

**IMPACT ASSESSEMENT OF THE PRESENCE OF ENGINEERED IRON
OXIDE NANOPARTICLES ON WASTEWATER EFFLUENT DISINFECTION**

By

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ABSTRACT

A previous study indicated that ~8.7% wt. Fe of the initially dosed engineered iron oxide nanoparticles coated with surfactants ($\text{ENP}_{\text{Fe-Surf}}$), as contaminants, was present in the effluent from the lab-scale sequencing batch reactors (SBRs). It was suggested that presence of $\text{ENP}_{\text{Fe-Surf}}$ might impact disinfection of wastewater effluents. Therefore, this study assessed the interactions among chlorine, $\text{ENP}_{\text{Fe-Surf}}$ and fecal coliforms (FC) in batch and plug flow reactor (PFR) disinfection processes using real wastewater effluent collected from a local wastewater treatment plant. Then, the characteristics of the settled SBR sludge incorporating $\text{ENP}_{\text{Fe-Surf}}$ were examined in order to validate the previous study aforementioned.

The results from batch disinfection studies showed that $\text{ENP}_{\text{Fe-Surf}}$ produced adverse (unfavorable) effects on FC disinfection. To achieve a 4-log FC removal, the required free chlorine residuals were 0.22, 0.18, and 0.10 mg $\text{Cl}_2 \text{ L}^{-1}$ for contact times of 24, 30, and 60 minutes in the absence of $\text{ENP}_{\text{Fe-Surf}}$. In comparison, when $\text{ENP}_{\text{Fe-Surf}}$ was present at 4.97 mg L^{-1} as Fe, the required free chlorine residual concentrations were 0.18, 0.14, and 0.07 mg $\text{Cl}_2 \text{ L}^{-1}$ for the same contact times, respectively, to achieve the same log removal of FC. More log FC removal was achieved for the same chlorine consumption in the absence of $\text{ENP}_{\text{Fe-Surf}}$ compared to values when it is present due to additional chlorine consumption (i.e., scavenging) with $\text{ENP}_{\text{Fe-Surf}}$.

The results from PFR disinfection studies showed scavenging effects of $\text{ENP}_{\text{Fe-Surf}}$ on chlorine as well: ~5% of the added chlorine concentration was still measured at pseudo steady state in the presence of $\text{ENP}_{\text{Fe-Surf}}$, while ~10% was observed in the absence of $\text{ENP}_{\text{Fe-Surf}}$. In general, FC log removals were proportional to CT values (the product of the residual disinfectant

concentration, C , in mg L^{-1} times the contact time, T , in min), whether or not $\text{ENP}_{\text{Fe-Surf}}$ was present. The same FC log removal at the same disinfection contact time was accomplished in PFR at lower concentrations of free chlorine residuals in the presence of $\text{ENP}_{\text{Fe-Surf}}$ compared to its absence. Again, this was due to scavenging effect of $\text{ENP}_{\text{Fe-Surf}}$ on chlorine, which reduces chlorine residuals available for FC disinfection and, thereby, poorer FC disinfection efficiency.

The settled activated sludge of the SBR run in the presence of $\text{ENP}_{\text{Fe-Surf}}$ had different characteristics from that of the control SBR run without $\text{ENP}_{\text{Fe-Surf}}$ present. It had unique iron-rich, hexagon-shaped compounds. This implies that the presence of $\text{ENP}_{\text{Fe-Surf}}$ modifies the physicochemical properties of the sludge and could alter the efficiency in the sludge management.

RESUMEN

Un estudio previo indicó que ~8.7% por peso en términos de Fe de la dosis inicial de nanopartículas de hierro oxidado recubiertas con un surfactante ($ENP_{Fe-Surf}$), como contaminantes, estuvo presente en el efluente de un reactor de lotes secuenciales a escala de laboratorio (SBR por sus siglas en inglés). Se sugirió que la presencia de $ENP_{Fe-Surf}$ puede impactar el proceso de desinfección del efluente de agua residual. En este estudio, las interacciones entre cloro, $ENP_{Fe-Surf}$ y los coliformes fecales (FC) fueron evaluadas en los procesos de desinfección de reactores por tanda y de flujo a pistón (PFR por sus siglas en inglés) utilizando efluente de agua residual de una planta de tratamiento de aguas residuales local. Por ende, las características de los lodos sedimentados en el SBR incorporando las $ENP_{Fe-Surf}$ fueron examinados para sustentar el estudio previamente mencionado.

Los resultados de desinfección por tanda mostraron que las $ENP_{Fe-Surf}$ produjeron efectos adversos (poco favorables) en la desinfección de los FC. En ausencia de $ENP_{Fe-Surf}$, para obtener una remoción logarítmica de 4-log en FC, fueron requeridos residuales de cloro de 0.22, 0.18 y 0.10 mg $Cl_2 L^{-1}$ para tiempos de contacto de 24, 30 y 60 minutos. En comparación, cuando hubo presencia de $ENP_{Fe-Surf}$ a 4.97 mg L^{-1} en términos de Fe, las concentraciones de cloros residuales requeridas fueron 0.18, 0.14 y 0.07 mg $Cl_2 L^{-1}$ para los mismos tiempos de contacto, respectivamente, para lograr la misma remoción logarítmica de FC en términos logarítmicos. Una mayor remoción de FC en términos logarítmicos fue obtenida para los mismos consumos de cloro en ausencia de $ENP_{Fe-Surf}$ en comparación con los valores cuando hubo presencia de $ENP_{Fe-Surf}$ debido al consumo adicional de cloro (por ejemplo, capturando) por parte de las $ENP_{Fe-Surf}$.

Los resultados de desinfección en PFR mostraron los efectos de captación de las $ENP_{Fe-Surf}$ en cloro: ~5% de las concentraciones de cloro añadidas fueron medidas durante los estados semi-estacionarios en presencia de $ENP_{Fe-Surf}$. En comparación, ~10% fue observado en ausencia de $ENP_{Fe-Surf}$. En general, las remociones de FC en términos logarítmicos fueron proporcionales a los valores de CT (el producto de la concentración de desinfectante residual, C en $mg L^{-1}$ multiplicado por el tiempo de contacto, T, en minutos) sin importar si las $ENP_{Fe-Surf}$ estuvieran presentes o no. La misma remoción de FC en términos logarítmicos en los mismos tiempos de desinfección fueron obtenidas en el PFR con menores concentraciones de cloros libres en presencia de $ENP_{Fe-Surf}$ comparado con la ausencia de $ENP_{Fe-Surf}$. Nuevamente, esto fue debido al efecto de captación de $ENP_{Fe-Surf}$ en cloro, reduciendo la cantidad de cloro residual disponible para desinfección de FC y por consecuencia, menor eficiencia en la desinfección de FC.

Los lodos activados sedimentados de los experimentos de SBR en presencia de $ENP_{Fe-Surf}$ tuvieron características diferentes comparadas a las del experimento control de SBR sin la presencia de $ENP_{Fe-Surf}$. Se obtuvieron unos compuestos únicos y ricos en hierro con formaciones hexagonales. Esto indicó que la presencia de $ENP_{Fe-Surf}$ modifica las propiedades físico-químicas de los lodos y pudiera alterar la eficiencia en el manejo de los lodos.

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To my Lord, family, and friends...

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LIST OF ABBREVIATIONS

BOD₅ = Biochemical Oxygen Demand (5-day)

CFU = Colony Forming Unit

CNT = Carbon Nanotubes

CT = Residual Free Chlorine Concentration x Contact Time

COD = Chemical Oxygen Demand

DI = Deionized Water

DNA = Deoxyribonucleic Acid

DOM = Dissolved Organic Matter

EBCT = Empty Bed Contact Time

EDAX = Energy-Dispersive X-ray Spectroscopy

ENP = Engineered Nanoparticle

ENP_{Fe-Surf} = Engineered Iron Oxide Nanoparticles Coated with Surfactants

EPA = Environmental Protection Agency

FC = Fecal Coliforms

MLSS = Mixed Liquor Suspended Solids

MLVSS = Mixed Liquor Volatile Suspended Solids

NERC = Natural Environmental Research Council

PFR = Plug Flow Reactor

RAS = Return Activated Sludge

RNA = Ribonucleic Acid

RTD = Residence Time Distribution

SBR = Sequencing Batch Reactor

SEM = Scanning Electron Microscope

TDS = Total Dissolved Solids

THB = Total Heterotrophic Bacteria

TP = Total Phosphorus

TSS = Total Suspended Solids

USGS = United States Geological Survey

WHO = World Health Organization

WWTP = Wastewater Treatment Plant

XRD = X-ray Diffractometer

1 INTRODUCTION

As an emerging field, nanotechnology has had a vast industrial and commercial increase, but it has also brought potential health and safety risks to mankind and the environment (Morose, 2010). Most of today's technologies are prepared for treating common and conventional types of pollutants. However, the environment and wastewater treatment plants (WWTP) are still not prepared or, at best, are seeking for new methods and techniques for emerging contaminants such as nanostructure materials. Since there are no standards or regulations for the appropriate discharge of nanostructure materials or engineered nanoparticles (ENPs) to the environment, most of the industries, businesses, , laboratories are currently discharging large amounts of used , waste ENPs. These discarded ENPs enter water bodies and/or WWTPs. Once the ENPs are in the water/sediment environment, their form, distribution between media, and ultimate transport will depend on a variety of physical influences (Weinberg et al., 2011), which will impact the wastewater treatment.

Wastewater treatment plants deal with different types of the components present in wastewater, such as natural organic matters, inorganic matters and microorganisms. The ENP presence as contaminants could become a problem since interactions between natural water components and ENPs might result in formation or break-up of aggregates based on the surface properties of the nanoparticles (Weinberg et al., 2011). The presence of ENPs is much of a concern as to what its effect is on the wastewater microorganisms, for example, fecal coliforms bacteria.

Although many nanostructure materials are already in the market, it is important to know how they will affect and benefit the environment and mankind, and also how they should be

treated and disposed of after their use. Additionally, ENPs effect on microorganisms in wastewater is starting to generate interrogatives and there are still few studies in this field. Therefore, this research focused on studies of the impact of the presence of engineered iron oxide nanoparticles as contaminants on chlorine consumption and fecal coliforms during wastewater effluent disinfection process. The results and discoveries from this study will provide scientific and engineering society a better and broader knowledge of the importance and consequences of the presence of ENPs on disinfection of microorganisms found in wastewater.

1.1 SCOPE & OBJECTIVES

Nanotechnology is a field of constant growth and development. As a consequence, ENPs continue to proliferate everywhere around us. Hence, it is essential to know and assess the effect and behavior of ENPs on public health and the environment. Despite such needs, very limited studies, if any, are available to assist engineers in dealing with nanoparticles sustainability.

Hwang et al. (2011) found that ~8.7 wt. % of the initially loaded engineered surfactant-coated iron-oxide nanoparticles ($ENP_{Fe-Surf}$) were present in the final effluent from a lab-scale sequencing batch reactor, resulting in significant increased effluent chemical oxygen demand (COD), turbidity, and apparent color. However, possible impacts of those $ENP_{Fe-Surf}$ present in the effluent on the subsequent disinfection process are unclear.

In this regard, the overall goal of this research was to understand the impact and behavior of $ENP_{Fe-Surf}$ on wastewater effluent disinfection. To accomplish this several lab-scale disinfection experiments in both batch and continuous-flow reactors were conducted. In addition, biochemical water quality characteristics were measured. To fulfill the previously mentioned

goal, this study aimed to perform disinfection experiments with wastewater effluent samples from the Mayagüez Wastewater Treatment Plant to evaluate impacts of the presence of $ENP_{Fe-Surf}$, as contaminants, on fecal coliforms removal and inactivation. A genomic test was also performed to assess microbial population dynamics in response to the presence of ENPs in wastewater effluent disinfection process. Finally, the physicochemical characteristics of effluent wastewater were evaluated to verify their relationship to the occurrence of fecal coliforms. Additionally, the effect of $ENP_{Fe-Surf}$ on activated sludge was also assessed to corroborate the findings from the previous studies.

2 LITERATURE REVIEW

2.1 WASTEWATER

Water is one of the most important compounds of planet and for mankind. Worldwide, there are about 780 million people that still lack access to clean or sanitate water sources (WHO, 2012). Over the last century, men have increased the manufacture of new products and goods thereby generating more wastes that end up in water bodies. These wastes can be in forms of solid, liquid, and gas emissions. The liquid emission or water already used by communities is also known as wastewater and it contains pollutants such as organics, inorganics, and microorganisms. As an example, high levels of inorganic pollutants can be found initially in these wastewaters and since they can be easily biodegraded, their impact on parameters such as, biochemical oxygen demand (BOD₅), total suspended solids (TSS) or COD could be in the tens to thousands mg/L. (Chan et al., 2009).

Depending on the wastewater quality characteristics and the required effluent quality characteristics, the degree of treatment will vary. These characteristics are classified between physical, chemical, and biological, depending on their nature (Reynolds and Richards, 1996). Therefore, in order to understand which treatment process best suits the selected wastewater, it is important to perform a bio-chemical analysis of the wastewater. The most important wastewater characteristics to consider are total dissolved solids (TDS), TSS, nitrogen, and phosphorus, alkalinity, sulfate, BOD₅, COD, and total organic carbon.

2.2 WASTEWATER TREATMENT

Wastewater treatment is the process in which wastewater passes through biological, chemical, and physical treatments with the purpose of purification. WWTPs must comply with some water quality standards proposed by the Clean Water Act. In 1972, the Clean Water Act established their main goal, which was to “restore and maintain the chemical, physical, and biological integrity of the Nation’s water” (EPA, 2011). The major aim of wastewater treatment is to remove as much of the suspended solids, organics, and nutrients as possible before the remaining water, called effluent, is discharged back to the environment (USGS, 2013). Thus, the wastewater treatment must comply with the primary and secondary regulatory standards that focus on the protection of public health and the enhancement of aesthetic quality, respectively.

Wastewater treatment processes often involve the following steps or stages preliminary, primary, and secondary treatments. Among these steps or stages, the wastewater undergoes different treatment methods like bar screen or bar-racks and grit removal, first clarifier, nitrification, denitrification, second clarifier, activated sludge process, and disinfection. Prior to the disinfection process, the effluent contains a range of pathogenic microorganisms, which pose a potential risk to the health of humans and the environment (EPA Victoria, 2002). Therefore, the wastewater treatment process will vary depending on final purposes of the use of the treated water (e.g., recreational water, drinking water). The motivation for this study to understand the effect of engineered nanoparticles presence in water to be disinfected.

2.2.1 PUERTO RICO AQUEDUCT AND SEWAGE AUTHORITY AND MAYAGÜEZ WASTEWATER TREATMENT PLANT

In Puerto Rico, the Puerto Rico Aqueduct and Sewage Authority (Autoridad de Acueductos y Alcantarillados de Puerto Rico, which abbreviation is AAA, in Spanish) is responsible for wastewater treatments and managements. Typically, wastewaters undergo preliminary, primary, and secondary treatments.

The first step in the preliminary treatment from the Mayagüez WWTP is the bar-racks with approximately 4.5-cm openings, which remove large solids such as wood particles, fats, and some plastics. In the grit chamber, big, hard, and solid inorganic materials are typically removed in a settling mode. Those removed inorganic materials like rocks, gravel and sand are dried and are typically taken to the municipal landfills. In the primary treatment, approximately 60% and 30% of SS and BOD, respectively, are removed in the primary sedimentation tank.

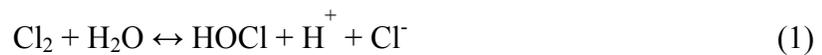
The secondary treatment starts with the activated sludge system followed by the secondary sedimentation. The supernatants from the primary sedimentation tank are sent to the activated sludge system. The Mayagüez WWTP has a modified activated sludge system where nutrients (nitrogen and phosphorus) removal is also achieved by putting the mixed liquors in separate aerobic and anaerobic basins in series. Following this, the activated sludge enters the secondary sedimentation tank.

The last step in the Mayagüez WWTP is the disinfection. Chlorine is injected in the Parshall flume to kill or make inactive the pathogenic microorganisms. The final disinfected wastewater effluent is discharged to the bay.

2.3 DISINFECTION

Disinfection is the most common method today globally utilized in most WWTPs and disinfection can control microbial health risks in reclaimed water and treated wastewater (Bouki et al., 2013) and it is effective at destroying every pathogenic microorganism up to a level where there is no threat to the human health. The process effectiveness will vary depending on the type of disinfection, whether it is chemical, physical or by irradiation, the applied disinfectant and its dosage, the water quality of the effluent (e.g., suspended solids, BOD, turbidity, nutrients, and pH), and environmental factors such as temperature. The most common chemical disinfectants are chlorine, ozone, chlorine dioxide, and sodium hypochlorite, while the most common physical disinfectants are the ultraviolet light and microfiltration. In order to use them as disinfectants, they must exhibit the following characteristics: 1) Destroy pathogens in a practical period of time, 2) Effective confronting concentration and composition fluctuations, 3) Non-toxic, 4) Cost-effective (Easy to store and transport), and 5) Persistent (Avoid re-contamination).

The most common chemical method, by far, for wastewater effluent disinfection prior to its discharge into receiving water bodies is chlorination. Due to the low cost, effectiveness and relative ease of use, chlorine has become the disinfectant most commonly used for water and wastewater treatment across the world (Silva et al., 2010). It can be applied either as hypochlorite salt or in gaseous form (Cl_2). Consequently, all forms of chlorine react rapidly with water to produce hypochlorous acid (HOCl):



Where, HOCl quickly dissociates to form the hypochlorite ion (OCl⁻) according to the following reaction:



Molecular Cl₂, HOCl, and OCl⁻ are the disinfectant agents or also known as the free chlorine residuals. The other types of disinfectant agents, known as combined chlorine residuals or chloamines, come into performance with the presence of ammonia in water, as shown in the following reactions:



For these, the combined chlorine residuals are NH₂Cl, NHCl₂, and NCl₃. The disinfecting ability of chlorine is due to its powerful oxidizing properties, which oxidize those microbial enzymes that are essential to the cells metabolic processes (Reynolds and Richards, 1996). Effective chlorine disinfection depends on the correct combination of pH, chlorine concentration, and contact time as well as the levels of ammonia and suspended solids (EPA Victoria, 2002).

After knowing how chlorine interacts when it is mixed with water, it is important to understand the characteristics of the effluent entering the disinfection process. Therefore, calculations of CT value and log inactivation are necessary, since they are the most significant factors in disinfection of microorganisms. The CT value is the product of the free chlorine residuals concentration and contact time in disinfection process. This is described by the following equation:

$$C \times T = CT \quad (6)$$

where C is the free chlorine residuals concentration in mg L^{-1} and T is the disinfectant contact time in minutes (EPA, 2003). CT value would then have a unit of min-mg L^{-1} .

In addition, the efficiency of disinfection can also be expressed by log inactivation value. Log inactivation is a method of evaluating the disinfection efficiency of microorganisms such as viruses, *Giardia lamblia*, and *Cryptosporidium* and is based on percentage of microorganisms removed by a specific process. As required by the EPA (2009), surface waters require a 3-log (99.9%) reduction for *Giardia lamblia* and ground water systems require 4-log (99.99%) reduction for viruses (EPA, 2003). Log inactivation value is the base 10 logarithmic of the ratio between microorganism concentrations in the disinfected water and the feed or effluent water (Asraf-Snir and Gitis, 2011). For this particular study, the log inactivation or log removals were calculated specifically for FCs and is described by the following equation:

$$\text{Log Removal} = -\log\left(\frac{N}{N_0}\right) \quad (7)$$

where N is equal to the FC count after the disinfection process and N_0 is the fecal coliform count in the effluent wastewater.

2.3.1 HOM EQUATION

Since the major focus of this study is disinfection, it is necessary to understand all of the topics that it embraces, like the application of the Hom equation in the disinfection experiments. In 1908, Harriete Chick pronounced the major percepts of disinfection kinetics and recognized

the similarity between microbial destruction by chemical reactions and disinfectants. This led to the formation of the Chick Law, as shown below:

$$\ln\left(\frac{N}{N_0}\right) = k^*t \quad (8)$$

where N is the number of microorganisms at contact time t , N_0 is the number of microorganisms at contact time of $t = 0$, k^* is the reaction rate constant and t is the contact time (Chick 1908; Lee and Nam, 2002).

Further, after evaluating and studying broadly the empirical equation proposed by Chick, Watson modified Chick's Law by including the relation between the disinfectant concentration, C and the rate constant of inactivation k^* , as follows:

$$k^* = -kC^n \quad (9)$$

where k is the constant of specific microorganisms, and n is the dilution coefficient (Watson, 1908; Lee and Nam, 2002). Based on this, the empirical Chick-Watson Law is described by the following equation:

$$\ln\left(\frac{N}{N_0}\right) = -kC^n t \quad (10)$$

Moreover, in 1972, Hom proposed a new disinfection model, in which the Chick-Watson equation was empirically generalized. The Hom equation provides a relationship between disinfectant concentration, contact time, and empirical correlations m and n ; if m equals 1 the Chick-Watson equation is attained (Haas and Joffe, 1994; WHO, 2004). As of this, the rate constant of inactivation k^* would turn into:

$$k^* = -kmNC^n t^{m-1} \quad \text{or} \quad \ln\left(\frac{N}{N_0}\right) = -kC^n t^m \quad (11)$$

where for this case k , m , and n are empirical correlations, C is the disinfectant concentration and N is the number of microorganisms at a contact time t .

With a logarithmic arrangement the Hom equation is obtained and presented below:

$$\ln\left(-\ln\frac{N}{N_0}\right) = \ln(k) + n\ln(C) + m\ln(t) \quad (12)$$

With this equation, correlations between disinfectant concentration, contact time, and microorganism's inactivation can be acquired (Haas and Joffe, 1994; WHO, 2004).

2.4 FECAL COLIFORMS

FC are a family of total coliforms that live in the intestines of warm-blooded animals (EPA, 2008). They are the most common and abundant microbiological pollutant in waters. These pathogenic microorganisms sizes are in the range of 1 to 2 μ m (Pachepsky et al., 2006). Among the standards for recreational water established by EPA (1986); is that the FC content of recreational waters shall not exceed a log mean of 200 colony forming unit (CFU) per 100 mL of a sample (Motamarri and Boccelli, 2012). Consequently, the effluent coming from secondary wastewater treatment process must be equipped with an additional treatment (e.g. chlorination) in order to minimize or eliminate the FC concentrations.

Ingestion or skin contact with waters containing high levels of coliforms will put into risk the human health and can increase the probability of developing waterborne diseases. Therefore, standard regulations have been established for FC concentrations in treated wastewaters. In order to permit the use of treated wastewater for contact recreation, FC should not exceed 100 to 1000

numbers per 100 mL (Chapra, 1997). Despite the importance of treating wastewaters for microorganism disinfection, there are still a lot of undeveloped countries lacking in wastewater treatment technologies and suffering from waterborne diseases. Besides, the removal of coliform bacteria achieved in disinfection procedures does not adequately reflect the removal of pathogenic viruses and protozoa due to their dissimilar resistance to disinfectants (Costán-Longares et al., 2008).

2.5 NANOSCIENCE & NANOPARTICLES

Nanoscience is the study of interactions of atoms, molecules, and objects in the nanometer scale (10^{-9} m) and has become a very active and vital area of research, which is rapidly developing and spreading to almost every field of technology areas as well as engineering and science disciplines (Islam and Miyazaki, 2009). Nanotechnology bases on the production and manipulation of such nano-scale objects. It started in the early 1980s with the invention of the scanning tunneling microscope, a computer imaging system with a surface probe (Miyazaki and Islam, 2007).

In nanotechnology, conventional or normal concepts of science lose their validity. At this size range, atoms, molecules, objects, and substances will have different properties compared to larger scale counterparts. Sub-microscopic objects (including nanoscopic) have properties that are affected by fluctuations around the average and become subject to the strange and unpredictable laws of quantum mechanics, while materials in the micron size level and above, have bulk (conventional) properties that obey the laws of classical science (Adams and Barbante,

2013). Therefore, nanomaterials will behave differently depending on the environments that surround them.

In nano-scale phenomena, a particle is known as a small definite quantity of matter that acts as a complete unit and interfaces with the surrounding environments. Particles can be found in any phases, like solid interacting with liquid or gas, liquid droplets in air, emulsions, bubbles in water, among others. They are classified depending on their sizes; for example, an ultra fine particle will have a size range from 1 to 100 nm, which can be classified as a nanoparticle. Nanoparticles are particles at least within one dimension smaller than 1 μm and theoretically as small as atomic and molecular length scales (~ 0.2 nm). They can have crystalline or amorphous form and their surfaces can act as carriers for gases or liquid droplets (Buzea et al., 2007). Nanoparticles may or may not exhibit size-related properties that differ significantly from those observed in their normal size.

