

EFFECT OF ENGINEERED IRON OXIDE NANOPARTICLES ON EFFLUENT WATER QUALITY FROM BIOLOGICAL WASTEWATER TREATMENT

by

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ABSTRACT

The production, use, and disposal of products containing nanoparticles may lead to their appearance in air, water, and soil, and subsequently in the human body. Because there is no existing regulation, large amounts of used or wasted engineered nanoparticles may be discharged into sewer systems and eventually enter wastewater treatment plants. Wastewater treatment plants can play an important role in controlling engineered nanoparticles release from the sources to the aquatic environmental receptors via treated effluent discharge, or to the terrestrial environments via sludge disposal to land.

Effluent wastewater quality from a lab-scale sequencing batch reactor (SBR) was evaluated with the different loadings of engineered iron oxide nanoparticles coated with a surfactant (ENP_{Fe-surf}). Lab-scale SBRs were run at different hydraulic retention times (HRT, 3 and 6 hrs), a 0.5-hr sedimentation, and a 0.5-hr decant/refill at a food-to-microorganisms ratio of 0.32 g BOD/g MLSS/day. The SBRs were stabilized by running up to the 6th cycle under the same experimental conditions. At the onset of the 7th-10th cycles, the treatment SBRs were loaded with ENP_{Fe-surf} at either 29.6 or 88.9 mg as soluble Fe per L of mixed liquors. Physiochemical influence of ENP on water quality parameters was tested, including pH, turbidity, and chemical oxygen demand, biological oxygen demand, and Fe concentrations.

Results showed that about ~8.7% of ENP_{Fe-surf} applied were present in the effluent stream of the treatment SBR regardless of ENP_{Fe-surf} loadings. The stable presence of ENP_{Fe-surf} was confirmed with the analyses of mean particle diameters and Fe concentrations in the effluent. Consequently, results showed that statistically significant ($p < 0.05$) increases were found for turbidity, apparent color and soluble chemical oxygen demand in the treatment SBR effluents,

compared to those in the effluents of the control SBRs that were run in parallel without $\text{ENP}_{\text{Fe-surf}}$ loadings. Biological oxygen demand concentrations were insignificantly higher in the effluents of the treatment SBRs than in those of the control SBRs. In general, these findings implied that $\text{ENP}_{\text{Fe-surf}}$ would be introduced into environmental receptors through the treated effluent and could potentially impact them.

Effluent suspended solid concentrations were not significantly different between the control and treatment SBRs. $\text{ENP}_{\text{Fe-surf}}$ loadings to the mixed liquors produced inhibitory respiration resulting in decreased oxygen uptake rate. Twice longer aeration time did not produce significant differences in any of water quality parameters. Such insignificant differences in water quality deterioration was attributed to the presence of the similar $\text{ENP}_{\text{Fe-surf}}$ concentrations in the effluent at 2.3 ± 0.3 and 2.7 ± 0.5 mg/L as Fe for the treatment SBRs run at hydraulic retention time of 3- and 6- hrs, respectively.

Low $\text{ENP}_{\text{Fe-surf}}$ concentrations (<10 mg/L as soluble Fe) in the SBR effluent were unlikely to produce significantly adversary effect on the removal and inactivation of fecal coliforms in disinfection process. Although greater ($p > 0.05$) chlorine demand was found proportionally to the $\text{ENP}_{\text{Fe-surf}}$ loadings, disinfection of fecal coliforms at the $\text{ENP}_{\text{Fe-surf}}$ concentrations lower than 3 mg/L as Fe was not affected, achieving 100% fecal coliform removals at the initial chlorine concentration of either 5.7 or 11.4 mg/L.

