NO₂/L. Nitrate is a product of the nitrification process and although most of the nitrogen is reduced to Nitrate, some bacteria will complete the process and create Nitrite. The large peaks are attributed to noise, especially during day 10 and have no statistical importance.



The phosphate value shown through the ion chromatography is also very minute, showing an average concentration of 0.4mg P/L as shown in the following graph.



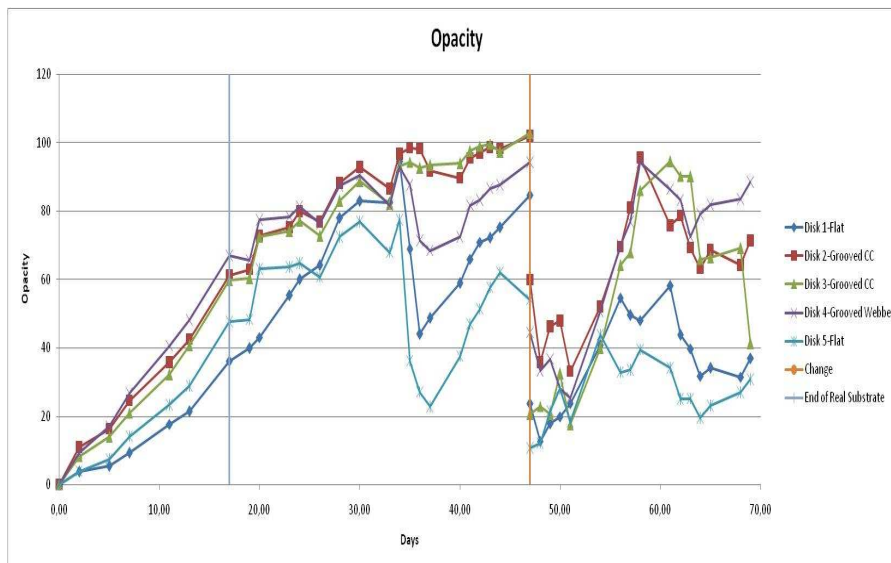
In the process of wastewater treatment, phosphorous is chemically removed in the tertiary process, which describes why the concentration level remains constant throughout the experiment. The higher concentration shown in the fast reactor from days 0-20 can be attributed to noise in the machine. The graph would be expected to have a constant concentration throughout the entirety of the experiment.

4.1.6 Opacity

The opacity is the darkness of the slide which corresponds to the amount of biofilm accumulated over the course of the experiment. It is measured through the use of the Epson scanner, and the Fortran program which calculates the average value of the opacity. As seen in the following graphs, the biofilm growth on each disk, as measured through opacity, was graphed with respect to day. These figures show that there was increasing biofilm growth over the course of the first 30 days. On day 17 the substrate was changed from clarified wastewater to synthetic substrate. This resulted in a noticeable change in the growth rate of the biofilm. Specifically a decrease in the rate of accumulation of biofilm which can be attributed to the fact that no new microorganisms are introduced when using synthetic substrate, while real

wastewater is awash in different microorganisms. The synthetic wastewater contains different concentrations of nutrients than the real wastewater, this causes the bacterial populations to shift, which could also be a reason for the different attachment rates.

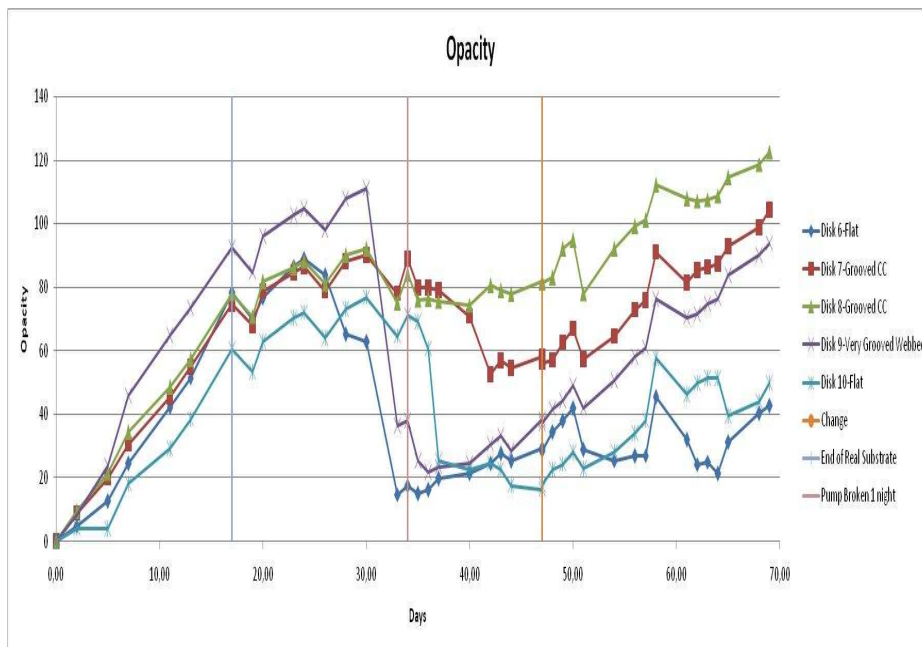
Disks 1-5 were initially in the slow reactor and disks 6-10 were initially in the fast reactor until day 47. On this day they were switched. In the slow reactor on day 36 a large detachment event occurred on the flat and webbed disks, while almost none of the biofilm from the concentrically grooved disks detached. It was thought that the more grooved the disks were, the more surface area the bacteria would be able to adhere to, and the less major detachment events would occur. The concentrically grooved disks all accumulated biofilm at an approximately even rate, the webbed disk suffered from major detachment, although not to the same extent as the flat disk, this can all be visualized in the following figure illustrating disks 1-5, with the divets in opacity at day 36.



On day 47 we switched the media from one tank to the other. Where these disks had been in the slow contactor, they were now in the fast one. The additional shear stresses introduced by the fast contactor caused all of the biofilm to clump in the center very thickly,

leaving almost all of the rest of the visibly free of biofilm. The biofilm the started to recover with all of the grooved slides accumulating rapidly while the flat disks never recovered from the loss.

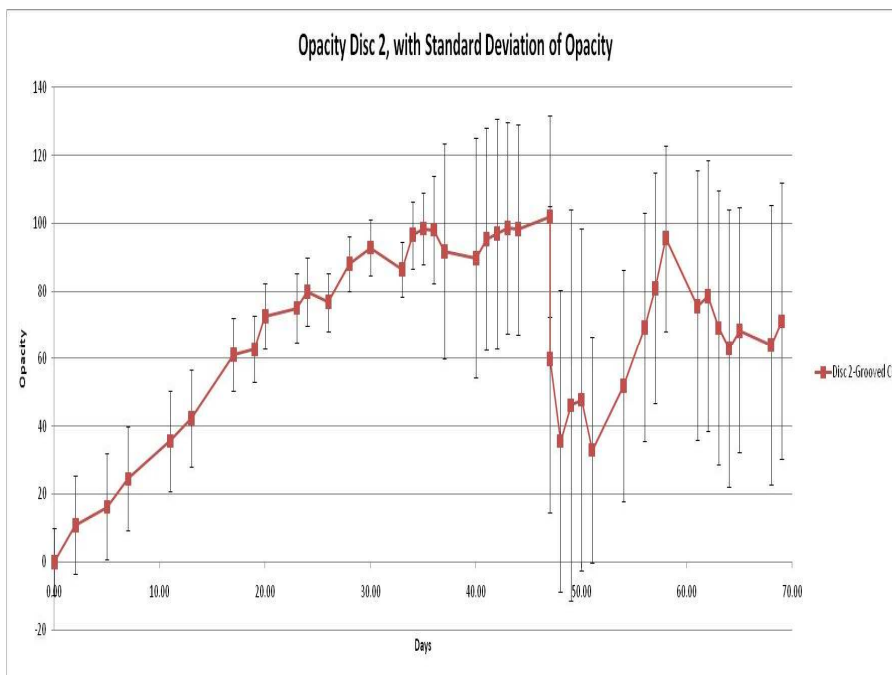
Disks 6-10 show very similar trends to disks 1-5. At day 34 there was a major detachment event for the flat disks and the webbed disk. This corresponded to an evening when the pump for the substrate became broken, killing some of the bacteria and allowing for the shear stresses to have more of an effect. The concentrically grooved disks were able to retain the attached biofilm almost entirely. Disks 6, 9 and 10 reached the lowest values for opacity on day 35 as shown in the following graph.



This shows the same trend as disks 1-5 in that the flat disks and the webbed disks had large detachment events, while the concentrically grooved disks allowed for the bacteria to remain attached much better. When we switched the bacteria from the fast reactor to the slower reactor,

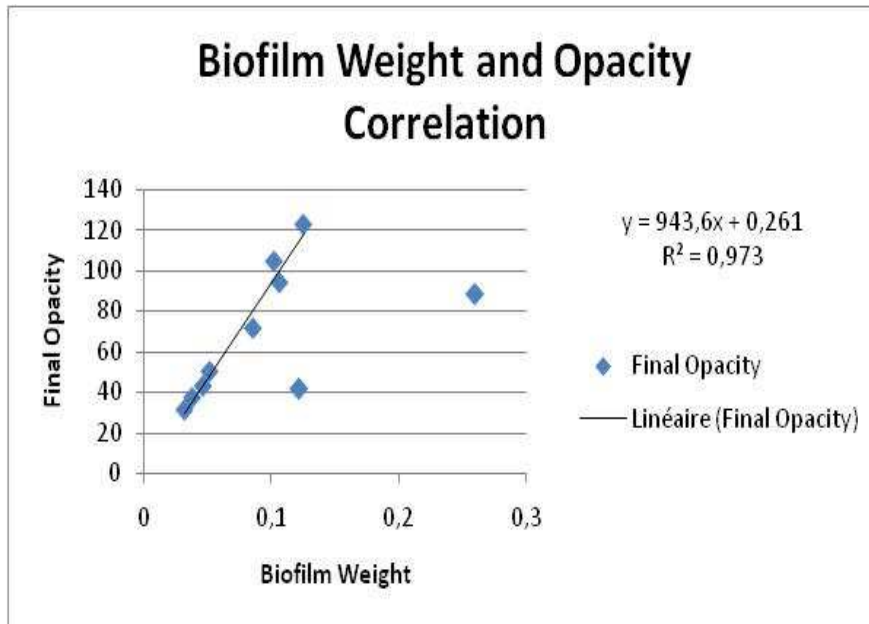
we do not see the major detachment event that you see in slides 1 through 5 since these slides were not experiencing higher shear stress. In fact the slower speeds allowed for more biofilm accumulation than was previously allowed for in the fast reactor. As expected the highest attachment was found in the concentrically grooved disks.

The standard deviation, which corresponds with the homogeneity of the biofilm thickness at varying points around the disk can be seen for disk 2 in the following figure, the large standard deviations seen in disk 2 were seen in all the disks once the detachment started to occur.



4.1.7 Biofilm Dry Mass/Opacity Correlation

A correlation can be made between the dry mass of the biofilm and the opacity. As can be seen in the following figure, this is a linear relationship with a high R^2 value.



There were only 2 outliers from the linear correlation, both of these were disks that had been moved from the slow reactor to the fast reactor. These are disks 3 and 4. The opacity and dry mass for these disks did not correlate because after all of the disks from the slow reactor were moved to the fast reactor on day 47, the biofilm immediately clumped up in the center. The greyscale program, as previously mentioned, averages the darkness of the disks from 38% of the radius to 98% of the radius. When the biofilm clumped in the center it fell within that 38% of the radius, and was not taken into account in the opacity measurement. Disks 1, 2, 5 were all fine because the clump has fallen off by the last day of imaging, whereas disks 3 and 4 still were clumped causing the biofilm mass to be higher than it would be were it correlated with the opacity reading.