2.5.1 NANOPARTICLES PRODUCTION

From a scientific point of view, nanotechnology can be defined as “materials and systems with structures and components exhibiting novel and significantly improved physical, chemical, and biological properties, as well as to the phenomena and processes enabled by the ability to control the material properties on the nano-scale size” (Miyazaki and Islam, 2007). Since studies have proven the advantages of applying nanotechnology in different fields, the production of nanomaterial has increased during the past years. Manufactured nanoparticles are currently being used in different areas such as biomedicine, electronics, catalysis and material sciences, environmental analysis and remediation, pharmaceuticals and cosmetics (Ju-Nam and Lead,

2008). In the environment, engineered nanoparticles have been extensively used for rapid or cost-effective cleanup of wastes. In addition, iron nanoparticles are very effective for the detoxification and transformation of a wide variety of common environmental pollutants and other nanosized materials, such as metalloporphyrinogens that have been tested for degradation of tetrachloroethylene, trichloroethylene, and carbon tetrachloride under anaerobic conditions (Dror et al, 2005; Brar et al., 2010). Examples of nanoparticles utilized for other nanoproducts are shown in Table 1.

Table 1: Examples of Nanoparticles Applied For Nanoproducts

Nanoparticles	Nanoproduct	Reference
Ag	Textiles containing nano-silver	Som et al., (2010)
TiO₂	Sunscreens with nano-titanium dioxides (TiO ₂) Windows coated with a nm-thin layer of TiO ₂	
CNTs	Batteries containing carbon nanotubes (CNTs)	
Pt, Pd	Automotive exhaust converters, catalysts	Bystrzejewska-Piotrowska et al., (2009)
SiO₂	Fire-proof glass, UV-protection, varnish, ceramics, electronics, pharmaceutical products, dentistry, polishing	
Fe₂O₃	Concrete additive	
Al	Metallic Coating	Brar et al., (2010)
Cu	Microelectronics	
CNTs	Used in a variety of composite materials	

Consequently, the increased use and production of nanomaterials introduce the nanoparticles intentionally/unintentionally into the waste streams and as a consequence to wastewater treatment facilities. But still, the impact that nanomaterials have on wastewater treatment, or conversely, the impact that wastewater treatment has on nanomaterials, is largely unknown (Brar

et al., 2010). Therefore, even though the production of nanoparticles have its advantages it is also important to understand how these manufactured nanoparticles will affect surroundings and human health. This is the motivation of this work.

2.5.2 IRON OXIDE NANOPARTICLES

Iron oxides exist in numerous forms in nature and can be prepared in the laboratory. In nature, the most common forms found are magnetite (Fe_3O_4), maghemite ($\gamma\text{-Fe}_2\text{O}_3$), and hematite ($\alpha\text{-Fe}_2\text{O}_3$) (Xu et al., 2012). In the laboratory, iron oxide nanoparticles can be prepared depending on their application. For example, the stability of iron oxide colloid suspensions could be greatly increased by modification of its surface with appropriate functional groups (Xu et al., 2012). In biomedical techniques, iron oxide is the material that is investigated the most, due to its superior biocompatibility with respect to other magnetic materials, both based on oxides or on pure metals (Figuerola et al., 2010). Due to their magnetic properties, it can be used as heat mediators in hyperthermia treatments, as magnetic guidance in drug delivery applications, and additionally as magnetic resonance imaging agents in diagnostic (Figuerola et al., 2010). Iron oxide nanoparticles can also be utilized in concrete as an additive to enhance structural strength and could also be added to wastewater streams for adsorption of heavy metals and reduction of hazardous substances (Bystrzejewska-Piotrowska et al., 2009; Hildebrand et al., 2009).

2.6 ENP ENVIRONMENTAL IMPACT

Nanoparticles released to the environment, deliberately or accidentally, disperse in the environment reaching water, soil, and the air. They can be released to the environment as bare or functionalized nanoparticles, embedded in a matrix or as aggregates (Bystrzejewska-Piotrowska et al., 2009). Therefore, after environmental dispersion in water, these nanoparticles will arrive at the wastewater treatment plants. Later on, wastewater treatment plants must implement treatments not only for the removal of organic matters and other conventional pollutants, but also for the emerging nanoparticles.

Since ENP inputs to the environment are not a naturally occurring event, they will act as pollutants and therefore, aggravate the environment in which they are being placed, for example, aquatic or soil environment. Consequently, an understanding of the presence, behavior, and effect of these nanoparticles in wastewater and wastewater sludge is essentially and timely (Brar et al., 2010).

ENPs environmental fate and transport could be influenced by several physicochemical properties (e.g., aggregation or size), which vary significantly between the materials and through aquatic systems. Aquatic chemistry dictates interactions between ENPs and natural water components. This might result in formation or break-up of aggregates based on the surface properties of the nanoparticles (Weinberg et al., 2011). The nanoparticles stability will be influenced by the surface properties and therefore, with more stability on the wastewater environment, there will be more toxicity for the aquatic life since the ENP will also remain for a longer period of time. In fact, if ENPs reside more time in the wastewaters, it will increase the probability to finally reach the wastewater sludge, more so through the agglomeration and/or

aggregation and settling mechanism. Subsequently, when the wastewater sludge is spread on agricultural fields as biosolids, concerns are raised due to possible leachability of ENPs into subsurface waters and groundwaters. (Brar et al., 2010).

The impact of ENPs on the environment is still largely unknown. Therefore, it is important to identify and quantify the sources of ENPs; secondly, after gaining more knowledge it is important to establish some standards and regulations for ENPs disposals; and third, examine the bioaccumulation factors that can be occurring on the wastewater treatment process.

2.7 ENVIRONMENTAL GENOMICS

Over ten thousands genes respond when an organism reacts to its environmental change. Genomics is the science of the identification and analysis of genomes, which are complete sets of an organism's deoxyribonucleic acid (DNA), and has greatly enhanced biotechnology (Madigan et al., 2010). Genomic investigations applied to the diversity of environmental bacteria are leading to understandings of the evolution of new forms of biological systems, ecological dynamics, and the detection of new functions that might be exploited for biomedical and biotechnological purposes (Deutschbauer et al., 2006).

There are different types of genomic studies or also known as “omic technologies”. For example, transcriptomics, which studies the part of the genetic code that is activated or inactivated in response to environmental change (NERC, 2005); metabolomics which studies the composite metabolites of a sample (or a cell), in ecotoxicology (Field et al., 2005); and proteomics, provides information of the dynamic protein expression in whole cells or tissue,

therefore providing a global analysis (Valenzuela et al., 2006). The environmental genomics, also known as metagenomics, is a genome-based analysis of entire microbial communities of complex living organisms in diverse environments or ecological contexts (Dupré and O'Malley, 2007). In other words it investigates how living organisms adapt to and are impacted by their environments (Field et al., 2005).

Instead of individual genomes ('monogenomes') or single gene markers, metagenomics starts with large amounts of the DNA collected from microbial communities in their natural environments in order to functional interactions, evolutionary relationships, and explore biodiversity (Dupré and O'Malley, 2007). The most common practice consists of extracting DNA from environmental samples and cloning it in large-insert libraries. These are screened for clone activity (particular functions expressed in the host cell) or specific gene sequences (Riesenfeld et al., 2004). The most common genes of interest include the ribosomal RNA genes (specifically the gene 16S rRNA). Sampled environments include agricultural soils, marine sediments, ocean waters of various temperatures and depths, the human gut and mouth, and also drinking-water valves which are human-made environments (Dupré and O'Malley, 2007).

In an environmental genomics study, Field et al. (2005) tested the hypothesis that microbes exist in definable communities in aquatic environments, and that this structure impacts their functional roles. They concluded that in aquatic habitats "nothing stands still", yet the collection and maintenance of accurate environmental description of samples is very important. Moreover, in conventional shotgun sequencing of microbial isolates, all shotgun fragments are derived from clones of the same genome. However, if the genomes of an environmental microbial community are to be analyzed, the ideal situation is to have a low diversity environment (Valenzuela et al., 2006). Such a system was found by analyzing the microbial communities inhabiting a site of

extreme acid mine drainage production, in which few organisms types were present (Bond et al., 2000). Thus, the environmental genomics is still an emerging discipline; therefore detailed assessment and research are necessary to acquire better conclusions on this study. Furthermore, genomics can help in the understanding of what controls the early development, from individual cells through the whole organism.

3 MATERIALS & METHODOLOGY

The methodology implemented for this study is discussed in this section. It consists of a general description of the materials utilized, the experimental approach, and bio-chemical and data analyses. Actual wastewater effluent was collected prior the disinfection process from the Mayagüez WWTP, located in Mayagüez, Puerto Rico, as shown in Figure 1.

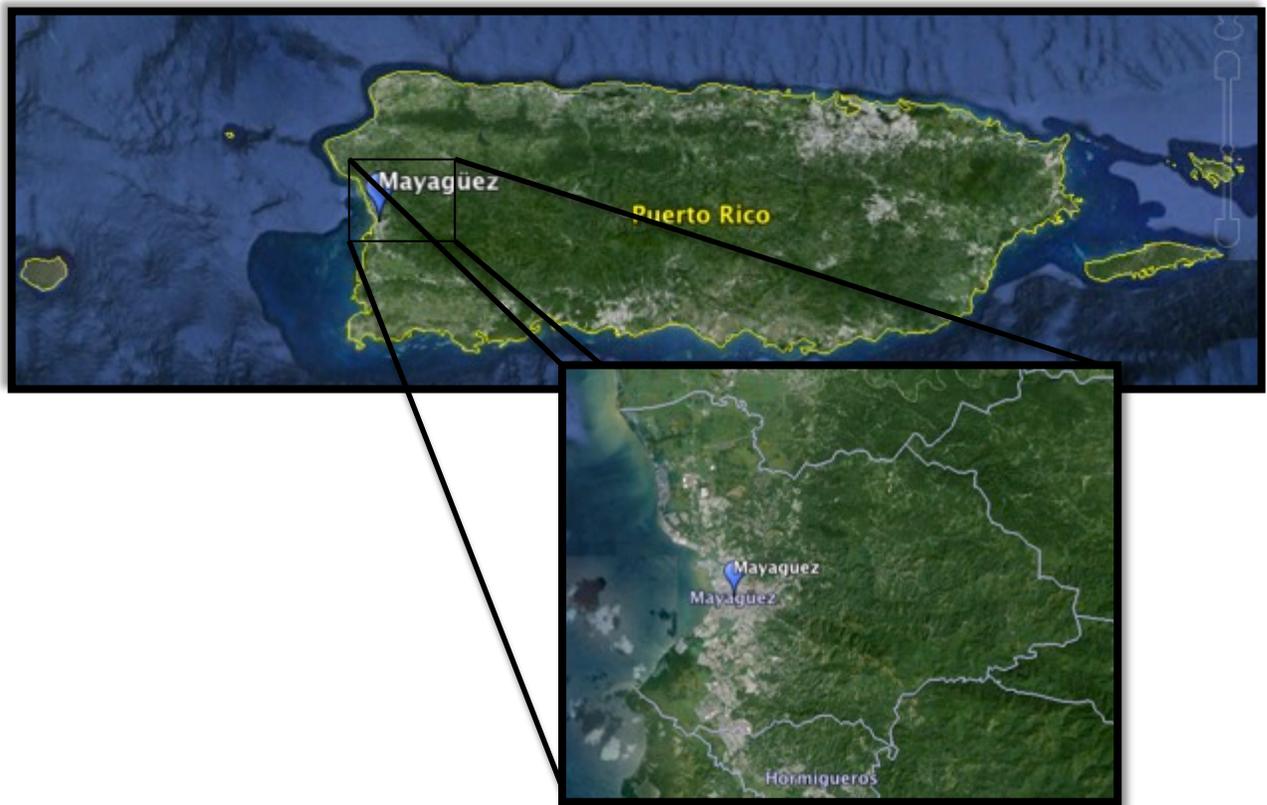


Figure 1: Study Area Located in Mayagüez, Puerto Rico (Source: Google Earth, 2014)

3.1 MATERIALS

3.1.1 WASTEWATER EFFLUENT

The wastewater effluent sample was collected in a Nalgene 10-liter container and was utilized within a 24 hour after sampling. It was also constantly aerated at a dissolved oxygen concentration of approximately 5 mg L^{-1} to maintain aseptic.

Figure 2 illustrates the treatment process used in Mayagüez WWTP where the wastewater samples were collected. The blue dot represents the area of the wastewater effluent collection, which is prior to the addition of chlorine for their disinfection process. In addition, Figure 3 shows a Mayagüez WWTP worker transferring the sampling effluent into the Nalgene 10-liter container in the wastewater effluent collection area.

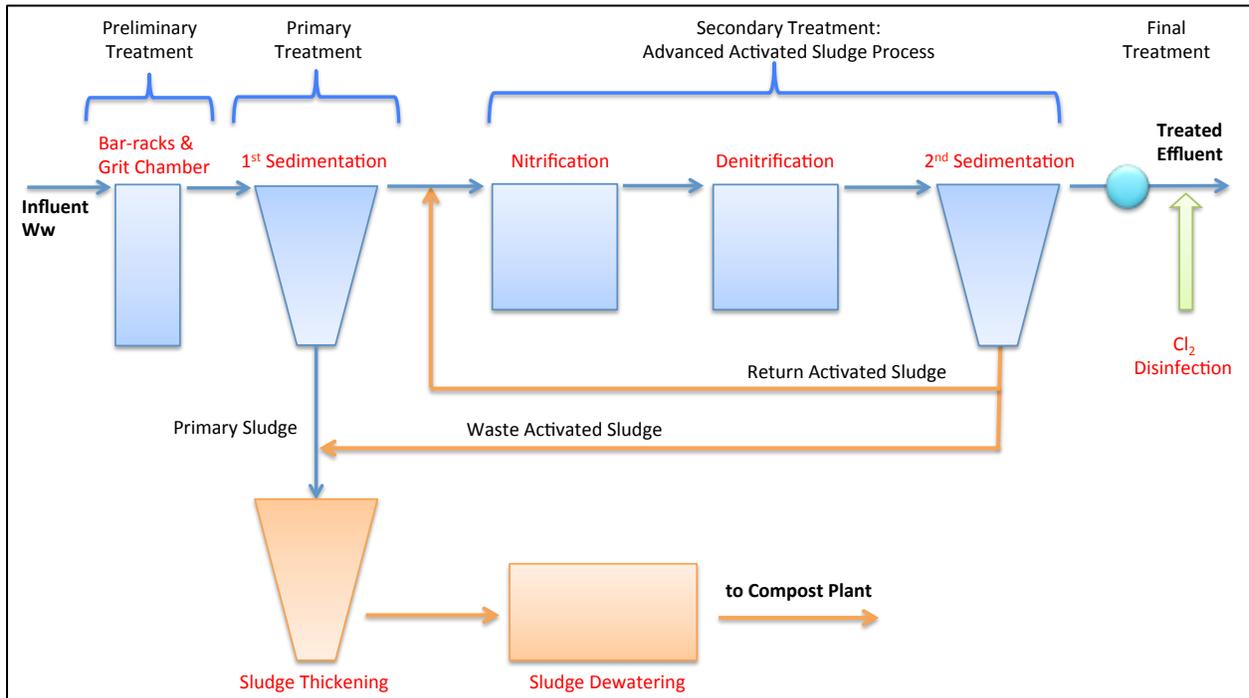


Figure 2: Process Diagram of the Mayagüez WWTP



Figure 3: Wastewater Effluent Collection at the Mayagüez WWTP

3.1.2 IRON OXIDE NANOPARTICLES

The nanoparticles used in this study were magnetite (Fe_3O_4) as illustrated in Figure 4. The $\text{ENP}_{\text{Fe-Surf}}$ were obtained from the Ferrotec Corporation (USA). Table 2 shows key characteristics of these ENPs.

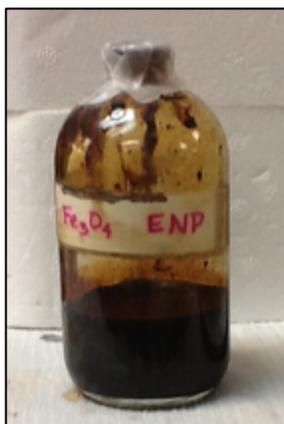


Figure 4: Solution of Engineered Iron Oxide Nanoparticles Coated with Surfactants

Table 2: Characteristics of Engineered Iron Oxide Nanoparticles Coated with Surfactant

Composition (% by volume)	Magnetite 2.8-3.5
	Surfactant 2-4
	Water 92.5 – 95.2
Appearance	Black Fluid
Carrier Liquid	Water
Saturation Magnetization	> 160 Gauss
Viscosity @27 °C	< 5 cP
Nominal Particle Diameter	10 nm
Initial Magnetic Susceptibility	> 0.45 (emu/g)/Oe
Density	1.17 g/mL
Surface Tension	> 34 dynes/cm
pH	> 10

3.1.3 OTHER MATERIALS

Other materials required for this study were are deionized water (DI), 10 mL vials, Erlenmeyer flasks (different sizes), pipets, Petri dishes, bacteriological membranes, m-FC and HPC broth medium (HACH[®]), filtration funnels, burner/lighter, air pump, sequencing batch reactors (SBRs, Phipps & Bird 2000 mL B-Ker^{2®} Lab Jars), and commercial bleach (Clorox[®], 6.15% Sodium Hypochlorite). Mixed liquor suspended solids (MLSS), influent wastewater, and raw activated sludge (RAS) were also used and collected from the Mayagüez WWTP.

3.2 METHODOLOGY

The impact of $ENP_{Fe-Surf}$ on chlorine consumption and FC removal in the disinfection was evaluated in different sets of lab-scale batch and continuous-flow reactors. Also, the interaction of $ENP_{Fe-Surf}$ with settled activated sludge was briefly studied in lab-scale SBR experiments. An SBR is a batch activated sludge treatment process. It consists of a sequence of fill, react, settle, and decant process occurring in the same tank (Tchobanoglous et al., 2014). This methodology consists of the general description of the procedures and system parameters applied during this study.

3.2.1 PHYSICOCHEMICAL ANALYSIS

The collected wastewater effluent samples were examined in order to determine the chemical, physical, and biological properties of the collected wastewater effluent. A summary of the analyses is shown in Table 3, including the equipment and method used.

Table 3: Instrument and Method for Physicochemical Analysis

Parameter	Instrument	Method
pH	pH Meter Model ORION720A+	Insert pH meter into sample
Total Phosphorus	HACH DR 2800	HACH Method 8190 - PhosVer 3 Phosphate Reagent Powder Pillow
Total Nitrogen	HACH DR 2800	HACH Method 10071 - Total Nitrogen Reagents A and B Powder Pillow
COD	HACH DR 2800	HACH Method 8000 - Ultralow range (0 to 40mg/L)
Conductivity	OAKTON Multi-parameter PCS Testr 35	Insert conductivity meter into sample
Chlorine	HACH Pocket Colorimeter II	HACH Method 8021 - DPD Free Chlorine Reagent

3.2.2 ENP_{Fe-Surf} IN BATCH DISINFECTION

A 2% wt. chlorine solution was prepared by diluting the commercial Clorox with DI water. For this experiment, the collected wastewater effluent from the Mayagüez WWTP was utilized. Test vials of 100 mL were filled with the wastewater effluent. A half of batch reactors were chlorinated and dosed with ENP_{Fe-Surf}, while the other half only had ENP_{Fe-Surf} as shown in Figure 5. Table 4 presents an example of the disinfection studies with the different amounts of chlorine and ENP_{Fe-Surf} concentrations added to each vial. The concentrations of chlorine and ENP_{Fe-Surf} used for further experiments were determined from this preliminary experiment, where both residual chlorine concentrations and FC after disinfection had been determined by the presence of the ENP_{Fe-Surf}. In such a way, the quantitative analysis were possible with the measurable residual chlorine concentrations and FC numbers.



Figure 5: View of Batch Disinfection Experiment

Table 4: Initial Chlorine Concentrations and ENP_{Fe-Surf} Amounts used for Batch Disinfection Experiment

Reactors	ENP_{Fe-Surf} Dosage (mg L⁻¹ as Fe)^a	Initial Cl₂ (mg L⁻¹)	Disinfection Volume of Wastewater Effluent (mL)
1	0.6	1-2	100
2	1.7	1-2	100
3	2.8	1-2	100
4	3.7	1-2	100
5	4.3	1-2	100
6	4.8	1-2	100
7	5.4	1-2	100
8	0	1-2	100
9	0.6	0	100
10	1.7	0	100
11	2.8	0	100
12	3.7	0	100
13	4.3	0	100
14	4.8	0	100
15	5.4	0	100
Effluent	0	0	100

After the addition of ENP_{Fe-Surf}, the vials were gently shaken and let stand for 30 minutes.

Then, the concentration of free chlorine residuals and FC were measured.

3.2.2.1 HOM EQUATION DISINFECTION

In an effort of understanding the influence of ENP_{Fe-Surf} on FC disinfection in wastewater effluent, additional batch experiment was performed. The Hom equation was applied in this case. A total of nine test vials and three different initial chlorine concentrations were used. In the same manner, 1.0, 1.5, and 3.0 % wt. chlorine solutions were prepared by diluting commercial bleach with DI water and the wastewater effluent from the Mayagüez WWTP was utilized. A group of three test vials were filled with wastewater effluent and were dosed at different chlorine

^a There is 66.2 mg Fe in 1 mL ENP_{Fe-Surf}

concentrations in the presence and absence of ENP_{Fe-Surf} (Table 5). As the reaction volume was 100 mL, the addition of 0.075 mL ENP_{Fe-Surf} was equivalent to 4.97 mg L⁻¹ as Fe.

Table 5: Batch Disinfection Arranged by Hom Equation in the Presence and Absence of ENP_{Fe-Surf}

Reactor	Initial Cl ₂ (mg L ⁻¹)	No ENP _{Fe-Surf}	Fixed ENP _{Fe-Surf} (0.075 mL)
		Contact Time (min)	
1	1.0	19	20
2		40	36
3		64	60
4	1.5	10	9
5		21	20
6		30	30
7	3.0	5	5
8		11	10
9		15	14

The vials were gently shaken and let stand for different contact times, ranging from 5 to 60 minutes. Then, the concentration of free chlorine residuals and the FC were measured.

3.2.3 ENP_{Fe-Surf} IN CONTINUOUS-FLOW PFR DISINFECTION

The impact of ENP_{Fe-Surf} in disinfection was further tested in a continuous-flow reactor, specifically an open channel plug flow reactor (PFR). The PFR was made of PVC tubes of 2 inches in diameter and 12 ft in length, as shown in Figure 6. For this experiment, ENP_{Fe-Surf}, chlorine and the wastewater effluent were pumped to the PFR run at the hydraulic retention time (HRT) of 15 to 30 minutes. The volumetric flow rates of ENP_{Fe-Surf} and chlorine were of 0.17

and 0.38 mL min^{-1} , respectively. These volumetric flow rates were selected in order to mimic the same application rates of $\text{ENP}_{\text{Fe-Surf}}$ and chlorine used in the previous batch experiments.

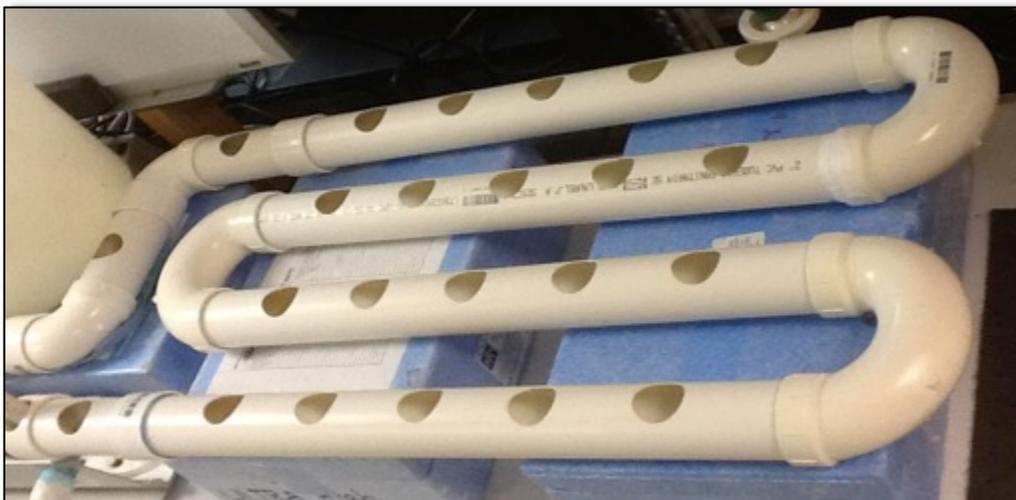


Figure 6: Views of Plug Flow Reactor

3.2.3.1 TRACER STUDY

Several tracer studies were implemented in order to corroborate if the PFR behaved within the requirements of a plug flow system and to verify the disinfection efficiency of the PFR. For this experiment, sodium chlorine (NaCl) was used as the tracer in DI water, and the volume and flow rate were varied to different empty bed contact times (EBCT) of approximately 15, 30, and 45 minutes. Since the volumetric flow rate was an adjusted parameter, the mean residence time of the PFR would be equal to the residence time. During the experiment total dissolved solids (TDS) was measured every 2 minutes and then the collected data were used to obtain the residence time distribution (RTD) curves. Table 6 presents the initial parameters used for the tracer studies.