RESUMEN

La producción, uso y el desecho de productos que contienen nanopartículas pueden llevar a la aparición de ellas en aire, agua, suelos y por consecuencia al cuerpo humano. Como no existe ninguna regulación, grandes cantidades de desperdicios de nanopartículas o nanopartículas usadas pueden ser descargadas en el sistema de alcantarillado y eventualmente entrar a las plantas de tratamientos de aguas usadas. Las plantas de tratamiento de aguas usadas pueden jugar un rol importante en controlar la liberación de estas nanopartículas de la fuente a los receptores ambientales acuáticos a través de la descarga de efluentes tratados, o para los ambientes terrestres a través de la disposición de lodos a la tierra.

La calidad del agua residual efluente de un reactor de lotes secuenciales a escala de laboratorio (SBR por sus siglas en inglés) fueron evaluadas con diferentes cargas de nanopartículas de hierro oxidado recubiertas con un surfactante ($\text{ENP}_{\text{Fe-surf}}$). Los SBRs a escala de laboratorio fueron corridos a diferentes tiempos de retención hidráulicos (HRT, 3 y 6 horas), media hora de sedimentación, y media hora para decantar y rellenar con una radio de comida-a-microorganismo de 0.32 g BOD/g MLSS/día. Los SBRs fueron estabilizados corriéndolos hasta el 6^{to} ciclo bajo las mismas condiciones experimentales. En el inicio de los 7^{mo}-10^{mo} ciclos, los SBRs tratados fueron cargados con $\text{ENP}_{\text{Fe-surf}}$ con 29.6 o 88.9 mg de hierro soluble por litro de licores mixtos. La influencia fisicoquímicas de las ENP en los parámetros de calidad del agua se puso a prueba, incluyendo pH, turbidez, y la demanda química de oxígeno, la demanda biológica de oxígeno, y las concentraciones de Fe.

Los resultados mostraron que aproximadamente ~8.7% de $\text{ENP}_{\text{Fe-surf}}$ aplicada estaban presentes en la corriente efluente del SBR de tratamiento independientemente de la cantidad de carga de $\text{ENP}_{\text{Fe-surf}}$. La presencia estable de $\text{ENP}_{\text{Fe-surf}}$ fue confirmada con los análisis de

diámetros de partícula medios y concentraciones de Fe en el efluente. En consecuencia, los resultados mostraron un aumento estadísticamente significativo ($p < 0.05$) para la turbidez, color aparente y la demanda química de oxígeno soluble en los efluentes SBR tratados, en comparación con aquellos en los efluentes de la de control SBRs que fueron corridos en paralelos sin cargas de $ENP_{Fe-surf}$. Concentraciones de demanda biológica de oxígeno no fueron significativamente mayor en los efluentes de los SBR tratados que en los de los controles. En general, estos hallazgos implican que las $ENP_{Fe-surf}$ van a ser introducidos al medio ambiente a través de los receptores de los efluentes tratados y potencialmente podría afectarlos.

Las concentraciones de los sólidos suspendidos de los efluentes no fueron significativamente diferentes entre los SBRs de control y tratados. Cargas de $ENP_{Fe-surf}$ a los licores mixtos produjeron respiración inhibidora lo que disminuyó la tasa de consumo de oxígeno. Dos veces más largo tiempo de aireación no produjo diferencias significativas en ninguno de los parámetros de calidad del agua. Tal deterioro de la calidad del agua se atribuyó a la presencia de las $ENP_{Fe-surf}$ en el efluente a 2.3 ± 0.3 y 2.7 ± 0.5 mg/L como Fe para las corridas de los SBRs tratados con un tiempo de retención de 3- y 6- horas, respectivamente.

Las bajas concentraciones de $ENP_{Fe-surf}$ (< 10 mg/L as soluble Fe) en el efluente de los SBRs fueron poco probable en producir efecto adversario significativo en la eliminación e inactivación de coliformes fecales en el proceso de desinfección. Aunque mayor demanda de cloro ($p > 0.05$) fue encontrada proporcional a la cantidad de carga de $ENP_{Fe-surf}$, a concentraciones de $ENP_{Fe-surf}$ más bajas de 3 mg/L como Fe la desinfección de coliformes fecales no fue afectada, alcanzando el 100% de remoción de coliformes fecales a concentraciones iniciales de cloro de 5.7 o 11.4 mg/L.