4.1.8 Compound Light Microscopy

Two typical pictures of the biofilm grown in the contactors as viewed through the light microscope can be seen in figures 6.1.1.1.1 and 6.1.1.1.2. When looking at the rotating

biological contactor disks under the microscope, the components of the biofilm were determined. The general compositions of the disks were very similar. The densities of the biofilm were significantly varied between the disks and have been discussed in section 4.1.6 Opacity from experiment A of the results section.

The most notable organisms found in the biofilm were that of filamentous bacteria. This was expected as filamentous bacteria is the major component of biofilm and is responsible for the nitrification and degradation of ammonium and organic particles, respectively. Under observation, there were several other components observed including rotifers, metazoa and nematodes. As mentioned in 2.11.3 and 2.11.4, these organisms were responsible for cleaning the biofilm by eating the bacteria. The ability to visually observe the organisms confirmed our results that healthy biofilm did grow on our disks.

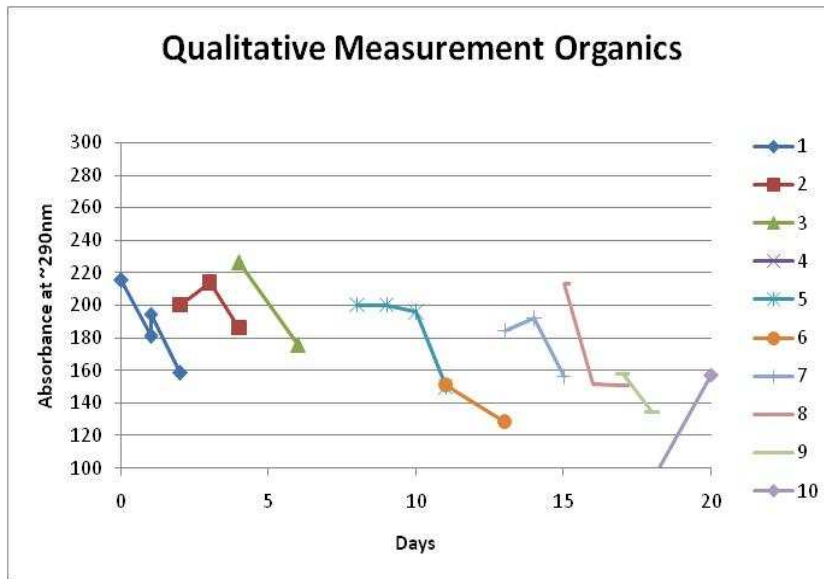
4.2 Results of experiment B, photocatalytic reactor

The following results summarize the tests performed on the photocatalytic reactor. The Fluorescence Spectroscopy, UV-Vis spectroscopy, TOC, Ammonium and Ion Chromatography were all tested from the water samples collected daily. The opacity results were gathered from the direct scanning of the titanium dioxide covered plates.

4.2.1 Fluorescence Spectroscopy

Similarly, for experiment B, the photocatalytic reactor, fluorescence spectroscopy was used to determine the concentration of organic compounds located in each of the samples, namely tryptophan and other aromatic structures. This nitrogen gets converted into nitrate, which would result in a lower concentration of tryptophan as the biofilm becomes more efficient.

The results from this test as seen in the following graph, show that the biofilm in the photocatalytic reactor was indeed maturing and were efficiently nitrifying the nitrogen.



The new batches of clarified water contained the highest concentration of organic nitrogen when compared to the old samples, collected daily. For the new samples, slight fluctuations in concentration were attributed to noise.

In comparison to experiment A, rotating biological contactors, the concentration of organic nitrogen was significantly lower, however, the same trend of nitrification was observed. The initial concentration of fresh clarified water showed the highest concentration of organic nitrogen and as time progressed, the bacteria were able to nitrify increasingly higher concentrations in the samples.

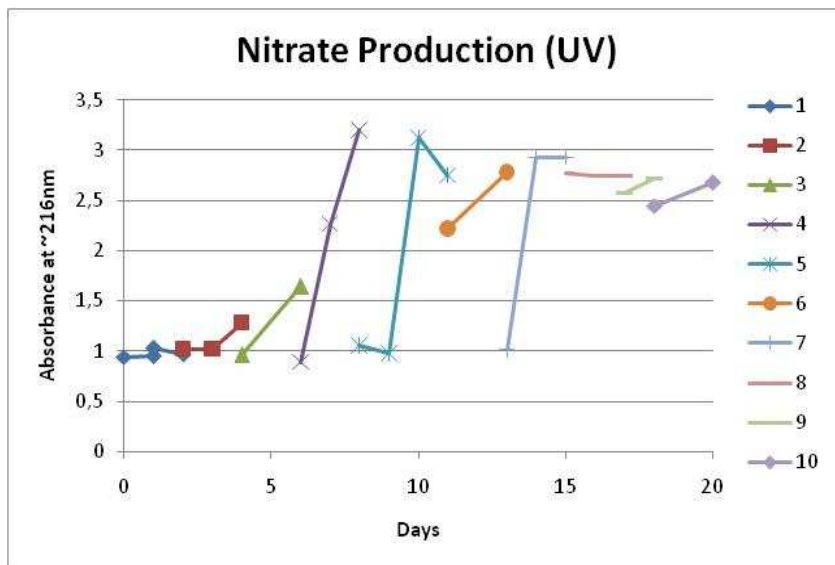
This test successfully shows that there was biofilm growth within the system; however, due to the nature of the test, we expected to see no growth on the titanium dioxide slides. Because it is impossible to prevent the growth of biofilm in other areas of the system, such as the tubes and around the edges of the reactor, we believe this is where the growth actually took

place. The best technique used to determine biofilm growth was to observe the glass slides under a microscope, in which minimal growth was found.

4.2.2 Ultraviolet-Visible Adsorption Spectroscopy

The UV-Vis spectroscopy that was performed on the photocatalytic reactor, also detected two separate peaks, one at 215 nm for the concentration of nitrate production and at 254nm for the concentration of Soluble COD.

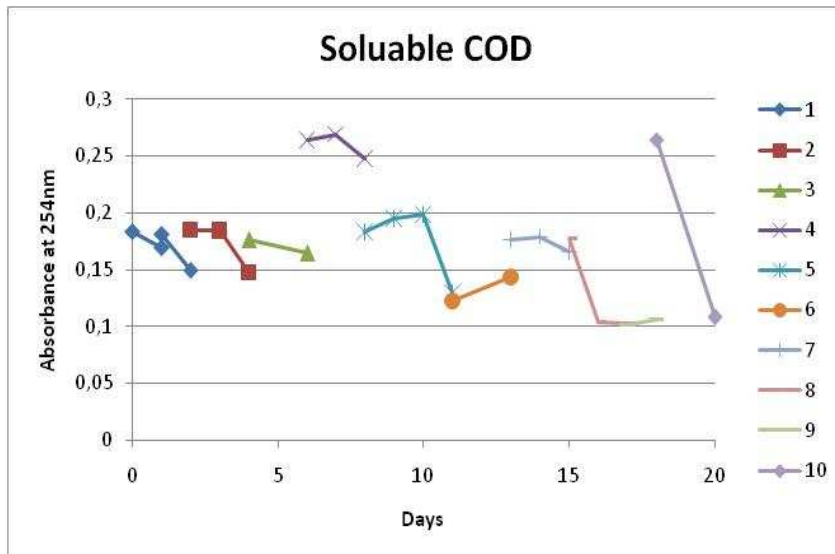
Similar to the results in the ammonium test, the UV spectroscopy showed that it took the biofilm a week before it was capable of nitrifying a significant amount of ammonium to oxidize it into nitrate, the compound that is detected through the UV spectrophotometer. Again, each color on the figure is representative of a different batch of clarified water, as seen in the following figure.



This figure did not show what was expected. Several data points did not fit the expected trend, namely, day 8 and 13. The concentration of nitrate on day 8 is greater than that on day 9, which leads to the conclusion that there were no viable biofilm in the reactor to nitrify the ammonium over that time period, or that the original container of the clarified wastewater had

some biofilm already forming inside of it, causing it to prematurely nitrify the ammonium. The trend on day 10, however, follows expectations due to the rapid increase of concentration of nitrate. Day 13 was also uncharacteristic of the trend, in which it appears that the nitrate production leveled off. The concentration does not appear to be large enough to have saturated the spectrophotometer. A hypothesis for the trend may be that the bacteria had nitrified all the viable ammonium in the sample. If there were not enough nutrients located in the clarified water, it would have caused the bacteria to die, thus stopping the nitrification process.

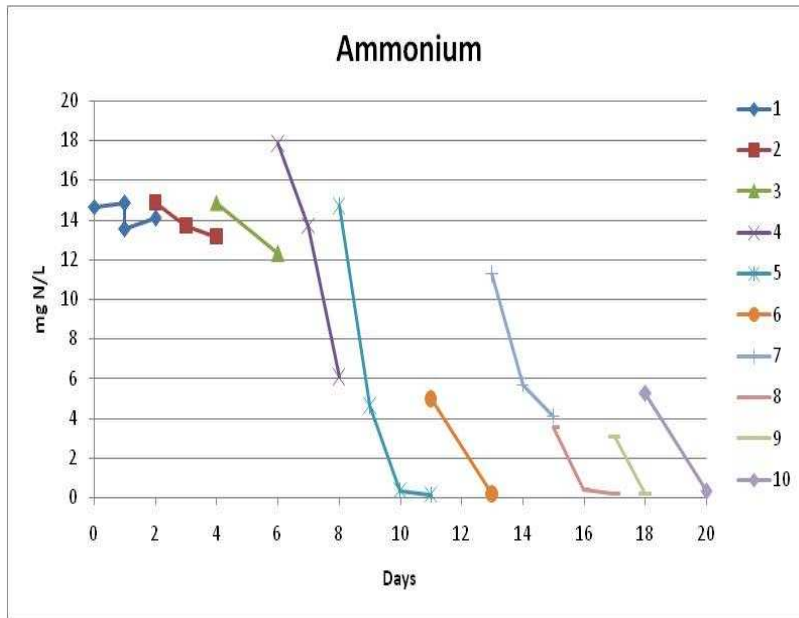
The soluble COD graph follows the general expectations for the anticipated trend. As previously mentioned, soluble COD is reference so the total amount of carbon in the sample at a given time. The Soluble COD value shown in the graph should be a constant value, which is true of the following figure.



The scale on the figure is also very small, and the slight fluctuations can be attributed to the noise of the spectrophotometer. The third batch of clarified wastewater, days 6-8 has a higher concentration of carbon in comparison to the other batches. Similarly, the wastewater used that day originally contained higher concentrations before placed into the reactor.

4.2.3 Ammonium Test

The ammonium test performed on the photocatalytic reactor was testing for the same nitrogen fixed ammonium as for experiment A on the rotating biological contactors. The following figure illustrates this process.



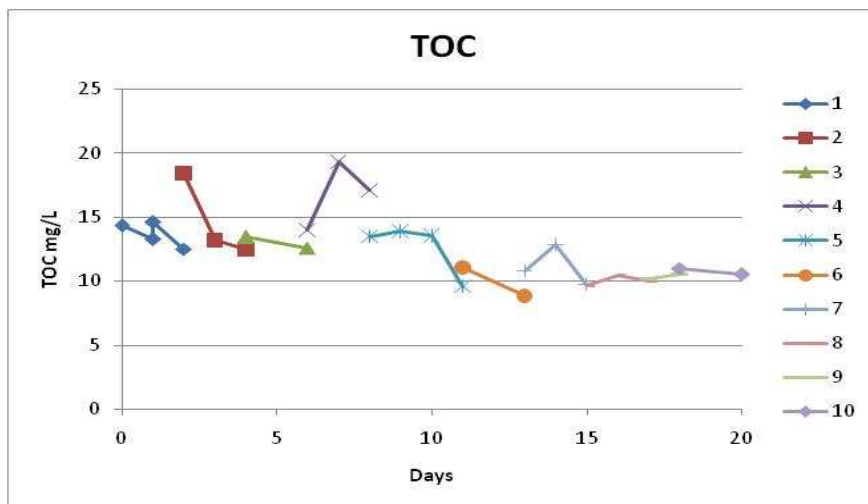
Each color is representative of a different batch of clarified water used, which is the time the new water is run through the system before switching out the water. The typical lifecycle of the clarified water was 2 days. The first point of each colored line was taken before the water entered the reactor, and the following points were collected after they had run through the tank for one or more days.