Table 6: Operating Parameters of Trace Study

Tracer Study	Time (min)	DI Water (mL)	Flow Rate (mL min ⁻¹)
1	13.0	2445	188.0
2	29.8	6389	214.7
3	45.0	6389	141.9

3.2.3.2 *ENP_{Fe-Surf}* & CHLORINE IN PLUG FLOW REACTOR

The reactor was equipped with four strainers (represented by magenta squares in Figure 7) to minimize any turbulence caused by influent and effluent, and to achieve a plug flow. The added wastewater effluent, chlorine, and ENP_{Fe-Surf} were thoroughly mixed at the entrance of the PFR, where the first strain provided a broader distribution between the ENP_{Fe-Surf} and chlorine among the wastewater effluent in the PFR. For the reactor to be an open channel, several holes were made on the top of the reactor, as shown in Figure 7. These holes were also used for samplings when needed. The chlorine contact time was adjusted by controlling either the flow rate at the influent or the reactor volume with effluent discharge structure.

Further, continuous-flow disinfection experiments were conducted, in which chlorine and ENP_{Fe-Surf} were added into DI water and wastewater effluent to assess and analyze the interaction among chlorine, ENP_{Fe-Surf}, and FCs.

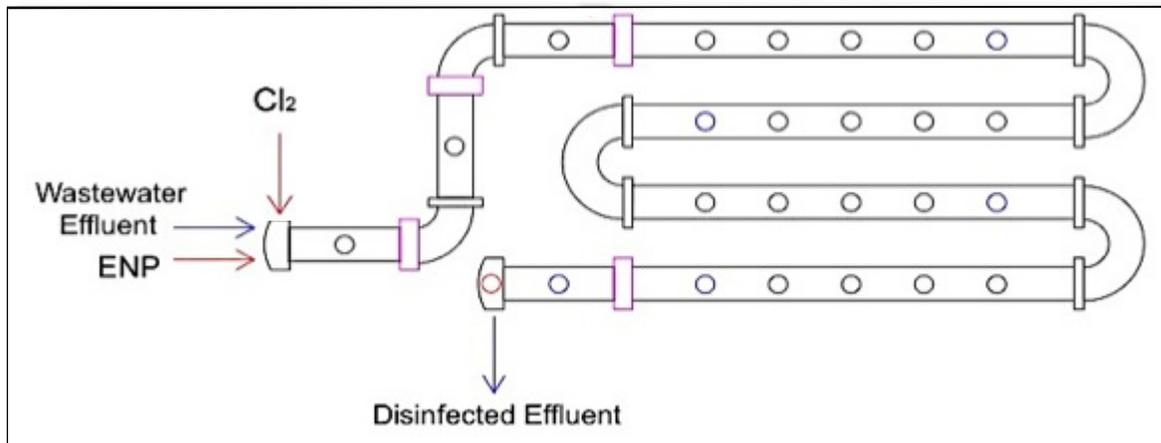


Figure 7: Top View of Plug Flow Reactor Used for Disinfection Process

Both influent and effluent samples were analyzed for bacteriological and physicochemical parameters, focusing on the analyses of free chlorine residual and FC. The results of this experiment are presented and discussed in the subsequent results section.

3.2.4 BACTERIOLOGICAL ANALYSIS

Three 500 mL Erlenmeyer were used as batch reactors and were filled with 400 mL of effluent wastewater. The first reactor had addition of chlorine only, the second reactor had addition of $ENP_{Fe-Surf}$ only, and the third reactor had addition of both. For the initial chlorine concentration to be 3 mg L^{-1} , a chlorine stock solution was prepared. On the other hand $ENP_{Fe-Surf}$ concentration was of 5 mg Fe L^{-1} to maintain the same concentrations used in Hom equation experiment. All reactors were conducted for a contact time of 30 minutes and then membrane filtration technique was performed for FC and, in some cases, total heterotrophic bacteria (THB). The filtered samples of FC were incubated on m-FC broth media for 24-h at $44.5 \text{ }^{\circ}\text{C}$ and those of

THB were on HPC broth media for 48-h at 30 °C. Figure 8 and Figure 9 show the colonies of FC and THB, respectively in the petri dishes containing membrane filters and growth media.

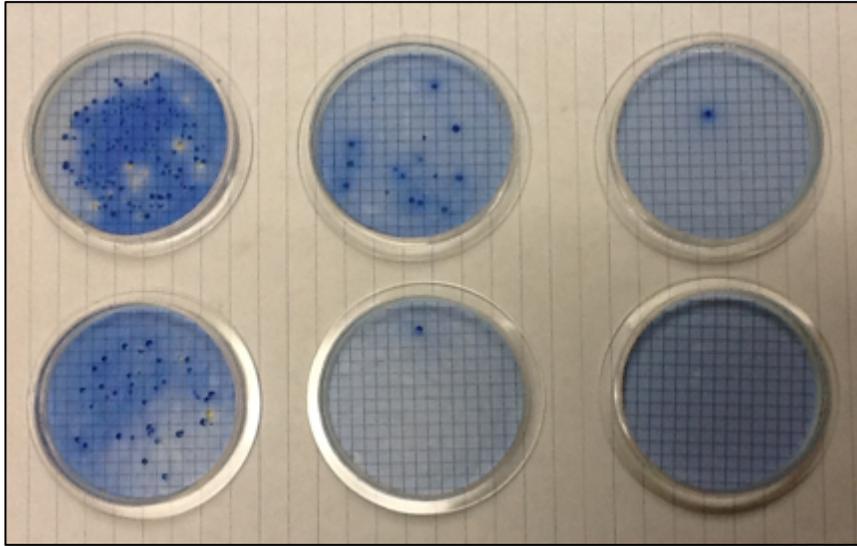


Figure 8: Views of Fecal Coliforms Growth and Colonies Formed



Figure 9: Views of Total Heterotrophic Bacteria Growth and Colonies Formed

Additionally, four filtered samples of each three reactors and the effluent wastewater were collected and stored at (-) 20 °C temperature for further metagenomic analysis to identify which microorganisms endure or get affected the most by the presence of the ENP_{Fe-Surf} and the addition of chlorine during and after the disinfection process. This was done to detect genes that will encode recognizable proteins and then to determine the phylogeny of the microorganisms present in the sample to which the detected genes belong.

Metagenomic analysis involved several procedures. First the metagenomic DNA of the samples were extracted using direct DNA extraction method. The concentration of the DNA was measured using a NanoDrop® spectrophotometer ND 100. Following this a polymerase chain reaction (PCR) procedure was conducted for scanning of the 16S rRNA gene using specific bacteria primers (27F and 1492R) and *E.coli* was used as the host bacteria for growth of colonies. A total of 96 clones were extracted from the host bacteria and were prepared for sequencing. For this process, additional product PCR purification procedure was performed (Figures 10 and 11) and DNA concentration was measured to verify if the process was executed correctly. Finally, the two samples, wastewater effluent before and after addition of ENP_{Fe-Surf}, were prepared in the plates containing 96 wells and the plates were sent for sequencing at MacroGen Inc.

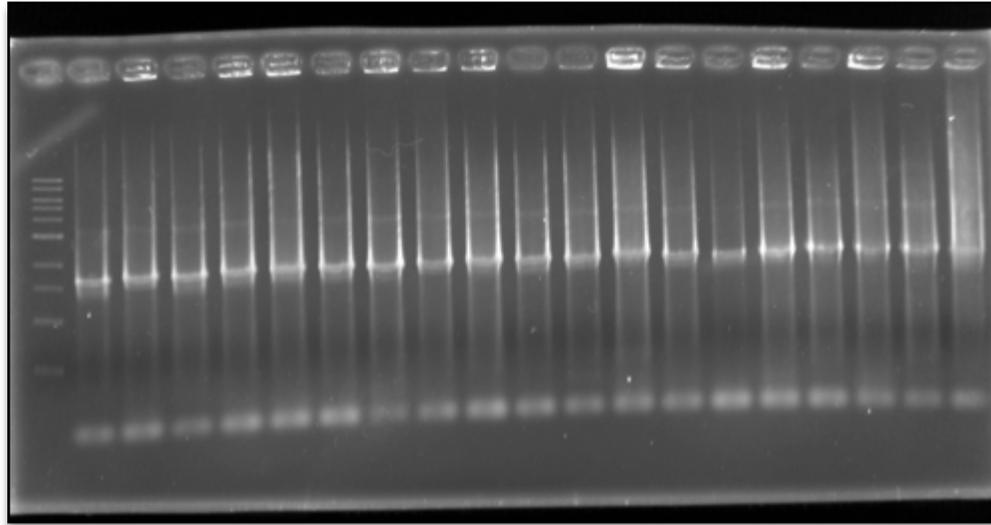


Figure 10: Gel Amplification of the Wastewater effluent before addition of ENP_{Fe-Surf}

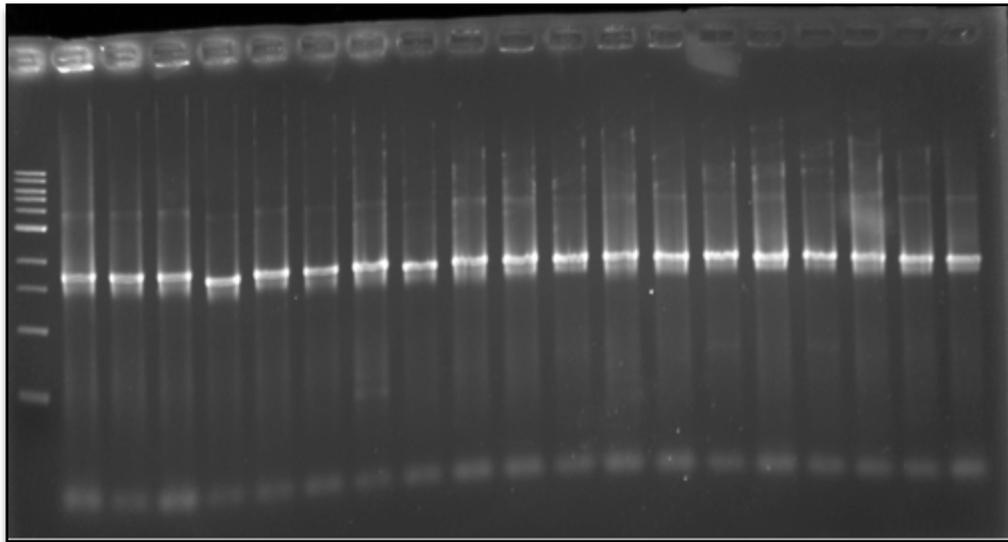


Figure 11: Gel Amplification of the Wastewater effluent after addition of ENP_{Fe-Surf}

For PFR disinfection bacteriological analysis, FC was quantified with the same method used for the batch experiments. Sampling was done at different contact times of 20, 30, and 45 minutes.

3.2.5 LAB-SCALE SEQUENCING BATCH REACTOR

The first SBR experiment consisted of 4 lab jars and a total of 10-cycles were executed. The first two reactors (labeled 1a and 1b) contained MLSS and raw wastewater (influent from primary settling tank), while the other two (labeled 2a and 2b) had MLSS, influent wastewater, and RAS from the Mayagüez WWTP, as shown in Figure 12. Following the protocol of the previous study (Hwang et al., 2011), 66.2 mg as Fe of ENP_{Fe-Surf} was added to the reactors 1b and 2b at the beginning of the 7th to 10th cycles.



Figure 12: Lab-Scale SBR Experiment (Left to right: reactors 1a, 1b, 2a and 2b)

Each cycle consisted of react for 3 hrs, settle for 0.5 hrs, and decant/refill process for 0.5 hrs and, for the reaction process, the reactor was equipped with aeration devices to maintain a dissolved oxygen concentration of $\sim 5 \text{ mg L}^{-1}$ (refer to Figure 13). At the end of each cycle samples were analyzed for sludge volume index (cm), pH, turbidity, COD, and BOD₅. During the 7th to 10th cycles, total iron was also measured for further iron analyses, visualizations, and comparisons. The influence of ENP_{Fe-Surf} on FC disinfection between the actual wastewater

effluent collected from the Mayagüez WWTP and the lab generated wastewater effluent from the aforementioned SBR was compared.

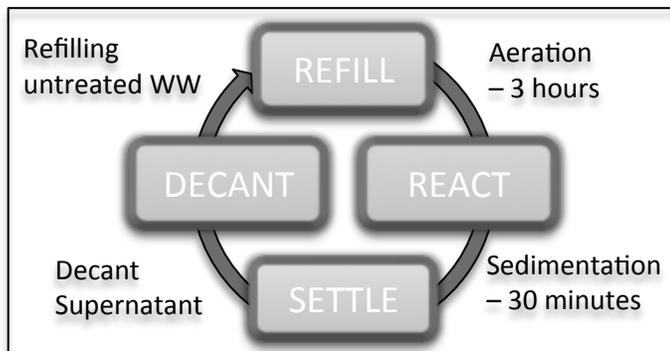


Figure 13: SBR Experiment Cycle Schematic

The second SBR experiment was conducted to corroborate the findings from the previous study (Hwang et al., 2011; Martínez, 2013) where an increase in COD, turbidity, and color intensity was observed due to the presence of ~8.7% wt. of the dosed $ENP_{Fe-Surf}$ in the effluent. As such, those studies implied that >90% wt. of the dosed $ENP_{Fe-Surf}$ would interact and be present in the sludge. However, the authors did not verify it.

Another batch experiment was conducted to comprehend potentially different chlorine interactions between aged and fresh $ENP_{Fe-Surf}$. For collection of the aged $ENP_{Fe-Surf}$, SBR experiment was conducted as shown in Figure 12 and the effluent was sampled. Six batch reactors in a 1-L graduated cylinder. The first three reactors had the supernatant collected from cycles 7th to 10th of the SBR reactor 2a which had been run as the control SBR, i.e., no $ENP_{Fe-Surf}$ addition. Then, fresh $ENP_{Fe-Surf}$ was added to each reactor to have iron concentrations of 5, 7.5, and 10 mg Fe L⁻¹. On the other hand, the other three reactors had the supernatants from the SBR reactor 2b in such a way to contain the same, but aged, iron concentrations that the first three

reactors had. All the reactors had the same initial chlorine concentration at $\sim 2 \text{ mg L}^{-1}$ and after 30 minutes the free chlorine residuals and iron concentrations were measured.

In order to visualize the microorganisms within the activated sludge in the SEM (JEOL JSM-6390 and JEOL JSM-5410LV), the samples were fixed with 2.5% glutaraldehyde phosphate buffer solution and rinsed with 0.1 mol L^{-1} phosphate buffer solution and centrifuged at 4000 rpm for 10 minutes. Rinsing and centrifugation were done three times. Following this, samples were dehydrated gradually with successive immersions in ethanol solutions at the increasing concentrations (50, 70, 80, 90, and 100%) and centrifuged each time at 400 rpm for 10 min. Subsequently, the samples were washed three times in 100% ethanol for dehydration and kept as it before the process of drying. The drying technique was conducted with CO_2 in the Electron Microscopic Science EMS 850. Finally, the samples were coated with gold to improve the electrical conductivity and thus better imaging and contrast of the samples.

Elemental compositions of the activated sludge samples were obtained utilizing an electron dispersive spectroscopy (EDAX). The EDAX chemical analysis identified the gold from the coating as the most abundant element as presented by the highest peak in all spectra. It is necessary to denote that the samples scanned by the EDAX were not standardized or calibrated for specific compounds of interest in the current study. For more accurate quantification, the equipment should be calibrated with the corresponding elements. The complete set of data is found in Appendix D.1 and D.2 contains the additional SEM images that were analyzed by the EDAX.

In addition, an X-ray diffractometer (XRD) was also used to identify minerals present within the activated sludge samples using a Siemens Diffraktometer D5000. The samples were prepared by pulverizing them using a mortar and pestle.

4 RESULTS & DISCUSSION

The collected data from both experimental setups (batch and continuous flow reactors) are described and discussed in this chapter. These studies aimed to understand the impact of ENP_{Fe-surf} on FC disinfection in the wastewater effluent.

4.1 WASTEWATER CHARACTERIZATION

It is important to recognize the characteristics of the materials employed. Therefore, during this research, the wastewater effluent was analyzed the same day it was collected. Table 7 presents the measured parameters of the wastewater effluent used in batch and PFR disinfection experiments.

Table 7: Characteristics of Wastewater Effluent

Parameter	Average ± St. Dev.
pH	7.5 ± 0.5 (n = 22)
Total Phosphorus (mg P L ⁻¹)	0.49 ± 0.7 (n = 17)
Total Nitrogen (mg N L ⁻¹)	8.4 ± 2.3 (n = 23)
COD (mg COD L ⁻¹)	10.5 ± 3.5 (n = 23)
Conductivity (μS cm ⁻¹)	1124 ± 801 (n = 23)

The values presented correspond to the averages with their standard deviations and the total samples measured (*n*). The wastewater effluent characteristics (Table 7) fell into the typical ranges of wastewater effluent quality after secondary treatment processes. According to Tchobanoglous et al. (2014), the effluent after activated sludge with biological nutrient removal

had ranges of pH, total phosphorus, total nitrogen, COD, and total dissolved solids in 6.5 ~ 8.5, 0.5 ~ 2.0 mg L⁻¹ as P, 5 ~ 10 mg L⁻¹ as N, 20 ~ 40, and 500 ~ 700 mg L⁻¹, respectively.

When the unit of total phosphorus was converted from mg L⁻¹ as PO₄³⁻ to mg L⁻¹ as P, the wastewater effluent used in the current study had a total phosphorus concentration at lower end of the typical values. However, COD in wastewater effluent was much lower than the typical values.

The average conductivity concentration reported in this study converted to TDS and the average TDS obtained was of 618.2 mg L⁻¹, which was in the typical TDS ranges. Appendix A presents more detailed values of the wastewater effluent characteristics.

4.2 PHYSICOCHEMICAL WASTEWATER CHARACTERISTICS CORRELATION WITH FECAL COLIFORMS

After the wastewater effluent was collected, the physicochemical characteristics such as pH, total phosphorus, total nitrogen, COD, and conductivity were measured. Further, in order to comprehend the relationship between FCs and physicochemical parameters, the Pearson correlations using Minitab[®] were calculated and are discussed in this section.

The physicochemical characteristics of wastewater effluent can influence the survival, growth or decay rate of fecal coliforms (Hong and Liang, 2010). This is because their metabolic activities depend whether the conditions available within the wastewater matrix are favorable or not. For example, the abundance of suspended solids in wastewater facilitated the growth of fecal coliform bacteria by providing different types of nutrients (Davies et al., 1995). Table 8 presents

the physicochemical wastewater characteristics and the fecal coliforms in the wastewater effluent collected from the Mayagüez WWTP during a five-day period.

Table 8: Physicochemical and Microbial Characteristics of Wastewater Effluent used for Batch Reactor Disinfection Experiments during a 5-Day Period

Day	pH	Total Phosphorus (mg P L ⁻¹)	Total Nitrogen (mg N L ⁻¹)	COD (mg L ⁻¹)	Conductivity (µS cm ⁻¹)	Fecal Coliforms (CFU/100 mL)	N/P ratio
1	8.3	0.9	15.6	8.9	897	35,000	17.4
2	7.9	0.2	6.2	9.7	611	0	27.7
3	7.9	0.4	6.4	12.8	4280	5,000	17.8
4	7.3	0.4	7.6	5.9	792	8,000	19.4
5	7.3	0.7	6.2	10.9	1108	103,000	8.84

In Table 9, the most relevant values obtained from the Pearson correlation between the wastewater effluent physicochemical characteristics and FC from Table 8 are presented. The Pearson correlations of the other characteristics are found in Appendix B. As shown, it can be seen that there existed a negative and a positive correlations between both FC with pH and FC with total phosphorus, respectively. A (-) 38.2% correlation value was obtained between FC and pH. The negative value represented an inversely proportional relationship. In general, this means that with higher pH levels the FC numbers were lower. This could be due to: (1) except for one value, most values were 7.3 and 7.9, which will not impose adverse impacts on FC growth (Hong et al., 2010); (2) if by means, there is any presence of toxic forms of oxygen, this may damage the cytoplasmic membrane allowing an entrance of hydroxyl ions which would increase the pH of the fecal coliforms and hence, inhibit their growth (Curtis et al., 1992).

Table 9: Pearson Correlation Coefficient between Total Phosphorus and Fecal Coliforms in Wastewater Effluent

	pH	Total Phosphorus
Total Phosphorus	0.234	1.000
Fecal Coliforms	-0.382	0.655

Mungray and Patel (2011) studied the coliforms removal in two types of reactors. During their study they found that there was a positive correlation (55%) between FC and total phosphorus. In the current study a positive relationship was also acquired, with a 65.5% correlation between FC and total phosphorus. Therefore, an increase in FC numbers was observed with high concentration of total phosphorus probably because they acted as nutrients for microorganisms survival/growth.

Additionally, a high correlation was found between total phosphorus and total nitrogen (75.1%). This means that ratios between total nitrogen and phosphorus (N/P ratios) can yield relevant information that can collaborate in the assessment of correlations. In the study by Chudoba et al. (2013), bacteria generally had higher demand of total phosphorus, but at the same time, at lower N/P ratios a positive environment for FC growth was available. This can support our results because at lower N/P ratios, higher total phosphorus content was available, as observed in Table 8.

4.3 ENP_{Fe-Surf} IN BATCH DISINFECTION

Several batch disinfection experiments were performed to assess interactions among ENP_{Fe-Surf}, chlorine, and microorganisms in the disinfection of wastewater effluent. It is important to note that the chlorine solutions for the experiments were made from the commercial bleach solution (6.15% NaOCl) and that there would be other components, like dissolved organic matter (DOM), in the water matrix, which interacts with those three components of interest in the current study (i.e. ENP_{Fe-Surf}, chlorine, and microorganisms).

4.3.1 IRON CONCENTRATION ORIGINATED FROM ENP_{Fe-Surf} IN PRESENCE AND ABSENCE OF CHLORINE

In this batch experiment, iron concentrations originated from ENP_{Fe-Surf} were determined at different chlorine concentrations in order to understand potential contribution of chlorine strength to iron leaching out of ENP_{Fe-Surf}. Three batch reactors containing 500 mL DI water were dosed with different chlorine concentrations but at a fixed concentration of ENP_{Fe-Surf}. Table 10 presents the corresponding iron concentration at different contact times with initial chlorine concentrations of 0, 1, and 5 mg L⁻¹.

Table 10: Iron Concentration (mg L⁻¹) leached without of ENP_{Fe-Surf} at Different Chlorine Concentrations in Wastewater Effluent

Time (min)	Reactor 1 (No Cl₂)	Reactor 2 (1 mg Cl₂ L⁻¹)	Reactor 3 (5 mg Cl₂ L⁻¹)
5	4.68	4.77	4.86
10	4.68	4.74	4.83
20	4.74	4.83	4.80
30	4.71	4.92	4.92
40	4.86	4.80	4.83

It is critical to quantify the mass of iron present in $ENP_{Fe-Surf}$ solution. For this, 0.01 mL of $ENP_{Fe-Surf}$ was added to 100 mL of DI water and iron concentration was measured after sonication. Results revealed that 1 mL of $ENP_{Fe-Surf}$ had 66.2 mg Fe based on the FerroVer method. Therefore, a conversion between iron concentration and $ENP_{Fe-Surf}$ volume was obtained as follows:

$$1 \text{ mL } ENP_{Fe-Surf} = \sim 66.2 \text{ mg Fe}$$

In general, iron concentrations originated from $ENP_{Fe-Surf}$ were slightly increased with an increase of contact times, as shown in Table 10. This could be explained with the fact that since the $ENP_{Fe-Surf}$ are coated with surfactant, there existed a high possibility of chlorine scavenging by the surfactant and consequently prohibiting the chlorine from direct reaction with the $ENP_{Fe-Surf}$. Tang and Lo (2013) found that surface coatings with organic functionalized surfactants, humic acids, and silicate coatings, can prevent chemical adsorption and redox reaction with the magnetic core of $ENP_{Fe-Surf}$, hence maintaining the nanoparticle magnetism. In addition, when nanoparticles are surface coated, an electrostatic repulsive force may originate among the nanoparticles in aqueous suspension providing much stabilization (Singh et al., 2011; Tang and Lo, 2013). This means that surface coating can provide stabilization within the solution they are submerged, making it harder for any other chemical to react with the magnetic nanoparticle itself and not with the surfactant. For that reason, it can be stated that the chlorine did not react directly with $ENP_{Fe-Surf}$ or required more time to react or greater concentration to exert the effect on iron solubilization.

In addition, slightly higher iron concentrations were found with greater initial chlorine concentrations. More available chlorine would react with $\text{ENP}_{\text{Fe-Surf}}$ to a greater extent and would let more irons leach out of $\text{ENP}_{\text{Fe-Surf}}$.

4.3.2 FRESH AND AGED $\text{ENP}_{\text{Fe-Surf}}$ VS. CHLORINE CONSUMPTION

The results from the additional batch experiment conducted in SBRs to understand potentially different chlorine interactions with either aged or fresh $\text{ENP}_{\text{Fe-Surf}}$ are discussed in this section. In Figure 14 the comparison between consumed chlorine by the fixed amounts of $\text{ENP}_{\text{Fe-Surf}}$ is presented.