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Dedicated to

My family and friends

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LIST OF ABBREVIATIONS

AAA = Autoridad de Acueductos y Alcantarillados

APHA = American Public Health Association

AWWA = American Water Works Association

BOD = Biological Oxygen Demand

BOD₅ = Five-day Biochemical Oxygen Demand

COD = Chemical Oxygen Demand

DI = Deionized Water

DLS = Dynamic Light Scattering

DO = Dissolved Oxygen

ENP = Engineered Nanoparticles

ENP_{Fe-surf} = Engineered Iron Oxide Nanoparticles coated with a Surfactant

EPA= Environmental Protection Agency

HRT = Hydraulic Retention Time

MLSS = Mixed Liquor Suspended Solids

MLVSS = Mixed Liquor Volatile Suspended Solids

NTU = Nephelometric Turbidity Units

OUR = Oxygen Uptake Rate

PRASA = Puerto Rico Aqueduct and Sewer Authority

sBOD = Soluble Biological Oxygen Demand

SBR = Sequencing Batch Reactor

sCOD= Soluble Chemical Oxygen Demand

SOUR = Specific Oxygen Uptake Rate

SS = Suspended Solids

SVI = Sludge Volume Index

TGA = Thermogravimetric Analysis

USGS = United States Geological Survey

WEF = Water Environment Federation

1. INTRODUCTION

Relevant background information about the nanoparticles and their use, biological wastewater treatment processes, and the different stages of such processes are introduced in this section. Furthermore, justification, scope and objectives of the research are also discussed.

1.1. BACKGROUND

Nanotechnology has widespread application from industrial sectors such as energy, catalysts, pigments, electronics, remediation, and fuel additives, to household commodities such as foods, cleaning and personal care products, cosmetics, and pharmaceutical formulations (Ju-Nam and Lead, 2008). Engineered nanoparticles (ENPs) would find their major route of release to the natural environment via domestic and industrial wastewater discharges (Nowack and Bucheli, 2006; Wiesner et al., 2006). Therefore, wastewater treatment plants can play an important role in controlling ENP release from the sources to the aquatic environmental receptors via treated effluent discharge, or to the terrestrial environments via sludge disposal or application to land (Brar et al., 2010). Wastewater contains a variety of constituents including microorganisms, natural organic matters, and clays. Stability of ENPs can on the water biochemistry exerted by those constituents and the characteristics of the ENPs (Brar et al., 2010). Presence of ENP may result in deterioration of effluent water quality. As we can see ENPs showed dissimilar effects on wastewater microbiology. It is likely that the type and functionality of ENPs govern their fate and implications in wastewater treatment processes.

If ENPs are stable and/or produce toxicity to treatment microorganisms, the effluent water quality will be deteriorated resulting in higher concentrations of chemical oxygen demand (COD), biochemical oxygen demand (BOD), and suspended solids (SS), to mention a few. If, on the contrary, the surrounding be enhanced or reduced depending biochemistry reduces ENP stability (e.g., heteroaggregation), so effluent water quality will be unaffected and toxicity of ENP to microorganisms in suspended growth may be negligible (Jarvie et al., 2010). Since many nanotechnology products have already been introduced to the market, it is necessary to find out how they will be treated and disposed of after the end of their original use (Eureka Alert, 2007).

For example, silver nanoparticles were tested for their effect on activated sludge microorganisms in a wastewater treatment system (Liang et al., 2010). The authors found no impacts of silver nanoparticles on heterotrophs, but inhibitory effects on nitrifying bacteria. Kiser et al. (2010) studied biosorption of seven nanoparticle suspensions, including fullerenes, titanium dioxide, and Ag, to heterotrophic wastewater biomass. The authors pointed out governing influence of ENP surface properties on biosorption, thereby their fate in the environment. Similarly, effects of surface functionality of silica nanoparticles on their fate during primary wastewater treatment (i.e., sedimentation) were evaluated (Jarvie et al., 2009). Tween-coated silica nanoparticles were flocculated rapidly and removed by sedimentation, whereas uncoated ones were not. However, ENP aggregation and precipitation to sludge may produce secondary impacts on sludge management processes such as sludge stabilization, composting, and landfill disposal.