The general trend of the lines shows that over the process of a few days the biofilm that was forming was able to nitrify the ammonium into nitrate. At the beginning of the experiment, days 1-6, there was not a lot of biofilm buildup and the colonies were only able to nitrify small quantities of ammonium. As the time progressed, day 7 through the duration of the experiment, the biofilm became very efficient and the concentration of ammonium dramatically reduced.

Although our opacity scale shows that not a very significant amount of biofilm grew on the titanium coated glass plates, this isn't representative of the whole system. The nature of the biofilm allows it to form on all surfaces that fit healthy environmental conditions, in this case on unwanted surfaces, which in turn allowed the substantial amount of nitrification to occur. Upon the cleaning of the reactor, significant biofilm buildup was found at the bottom of the reactor, which as suggested, would have been responsible for the majority of the nitrification process.

4.2.4 Total Organic Carbon Testing

The total organic carbon test is a quantitative measurement of the organic carbon in a sample. The total organic carbon measurement was performed on the samples taken from the catalytic reactor because of the use of clarified water. These samples contain a much smaller percentage of organics, and this test allows for much more precise quantification of the organic compounds, primarily carbon, present in the samples. As can be seen in the following graph, the trend shows significant decomposition of the organic compounds, noted by the downward curve of the lines. Each new line, depicted by a different color is a new substrate feed that was used in the reactor. Each curve begins high and as the biofilm decomposes the carbon, the concentration level drops.



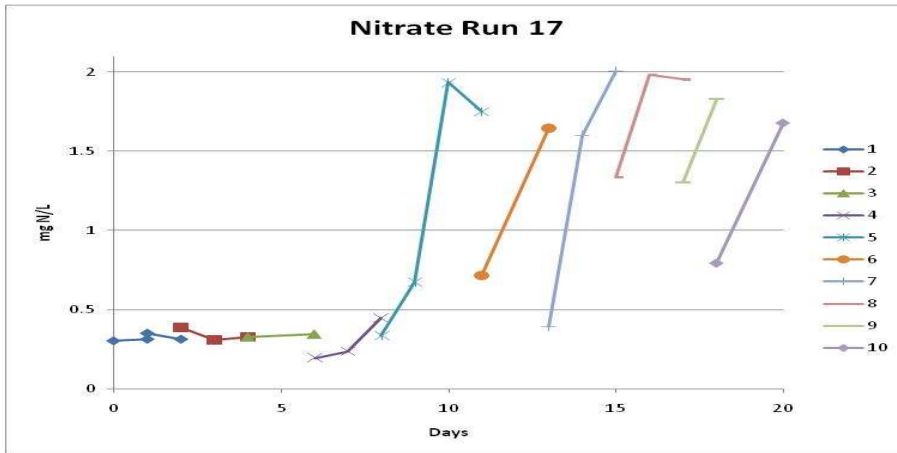
On day 15 we obtained a new batch of clarified water from the Nancy wastewater treatment facility, which was apparently very low in organic carbon. This is noted by the low initial concentration of the sample. The clarified water that was obtained for the photocatalytic reactor was taken after the secondary phase of treatment at that facility. This corresponds to a high level of decomposition happening at the plant and not much left for our bacteria to decompose.

There are 2 data points, the 7th and the 14th that do not follow the general trend. The one from the 14th could potentially be explained by the pump breaking the night before and not introducing any new substrate into the reactor. This caused the water to stop flowing over our biofilm, potentially hindering their growth and their ability to decompose the organic compounds. On the 7th data point the concentration of the organic compound increases. With no direct human error to report, the reason for this error is unknown.

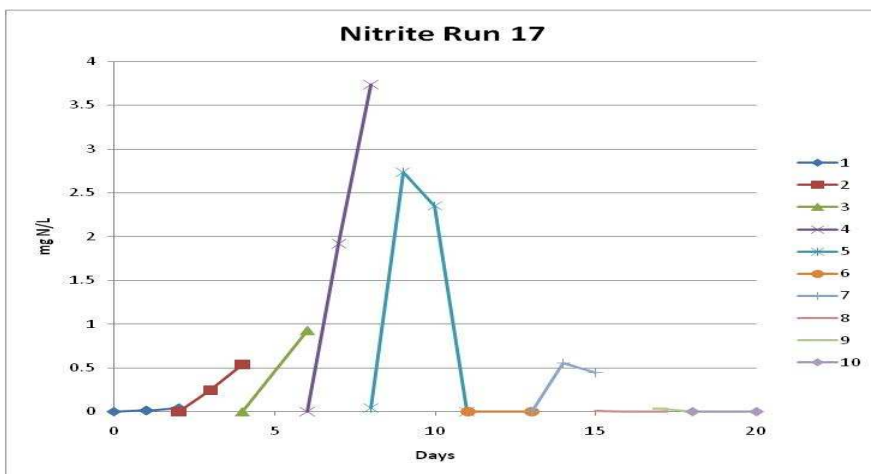
4.2.5 Ion Chromatography

The results of the ion chromatography for the photocatalytic reactor were very similar for those from the rotating biological contactor experiments. Three compounds in particular were observed, including nitrate, nitrite and phosphate. Again, similar to experiment A, the rotating biological contactors, sulfate and chloride were also present in the sample, however, they were not considered for this experiment.

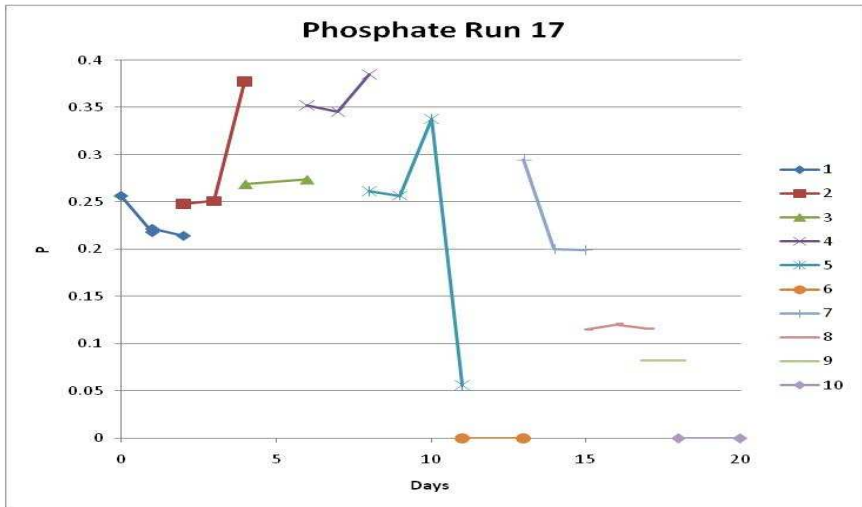
The concentration of nitrate in our samples followed the trend seen in the Ultraviolet-visible spectroscopy for experiment A. The concentration of nitrate took 6 days to mature before it was able to efficiently nitrify the present ammonium in the samples.



This trend is identical to that of nitrite, however, it should be noted that both concentrations, nitrate and nitrite are found in very small concentrations. Due to the use of clarified water in the photocatalytic reactor, the concentration of initial ammonium is very low. The process of nitrification first reduces ammonium to nitrite, and then the bacteria work to convert that nitrite to nitrate. Although the process is very efficient, a steady concentration of nitrite is present in samples.

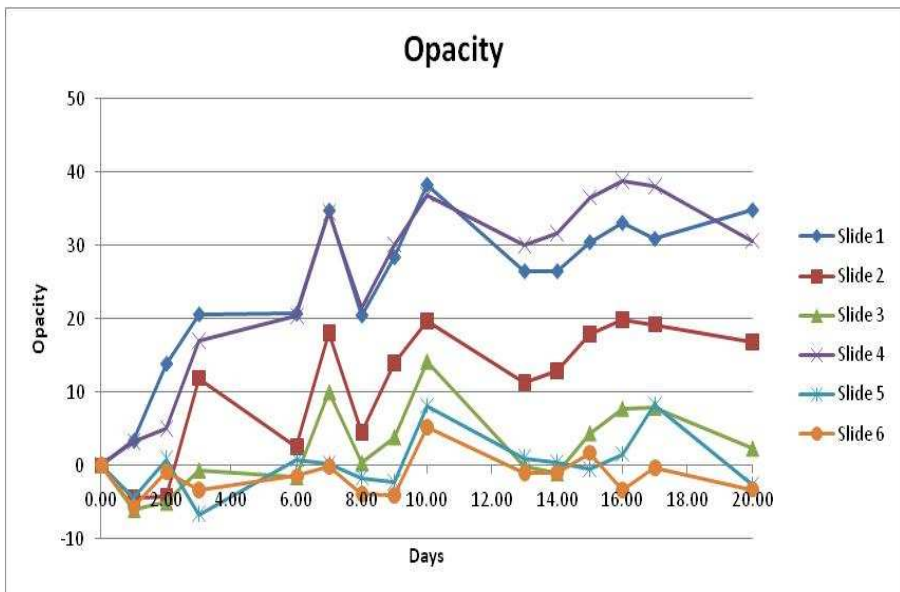


The phosphate concentration was even smaller than that of nitrate and nitrite showing an average concentration of 0.3mg P/L. Again, this is to be expected because although there are some strains of bacteria that can remove phosphate from the wastewater, much of the phosphate is chemically removed in the tertiary phase of treatment.



4.2.6 Opacity

The opacity readings conducted on the samples taken from the photocatalytic reactor, found in the following figure, were found to be inconclusive because the low density of biofilm that attached due to the time constraints were not able to be imaged through the scanner and the formation of rust colored ions on slides 1, 2, and 4 caused false-positive results. As can be seen for slides 3, 5 and 6, the opacity of the slides did not deviate far enough from the baseline to show biofilm growth.



4.2.7 Microscopy (Scanning Electron and Compound Light)

The SEM graphs of the ions found in the slides can be found in figures 6.1.2.2.1 through 6.1.2.2.4 for slides 1 through 4, while the SEM images of these slides can be found in figures 6.1.2.2.5 through 6.1.2.2.8 for slides 1 through 4, in the appendix. The slides were visualized using SEM because a discoloration was noticed on the titanium dioxide deposits. The titanium dioxide deposits were originally a whitish-blue in color when placed in the reactor, yet at the end of the experiment they were a rust orange color. The SEM did not detect large amounts of iron formation, but even with small amounts of iron build-up, discoloration can be seen. It is believed that it was in fact iron that was present on the slides.

Pieces of the glass slides that were coated with titanium dioxide from the photocatalytic reactor were visualized and analyzed for ion content using Scanning Electron Microscopy (SEM). The fragments of the glass slides were found to be composed primarily of silicon and titanium with high concentrations of oxygen, a bit of calcium and basal levels of carbon, iron, sodium, magnesium, aluminum, and gold due to the initial coating of the slides. Since the slides are composed of glass, which is composed of silicon, and were coated in titanium dioxide, the concentrations of titanium, silicon and oxygen were as expected. To be imaged by the SEM the slides were coated in gold. The other compounds were either generated from the photocatalytic reactions, or found in the wastewater.