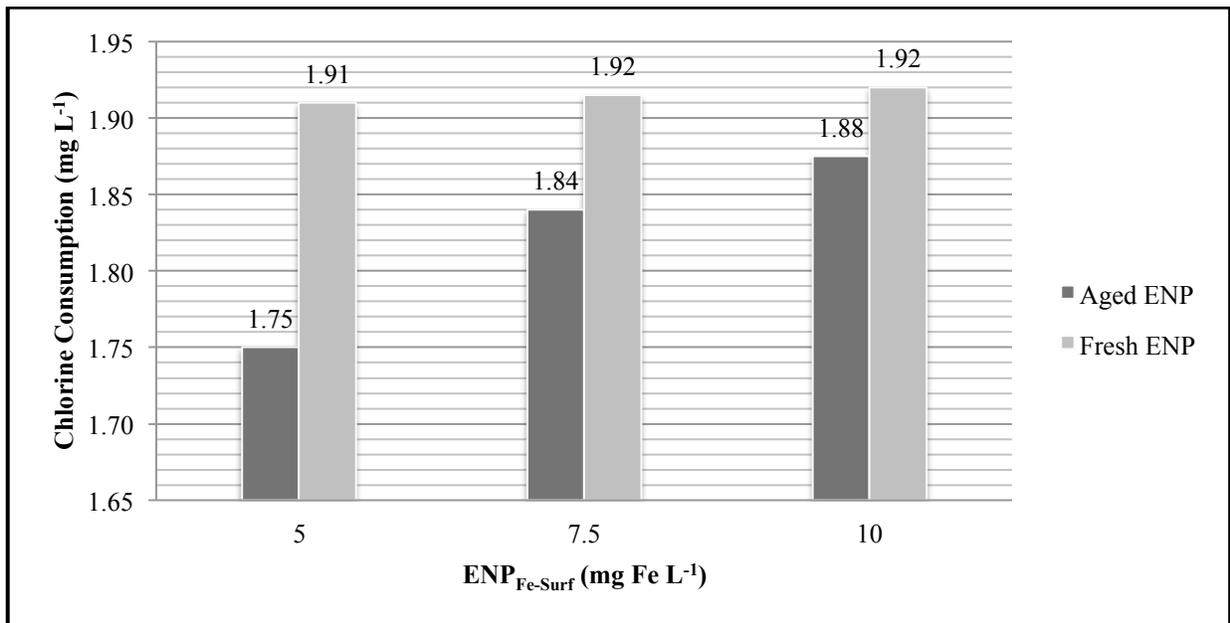


Figure 14: Chlorine Consumption by Aged and Fresh $\text{ENP}_{\text{Fe-Surf}}$

As shown in Figure 14, chlorine was consumed more with the fresh $\text{ENP}_{\text{Fe-Surf}}$ than the aged $\text{ENP}_{\text{Fe-Surf}}$. Certainly, when the $\text{ENP}_{\text{Fe-Surf}}$ is fresh so that surfactant coating is also fresh, it is expected that greater extent of chlorine would be scavenged by fresh $\text{ENP}_{\text{Fe-Surf}}$. Since the aged

ENP_{Fe-Surf} had been through the biological SBR process, microorganisms and chemical components present in the activated sludge could modify (if not consume) part of the properties of the surfactant.

Additionally, as shown in Figure 14, the aged and fresh ENP_{Fe-Surf} exerted consumption in a different manner. Fresh ENP_{Fe-Surf} maintained constant chlorine consumption regardless of the strength of the ENP_{Fe-Surf} dosages. In comparison, the aged ENP_{Fe-Surf} consumed more chlorine at greater ENP_{Fe-Surf} dosages. Hence, it is construed that the surfactants coated on the ENP played a controlling role in chlorine consumption when the fresh ENP_{Fe-Surf} was added. Having been through the SBR process, the aged ENP_{Fe-Surf} is believed to have lesser surfactants on the ENP_{Fe-Surf} so that the iron itself seemed to take a controlling role in chlorine consumption. Lu et al. (2007) reported that the surfactants acted not only as protective shells but also for functionalization with specific components, such as various drugs, specific binding sites and catalytically active species, or other functional groups.

Results of the chlorine consumptions with either the aged or fresh ENP_{Fe-Surf} justified the disinfection experiments where the fresh ENP_{Fe-Surf} was dosed at $\sim 10 \text{ mg L}^{-1}$ as Fe to simulate the ENP_{Fe-Surf} concentration at $\sim 9 \text{ mg L}^{-1}$ as Fe found in the SBR effluent from the experiment done by Martínez (2013) and Hwang et al. (2011). As shown in Figure 14, similar chlorine consumption was found at ENP_{Fe-Surf} dosage of 10 mg L^{-1} regardless of the aged or fresh ENP_{Fe-Surf}. The difference in chlorine consumption between the aged or fresh ENP_{Fe-Surf} was only by 2% (i.e., $1.88 \text{ vs. } 1.92 \text{ mg L}^{-1}$).

4.3.3 ENP_{Fe-Surf} IMPACT ON FECAL COLIFORMS DISINFECTION IN BATCH REACTOR

The importance of the free chlorine residual concentration and contact time in FC disinfection in the presence and absence of ENP_{Fe-Surf} was investigated in additional batch experiment. The Hom equation was applied for this purpose. The FC log removal, CT value, and chlorine consumption were calculated and discussed in this section.

As presented in the methodology section, several vials were used as batch reactors and a set of them had chlorine with addition of ENP_{Fe-Surf} while the other set only had chlorine addition. For both cases, the reactors had different contact times and FC log removal under different CT values was calculated. Figure 15 presents the FC log removal with different CT values at different chlorine doses in the presence and absence of ENP_{Fe-Surf}.

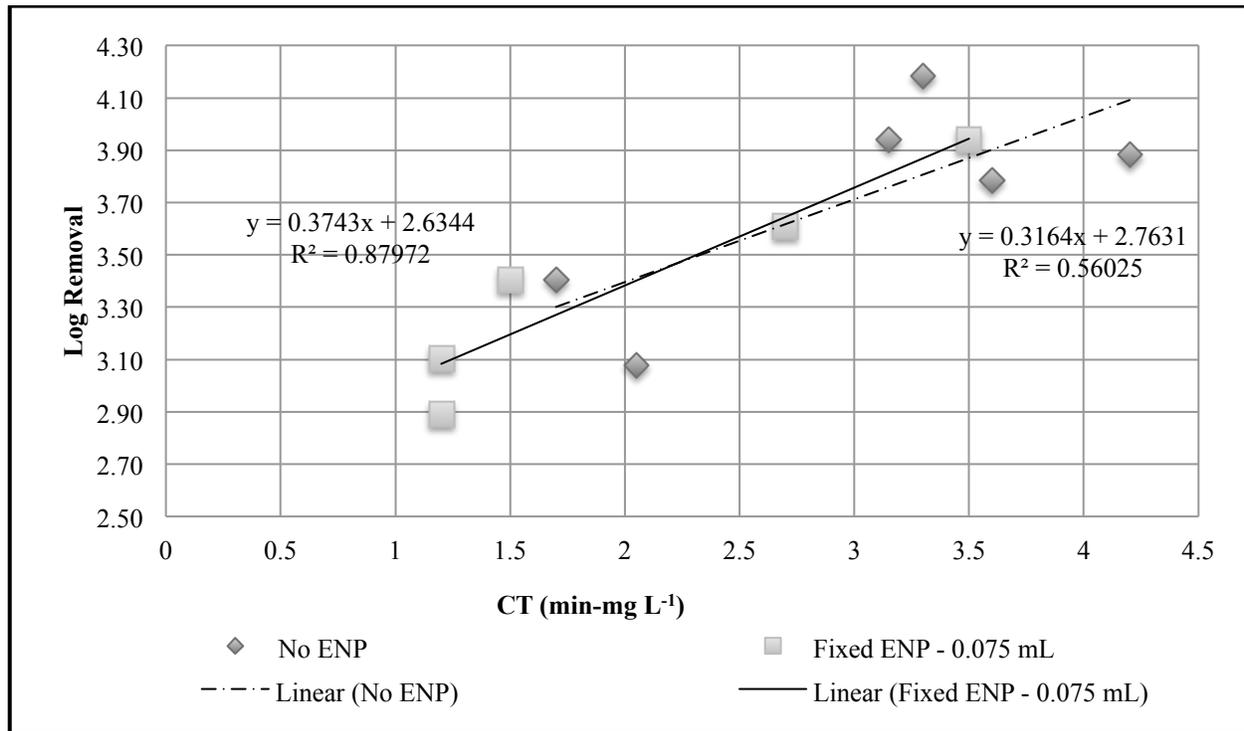


Figure 15: Fecal Coliform Log Removal vs. Contact Time in Disinfection Arranged by Hom Equation

As shown in Figure 15 the presence of ENP_{Fe-Surf} (represented by the red points) caused a decrease in FC log removal compared to the case where no ENP_{Fe-Surf} was added (represented by the blue points). Yet, acceptable FC log removals were acquired and with increasing CT values the log removals also increased in both cases.

As is required for disinfection, chlorine is added to achieve 3 and 4 log removals of the microorganisms (viruses and *Giardia lamblia*) present in the final effluent wastewater of a WWTP or other water treatment processes (EPA, 2009). For this study, when chlorine was dosed in the presence of ENP_{Fe-Surf}, chlorine would have been scavenged and consequently, less chlorine would have been available for FC disinfection. This resulted in lower FC removal than the case without ENP_{Fe-Surf} presence. These findings open an additional investigation to be done for quantitative assessment of competitive reactions of chlorine between FC and ENP_{Fe-Surf}. Taking advantage of chemical competitions in redox reaction has been successfully utilized in the literature. For example, Hwang et al. (2010) used chloroform and isopropanol as competitive scavengers for superoxide radicals and hydroxyl radicals, respectively, in a Fenton-like degradation of Methyl *tert*-butyl ether.

Toxic effect of ENP_{Fe-Surf} on FC removal should not be overlooked. Several studies have shown that since nanoparticles are so small in size they can: (1) stick to cellular membrane (Bystrzejewska-Piotrowska et al., 2009), (2) impact and harm microorganisms by entering through inhalation or ingestion, hence, locating within the cell body (Tang and Lo, 2013) and (3) cells can also take up inorganic ENPs and internalize them (Nowack and Bucheli, 2007). For example, Nowack and Bucheli (2007) documented two cases of nanoparticles internalization in the cell. First, CeO₂ nanoparticles were adsorbed onto *E. coli* cell walls, but the internalization was not visualized due to limitations in microscopic methods. Second, the ZnO nanoparticles

internalized in bacteria. Based on these studies, if the surfactant from the ENPs were deteriorated or consumed by chlorine, a high possibility could exist for ENPs themselves to cause toxic effects and therefore contribute to FC removal as well.

Chlorine consumption (or demand) was also calculated and is shown in Table 11. Figure 16 presents the comparison of FC log removal with chlorine consumption, depending on the absence or presence of ENP_{Fe-Surf}. As observed in Figure 16, a greater FC log removal was achieved for the same chlorine consumption in the absence of ENP_{Fe-Surf} than in the presence of ENP_{Fe-Surf}. In addition, when ENP_{Fe-Surf} is absent almost the same FC log removal was found regardless of the magnitude of chlorine consumption. On the contrary, in the presence of ENP_{Fe-Surf}, FC log removal increased with an increase of chlorine consumption. These data were in agreement with the results of ENP_{Fe-Surf} scavenging of chlorine.

Table 11: Chlorine Consumption in Disinfection Arranged by the Hom Equation

Reactor	Initial Cl ₂ Dosage (mg L ⁻¹)	No ENP _{Fe-Surf}		Fixed ENP _{Fe-Surf} (0.075 mL)	
		Time (min)	Cl ₂ Consumption (mg L ⁻¹)	Time (min)	Cl ₂ Consumption (mg L ⁻¹)
1	1.0	19	0.88	20	0.94
2		40	0.90	36	0.96
3		64	0.91	60	0.98
4	1.5	10	1.33	9	1.41
5		21	1.35	20	1.44
6		30	1.38	30	1.45
7	3.0	5	2.59	5	2.67
8		11	2.70	10	2.73
9		15	2.72	14	2.75

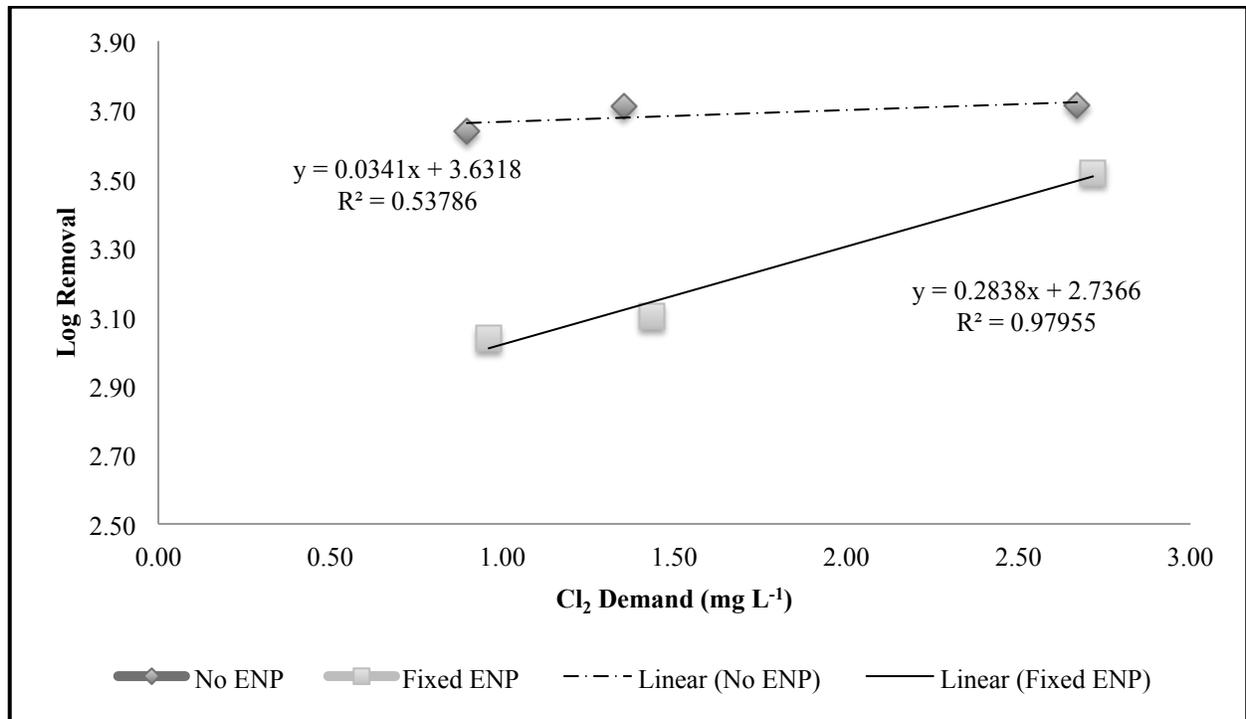


Figure 16: Average FC Log Removal and Average Chlorine Demand in Disinfection

In addition, the disinfection kinetics on the Hom equation can be used to obtain required free chlorine residual concentration at the fixed contact time to achieve a certain level of FC removal. As shown in Figure 17, to achieve a FC 4-log removal, the required free chlorine residuals was of 0.22, 0.18, and 0.10 mg Cl₂ L⁻¹ for contact times of 24, 30, and 60 minutes in the absence of ENP_{Fe-Surf}.

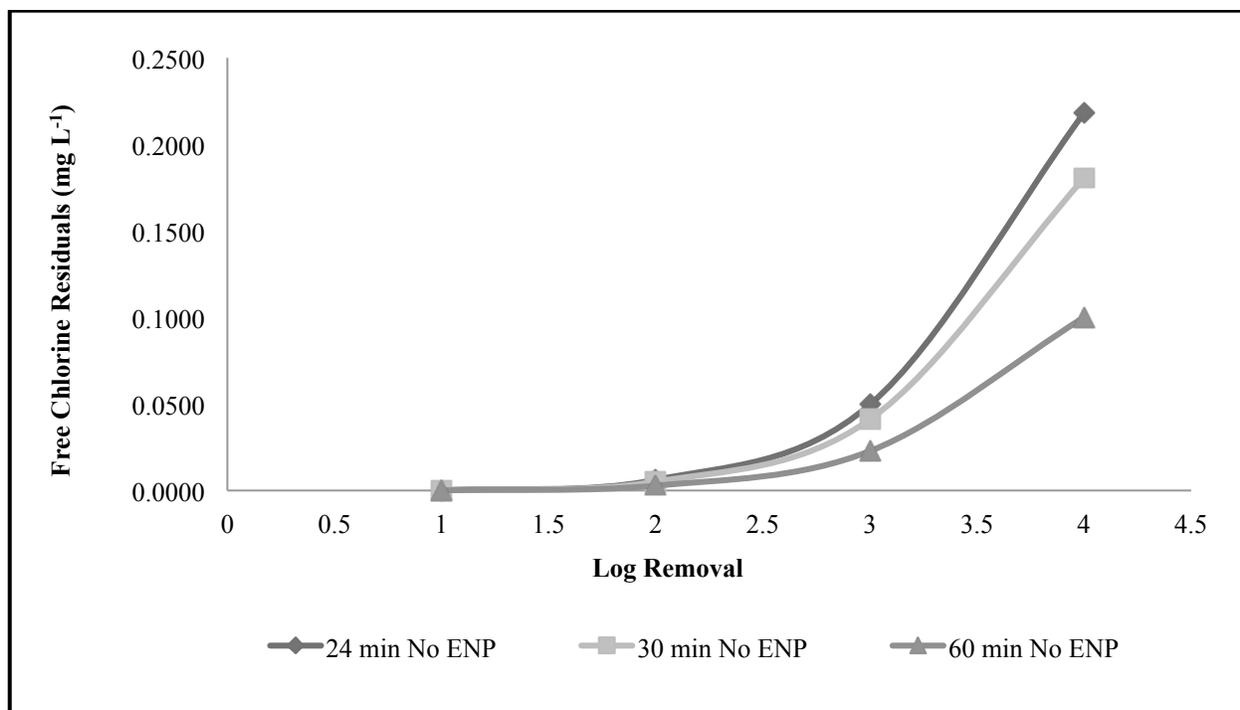


Figure 17: Free Chlorine Residuals vs. FC Log Removal Without ENP_{Fe-Surf} (0.075 mL)

In case where ENP_{Fe-Surf} was present, the required free chlorine residuals concentrations were obtained to be 0.18, 0.14, and 0.07 mg Cl₂ L⁻¹ for the contact times of 24, 30, and 60 minutes, respectively to achieve 4-log removal of FC (Figure 18). Again, as ENP_{Fe-Surf} scavenged chlorine, lowered free chlorine residuals would be present than the case without ENP_{Fe-Surf} for the same FC log removal at the same contact time.

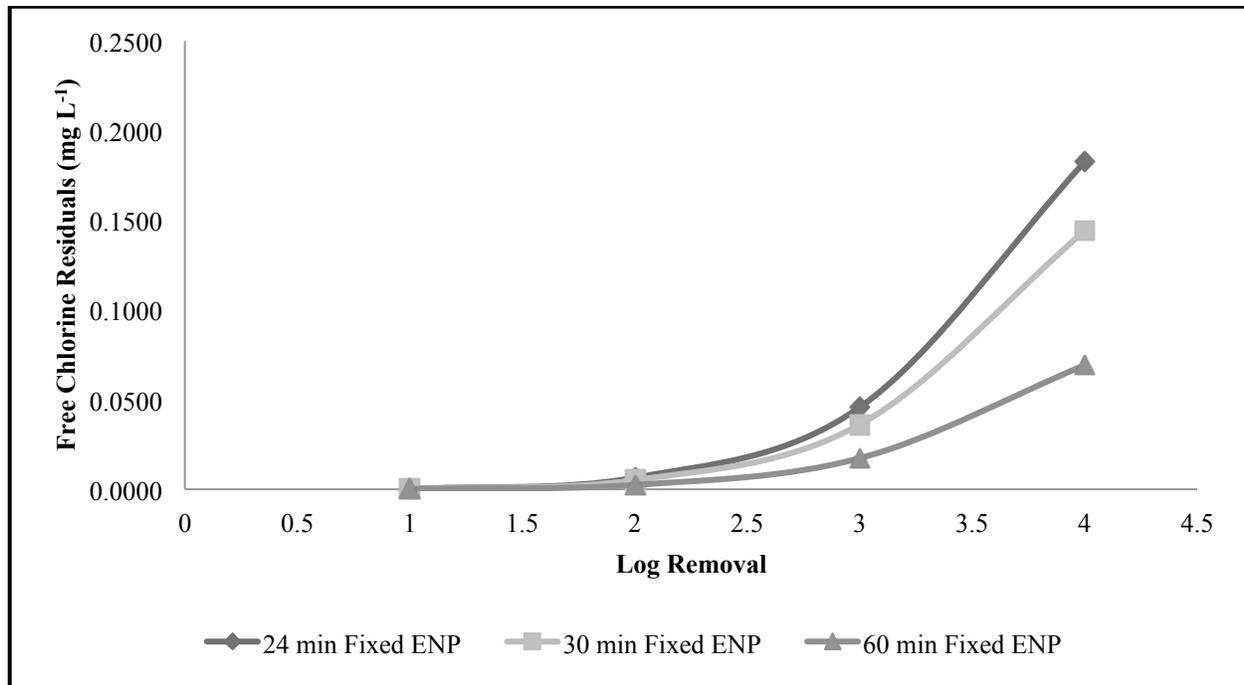


Figure 18: Free Chlorine Residuals vs. FC Log Removal With Fixed ENP_{Fe-Surf}

4.4 ENP_{Fe-Surf} IN CONTINUOUS-FLOW PFR DISINFECTION

In addition to batch disinfection experiments, plug-flow disinfection experiments were conducted to assess and understand how ENP_{Fe-Surf} behave and impact disinfection processes of wastewater effluent in a continuous flow disinfection. The plug-flow experimental results are presented and discussed in the following subchapters.

4.4.1 TRACER STUDY

After the PFR was built, it was necessary to evaluate if it satisfied the characteristics of an ideal plug-flow reactor and its disinfection capacity. For the reactor to behave as an ideal plug flow reactor, all atoms of the substance leaving the reactor have already been the same amount of

time inside the reactor (Fogler, 2005). Therefore, in order to characterize the reactor, several step input tracer studies at different hydraulic retention times (HRT) were conducted.

Step input tracer studies consists of continuous addition of a tracer within time, until the concentration measured at the output of the reactor reaches steady state level (Fogler, 2005; Asraf-Snir and Gitis, 2011). In addition, the residence time distribution (RTD) curve of a step input tracer study will illustrate a constant concentration from time $t = 0$ to the HRT, then close to the HRT a step increase will be observed and afterwards of the HRT a constant concentration will be observed and maintained within time. The flow rates and volumes were adjusted for specific HRTs of 13, 30, and 45 minutes , the resulting RTD of each tracer study are presented in Figure 19.

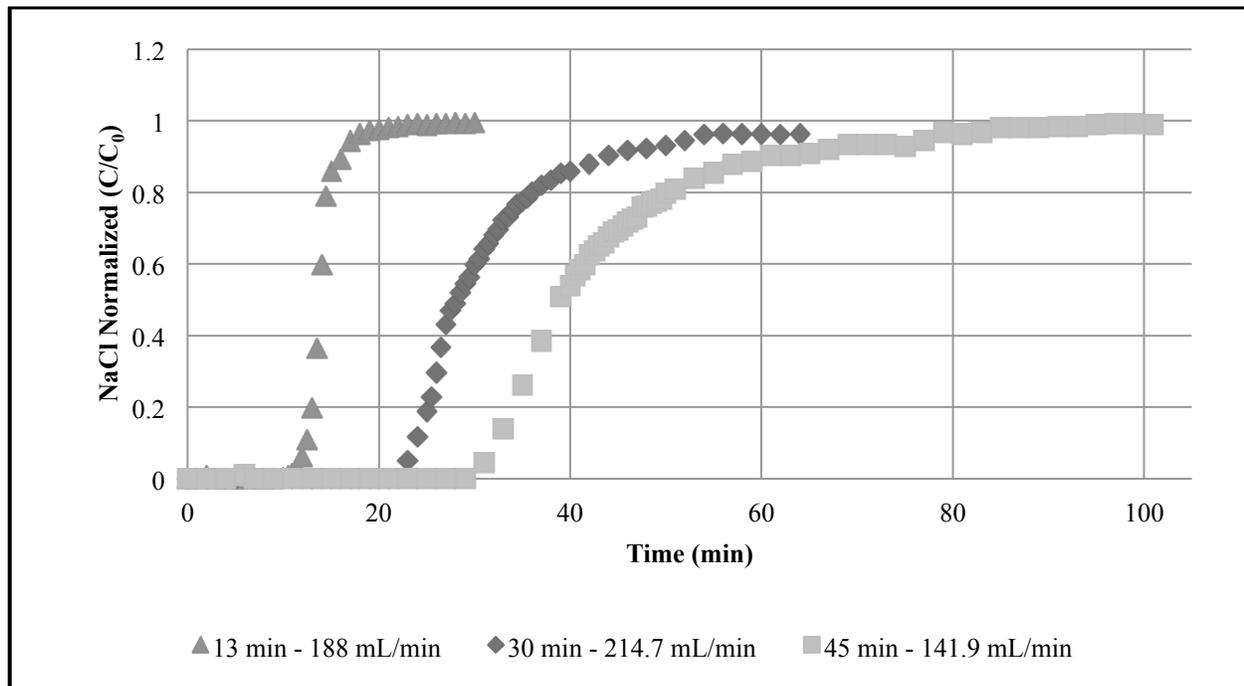


Figure 19: RTD Curve of the Tracer Study - NaCl Normalized Concentration at Different HRT and Flow Rates

In the 13 minutes HRT tracer study, a t_{10}/t_{90} ratio of 78.1% was reached. For the other two tracer studies, 30 and 45 minutes HRTs, the t_{10}/t_{90} ratios were of 54.5 and 54.1 %, respectively. In order to behave as an ideal PFR, the t_{10}/t_{90} ratio should be close to 100%. But it is important to denote that, in reality, PFRs are far from behaving as ideal PFRs, for that reason tracer studies need to be done (Asraf-Snir and Gitis, 2011). Based on these percentages, it can be stated that the PFR constructed for this study had close characteristics and behavior of an ideal PFR but at shorter HRTs. The 13-minute HRT tracer study projected that the PFR could provide pseudo-ideal plug-flow disinfection characteristics, since a higher t_{10}/t_{90} ratio was acquired. However, considering practical disinfection contact time, the remaining PFR experiments were performed at an HRT of 30 minutes.

4.4.2 CHLORINE BREAKTHROUGH IN DI WATER AND WASTEWATER EFFLUENT

Hydrodynamic chlorine disinfection characteristics were tested first with DI water and then with wastewater effluent. The former experiment was served as the blank, whereas the latter as the control.

It was hypothesized that one factor causing a chlorine decay of ~50% in DI water as shown in Figure 20 could be the pipe wall. Pipe wall decay can be influenced by the pipe material and initial chlorine concentration. Hallam et al. (2002) revealed that the wall decay for chlorine would depend on the pipe material. For PVC pipe material they found a strong inverse relationship between the initial chlorine concentration and the wall decay, but this was for initial chlorine concentrations of 0.15 up to 0.3 mg L⁻¹. Therefore, the hypothesis was rejected, because as soon as chlorine enters the plug-flow reactor, very low reactions between the PVC pipe and

the initial chlorine concentrations would be occurring. In addition, Al-Jasser (2005) found that PVC and polyethylene pipes does not affect the chlorine decay, therefore chlorine decay in PFR would be due to other factors reacting or consuming chlorine (e.g., auto-decomposition).

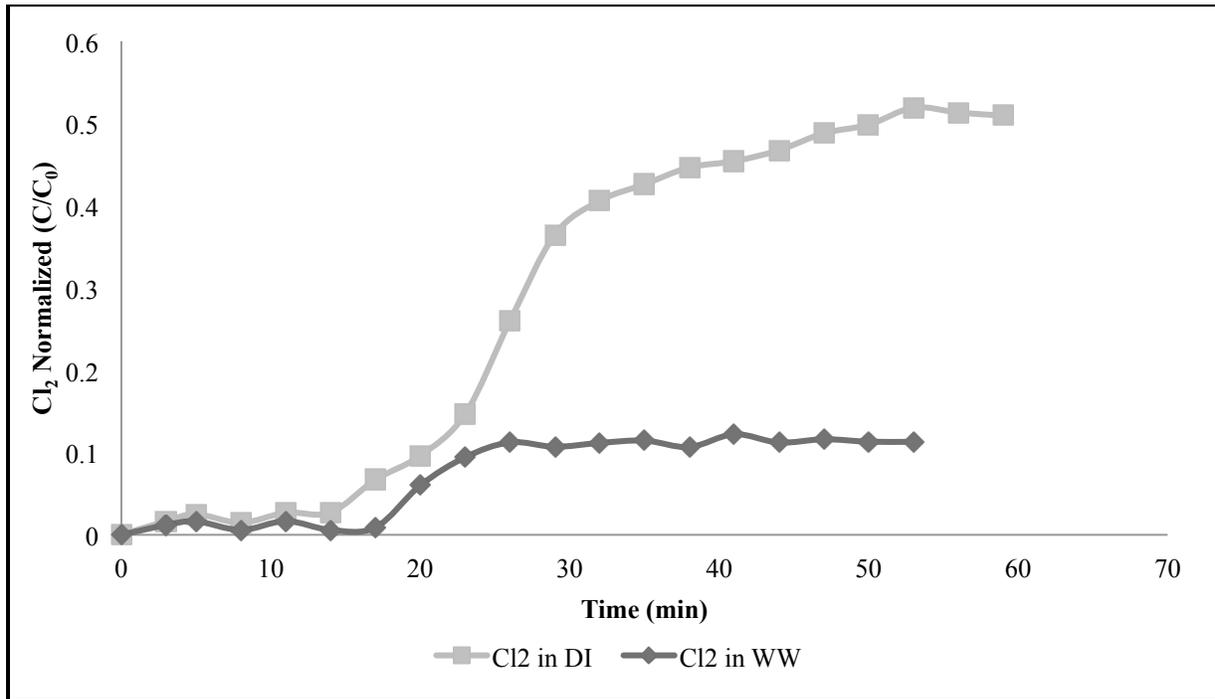


Figure 20: RTD Curve of Cl₂ in DI and WW (HRT – 30 minutes)

A higher chlorine decay (~90%) occurred in wastewater effluent than in DI water. This was expected to occur since wastewater effluent contained various organic and inorganic compounds that consumed chlorine. Constituents that could react with chlorine could be ammonia compounds, corrosion by-products, organic impurities, and unremoved metallic compounds (Al-Jasser, 2005). These constituents will induce more chlorine consumption and, in a worst case scenario, higher chlorine dosage will be necessary. Therefore, the enormous discrepancy between chlorine in DI and chlorine in wastewater effluent occurred because of the chlorine consumption by interactions with the constituents in wastewater effluent (Figure 20).

4.4.3 ENP_{Fe-Surf} BREAKTHROUGH IN DI WATER AND WASTEWATER EFFLUENT

Similar to the aforementioned study on the chlorine RTD in PFR, hydrodynamic characteristics of ENP_{Fe-Surf} was evaluated in DI water and wastewater effluent. As shown in Figure 21, the RTD curves of ENP_{Fe-Surf} were very similar regardless of the type of water to which ENP_{Fe-Surf} was dosed. At pseudo steady-state, ~70 and 80% of ENP_{Fe-Surf} exited in the effluent when ENP_{Fe-Surf} was added to DI water and wastewater effluent, respectively. It was noticed that ENP_{Fe-Surf} covered on the PFR wall to minor extent. This would account for ~70-80% recovery of ENP_{Fe-Surf} in the PFR. A slightly higher ENP_{Fe-Surf} concentration could be attributed to the properties of ENP_{Fe-Surf} interacting with DOM present in wastewater effluent. DOM is known for its characteristics of facilitating pollutant transport in water (Zhang and Zhang, 2010).

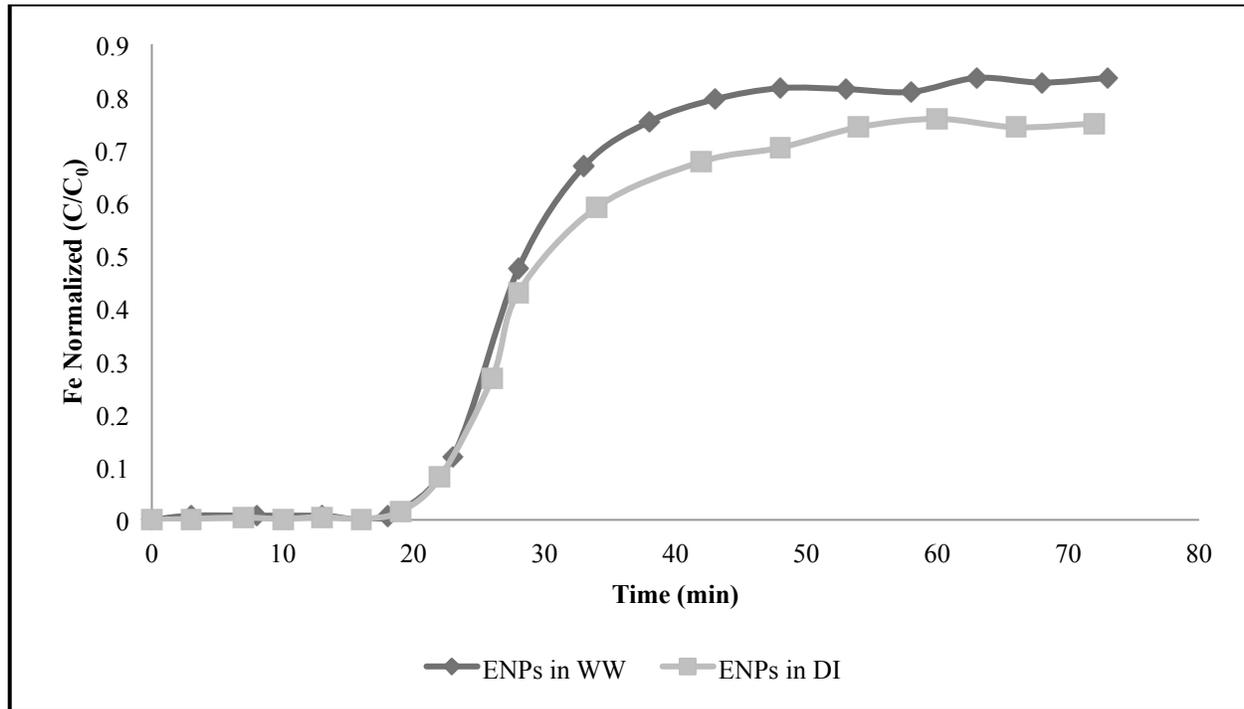


Figure 21: RTD Curve of ENP_{Fe-Surf} in DI and WW (HRT – 30 minutes)

It is also important to clarify the reasons for slight difference in $ENP_{Fe-Surf}$ RTD between in DI water and in wastewater effluent. Tang and Lo (2013) discussed the different properties of magnetic nanoparticles and mentioned that surfactants could enhance significantly the stability of magnetic nanoparticles suspension by modifying the outermost layer of the particle or by conserving the particle charge (Brar et al., 2010). In addition, surfactant could also prevent aggregation among the nanoparticles. In the continuous flow experiment, $ENP_{Fe-Surf}$ would disperse rapidly throughout DI water and wastewater effluent. However, $ENP_{Fe-Surf}$ would interact with various constituents present in wastewater effluent, including microbial degradation of the surfactants or DOM. As it is known, DOM can react with heavy metal and form some complex by-products (Yoon et al., 2009). For example, in a study conducted by Wang et al. (2003), they found that DOM contained weak acid sites, which can react with some heavy metal ions and produced metal DOM-complexes. Then, the magnetite ENPs could have agglomerated each other. This potentially can enhance their transport throughout the PFR and consequently higher amounts of ENPs would measure as found.

4.4.4 BREAKTHROUGH OF CHLORINE - $ENP_{Fe-Surf}$ IN DI WATER AND WASTEWATER EFFLUENT

Now that individual hydrodynamic behavior of chlorine and $ENP_{Fe-Surf}$ was understood in either DI water or wastewater effluent, it was of significant importance to assess how they interacted each other when presented in the PFR system together. Several experiments were conducted in the co-presence of chlorine and $ENP_{Fe-Surf}$ in DI water and wastewater effluent.

Figure 22 depicts the RTD curve for both the chlorine and $ENP_{Fe-Surf}$ in DI water. As shown, the hydrodynamic behaviors of the co-presented chlorine and $ENP_{Fe-Surf}$ were very different from

those observed when presented individually (Figures 20 and 21). For chlorine in DI water, ~50% of the dosed concentration was measured at pseudo steady state when presented without ENP_{Fe-Surf} (Figure 20), whereas ~35% was found with the co-presence of ENP_{Fe-Surf} (Figure 22). However, ENP_{Fe-Surf} concentration was increased when dosed together with chlorine. ~75% of the dosed ENP_{Fe-Surf} was measured at pseudo steady state when dosed in DI water in the absence of chlorine (Figure 21), whereas ~90% was in the presence of chlorine (Figure 22).

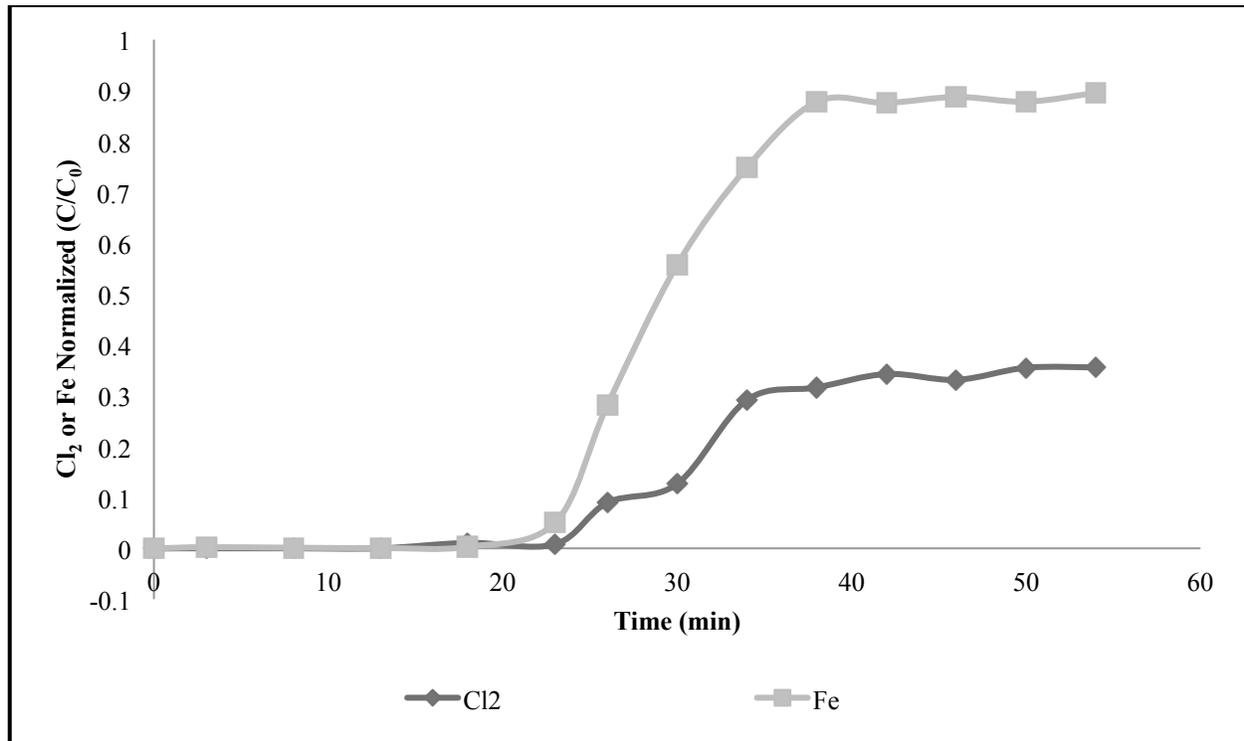


Figure 22: RTD Curve of Co-Present ENP_{Fe-Surf} and Cl₂ in DI Water (HRT – 30 minutes)

As previously mentioned, chlorine is a chemical compound that degrades easily with time, when exposed to light, reacts rapidly when interacts with other chemical compounds. In the experiment, when chlorine was added to water it would start to degrade and produce its common byproducts but at the same time it would be scavenged by ENP_{Fe-Surf}. As such, the chlorine demand would increase and lower chlorine concentration would leave the PFR system.

A slight increase in $ENP_{Fe-Surf}$ concentration was due potentially to degradation of the surfactants by chlorine. As a result, lesser amount of $ENP_{Fe-Surf}$ would have been lost by adsorption to the PFR wall.

The results from the case where wastewater effluent was utilized in the PFR experiments in the co-presence of chlorine and $ENP_{Fe-Surf}$ are presented in Figure 23. As shown, compared to the case of $Cl_2-ENP_{Fe-Surf}$ in DI water, lower concentrations of both chlorine and $ENP_{Fe-Surf}$ were observed in wastewater effluent.

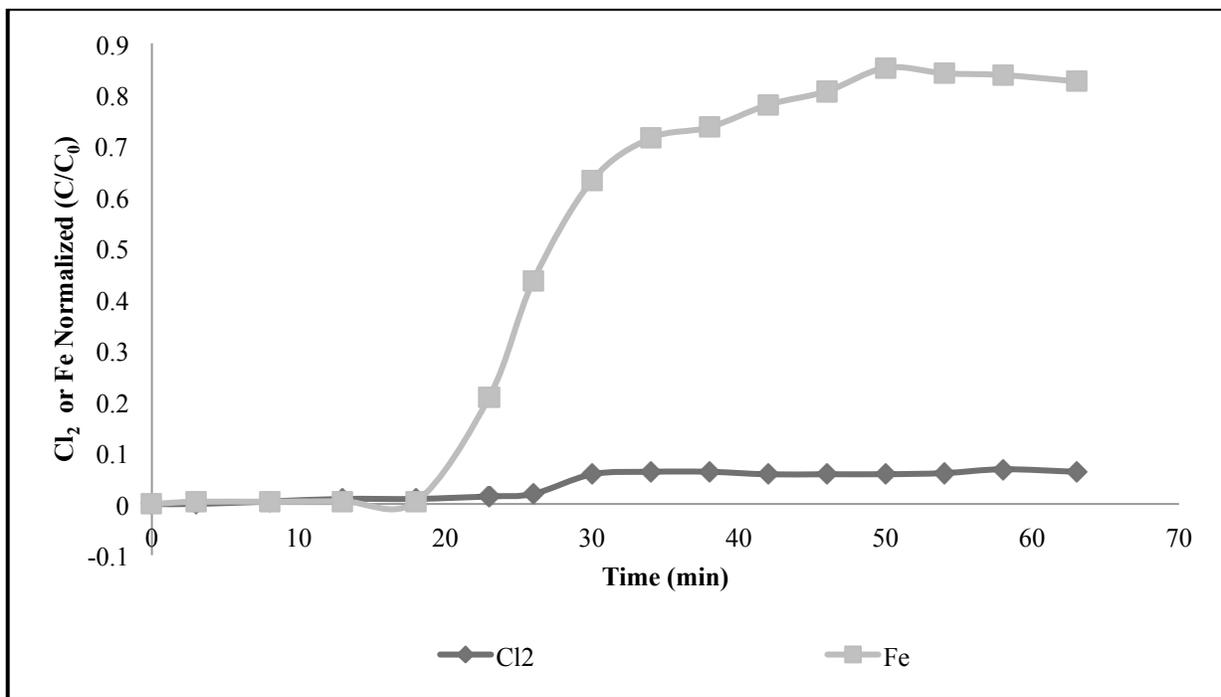


Figure 23: RTD Curve of Co-Present $ENP_{Fe-Surf}$ and Cl_2 in Wastewater Effluent (HRT - 30 minutes)

Similar to the case in DI water, the co-presence of another component, either chlorine or $ENP_{Fe-Surf}$, also decreased the concentration of the other in wastewater effluent. ~5% of the added chlorine concentration was measured at pseudo steady state in the presence of $ENP_{Fe-Surf}$ (Figure 23). In comparison, ~10% was observed in the absence of $ENP_{Fe-Surf}$ (Figure 20).

In comparison to the case with DI water, slightly lower $ENP_{Fe-Surf}$ was measured in wastewater effluent at pseudo steady state (~90% in Figure 22 vs. ~85% in Figure 23). However, earlier breakthrough of $ENP_{Fe-Surf}$ was made in wastewater effluent. It is believed that chlorine degraded the surfactants resulting in bare ENP that interacted with DOM in wastewater effluent. As discussed previously, DOM is known to facilitate pollutant transport (Zhang and Zhang, 2010).

A slightly increased $ENP_{Fe-Surf}$ concentration was found in the presence of chlorine in wastewater effluent at pseudo steady state (~85% in Figure 23) than in the absence of chlorine (<85% in Figure 21). These results could have also been attributed to surfactants degradation resulting in enhanced transport of ENP and DOM.

4.4.5 $ENP_{Fe-Surf}$ IMPACT ON FECAL COLIFORMS DISINFECTION IN PFR

The impact of $ENP_{Fe-Surf}$ on FC disinfection in batch experiment was already discussed in previous subchapters. In this chapter, two continuous flow disinfection experiments were run in PFR, with and without the addition of $ENP_{Fe-Surf}$, focusing on FC disinfection. It is important to denote that these experiments were run in replicate on different days. Prior to the PFR run, fresh wastewater effluent was collected from the Mayagüez WWTP. Therefore, there was a little dissimilarity in the characteristics of wastewater effluent as shown in Table 12.

Table 12: Physicochemical and Microbial Characteristics of Wastewater Effluent used for the Days of PFR Experiments

Analysis Type	Samples	Day	
		1st	2nd
pH	1	7.8	8.2
	2	7.8	8.2
Total Phosphorus (mg PO ₄ ⁻³ L ⁻¹)	1	0.13	0.51
	2	0.14	0.51
Total Nitrogen (mg N L ⁻¹)	1	9.7	13.6
	2	9.5	11.3
COD (mg COD L ⁻¹)	1	10.4	12.0
	2	10.5	12.3
Conductivity (μS cm ⁻¹)	1	856	977
	2	857	976
Fecal Coliforms (10 ³ CFU/100 mL)	1	21	43

The results of FC log removal and CT values from the PFR experiment containing only chlorine are presented in Figure 24. It is noted that when 100% FC removal was achieved, the FC log removal was conservatively assigned to be 4-log removal. Likewise, when no FC removal was found (i.e., 0% removal), 0.0001-log removal was assumed taking into consideration the rule of log conversion.

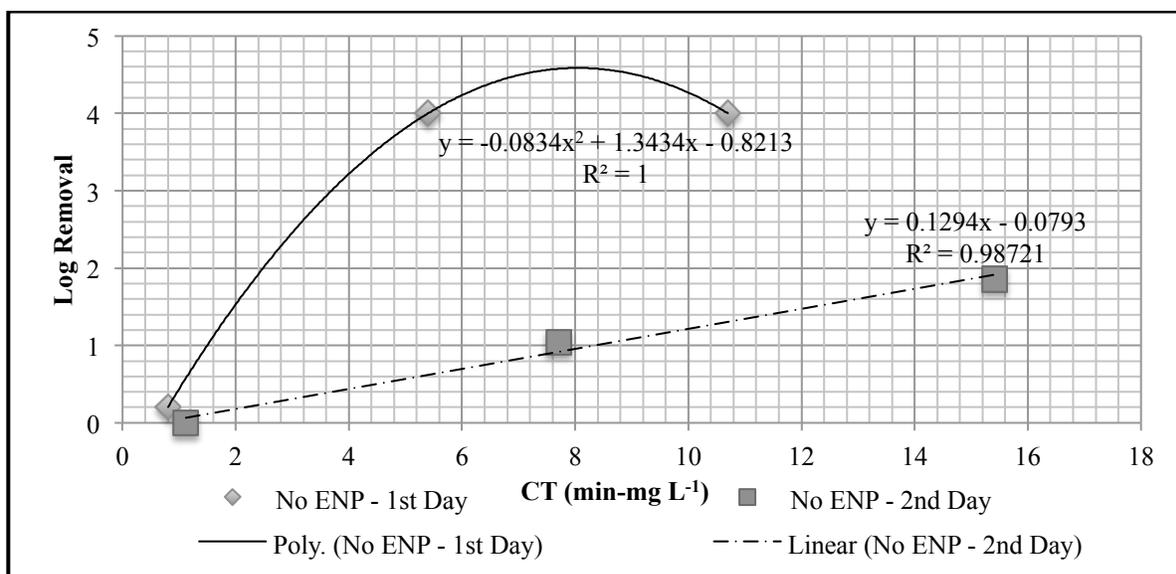


Figure 24: FC Log Removal vs. CT values in Cl₂ Only PFR Experiment

As can be seen in Figure 24, FC log removals are mostly directly proportional to CT values in either a linear or a curvilinear relationship. The linear and curvilinear regression values of 99% and 100% were obtained for the second and first day PFR experiments, respectively. The curvilinear behavior can imply that after ~5 CT value the maximum or suitable FC log removal (>4-log removal) was reached in disinfection in the specific wastewater effluent collected. For the linear regression, CT value greater than 16 would be necessary to achieve even a lower FC log removal at 2.

Judged by the discrepancy observed in FC log removal physicochemical characteristics of wastewater effluent must have played an influence in the decrease of FC log removal on the second day. As observed in Table 12, higher total phosphorus was quantified in the wastewater effluent collected on the second day than in wastewater effluent on the first day. In agreement with the strong Pearson's correlation coefficient (0.655) between TP and FC numbers (Table 9 in the Chapter 4.2), FC numbers in wastewater effluent were twice more on the second day (43×10^3 CFU/100 mL) than on the first day (21×10^3 CFU/100 mL). Total phosphorus serves as nutrients for pathogens (Chudoba et al., 2013) and also TP and FC tend to be bounded by particles facilitating the transport of FC (Hong et al., 2010).