The structure of the deposits can be seen from the light microscope and SEM images. The PC500 and the P25 deposits looked distinctly different from one another, but similar to the other slide which had been coated with the same material. These slides were looked at under the light microscope to determine if there was a bacterial population growing on them. In figures 6.1.2.1.5 and 6.1.2.1.6 of slides 5 and 6 which were uncoated with titanium dioxide, it is clear to

see the bacterial populations growing on the slides, and as expected, there was a larger amount of bacteria to be found on slide 6 than on slide 5. In slides 1-4, figures 6.1.2.1.1-6.1.2.1.4, no bacteria could be visualized in the microscope, but this was due to the difficulty in visualizing through the opaque titanium dioxide deposits, and it is uncertain still whether or not bacteria was or could have been found there.

4.2 Results of experiment C, stationary TiO₂ deposited slides.

The biofilm that was grown on the uncoated slides was easily visualized. In all of the uncoated slides that were exposed to the wastewater biofilm growth was present. There was no growth seen on the slides that were exposed only to tap water. The titanium dioxide slides were more difficult to visualize using optical microscopy. The density of the titanium dioxide obscured clear visualization of the biofilm on those slides. The staining procedure allowed for visualization of the stain on some of the slides, but most of what was seen was the titanium dioxide. When these titanium dioxide coated slides were initially removed from the wastewater stained biofilm could clearly be seen on them, but after washing with water, it was not as clearly visualizable. Biofilm could be visualized in the wells of the plates containing wastewater, especially in those containing the wastewater that was before secondary treatment. Other than that there was little difference between the visual amount of biofilm grown on either of the two types of wastewater treated slides. Both the safranin and the crystal violet stained components of the biofilm on all slides where biofilm was identified.

5 Conclusions

5.1 Effect of Shear Forces and surface structure

Shear forces played a substantial role in the development and detachment of the biofilm. The forces applied to the slow reactor were able to produce a thicker film, as shown through the opacity measurements. This contactor found an efficient balance between detachment and regrowth and throughout the entirety of the experiment; there were no severe detachment periods. The fast reactor was able to accumulate more initial growth, although this set those disks up for a very severe detachment period as the biomass was easily detached. The detachment period was found to be extremely detrimental to the biofilm as it was never able to fully recover.

At the stage when the rotating disks were switched into opposite contactors, a similar conclusion was made. The shear forces of the fast tank acting upon the original slow rotating disks, disks 1-5, were too forceful to continue biofilm development. Nearly all the biofilm detached, and even after several weeks it was unable to reach the level of thickness that the original fast rotating disks, disks 6-10, were able to achieve. The opposite is true for disks 6-10, who went from the fast contactor to the slow. Because the bacterial populations were exposed to the forces in the fast reactor the development of their EPS structure was more sustainable in the slow contactor resulting in minimal detachment. This resulted in the subsequent biofilm reaching a thickness greater than the original disks 1-5 that began on the slow reactor.

Differences in the opacity and thickness of the disks in each of the RBCs were observed, however, the results of the tests performed on the samples are not so conclusive. The UV-Visible spectroscopy as well as the ammonium test shows that both reactors were equivalent in their ability to nitrify ammonium. In regards to the UV testing, both contactors saturated the spectrophotometer. The results are similar when reading the graphs of the fluorescent

spectroscopy and the COD in that both RBCs had very similar results in regards to their ability to degrade organic compounds. Both reactors were capable of growing efficient biofilm. In order to determine which reactor was more capable, the test would have to run over a longer period of time to track more detachment and more regrowth periods.

Surface structure was another parameter that was studied. The opacity of the disks strongly varied with respect to disk surface structure, especially after the first detachment event. It was determined that the concentrically grooved disks were able to retain the attached biofilm most efficiently. The four concentrically grooved disks, disks 2, 3, 7 and 8 were consistently found to have the most opacity throughout the duration of the experiment. Disks 2 and 3 did see a very large detachment period when switched from the slow reactor to the fast reactor, however, those two disks were also the most efficient at redeveloping biofilm.

5.2 Effect of photocatalysis, titanium dioxide and UV light

The photocatalytic reactor was inconclusive in regards to bacterial growth. The opacity test and visual observation using compound light microscopy were the best techniques to determine the amount of growth on the slides. However, due to the iron formation on the glass slides while in the reactor and the short time span of the experiment, the opacity measurements did not correlate to biofilm development. The iron caused false-positives on the opacity reading because it appeared as though the film was getting thicker and the opacity increased. The iron caused a rust colored discoloration which was darker than the original white coating of the titanium dioxide. Similarly, because of this coloration, it was hard to observe the slides under the light microscope to detect microorganisms and filamentous bacteria. The detection of small areas of biofilm growth on slide 6, the uncoated slide shielded from the UV light was noted. There was more minimal growth on slide 5, the uncoated slide subject to the UV light, which showed that

the wavelength of the UV light was long to kill the bacteria. The other four slides were inconclusive about the growth of biofilm because the titanium dioxide coating was too opaque to visualize bacteria with the available light source.

The nitrification process completed by experiment B was tested using UV-visible spectroscopy and ammonium tests. These tests concluded that nitrification did occur. The high levels of nitrification were most likely due to the buildup of biofilm on the outer surfaces (tubing, holding tank, etc) of the reactor than the slides, since such minimal growth was detached. Biofilm can develop easily and the whole system has the ability to house biofilm, making it impossible to pinpoint an exact location for growth. While our results do show the maturation of biofilm, it does not necessarily prove that the growth was found on the slides.

The staining of the slides did not help with the determination of biofilm formation in Experiment C. The staining was useful in clearly showing the biofilm that was growing planktonically in the media, but less useful in identifying biofilm that had formed on the titanium dioxide coated slides due to the inherent opacity of the titanium dioxide. A future recommendation would be to allow the biofilm to grow very thickly before removing it from the UV light so that a visual (without the microscope) observation on biofilm formation can be made.

6 Appendices

6.1 Figures:

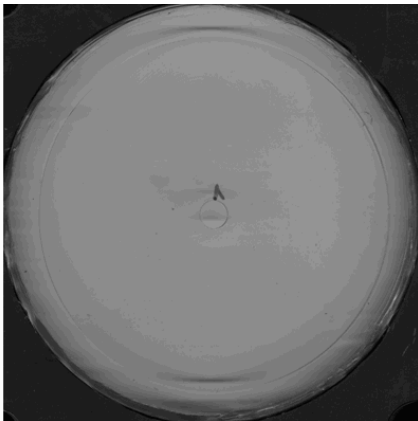
6.1.1 Experiment A, rotating biological contactors

The following figures illustrate additional results of the experiments performed on experiment A throughout the duration of the experiment.

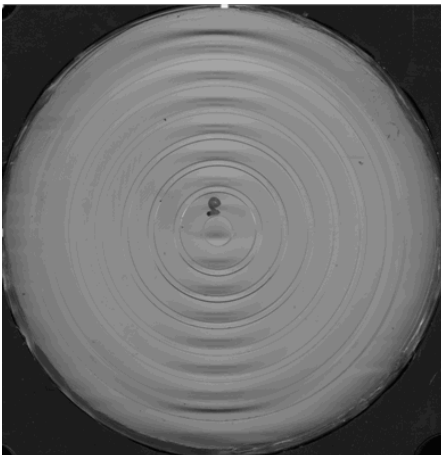
6.1.1.1 Disk Shape

The following figures show the characteristic surfaces of the disks used throughout experiment A, rotating biological contactors.

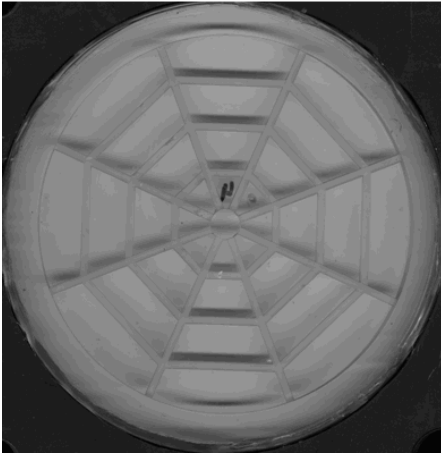
6.1.1.1.1 Flat



6.1.1.1.2 Grooved Concentric Circles



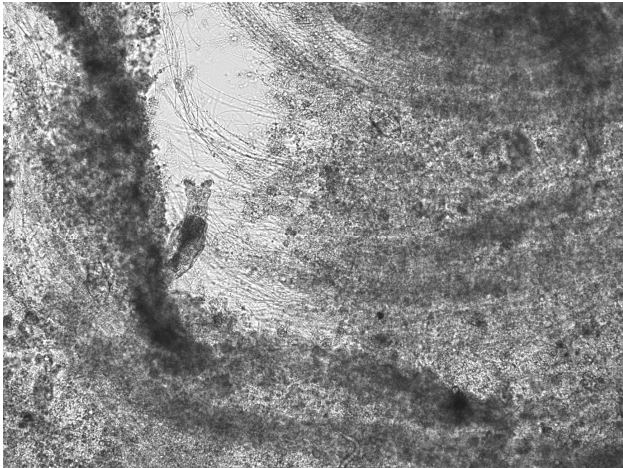
6.1.1.1.3 Grooved Webbed



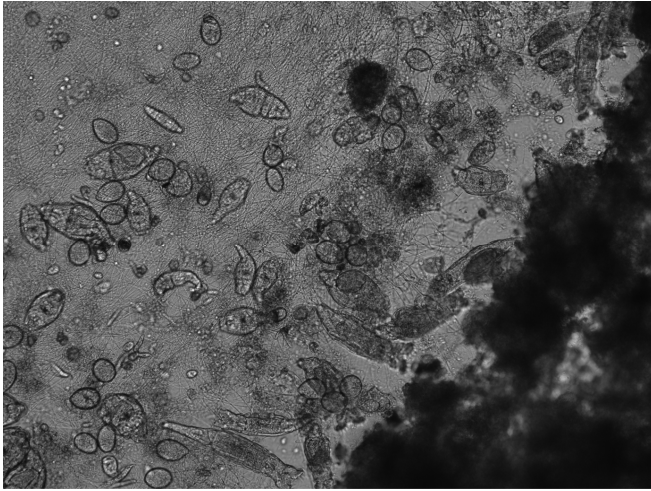
6.1.1.2 Compound Light Microscopy

The following images were taken from the rotating biological contactor with the use light microscope. Filamentous bacteria, metazoan, protozoa and rotifers were observed using this technique.

6.1.1.2.1 Disk 3, 10X



6.1.1.2.2 Disk 8, 10X



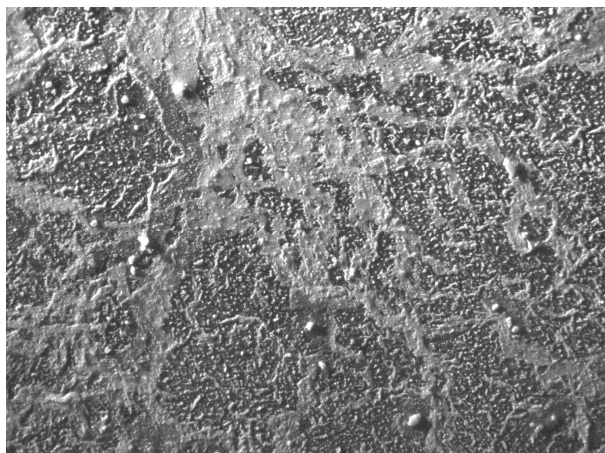
6.1.2 Experiment B, photocatalytic reactor

The following graphs illustrate the results of the experiments performed on run 17 throughout the duration of the experiment.