Figure 25 presents the FC log removal approximations depending on CT values for disinfection in presence of $ENP_{Fe-Surf}$. Similar to Figure 24, with higher CT values the FC log removals increased.

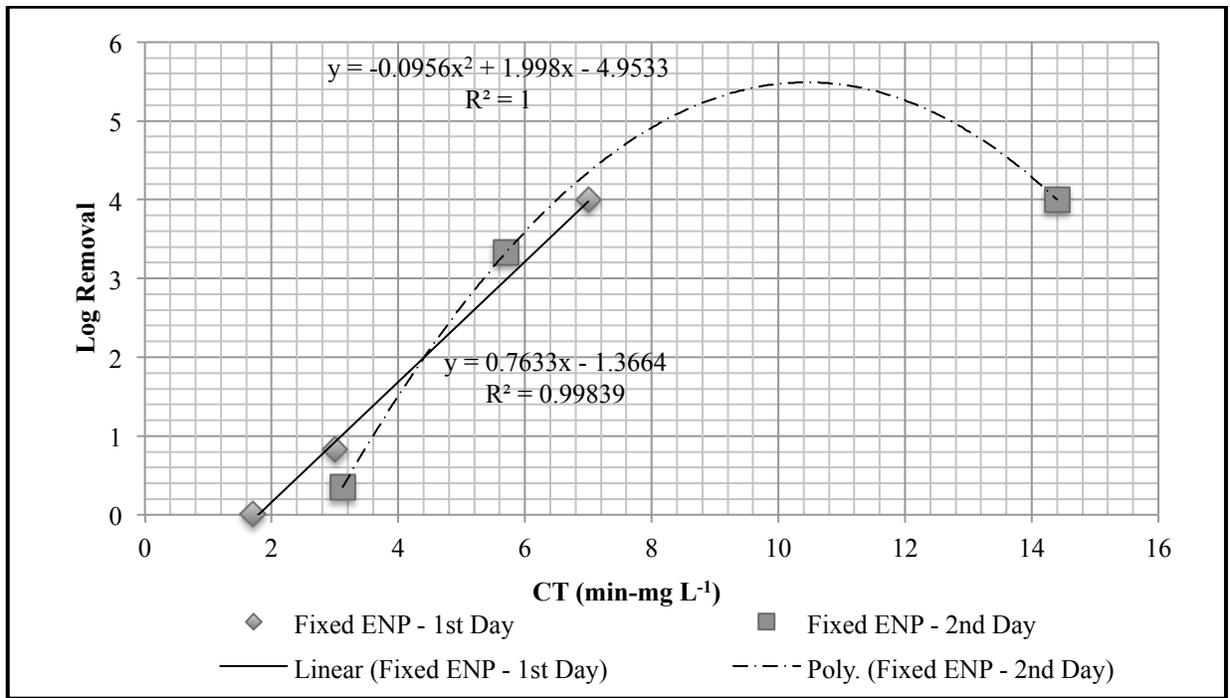


Figure 25: FC Log Removal vs. CT values in Cl₂-ENP_{Fe-Surf} PFR Experiment

In the batch disinfection (Chapter 4.3), it was found that the presence of ENP_{Fe-Surf} made more chlorine consumption leaving lower free chlorine residuals to achieve the same FC log removal at the same contact time than the absence of ENP_{Fe-Surf}. The results from the PFR disinfection were not in agreement with those from the batch disinfection. FC removals as a function of CT values were plotted only with the accountable results after exclusion of those assumed data points in Figures 24 and 25. As shown in Figure 26, the presence of ENP_{Fe-Surf} required a smaller CT for FC disinfection in the PFR than in the PFR in the absence of ENP_{Fe-Surf}. In other simpler words, the same FC log removal at the same disinfection contact time would be accomplished at lower concentrations of free chlorine residuals in the presence of ENP_{Fe-Surf} than in the absence of ENP_{Fe-surf}. This was in good agreement with the previous experimental results that showed lower concentrations of free chlorine residuals due to scavenging effect of ENP_{Fe-surf} on chlorine.

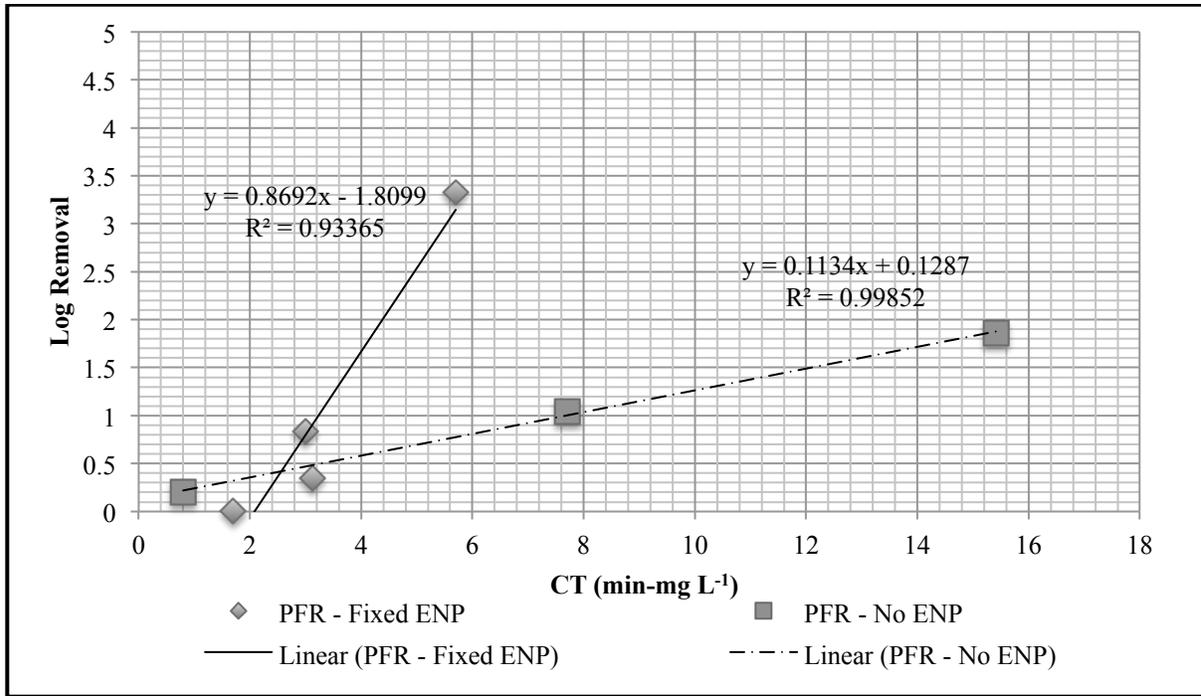


Figure 26: Comparison of FC Log Removal in the Presence and Absence of ENP_{Fe-Surf} in PFR

In an ideal case, FC disinfection can be expressed in a different manner depending on the reactor type and rate as shown in Table 13 (Tchobanoglous et al., 2014). Accordingly, the same FC disinfection kinetics is applied for both batch reactor and PFR.

Table 13: Disinfection kinetic expressions

Reactor type	Zero order	First order
Batch	$kt = C_o - C$	$kt = \ln(C_o/C)$
CSTR	$kt = C_o - C$	$kt = (C_o/C) - 1$
PFR	$kt = C_o - C$	$kt = \ln(C_o/C)$
CSTRs (in series)	$kt_{total} = C_o - C_n$	$kt_{total} = n[(C_o/C_n)^{1/n} - 1]$

Note: k, reaction rate order constant; t, contact time; n, the number of reactors; and C_o and C, initial and final concentrations, respectively.

As previously described with the tracer study, the PFR had t_{10}/t_{90} ratios of 0.781, 0.545, and 0.541 at HRT's of 13, 30, and 45 minutes despite 4 strains installed along with the reactor length. In order to behave as an ideal PFR, the t_{10}/t_{90} ratio should be close to 1. Therefore, lower FC removals achieved from PFR (Figure 26) than batch reactors (Figure 15) were due to small t_{10}/t_{90} ratios resulting in poor disinfection efficiency. For example, using the reaction expressions in Table 13 and assuming FC disinfection follows the Chick's Law in the first reaction order constant, k of 0.46 min^{-1} (for example), 3-log removal of FC disinfection takes 15 and 2,172 minutes in a PFR (or batch reactor) and a continuously stirred tank reactor, respectively. It is expected that a better PFR configuration with an ideal t_{10}/t_{90} ratio of 1 would result in the similar (if not the same) FC disinfection efficiency that the batch disinfection achieved.

4.5 METAGENOMICS

The impact of $\text{ENP}_{\text{Fe-Surf}}$ to THB was also evaluated and compared with the FCs and the results are illustrated in Figure 27. It is observed that chlorine was effective in the removal of both FC and THB, regardless of the presence of $\text{ENP}_{\text{Fe-Surf}}$. For the case where only $\text{ENP}_{\text{Fe-Surf}}$ was added, a greater removal was achieved for the FCs than the THB. Therefore, metagenomic analysis was conducted to better understand this behavior and the results are presented in Figures 28 to 31.

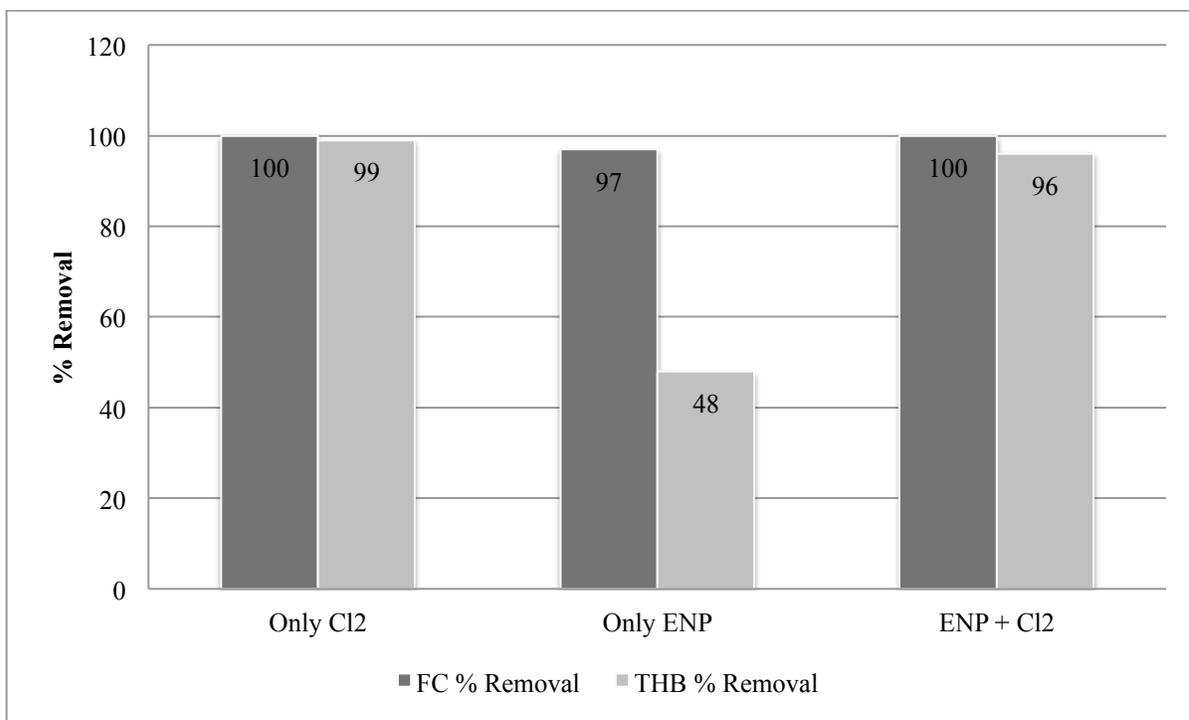


Figure 27: FC and THB Percent Removal Comparison

From the metagenomic analysis, biodiversity in microbial DNA communities were explored and the sequencing results presented different bacteria classifications in each sample. The phylum classification is a taxonomic rank between the domain and the class ranks. The lower rank represents a greater similarity and a lesser numbers of bacteria. The results in Figures 28 and 29 are the phylum classifications found in the wastewater effluent before and after addition of $ENP_{Fe-Surf}$, respectively. It can be observed that after the $ENP_{Fe-Surf}$ were added (Figure 29) the phylum of Proteobacteria, Bacteroidetes, Fusobacteria, Verrucomicrobia, and Actinobacteria decreased in diversity, whereas Chlamydiae, Deinococcus-thermus, Euryarcheote, Planctomycetes, Firmicutes, and Crenarcheota phylum diversity increased. This means that the impact $ENP_{Fe-Surf}$ had on bacteria varied depending on the type of bacteria present.

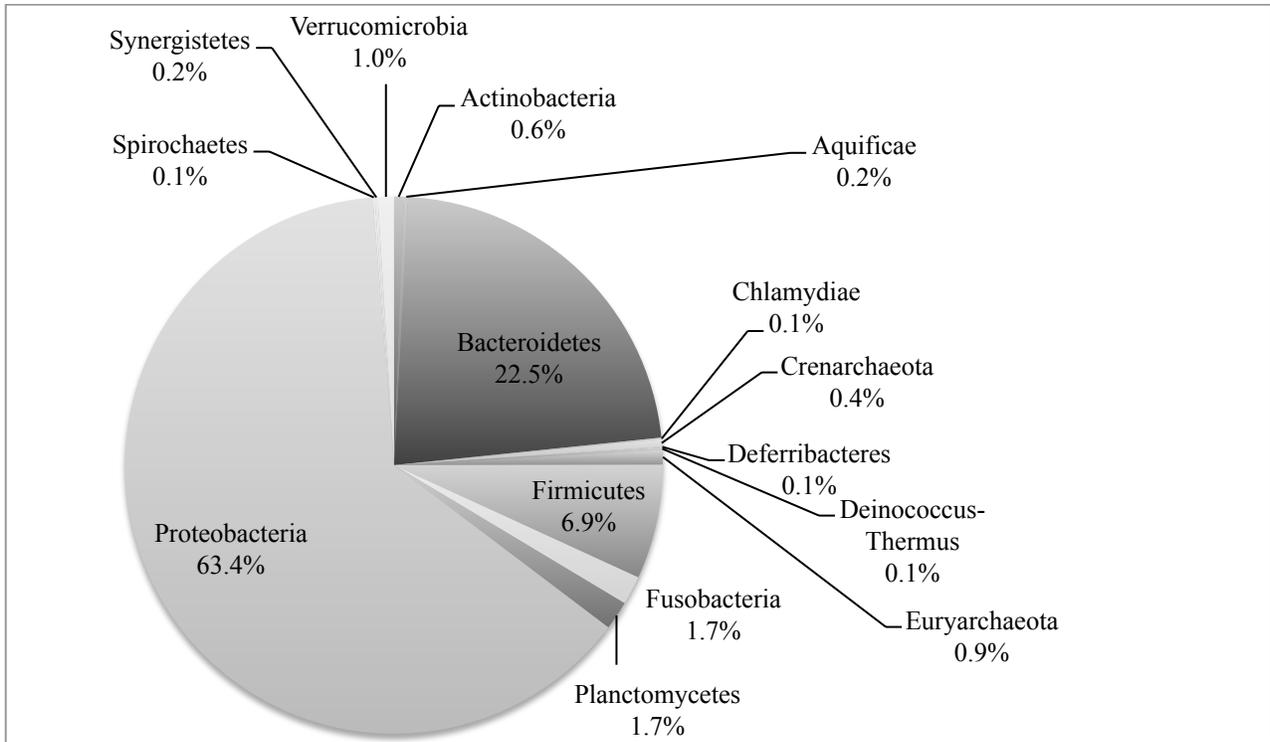


Figure 28: Phylum Classification of WWE before ENP_{Fe-Surf} Addition

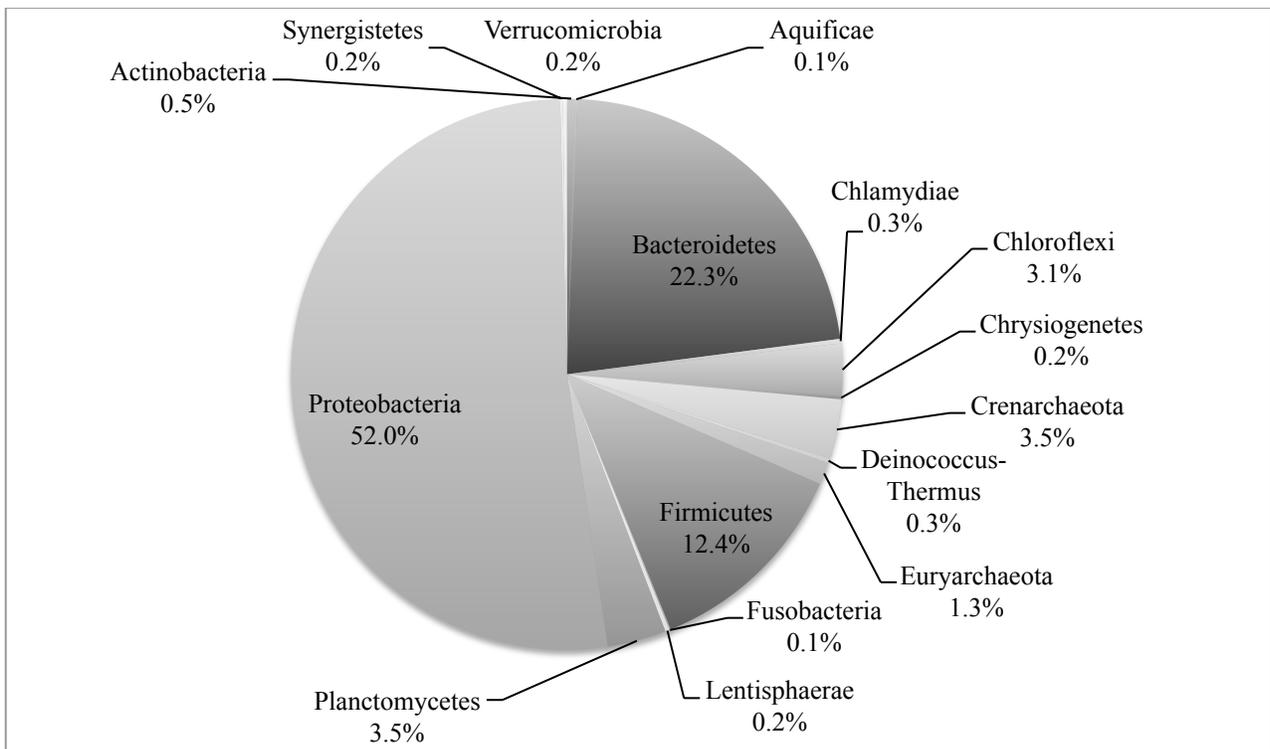


Figure 29: Phylum Classification of WWE after ENP_{Fe-Surf} Addition

In order to obtain better specifications of which bacteria were present or absent in the samples, for example fecal coliforms, the family classification was analyzed for the same cases of wastewater effluent before and after ENP_{Fe-Surf} addition. As shown in Figure 30, a total of 22 families of bacteria presented a significant percent diversity and 5% diversity of Enterobacteriaceae, also called coliforms, were found within the families. In contrast, after ENP_{Fe-Surf} were added the number of families with significant percent diversity decreased to 15 families and specifically the diversity of Enterobacteriaceae decreased to 0.46% diversity (Figure 31). It can also be observed that for other families the presence of ENP_{Fe-Surf} did not affect them. Instead, it increased their diversity. For example Campylobacteraceae family increased from 6 % to 9 % diversity.

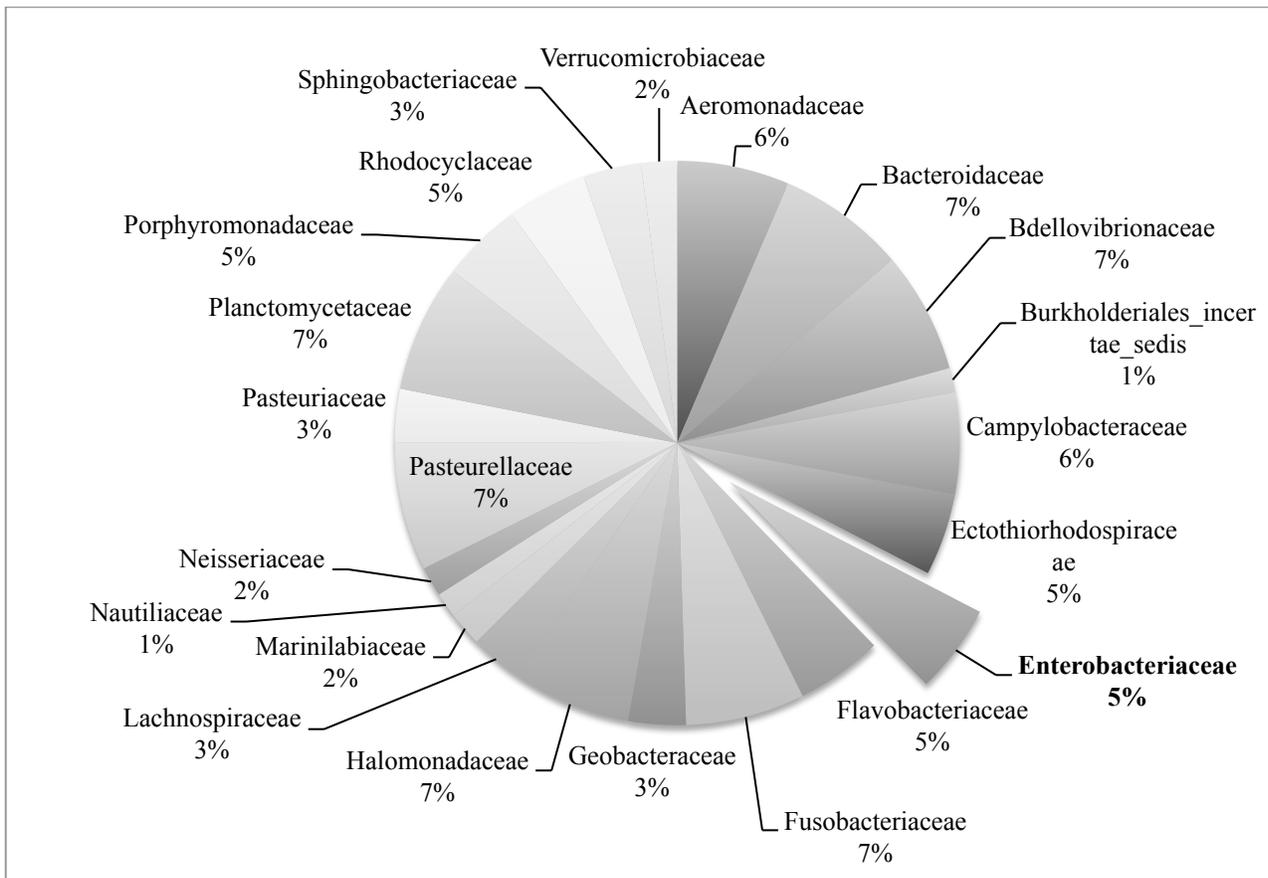


Figure 30: Family Classification of WWE before ENP_{Fe-Surf} Addition

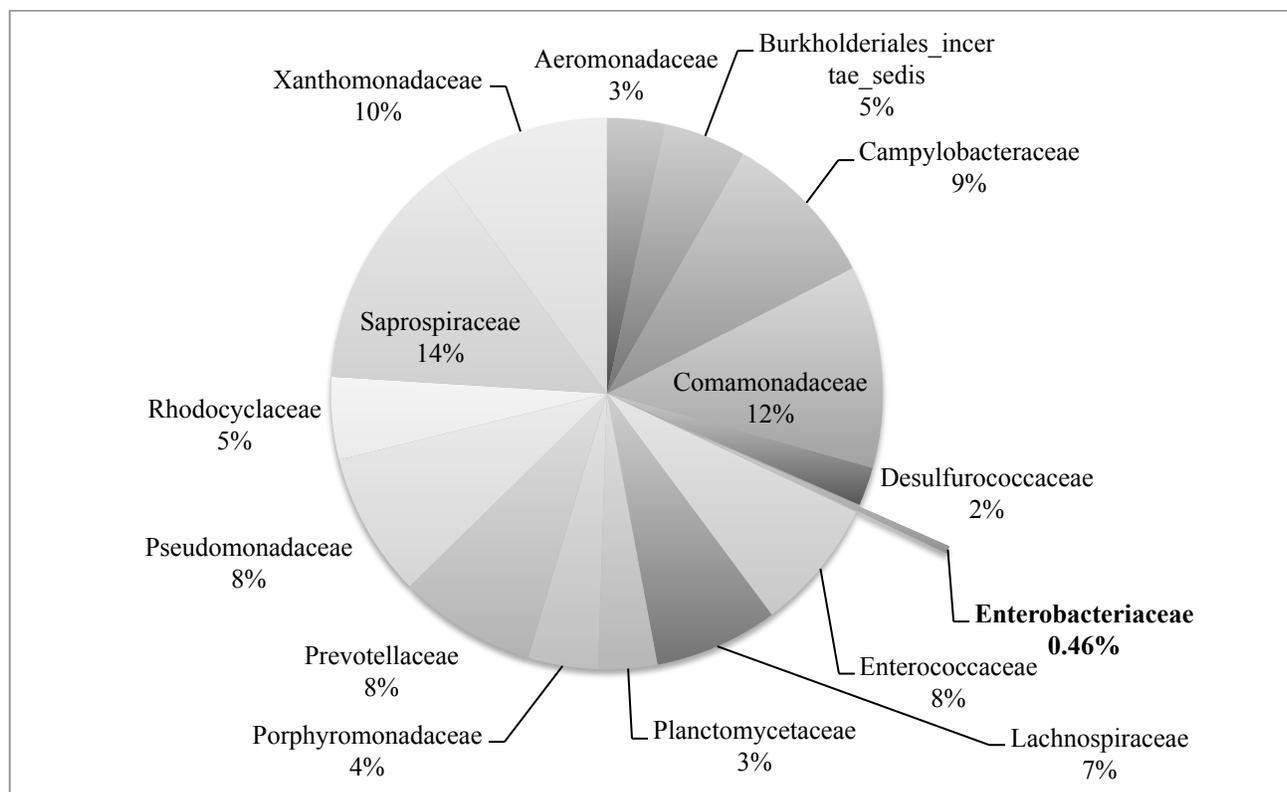


Figure 31: Family Classification of WWE after ENP_{Fe-Surf} Addition

From these results, it can be noted that the presence of ENP_{Fe-Surf} can impact both the growth and removal of microorganisms in the wastewater effluent. However, in order to obtain a more specific understanding on the mechanisms encountered between the ENP_{Fe-Surf} and the microorganisms, further studies are still necessary to conduct with less diverse sample matrix. As stated by Valenzuela et al. (2006), in order to obtain better analysis of an environmental sample community the ideal situation is to have low diversity environment. Similarly, few complete microbial genomes were assembled from the samples collected in the Sargasso Sea where immense diverse microbial communities were present (Venter et al., 2004).

4.6 ENP_{Fe-Surf} IMPACT ON SETTLED ACTIVATED SLUDGE

In order to have a broader understanding of the ENP_{Fe-Surf} impact on the settled activated sludge system, lab-scale SBR experiments were employed. Samples collected at the end of each cycle were then taken for SEM, EDAX, and XRD analysis.

The SEM was utilized for acquiring a better visualization of the incorporation of ENP_{Fe-Surf} the settled activated sludge. In Figures 32 to 37, SEM images of the control and treatment samples are shown, respectively. It is also included the EDAX elemental composition analyses of the scanned areas in each SEM image and other areas of each sample. The XRD was used to examine mineralogical characteristics of the sludge. In Figures 38 to 41 the XRD spectra of the control and treatment are also shown.

Table 14 contains the percentages of the elements analyzed by the EDAX in the settled activated sludge sample of the control reactor, shown in Figures 32, 33, and 34. In spectra 1, the highest atomic percentages were iron with 39.47 atomic^b % and silicon with 30.32 atomic %. Spectra 2 detected iron to be the highest element with a 42.41 atomic %, followed by aluminum with a 20.38 atomic %, cobalt with an 18.22 atomic %. Spectra 3 identified that the highest atomic percentages were accounted for silicon, aluminum, and iron with a 41.11, 20.40, and 18.65 atomic %, respectively. In general, the EDAX chemical analyses provided evidence that the most abundant elements within the control sample were mostly iron, silicon, and aluminum, but depending on the scanned area the abundance or presence of an element varied.

^b Atomic percentages are presented as the reference percentages since it provides more accurate values than the weight percentages.

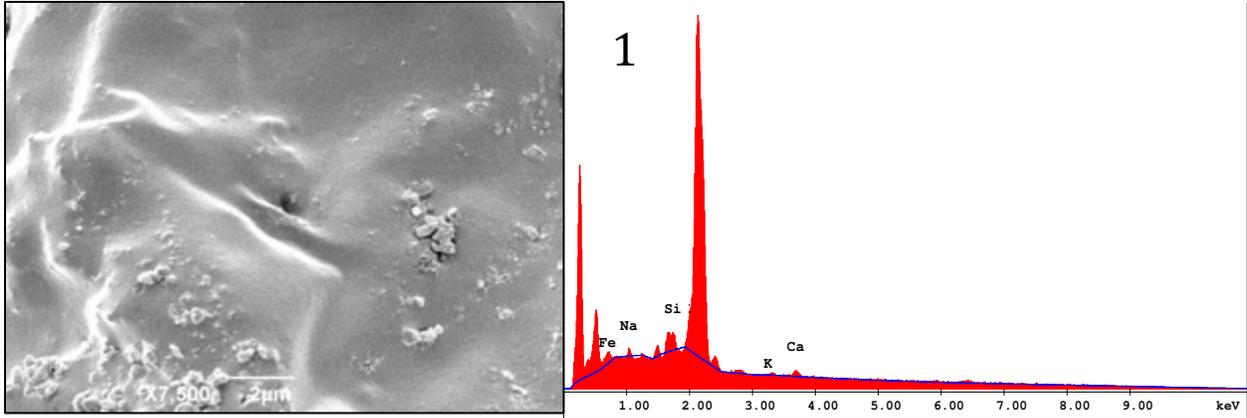


Figure 32: SEM and EDAX Analysis of the Settled Activated Sludge from the Control SBR (Spectra 1)

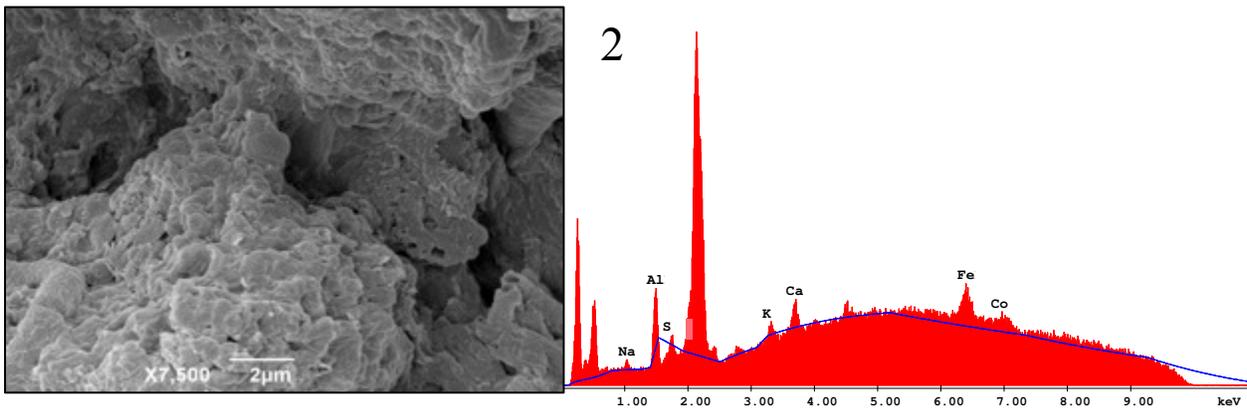


Figure 33: SEM and EDAX Analysis of the Settled Activated Sludge from the Control SBR (Spectra 2)

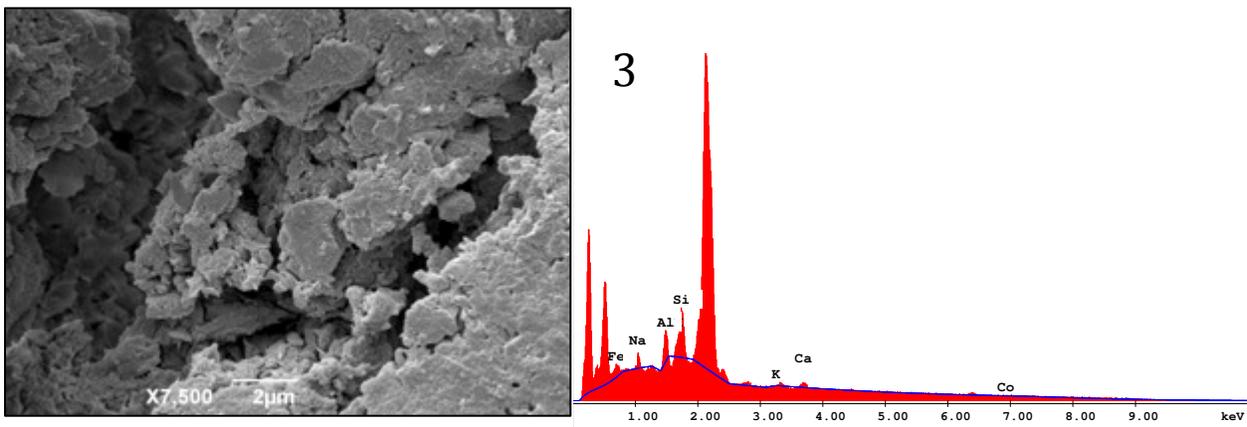


Figure 34: SEM and EDAX Analysis of the Settled Activated Sludge from the Control SBR (Spectra 3)

Table 14: EDAX Quantification of the Settled Activated Sludge from the Control SBR

Element	Spectra 1		Spectra 2		Spectra 3	
	Weight %	Atomic %	Weight %	Atomic %	Weight %	Atomic %
Na	8.92	15.52 ^b	2.94	5.92	9.41	13.54
Al	-	-	11.88	20.38	16.63	20.40
Si	21.3	30.32	0.91	1.50	34.90	41.11
K	3.08	3.15	2.21	2.62	2.21	1.87
Ca	11.57	11.54	7.74	8.95	5.37	4.44
Fe	55.13	39.47	51.14	42.41	31.49	18.65
Co	-	-	23.18	18.22	0	0
Total	100.00	100.00	100.00	100.00	100.00	100.00

To distinguish the differences between the control and the treatment reactors, Figures 35 to 38 present the SEM images of the activated sludge sample from the treatment reactor. As shown, the presence of a particular material arrangement in hexagonal structure was observed. This shape of the materials was not found in any of the scanned areas of the control reactor sample. Then again, the aim with the SEM analysis was to obtain a visualization of the previously added ENP_{Fe-Surf} or any other type of formation produced by them. Judged by the size, they were not ENP_{Fe-Surf} themselves. Rather, they might be the byproducts that ENP_{Fe-Surf} induced to form. The EDAX as shown in Table 15 disclosed that the most abundant element was iron ranging 19.86 - 51.56 atomic %, which in fact were slightly higher than those found in the control samples (18.65 ~ 42.41 atomic %). Following iron, sodium and silicon accounted for 9.03-29.63 and 3.83-24.07 atomic %, respectively.

^b The elements in higher abundance are marked in red.

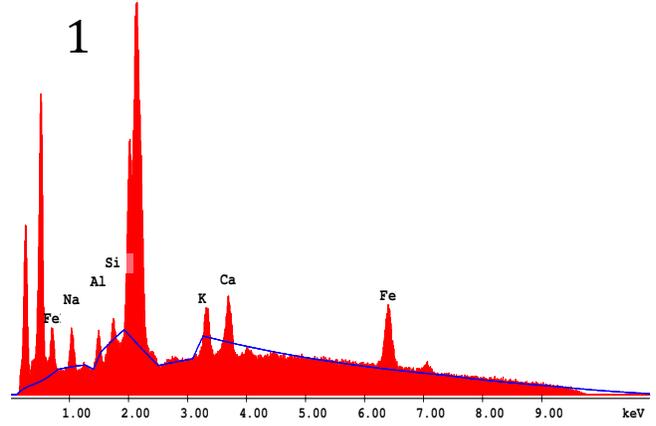
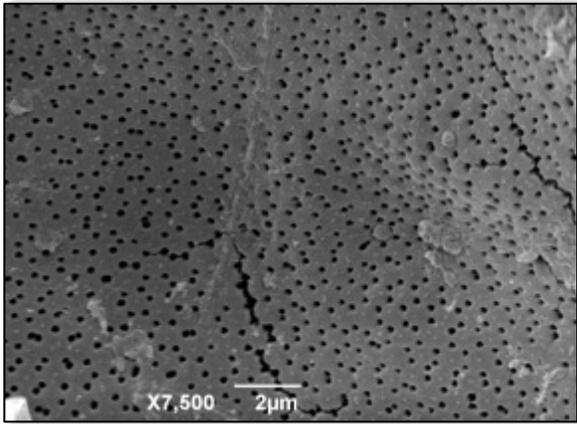


Figure 35: SEM and EDAX Analysis of the Settled Activated Sludge from the Treatment SBR (Spectra 1)

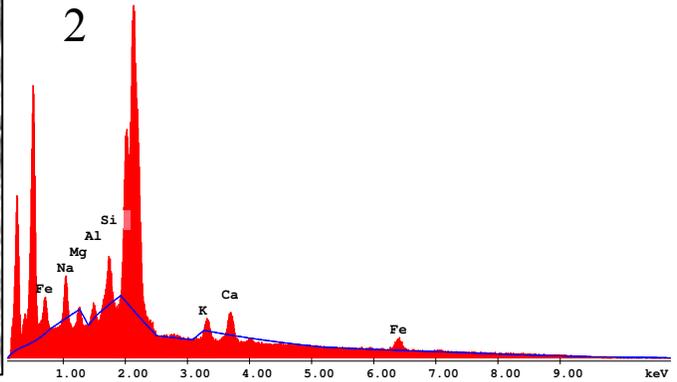
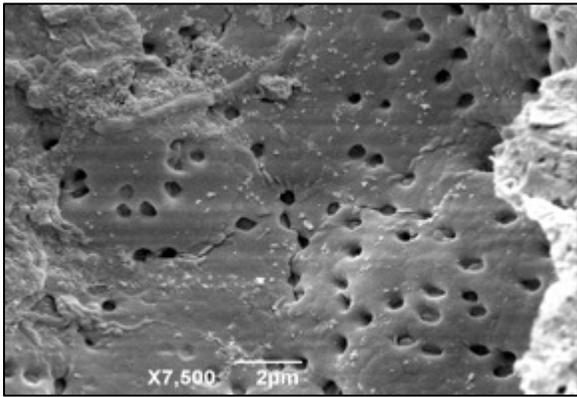


Figure 36: SEM and EDAX Analysis of the Settled Activated Sludge from the Treatment SBR (Spectra 2)

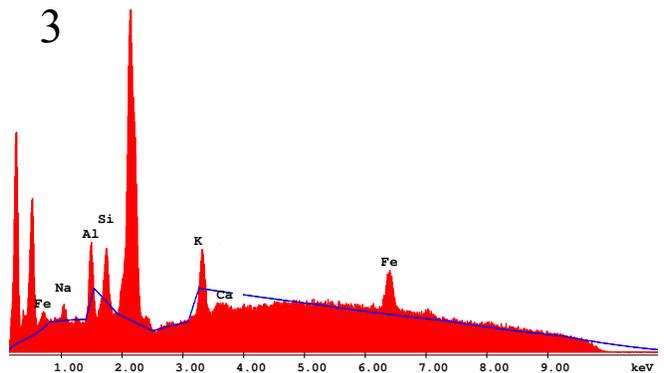
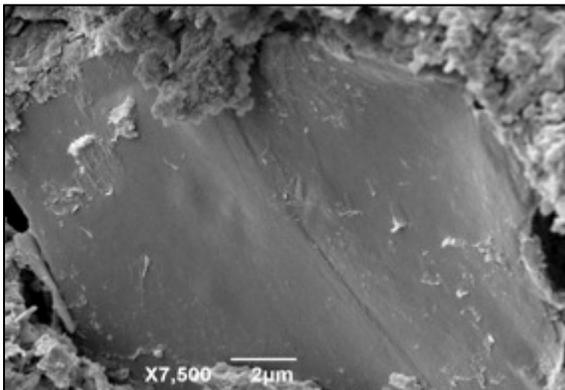


Figure 37: SEM and EDAX Analysis of the Settled Activated Sludge from the Treatment SBR (Spectra 3)

Table 15: EDAX Quantification of the Settled Activated Sludge from the Treatment SBR

Element	Spectra 1		Spectra 2		Spectra 3	
	Weight %	Atomic %	Weight %	Atomic %	Weight %	Atomic %
Na	10.06	19.06	19.86	29.63	5.02	9.03
Al	4.05	6.54	5.45	6.93	11.31	17.33
Si	2.47	3.83	19.71	24.07	11.65	17.16
K	6.09	6.79	5.85	5.85	9.88	10.45
Ca	11.24	12.22	16.80	16.80	0	0
Fe	66.09	51.56	32.34	19.86	62.14	46.03
Mg	-	-	0	0	-	-
Total	100.00	100.00	100.00	100.00	100.00	100.00

A study conducted by Ivanov and Chu (2008) revealed formation of biocementation by ammonifying and iron-reducing bacteria depending on the conditions available in the soil. For ammonifying bacteria the presence of urea and soluble calcium salts creates an enzymatic reaction that increases the pH, which produces hydrocarbonates and precipitates calcium as calcium carbonate. On the other hand, the iron-reducing bacteria in the presence of ferric minerals and anaerobic conditions changed to aerobic conditions can lead to the production of ferrous solutions and precipitation of insoluble ferrous, ferric salts and iron hydroxides into the soil (Ivanov et al., 2004; Ivanov and Chu, 2008).

Therefore, since the pHs measured during the SBR experiment were in the range within 8.5 to 8.9 (Appendix D.1) and from the EDAX the presence of calcium was higher, biocementation formation from ammonifying bacteria could occur. Additionally, during the SBR experiment aerobic environment was prearranged and the addition of ENP_{Fe-Surf} to the SBRs could also generate the essential conditions for iron-reducing bacteria to produce binding between the sludge particles. In other words, this means that there was a high probability that the hexagonal material could be produced by the added ENP_{Fe-Surf} or any calcium component available within

the settled activated sludge sample. Thus, further study is required to better understand and identify the formation of the hexagonal arrangement material.

The raw spectra of the control and treatment samples of the XRD analysis are shown in Figure 38. The control reactor sample is represented by the green spectra while the red and blue spectra correspond to the treatment reactor samples 1 and 2, respectively. In general both the control and treatment spectra showed a similar pattern.

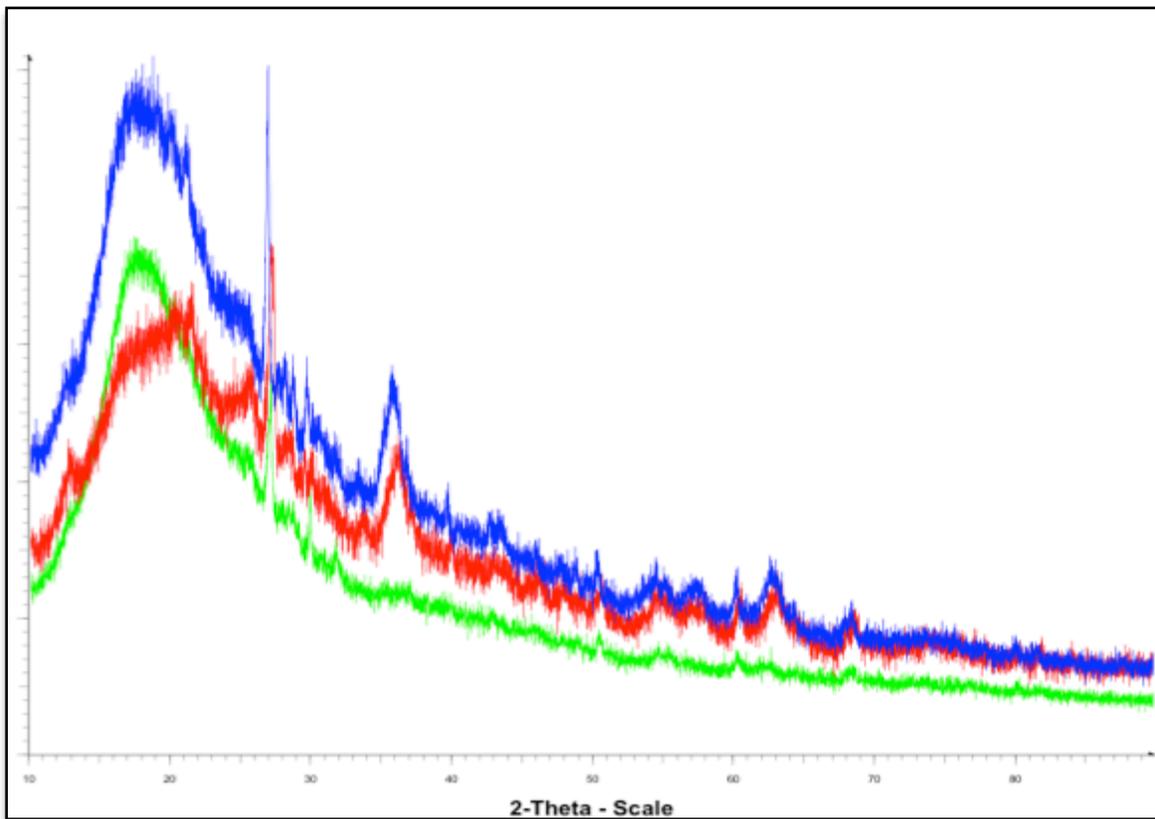


Figure 38: XRD Raw Spectra of Control and Treatment SBR Sludge Samples

For better identification of the minerals in the samples, the background spectra were eliminated and the results are shown in Figures 39-41. In Figure 39 it can be observed that the control SBR sludge had a much stronger intensity narrow peak of CaCO_3 at $2\theta = 30^\circ$ than the

treatment SBR 1 and 2 sludge's that were run in the presence of $\text{ENP}_{\text{Fe-Surf}}$ (Figure 40 and 41). Instead, the treatment SBR 1 and 2 sludge had unique wide peaks of CaCO_3 at $2\theta = 36^\circ$ and 63° . These additional calcites might be formed through a microbial precipitation process in aid of $\text{ENP}_{\text{Fe-Surf}}$ during the treatment SBR reaction. Taking into consideration the peak width, the CaCO_3 at $2\theta = 30^\circ$ in the control SBR sludge was in crystalline phase, whereas the CaCO_3 at $2\theta = 36^\circ$ and 63° in the treatment SBR1 sludge was in amorphous phase (Waseda et al., 2011).

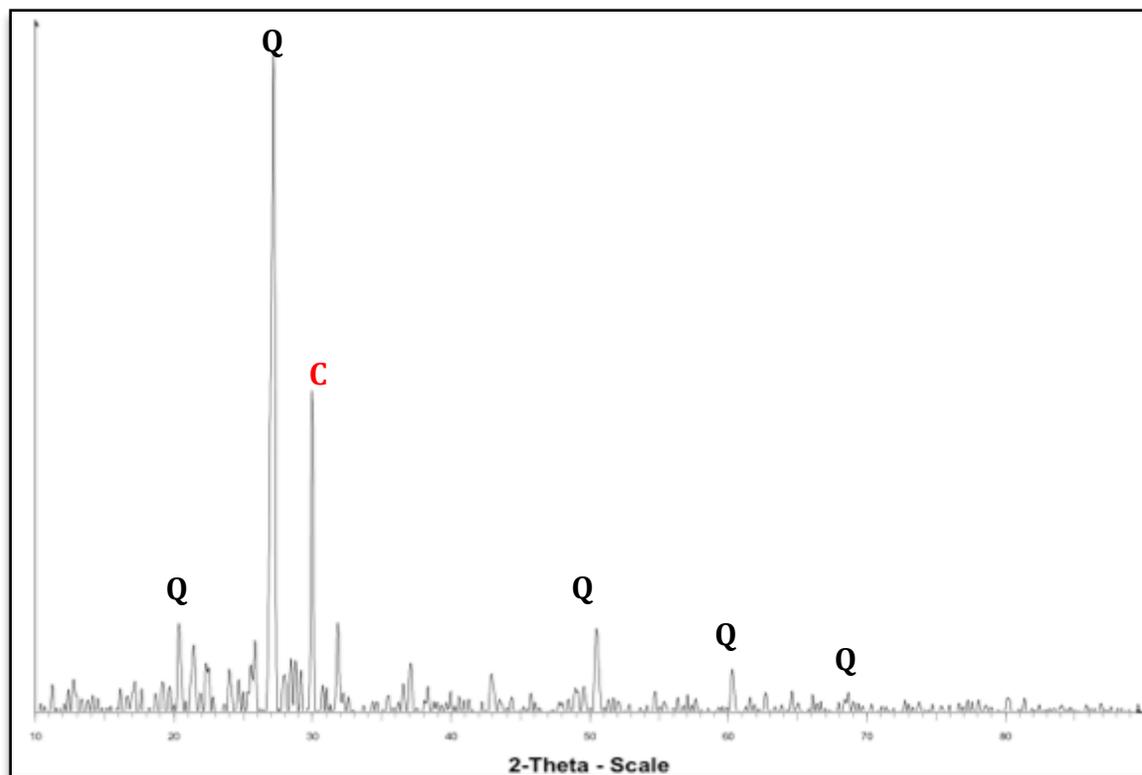


Figure 39: XRD Modified Spectra of the Control SBR Sludge Sample^c

^c Letter's "Q" and "C" stand for quartz and calcite, respectively.