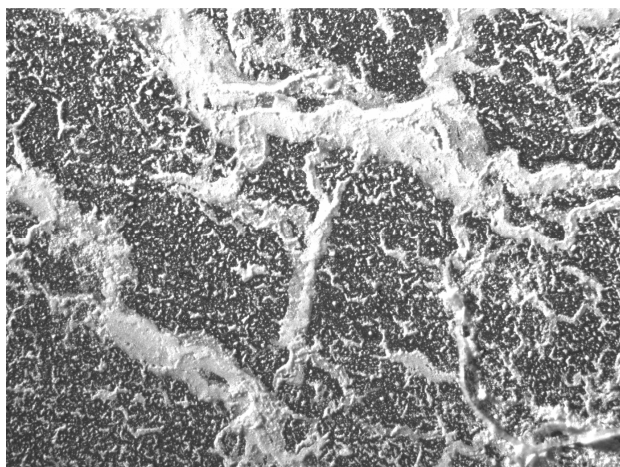
6.1.2.1 Compound Light Microscopy

The following images depict the slides used in the photocatalytic reactor at the conclusion of the experiment using the light microscope.

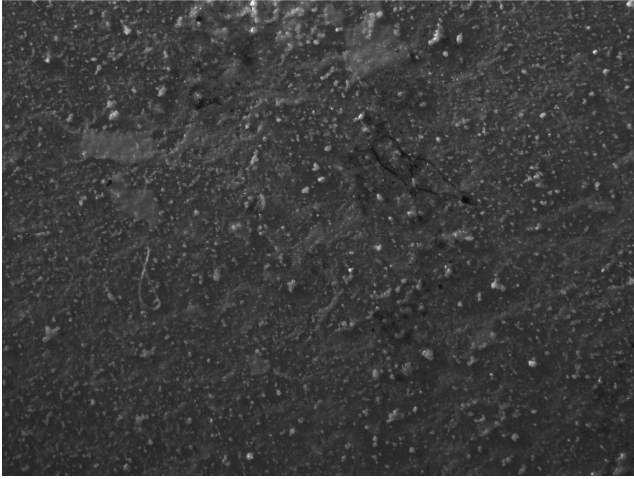
6.1.2.1.1 Slide 1, 10X, PC500



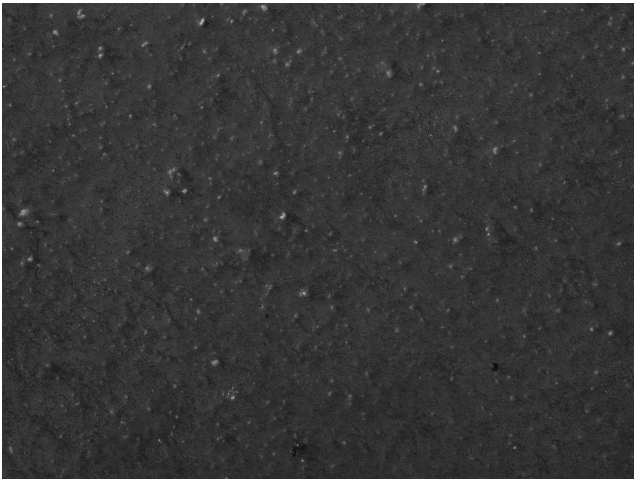
6.1.2.1.2 Slide 2, 10X, PC500



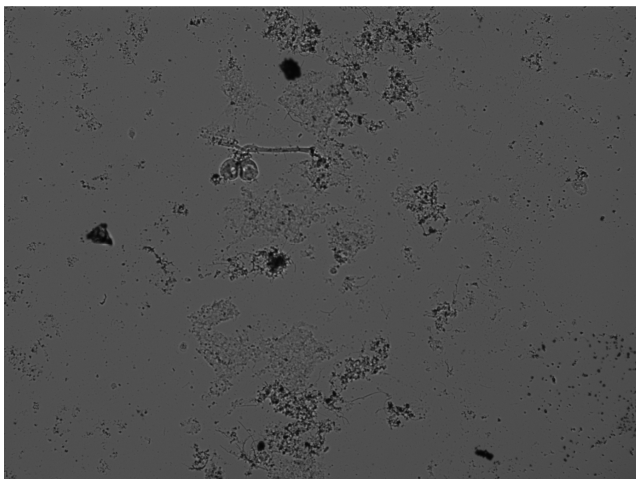
6.1.2.1.3 Slide 3, 10X, P25



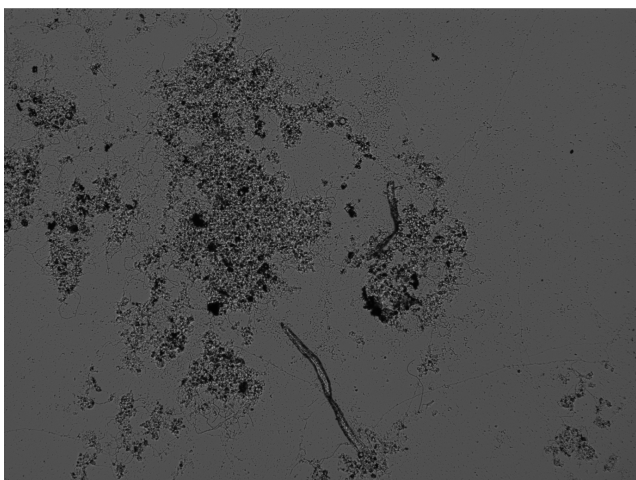
6.1.2.1.4 Slide 4, 10X, P25



6.1.2.1.5 Slide 5, 10X



6.1.2.1.6 Slide 6, 10X



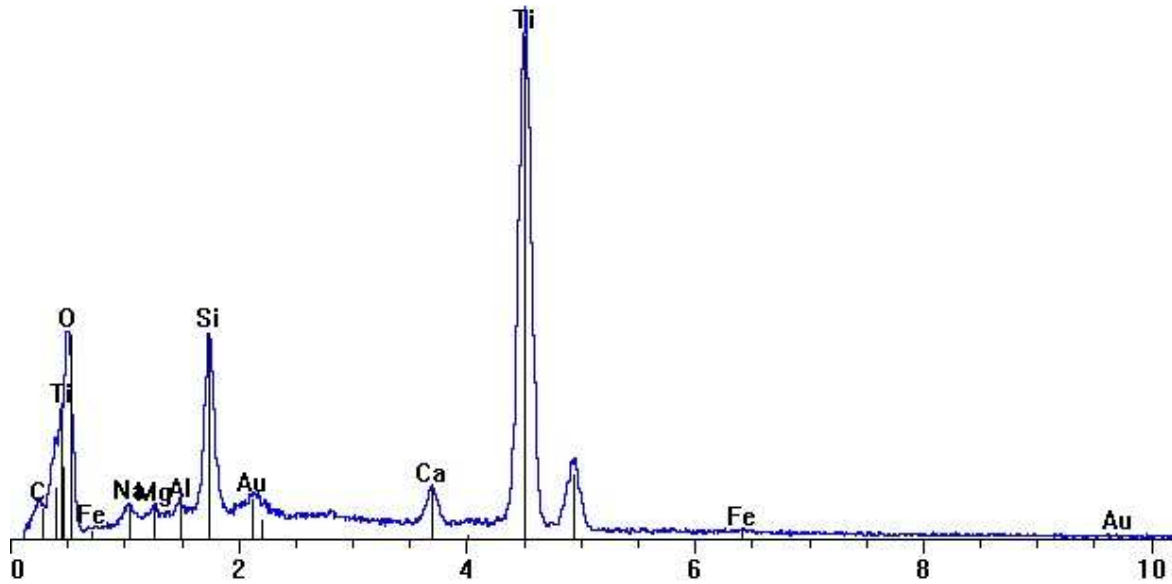
6.1.2.2 Scanning Electron Microscopy

The following images depict the slides used in the photocatalytic reactor at the conclusion of the experiment using the SEM.