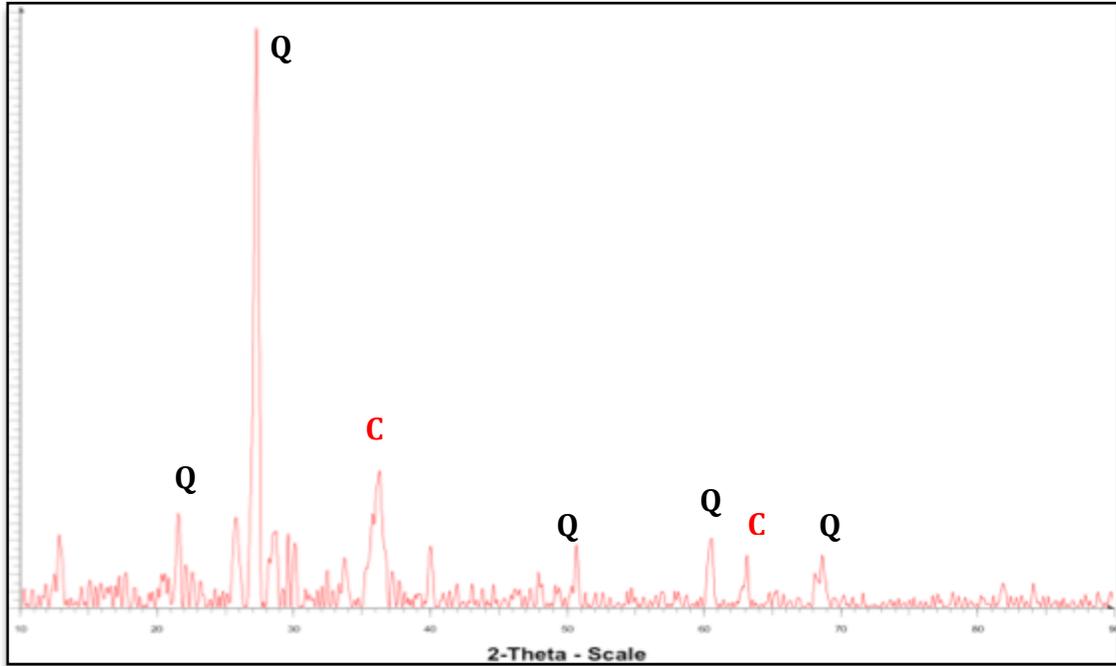


Figure 40: XRD Modified Spectra of the Treatment 1 SBR Sludge Sample

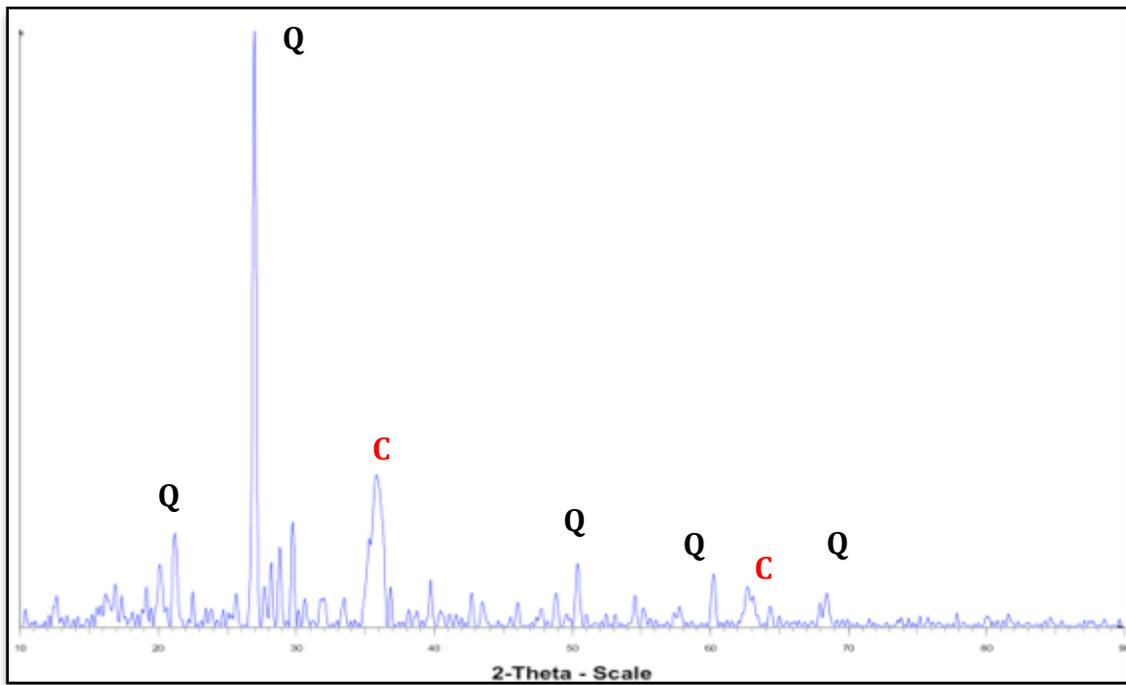


Figure 41: XRD Modified Spectra of the Treatment 2 SBR Sludge Sample

5 CONCLUSIONS & RECOMMENDATIONS

The objectives of this study were accomplished. It was demonstrated that the presence of engineered nanoparticles (ENPs) in wastewater affects its disinfection in an adverse way. This was demonstrated both thru batch and continuous flow reactors experiments using wastewater samples collected from Mayagüez (PR) wastewater treatment plant.

It was found that the characteristics of wastewater effluent fluctuated and this is expected. However, it was determined that there exists a good relationship between TP concentration and FC numbers with the Pearson's correlation coefficient of 0.655.

Batch and PFR disinfection experiments were performed to assess potential effect of $ENP_{Fe-Surf}$ as contaminants on FC removal in wastewater effluent collected from a local WWTP. The following conclusions can be made based on the results from the batch disinfection experiments:

- To achieve a 4-log FC removal, the required free chlorine residuals was 0.22, 0.18, and 0.10 mg $Cl_2 L^{-1}$ for contact times of 24, 30, and 60 minutes in the absence of $ENP_{Fe-Surf}$. In case where $ENP_{Fe-Surf}$ was present, the required free chlorine concentrations were 0.18, 0.14, and 0.07 mg $Cl_2 L^{-1}$ for the same contact times, respectively, to achieve the same log removal of FC.
- Slightly greater iron concentrations were leached out of $ENP_{Fe-Surf}$ with higher chlorine concentrations.
- A greater FC log removal was achieved for the same chlorine consumption in the absence of $ENP_{Fe-Surf}$ than in the presence of $ENP_{Fe-Surf}$. In addition, almost the same FC log removal was found regardless of the magnitude of chlorine consumption. On the

contrary, in the presence of ENP_{Fe-Surf}, FC log removal increased with an increase of chlorine consumption.

- As such, ENP_{Fe-Surf} increased chlorine consumption so that greater CT values were required for 4-log FC removal in the presence of ENP_{Fe-Surf}.

From the results of the PFR disinfection, conclusions can be derived as follows:

- Scavenging effect of ENP_{Fe-Surf} on chlorine was also observed. ~5% of the added chlorine concentration was measured at pseudo steady state in the presence of ENP_{Fe-Surf}. In comparison, ~10% was observed in the absence of ENP_{Fe-Surf}.
- A slightly increased ENP_{Fe-Surf} concentration was found in the presence of chlorine in wastewater effluent at pseudo steady state (~85%) than in the absence of chlorine (<85%), possibly due to degradation of ENP_{Fe-Surf} by chlorine resulting in increase of iron concentration leached out of ENP.
- In general, FC log removals were proportional to CT values whether or not ENP_{Fe-Surf} was present.
- The same FC log removal at the same disinfection contact time was accomplished in PFR at lower concentrations of free chlorine residuals in the presence of ENP_{Fe-Surf} than in the absence of ENP_{Fe-Surf}. Similar to other results, this was due to scavenging effect of ENP_{Fe-Surf} on chlorine.

The metagenomic analysis presented population diversity within the wastewater effluent before and after ENP_{Fe-Surf} addition. The ENP_{Fe-Surf} had adverse effects on FCs, specifically coliforms family, since approximately 0.46% diversity was found in the wastewater effluent with the addition of ENP_{Fe-Surf} after a 30-minute contact time.

The settled activated sludge collected from the lab-scale SBR systems that were run with the addition of ENP_{Fe-Surf} had unique hexagonal structures as shown by SEM. Because of their size, they were not ENP_{Fe-Surf} themselves. Rather, they might be byproducts or clusters of ENP_{Fe-Surf} induced by conditions in wastewater. EDAX analysis revealed the presence of higher iron concentration in comparison to the settled sludge from the control SBRs run without the addition of ENP_{Fe-Surf}.

Further investigations are needed in the following areas:

- Biochemical mechanisms governing the intermingled interactions among chlorine, FC, and ENP_{Fe-Surf}.
- Broader research on metagenomic mechanisms on different environments.
- More quantitative determination of hexagonal structures appearing in the settled sludge from the SBR reactors with the addition of ENP_{Fe-Surf}.
- Total maximum daily load of ENP_{Fe-Surf} that can be permitted for achieving required levels of FC removal.
- Future standardization of the regulations concerning release of nanoparticles in wastewater treatment plants.

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7 APPENDIXES

A. WASTEWATER EFFLUENT CHARACTERIZATION

The wastewater effluent was regularly collected in the morning time during summer and fall seasons in the years 2012 and 2013. It is important to take into account that the wastewater characteristics were always different regardless of the seasons and weather, wet or dry. In some cases, unusual factors like the cleaning of the sedimentation tanks could have interfered with the wastewater effluent characteristic of that day (personal communication with WWTP workers). In general, pH, COD, total nitrogen, total phosphorus, and conductivity (Tables 16 to 20) were the five main water quality parameters analyzed and mostly the samples were measured twice for better precision and accuracy.

A.1 pH

Table 16: pH in Wastewater Effluent

Date	pH Reading	Average					
27-Aug-12	-	7.1	7.1	7-Nov-12	7.3	7.4	7.4
29-Aug-12	-	7.9	7.9	12-Nov-12	7.3	7.3	7.3
5-Sep-12	8.0	8.1	8.1	27-Jun-13	7.5	7.4	7.5
11-Sep-12	7.5	7.7	7.6	8-Jul-13	7.1	7.2	7.2
17-Sep-12	7.1	7.1	7.1	11-Jul-13	6.9	7.0	7.0
26-Sep-12	7.0	7.0	7.0	18-Jul-13	6.9	6.9	6.9
1-Oct-12	7.0	7.0	7.0	3-Oct-13	7.5	7.5	7.5
10-Oct-12	7.4	7.2	7.3	9-Oct-13	6.9	7.0	7.0
17-Oct-12	8.3	8.2	8.3	26-Nov-13	8.1	8.1	8.1
25-Oct-12	7.9	7.9	7.9	11-Dec-13	7.8	7.8	7.8
31-Oct-12	7.9	7.9	7.9	13-Dec-13	8.2	8.2	8.2

A.2 CHEMICAL OXYGEN DEMAND

Table 17: COD in Wastewater Effluent

Date	COD (mg/L)		Average
27-Aug-12	11.0	14.0	12.5
29-Aug-12	6.0	8.0	7.0
5-Sep-12	6.2	12.8	9.5
11-Sep-12	5.5	5.1	5.3
17-Sep-12	4.7	6.0	5.4
26-Sep-12	7.6	4.5	6.1
1-Oct-12	5.0	5.2	5.1
10-Oct-12	11.9	12.2	12.1
17-Oct-12	9.0	8.7	8.9
25-Oct-12	9.4	10.0	9.7
31-Oct-12	12.0	13.6	12.8
7-Nov-12	3.7	8.1	5.9
12-Nov-12	12.6	9.2	10.9
27-Jun-13	12.6	12.5	12.6
8-Jul-13	13.3	11.5	12.4
11-Jul-13	11.9	12.2	12.1
18-Jul-13	14.8	13.4	14.1
3-Oct-13	11.1	9.3	10.2
9-Oct-13	13.9	15.5	14.7
7-Nov-13	16.8	19.9	18.4
26-Nov-13	15.2	13.2	14.2
11-Dec-13	10.4	10.5	10.5
13-Dec-13	12.0	12.3	12.2

A.3 TOTAL PHOSPHORUS

Table 18: Total Phosphorus in Wastewater Effluent

Date	Total Phosphorus (mg P/L)		Average
1-Oct-12	0.40	0.41	0.4
10-Oct-12	0.32	0.31	0.3
17-Oct-12	0.88	0.90	0.9
25-Oct-12	0.21	0.23	0.2
31-Oct-12	0.39	0.33	0.4
7-Nov-12	0.39	0.39	0.4
12-Nov-12	0.69	0.72	0.7
27-Jun-13	0.42	0.39	0.4
8-Jul-13	0.42	0.41	0.4
11-Jul-13	0.57	0.57	0.6
18-Jul-13	0.91	0.90	0.9
3-Oct-13	0.65	0.68	0.7
9-Oct-13	0.39	0.37	0.4
7-Nov-13	0.33	0.29	0.3
26-Nov-13	0.72	0.72	0.7
11-Dec-13	0.13	0.14	0.1
13-Dec-13	0.40	0.52	0.5

A.4 TOTAL NITROGEN

Table 19: Total Nitrogen in Wastewater Effluent

Date	Total Nitrogen (mg N/L)		Average
27-Aug-12	-	8.3	8.3
29-Aug-12	-	8.6	8.6
5-Sep-12	7.0	4.8	5.9
11-Sep-12	8.9	9.1	9.0
17-Sep-12	7.8	7.6	7.7
26-Sep-12	9.8	9.1	9.5
1-Oct-12	13.6	4.9	9.3
10-Oct-12	12.2	6.3	9.3
17-Oct-12	7.7	23.4	15.6
25-Oct-12	5.9	6.4	6.2
31-Oct-12	6.6	6.2	6.4
7-Nov-12	7.7	7.5	7.6
12-Nov-12	6.4	6.0	6.2
27-Jun-13	7.2	7.8	7.5
8-Jul-13	5.9	6.5	6.2
11-Jul-13	7.6	7.5	7.6
18-Jul-13	8.1	7.9	8.0
3-Oct-13	9.6	9.4	9.5
9-Oct-13	5.0	5.0	5.0
7-Nov-13	9.3	9.1	9.2
26-Nov-13	9.2	10.5	9.9
11-Dec-13	9.7	9.5	9.6
13-Dec-13	13.6	11.3	12.5

A.5 CONDUCTIVITY

Table 20: Conductivity in Wastewater Effluent

Date	Conductivity ($\mu\text{S cm}^{-1}$)		Average
27-Aug-12	1506	-	1506
29-Aug-12	1454	1436	1445
5-Sep-12	1490	1486	1488
11-Sep-12	1934	1930	1932
17-Sep-12	1770	1770	1770
26-Sep-12	1438	1454	1446
1-Oct-12	747	718	733
10-Oct-12	680	674	677
17-Oct-12	899	894	897
25-Oct-12	611	610	611
31-Oct-12	4,260	4,300	4280
7-Nov-12	791	793	792
12-Nov-12	1,107	1,109	1108
27-Jun-13	684	689	687
8-Jul-13	682	689	686
11-Jul-13	773	774	774
18-Jul-13	686	717	702
3-Oct-13	565	-	565
9-Oct-13	511	512	512
7-Nov-13	629	594	612
26-Nov-13	807	806	807
11-Dec-13	856	857	857
13-Dec-13	977	976	977

B. BATCH EXPERIMENTS

Minitab program was used to obtain Pearson's correlation coefficients among the physicochemical characteristics of wastewater effluent. Results are presented in Table 21. The values that had higher correlations are marked in bold.

Table 21: Pearson Correlation Coefficients among the Physicochemical Characteristics of Wastewater Effluent

	pH	Total Phosphorus	Total Nitrogen	COD	Conductivity
Total Phosphorus	0.234	1.000			
Total Nitrogen	0.663	0.751	1.000		
COD	0.269	-0.033	-0.278	1.000	
Conductivity	0.168	-0.227	-0.26	0.718	1.000
Fecal Coliforms	-0.382	0.655	0.013	0.205	-0.222

C. PFR EXPERIMENTS

The respective FC log removals and CT values from the PFR experiments are shown in Table 22. It is important to note that there was no fecal coliform growth on the growth media for 2HRT. However, 4-log removal was conservatively assumed for comparison purposes.

Table 22: FC Log Removals and CT Values from PFR Disinfection Experiments

Day	ENP _{Fe-Surf}				No ENP _{Fe-Surf}			
	1st		2nd		1st		2nd	
Time (min)	CT (min-mg L ⁻¹)	FC Log Removal	CT (min-mg L ⁻¹)	FC Log Removal	CT (min-mg L ⁻¹)	FC Log Removal	CT (min-mg L ⁻¹)	FC Log Removal
t ₁₀ = 24	1.7	0.002	3.12	0.35	0.8	0.2	1.12	~ 0.0001
HRT = 29	3.0	0.83	5.7	3.33	5.4	> 4	7.71	1.04
2HRT = 58	7.0	> 4	14.4	> 4	10.7	> 4	15.42	1.86

D. ACTIVATED SLUDGE

D.1 ENP_{Fe-Surf} IN ACTIVATED SLUDGE

The data collected from the 10th cycle SBR experiment are presented, Table 23 and Table 24. For all 4 reactors, iron concentration was measured after the addition of ENP_{Fe-Surf} after cycle 7th.

Table 23: Experimental Data from SBR with Influent & MLSS

Reactors	a (control)						b (treatment = Fixed ENP at 66.2 mg L ⁻¹ Fe)								
Cycle	RAS (cm)	pH	Turbidity (NTU)	COD (mg/L)	BOD (mg/L)		Fe (mg/L)	RAS (cm)	pH	Turbidity (NTU)	COD (mg/L)	BOD (mg/L)		Fe (mg/L) Dill: 50	Fe (mg/L)
					R1	R2						R1	R2		
1	1.5	9.07	32.6	93	8.31	6.82	-	1.0	8.83	29.2	85	8.55	6.76	-	-
2	1.4	8.87	16.9	99	7.62	6.71	-	1.2	8.63	24.7	97	8.61	7.28	-	-
3	1.4	9.20	30.3	100	7.68	6.36	-	1.3	9.09	45.7	120	7.55	6.01	-	-
4	1.3	8.66	32.3	109	7.32	5.94	-	1.3	8.70	37.5	111	7.46	5.68	-	-
5	1.4	8.86	28.0	93	7.52	5.94	-	1.3	8.88	37.3	104	7.47	5.77	-	-
6	1.3	9.00	33.0	109	8.53	6.96	-	1.3	9.03	34.5	108	8.42	7.25	-	-
7	1.2	8.86	27.3	90	8.53	7.29	0.06	1.1	8.89	179	275	7.65	6.96	2.21	110.5
8	1.6	9.02	15.2	76	8.50	7.12	0.08	1.4	9.01	199	290	8.06	7.63	2.48	124
9	1.4	8.52	17.6	53	8.47	7.66	0.12	1.4	8.59	189	254	8.42	7.47	2.39	119.5
10	1.4	8.74	12.8	21	8.46	7.59	0.10	1.4	8.81	206	252	8.39	7.11	2.86	143

Table 24: Experimental Data from SBR with Influent, MLSS & RAS

2	Influent + MLSS + RAS														
Reactors	a (control)						b (treatment = Fixed ENP at 66.2 mg L ⁻¹ Fe)								
Cycle	RAS (cm)	pH	Turbidity (NTU)	COD (mg/L)	BOD (mg/L)		Fe (mg/L)	RAS (cm)	pH	Turbidity (NTU)	COD (mg/L)	BOD (mg/L)		Fe (mg/L) Dil: 50	Fe (mg/L)
					R1	R2						R1	R2		
1	4.3	8.61	5.45	21	7.57	6.82	-	4.25	8.53	5.15	18	7.64	7.40	-	-
2	4.5	8.32	4.60	28	7.39	6.68	-	4.20	8.12	5.17	13	7.73	7.58	-	-
3	4.5	8.36	11.1	43	6.91	0.79	-	4.50	8.33	11.7	45	5.14	0.30	-	-
4	4.4	8.57	5.75	29	7.10	6.06	-	4.60	8.71	7.53	40	6.91	5.26	-	-
5	4.5	8.65	7.21	31	6.89	5.68	-	4.60	8.78	6.57	27	7.38	5.72	-	-
6	4.7	8.08	9.40	44	8.52	8.50	-	4.80	8.13	10.3	44	8.57	8.59	-	-
7	4.9	8.70	5.07	22	8.55	8.66	0.04	5.10	8.69	105	202	8.51	7.92	1.50	75
8	4.9	8.40	10.1	49	8.50	7.82	0.08	4.80	8.36	147	238	8.49	7.75	2.32	116
9	5.0	8.67	5.40	23	8.53	8.51	0.05	5.10	8.73	137	214	8.47	7.78	2.06	103
10	5.1	8.82	3.42	71	8.47	8.40	0.04	5.00	8.86	144	289	8.48	7.66	2.60	130

D.2 ADDITIONAL SEM IMAGES OF ACTIVATED SLUDGE

In this study, two different scanning electron microscopes were utilized. The JEOL JSM 5410LV – 25kV from the Department of Biology and the JEOL JSM-6390 from the Chemical Engineering Department at the University of Puerto Rico in Mayagüez. Additional SEM images for the control and treatment SBRs are shown in Figures 42 and 43, respectively.

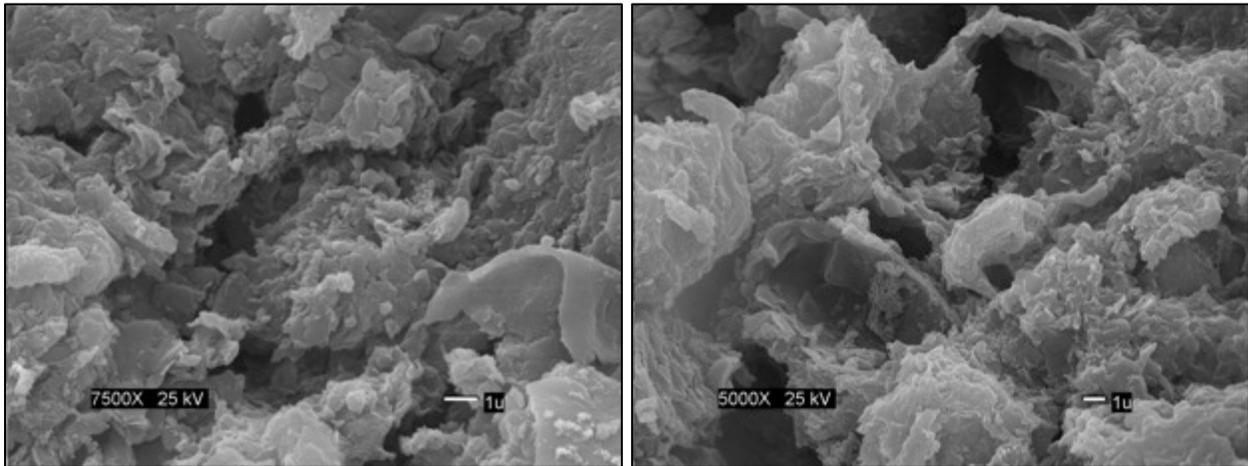


Figure 42: SEM Image of Different Scanned Areas of the Activated Sludge Samples from the Control Reactor (JEOL JSM-6390)

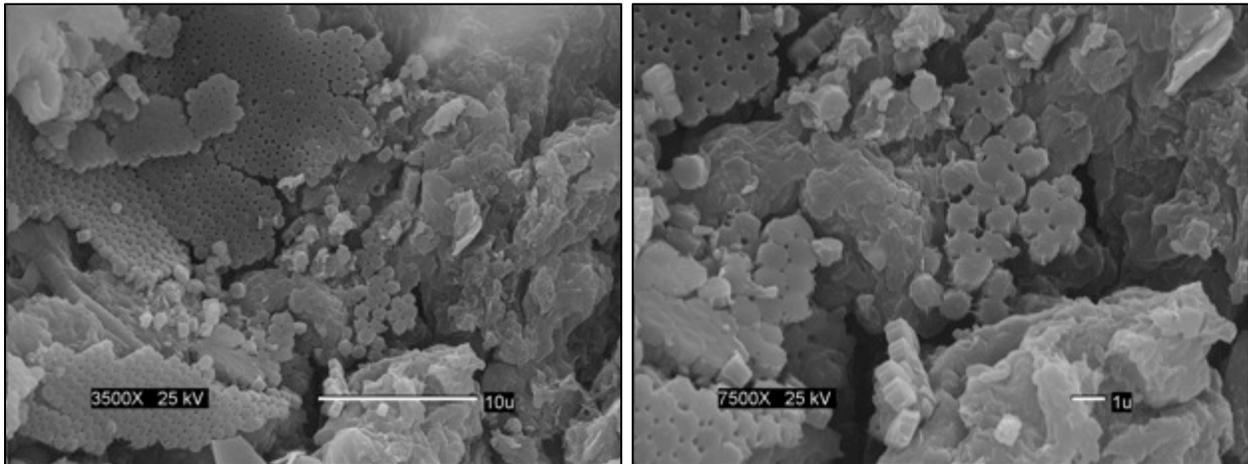


Figure 43: SEM Image of Different Scanned Areas of the Activated Sludge Samples from the Treatment Reactor (JEOL JSM-6390)