6.1.2.2.1 Ion Composition, Slide 1, PC500

slide_1_4_S001.pgt

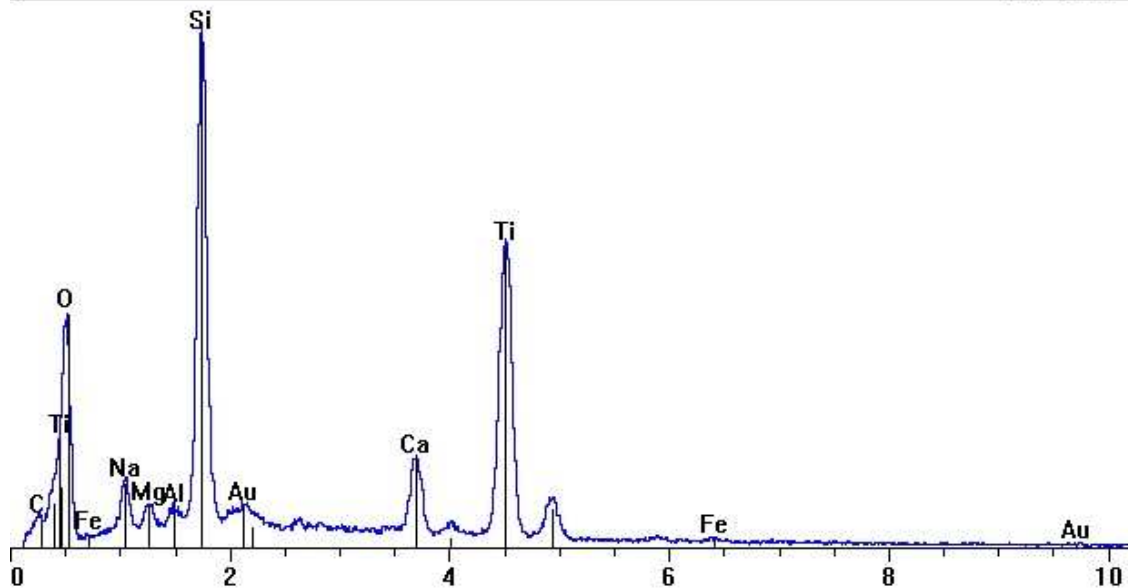
FS: 3200



6.1.2.2.2 Ion composition, Slide 2, PC500

slide_2_3_S001.pgt

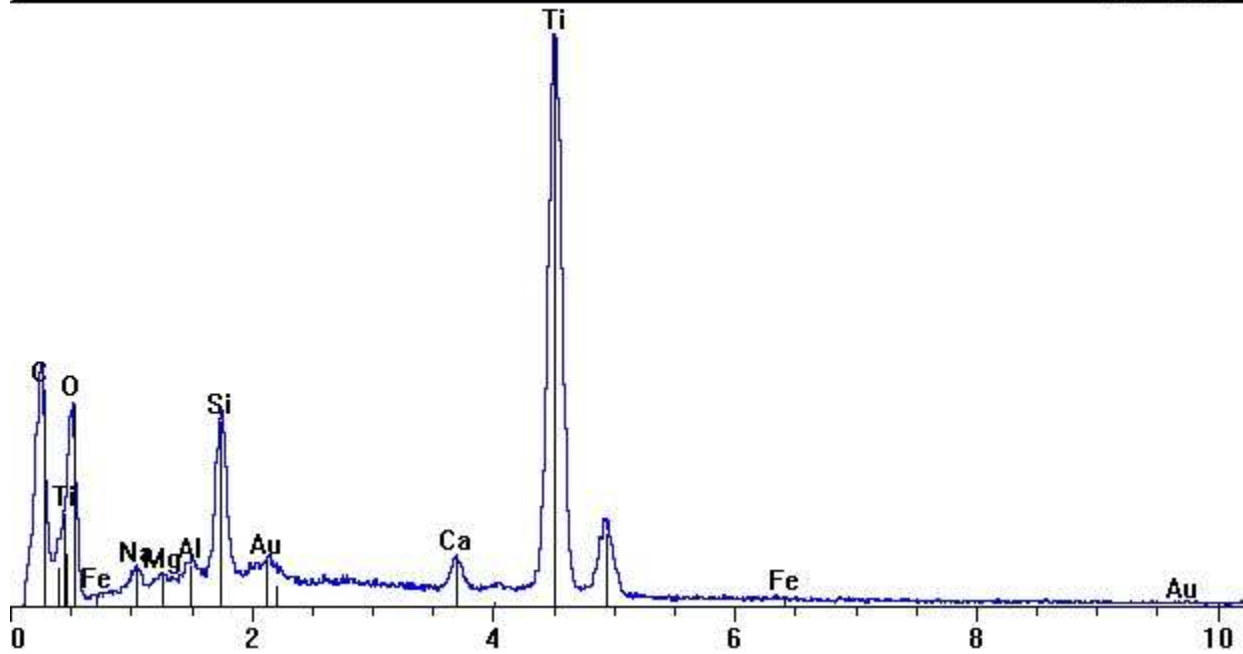
FS: 3200



6.1.2.2.3 Ion Composition, Slide 3, P25

slide_3_4_S001.pgt

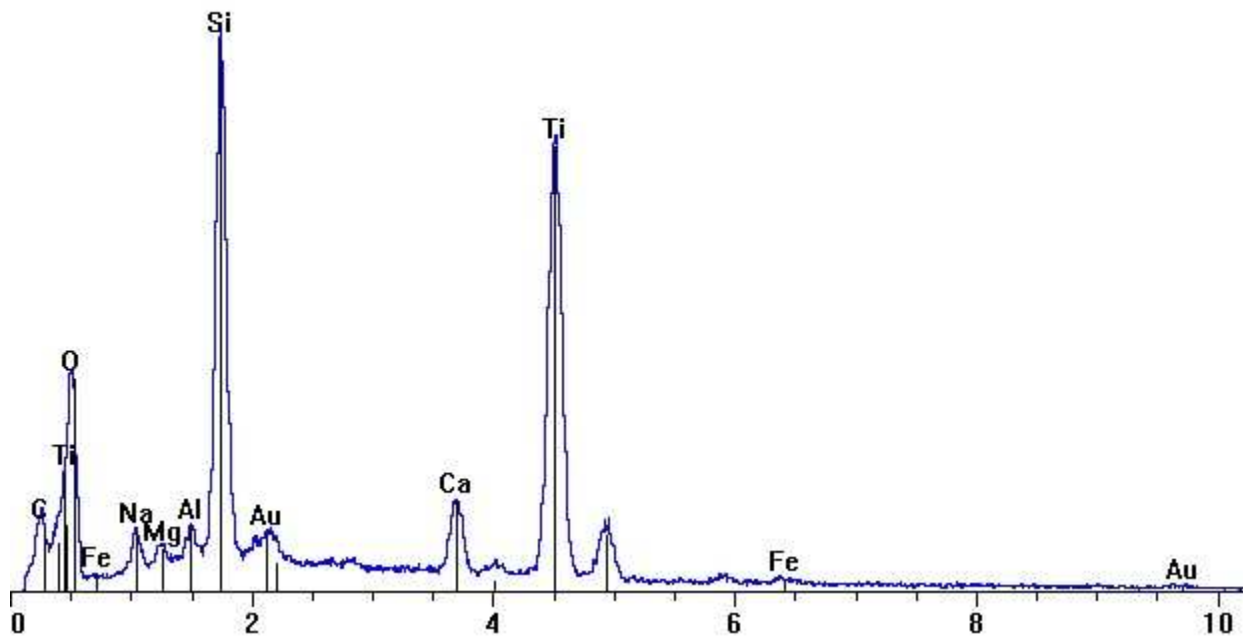
FS: 3600



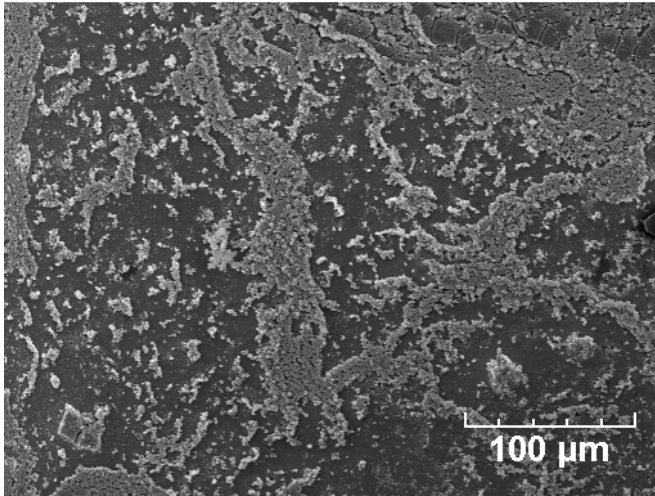
6.1.2.2.4 Ion Composition, Slide 4, P25

slide_4_3_S001.pgt

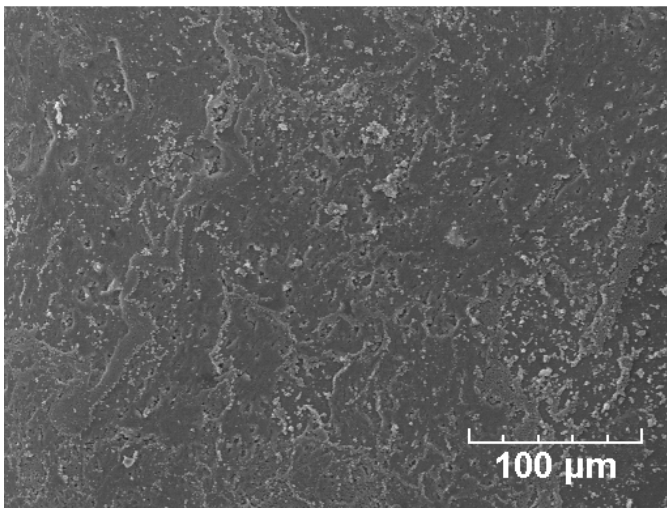
FS: 3200



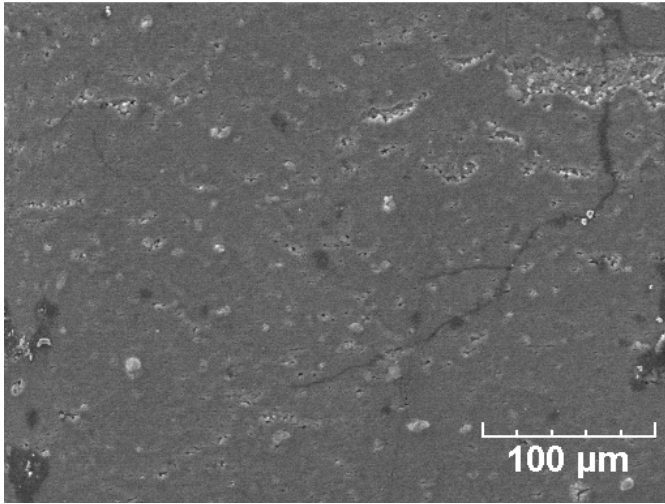
6.1.2.2.5 Image, Slide 1, PC500



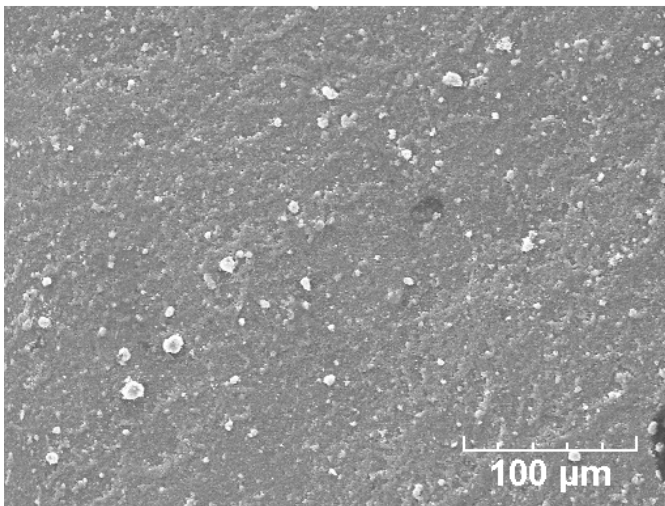
6.1.2.2.6 Image, Slide 2, PC500



6.1.2.2.7 Image, Slide 3, P25



6.1.2.2.8 Image, Slide 4, P25



6.2 Procedures:

6.2.1 Housekeeping for the Rotating Biological Contactors:

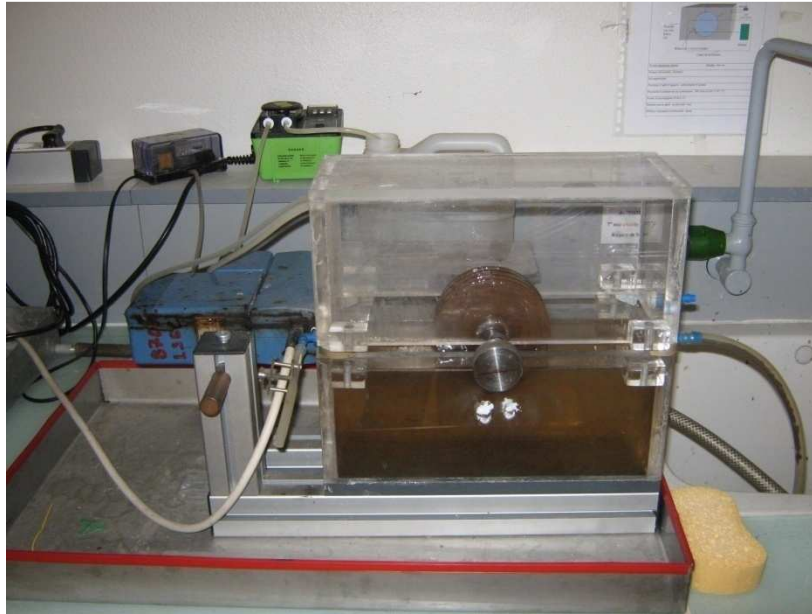
In order to ensure that the RBCs are providing the biofilm with enough nourishment, as well as having the tanks clean and efficient for accurate data, a daily housekeeping protocol was followed. These tasks changed out the synthetic substrate that replenished the tanks, removed the biofilm that had detached due to shear forces and removed the build-up of biofilm in undesirable locations, notably the plastic tubing



Procedure for the housekeeping of the slow RBC:

1. Turn off the pump and the motor attached to the small RBC.
2. Remove the empty substrate container from the counter and replace it with the freshly prepared substrate following the guidelines specified in “synthetic substrate preparation”.
3. Detach the rubber tubing connecting the pump to the substrate container and to the contactor and run under water to remove the buildup of biofilm. At this point, also remove the tubing for the air as well.
4. Reattach the tubing and put it back on the pump.
5. Remove the lid of the contactor, unscrew the back screw of the axel on the contactor and take out the biofilm disks and place into the vice on the table.
6. Remove the outer screw and carefully remove the biofilm disk.

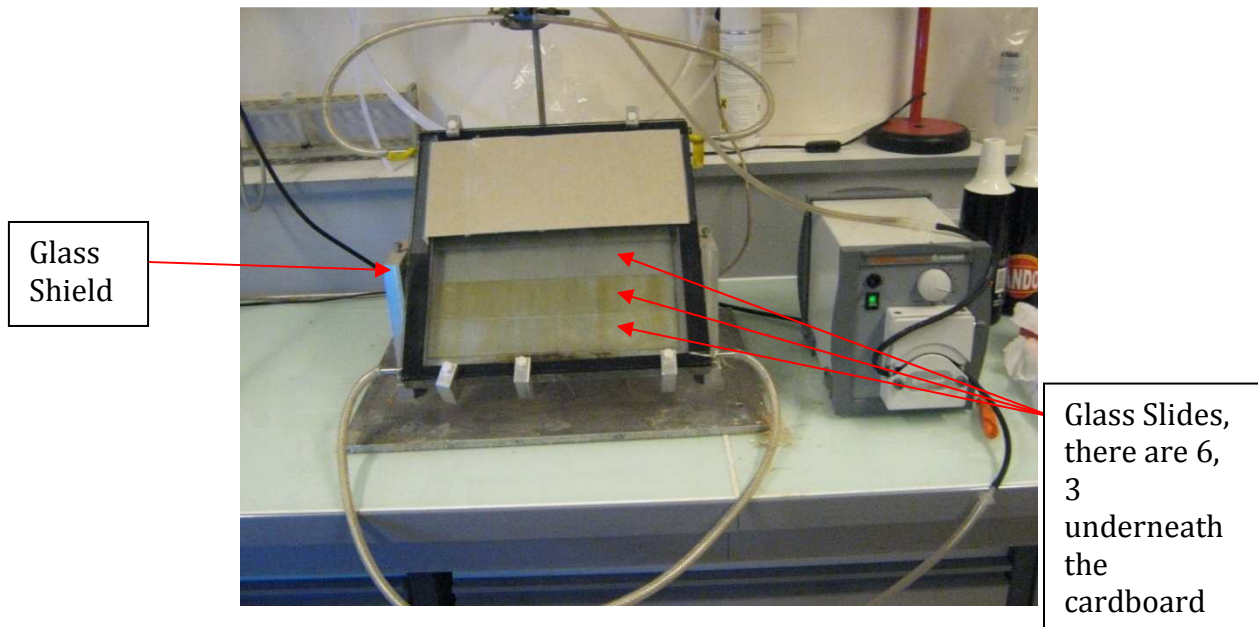
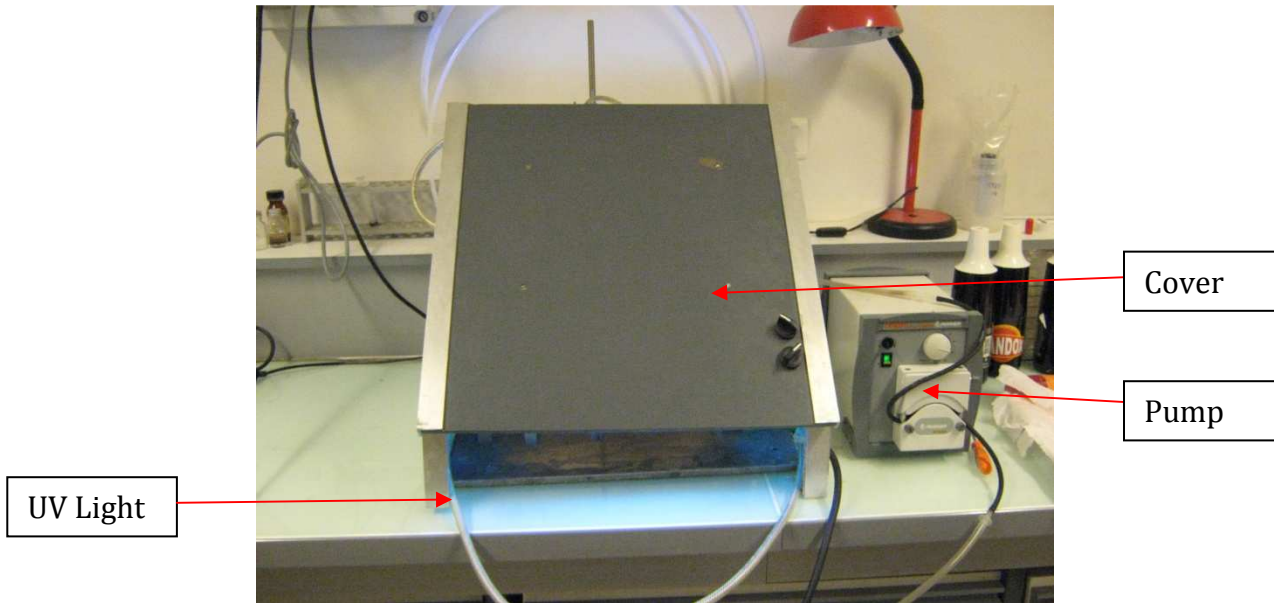
7. Use a dry paper towel to wipe away new growth of the biofilm on the backside of the disk.
8. Place into a Tupperware container to prevent the biofilm from drying out and for protection while carrying it to the scanner.
9. At this point follow the procedure outlined in “biofilm scanning”.
10. Once complete, Use a sieve to remove the detached biofilm left in the tank.
11. Collect the sample of the solution in the tank and label it with the date and the name of the contactor.
12. Place the disks back on the axel in descending order. It should be the same order with which it was first found and reattach it to the motor. Tighten the screw into place. Place the cover back over the contactor.
13. Place the rubber tubing back into both the new substrate container and in the hole of the contactor lid. Place the air tube back in the contactor lid as well and turn on both the pump and the motor to start the contactor again.

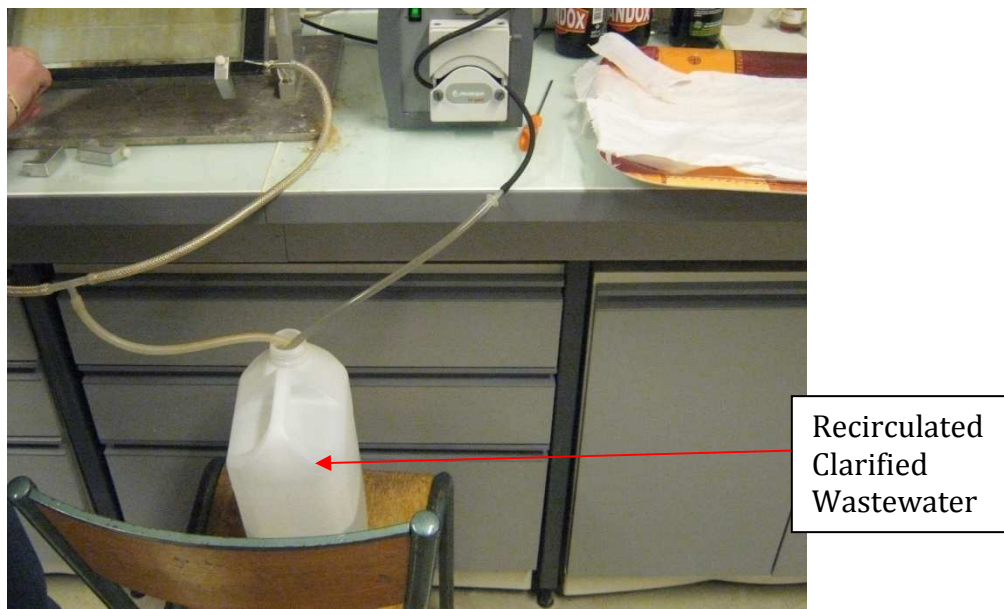


Procedure for the housekeeping for the fast reactor:

1. Follow the same procedure of the slow reactor, steps 1-4.
2. To remove the cover of the fast contactor, unscrew the four screws at the top of the container.
3. Remove the rubber band surrounding the axel and remove the biofilm disks. Place on the counter balancing on the large circular edge and carefully remove the disks.
4. Continue to follow the protocol of the slow reactor, steps 7-11.
5. Place the axel back into the tank and place the rubber band back on the axel.
6. Place the cover back on and tighten the screws to form a tight hold.

7. Place the rubber tubing back into both the new substrate container and in the hole of the contactor lid. Place the air tube back in the contactor lid as well and turn on both the pump and the motor to start the contactor again.





Procedure for the housekeeping for the photocatalytic reactor:

1. Turn off the UV light and unplug the electricity from the cover and the pump.
2. Remove the UV light shield from the reactor and set aside.
3. Remove the glass shield from the top of the reactor by unscrewing the clamps and set aside.
4. Remove the glass plates from the reactor. Place them face up on paper towels in preparation for their scanning.
5. Collect a sample of the clarified water by filtering it through a coffee filter. Label the bottle with run17, the date, and the description 'old'.
6. Every other day, exchange out the clarified water by dumping the old water in the sink after the sample was collected.
7. Measure 4L of fresh clarified water from the fridge.
8. Take another sample of the new clarified water by filtering it through a coffee filter. Label the bottle with run 17, the date, and the description 'new'.
9. After the scanning, place the plates back on the reactor in the same order as before.
10. Place the glass shield back on the reactor; be sure the cardboard is on the top half of the reactor.
11. Place the clarified water back where it was situated; plug back in the cords for the pump and UV light.
12. Replace the UV light; turn it on, as well as the pump.

6.2.2 Synthetic substrate preparation:

The biofilm was seeded with wastewater from the Nancy, France wastewater treatment plant for the first 15 days. After this point in time, synthetic wastewater was introduced. The synthetic wastewater was prepared from di-Sodium Phosphate, Ammonium Chloride, Sugar, Tap Water, and Viadox, a solution of meat and yeast extracts.

Procedure for the preparation of the synthetic substrate concentrate:

1. Fill a 1000mL volumetric flask with approximately 400mL of tap water.
2. Add 1 cube of sugar, 0.25g di-sodium hydrogen phosphate, and 5g of ammonium chloride.
3. Stir using a magnetic stir bar and a magnetic plate until all of the solids have dissolved.
4. Add 100g of Viadox
5. Fill to 1000mL with tap water
6. Stir until uniform
7. Using a funnel transfer to 1L plastic bottle
8. Store in refrigerator.

To make properly diluted substrate dilute the concentrate in the ratio of 50mL of concentrate to 4L of tap water. (12.5 mL concentrate/L tap water) Mondays through Thursdays 4L of properly diluted synthetic substrate was prepared, while on Fridays 6L of properly diluted synthetic substrate was prepared.

6.2.3 Biofilm Scanning:

An Epson Perfection 4490 Photo Flat-bed scanner was used to scan the development of the biofilm daily. A frame was placed on the scanner to ensure accurate alignment, providing each disk with an identical field of view. It was also used as a protective surface to prevent wear to the scanner. Styrofoam was placed on both ends of the scanner surface to protect the biofilm from touching to the top of the scanner as it was closed. The amount of light was kept constant within the room, keeping the overhead light off so as to not fade the images. The program EPSON SCAN was used to collect images of the disks. Eleven images were collected per day including one blank image, five images from the slow contactor and five images from the fast contactor. The program saves these images in the TIFF file format in a designated folder. The scanning process was conducted in an efficient manner to minimize the amount of time that the biofilms are out of the RBC and the extent to which they dry out.

Procedure followed for scanning the biofilm disks:

1. Turn on computer and scanner.
2. Turn off light in main scanner room and turn on the light in the secondary room (minimize light interference)
3. Open program Epson SCAN
4. Use Mode Professional, Parametrage 1 for run 16
5. Check the alignment of the frame on the scanner bed. It will be appropriately adjusted if the far edge is positioned at the second notch on the left top corner of the scanner.
6. Begin by clicking on the *Apercu* button for a short scan to recheck alignment.
7. If aligned properly, click on the Numeriser button for a full scan of the selected area.
8. A box will pop up after clicking Numeriser, make sure the file name begins with run#_date(yymmdd) and that it is set to file 001, then hit OK [*same thing?*] and the image will be scanned and saved. Each day the blank image will be set as number 001.
9. Beginning with disk number 1, remove the disk from Tupperware, again wipe the backside with a paper towel and place it on the scanner in the center of the frame with the biofilm facing upwards.
10. Scan the disk by clicking *Numeriser*, the file names will ascend in numerical order and press OK. The image will be scanned and saved to the file.
11. When switching from one disk to the other, wipe the scanner with a dry paper towel to remove any residual moisture. Recheck for alignment of the frame after each run as well.
12. Place the disks back in the box after scanning.
13. Once all five disks from a single contactor are imaged, place the disks back into the RBC in original order. Prepare the contactor and allow it to run again.
14. After scanning all of the disks from the slow RBC, continue to scan the disks from the fast RBC by following steps 9-14. In total there will be 11 files per day.
15. Exit the program and turn off the computer, scanner, and light in the secondary room.

Procedure for the scanning of the titanium dioxide glass plates:

1. Turn on computer and scanner.
2. Turn off light in main scanner room and turn on the light in the secondary room (minimize light interference).
3. Open program Epson SCAN
4. Use Mode Professional, Parametrage 8 for run 18. This will alter the parameter of the screen.
5. Remove the frame used for run16 and replace it with two glass slides, appropriately labeled. The slides will go into the front corners of the scanner in the same alignment.
6. Begin by clicking on the *Apercu* button for a short scan to check the alignment.
7. If aligned properly, click on the Numeriser button for a full scan of the selected area.

8. A box will pop up after clicking Numeriser, make sure the file name begins with run17_date(yymmdd) and that it is set to file 001, then hit OK and the image will be scanned and saved. Each day the blank image will be set as number 001.
9. Wipe the back of the plate and scan the plate by placing it face down on the glass slides.
10. Scan the disk by clicking *Numeiser*, the file names will ascend in numerical order and press OK. The image will be scanned and saved to the file.
11. Place the disks back in the box after scanning.
12. Once all six plates are imaged, place the disks back into reactor in original order.
13. Exit the program and turn off the computer, scanner, and light in the secondary room.

6.2.4 Preparation of Titanium Dioxide Deposited Glass Slides

Titanium Dioxide was deposited on glass slides. To do this a solution of titanium dioxide in water was prepared.

Step by step instructions for titanium dioxide solution preparation:

1. Mix in a 100ml volumetric flask, 0.4 g of titanium dioxide
2. Fill to 100ml with pure water.
3. Mix by inverting
4. Adjust pH of solution to about 3 from about 7 using pH strips (PH-fix-0-14, Art. Nr 92110 Machery-Nagel (MN)) and 1M HNO₃
5. Sonicate for 15minutes.
6. Use, cover and save, when ready to reuse, sonicate for 10 min to break up any clumps of titanium dioxide that have formed.

The glass slides were then prepared for the depositing of the titanium dioxide solution by washing with acetone, labeling and weighing each slide.

To deposit the titanium dioxide solution, scotch tape was placed around each of the glass slides. The solution was then spread evenly over the slide using a pipette. The slides were allowed to dry for 4 to 5 hours. Once the slides were dry they were placed in an oven to calcine. The oven heated the slides up to 100°C from room temperature for 1 hour, then stayed at 100 °C for 1 hour, then heated the slides up to 475°C for one hour, and stayed at 475°C for 5 hours. Then the oven turned off. The slides were allowed to cool in the oven overnight and were retrieved, re-taped; solution was spread, dried and heated, until 4 layers were added.

6.2.5 Ammonium Measurement:

The concentration of ammonium bound nitrogen was tested and recorded twice a week for the substrate sample and the water samples for each of the contactors. Using the Hamilton digital diluter, samples were diluted 10 fold before being analyzed by the Hach Spectrophotometer. A mineral stabilizer, polyvinyl alcohol and Reactif de Nessler were added to the samples in order to begin the necessary chemical reaction. Nessler's reagent is a 0.09 mol/L solution of potassium tetraiodomercurate (II) in 2.5 mol/L potassium hydroxide. After introducing the chemical to the solution, the tetraiodomercurate (II) reacts with the ammonium in the sample producing ammonium ions and causing a change in color. There is a direct correlation between the color intensity and the concentration of ammonium ions in the sample. This color alteration is then read by the spectrophotometer, multiplied by 3.6517 and again multiplied by 10 to account for the dilution factor.

Step by step procedure for the Ammonium Measurement:

1. Using the Hamilton Digital Diluter, dilute the daily substrate sample and the two samples from the contactors 10 fold.
2. The values on the machine should be set at 90 X 50.
3. Place the tube into the sample and hit the down arrow to suck up the appropriate volume of sample and pure H₂O.
4. Place the tube into a clean test tube and press the down arrow, injecting 5mL on diluted sample into the test tube.
5. Repeat steps 3 and 4 to attain 10mL of the diluted sample.
6. Add two drops of the mineral stabilizer into the diluted sample.
7. Add two drops of the polyvinyl alcohol into the diluted sample.
8. Add 400uL of the Reactif de Nessler into the diluted sample.
9. Cover sample and invert to mix.
10. To measure the ammonium concentration using a spectrophotometer. Select the wavelength of 425nm.
11. Zero with the blank control. Each tube is to be test 4 times, turning the test tube a quarter turn to assure an accurate reading. If there is a negative reading while setting the zero, press the zero button again and the process over.
12. To test the samples, use the same process as the blank, recording a measurement in quarter turn intervals. If the standard deviation is large, record 8 measurements by turning the tube an 8th of the way and discard the data from the quarter turns.
13. The trend line generated of the ammonium bound nitrogen concentration = $3.6517 * (\text{percent absorbency})$ with an R^2 value of 0.999. This equation is used to calculate the concentration of ammonium bound nitrogen in the sample.

6.2.6 Chemical Oxygen Demand measurement:

COD measurements are used to determine the amount of organic compounds in water. The reactions that take place during this test oxidize all or nearly all of the organic compounds into carbon dioxide, ammonium, and water. A strong oxidizing agent is used to oxidize all of the organic compounds. Potassium dichromate is a strong oxidizing agent in acidic conditions and the one that was used here. The dichromate is reduced to the chromic ion by this reaction, which absorbs strongly in the 600nm region, while the dichromate does not absorb strongly in the 600nm region. The absorption is then detected by the spectrophotometer.

Step by step instructions for COD measurement are:

1. Turn on power of Aqualytic CSB/COD reactor AL 31.
2. Set it to 148°C
3. Inject 2ml of sample (a substrate sample taken from one of the reactors or a sample of the fresh substrate added that day) into a test tube. Inject 2ml of de-ionized water into the test tube that will serve as the control.
4. Add 1.5ml of Solution Digestion COD range 0-750 O₂/L. (the solution digestion is composed of water, potassium dichromate, and mercury sulfate)
5. Add 3.5 ml of sulfate solution. (Concentrated sulfuric acid and silver sulfate.)
6. Cover sample and invert to mix.
7. Place test tubes in reactor.
8. After 2 hours allow the samples to cool to room temperature.
9. To measure COD using spectrophotometer, select a wavelength of 620nm.
10. Zero with the control, turning the test tube, quarters of the way in a full circle, measuring 4 times, to assure that all readings are similar, and that there are not major discrepancies due to messed up glassware. If there is a negative reading while setting the zero, hit zero again and start turning in quarters again.
11. If the standard deviation is large, turn the test tubes, an eighth of the way in a full circle, measuring 8 times and discard the data from the quarter turns.
12. Use the Curve Calibration Graph for the Solution Digestion COD range 0-750 O₂/L to find how the percent absorbency at 620nm correlates to the COD in mg O₂/L. The trend line generated is that the COD = 2,953*(percent absorbency) with an R² value of 0.998. This equation is used to calculate the COD.

6.2.7 Ultraviolet-Visible Adsorption and Fluorescence Spectroscopy:

The UV- visible adsorption spectroscopy and the Fluorescence spectroscopy is used to determine which analyte components are present in the samples of wastewater collected from the two RBCs and the reactor. Peaks present on the spectrum each correlate to a different analyte. In regards to the UV analysis, peaks were present at 215nm and 254nm, which corresponds to Nitrate and aromatic molecules and soluble COD. In the results it was found that the fast reactor and the slow reactor would show a greater peak at the 215nm due to the presence of the biofilm. The biofilm changes the ammonium, found in high quantities in wastewater, into Nitrate. The system can be saturated however, which peaks at **(3000)**. For the fluorescence spectroscopy, peaks were noted at 285nm and 400nm, corresponding to Tryptophan (or other aromatic proteins) and optical brighteners found in detergents, respectively.

Step by step procedure for UV-visible spectroscopy:

1. Sign onto the computer using the name Eccma9.
2. Turn on the power of the spectrophotometer and press the validate button to bring the autotest.
3. The spectrometer will prompt to print (impremier) hit escape on the panel.
4. Change the configuration to Laison RS232 and press validate.
5. At the conclusion of the autotest turn on the computer and open the LabPowerJ program.
6. Select the Methods on the upperleft side of the screen and select balayage de spectre.
7. Select the Editer on the bottom left and change the parameters to read 200nm min and 600nm max. Press okay when completed.
8. Fill the Quartz cuvet with DIH₂O and put into the spectrophotometer.
9. The computer will prompt a baseline reading, press ok.
10. Put in sample number 1 and click the M button in the top bar.
11. Once complete, select fishier and export the file into excel.
12. Save into folder entitled run16 for the contactors and folder run17 for the reactor. Save the file in the format, UV-reactor/substrate/run-day-month.
13. Continue process #9-#11 for the remainder of the samples.

Step by step procedure for the Fluorescence Spectroscopy:

1. Sign onto the computer using the name Pons.
2. Turn on the fluorescence spectrophotometer and be sure the light for the lamp turns on.
3. Open the program FLSolution2.0 from the desktop.
4. Set a baseline by selecting methods, eau-sync and click okay.
5. Select the instrument screen and put it on synchronomous.
6. Select the Data mode and change it to fluorescence. Set the three following parameters to 280nm, 230nm and 600nm respectfully.
7. Fill a plastic cuvet with DIH₂O.
8. Click on the spectrophotometer key and select adjust zero
9. Fill the plastic cuvet with sample 1. Select sample from the bar on the right and name sample in the format of FL-reactor/substrate/run-day-month.

10. Click measure and let the spectrophotometer run.
11. Select Fishier – le copieux – and save the samples into the corresponding folder, either run16 or run17.
12. Close all windows and follow process #9-#11 for the remainder of the samples.

6.2.8 TOC and Ion Chromatography:

Steve Pontvianne, the technician at ENSIC, performed the TOC and ion chromatography tests. These tests required a username and password in order to be accessed. Samples were prepared for the tests, and the instructions have been included below.

Step by step procedure for the preparation of samples for TOC:

1. Measure 30 to 40 ml of sample.
2. Filter the sample using a coffee filter.
3. Store samples in a refrigerator until testing.

Step by step procedure for the preparation of samples for Ion Chromatography:

1. Measure 1.5 ml of sample using a syringe.
2. Filter using a 0.45 μ m syringe filter.
3. Store in vials in a refrigerator until testing.

6.2.9 Biofilm Dry Mass:

Biofilm dry mass was calculated to develop the correlation between biomass and opacity.

Step by step procedure for the determination of biofilm dry mass:

1. Weight and dry over night in a drying oven pie tins and filter paper.
2. Using a spatula scrape off all of the biomass from the disk onto the filter paper.
3. Vacuum filter the filter paper, while rinsing the rest of the biomass off of the disk into the filter.
4. Vacuum filter until dry.
5. Place filter paper with biomass into pie tin and pie tin set up into drying oven overnight.
6. Measure weight of biomass, filter paper and pie tin together.

6.2.10 Microscopy:

Light Microscopy:

Microscopy was used to visually observe the growth of the biofilm on both the rotating biological contactors and the photocatalytic reactor. The samples were looked at under an optical microscope at varying magnifications, 5X, 10X, 20X, and 63X. A sample was collected of the biofilm that had detached from the mass on the disks and at the end of the experiment before the dry mass procedure was followed.

Scanning Electron:

The glass slides from experiment B were broken into shards of the appropriate size to fit in the holder for the SEM. Professor Pons coated them in gold for imaging. Several shards from each slide was viewed. Marie Noelle Pons conducted the SEM to obtain our results.

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