Magnetic ENPs are one of the mostly used ENPs and occur in environmental receptors (Calero-DdelC et al., 2006; Buzea et al., 2007). However, little has been done to assess the fate

and behavior of magnetic ENPs in the secondary wastewater treatment processes where biological activated systems are most common (Tchobanoglous et al., 2003).

1.2. JUSTIFICATION

Due to the expanding application of nanotechnology in recent years, there have been many important implications that have come into the scene in different areas of the environment such as air, water, and soil. Unlike other common metals and chemicals of which environmental impacts are well known, nano-sized materials have not been studied enough to understand their impacts on our environment. Because there is no existing regulation, large amounts of used or waste ENPs may be discharged into sewer systems and eventually enter wastewater treatment plants.

Presence of ENP may result in deterioration of effluent water quality (increase of effluent COD and SS) and potential toxicity to treatment microorganisms. If the surrounding biochemistry reduces the stability of the ENPs, effluent water quality will not be affected (Jarvie et al., 2010). However, nanoparticle aggregation and precipitation to the sludge will produce secondary concerns in sludge stabilization, composting, and landfilling.

Despite such unknown fate mechanisms and resulting impacts, few studies have been conducted to understand the impact of ENPs in biological wastewater treatment processes. Since many nanotechnology products have already been introduced to the market, it is necessary to find out how they will be treated and disposed of after the end of their use (Eureka Alert, 2007). Therefore, this research explored the effect of the presence of engineered surfactant-coated iron-oxide nanoparticles ($\text{ENP}_{\text{Fe-surf}}$), as a pollutant, on a biological wastewater treatment system.

For this, lab-scale sequencing batch reactors (SBRs) were run at two different hydraulic retention times (HRT, 3 hrs and 6 hrs) and with two different $\text{ENP}_{\text{Fe-surf}}$ loadings (29.6 and 88.9 mg as Fe per L of mixed liquor). Results and findings from this study, which was the first of its kind, will provide science and engineering communities with a better understanding of the significance and implications of the presence of ENPs in biological wastewater treatment processes.

2. SCOPE AND OBJECTIVES

Nanoscience is a technology in continuous growth and nanoparticles are ever present in today's and future products. For that reason, investigation of the effects it will or may have on our lives is of great interest and concern. Nevertheless, the effect that this technology has on our environment has not been studied in depth.

2.1. SCOPE

The overall goal of this research has been to understand the behavior and the effect of ENP_{Fe-surf}, specifically the engineered iron oxide nanoparticles, in biological wastewater treatment systems. To meet this goal, a lab-scale simulation of SBRs was conducted.

2.2.OBJECTIVES

To meet the aforementioned goal, this study specifically aims to:

- Operate lab-scale SBRs to evaluate ENP_{Fe-surf} impacts on the effluent wastewater quality parameters; and
- Assess potential impacts of ENP_{Fe-surf} on the water quality parameters, such as, COD, BOD, SS, turbidity, and color intensity; and
- Preliminarily evaluate the impacts of stable ENP_{Fe-surf} present in the effluent on wastewater disinfection efficiency.

3. LITERATURE REVIEW

A review of previous works related to wastewater, treatment of wastewater, nanotechnology and engineered nanoparticles is provided in this chapter. Previous works on ENP production, environmental impact of metals on water quality was also reviewed in this chapter.

3.1. WASTEWATER

Human activities in communities produce wastes via liquid, solid and air emissions. The liquid waste, best known as wastewater, is the used water discharged from the community after it has been used. Before 1940, most of the wastewater was produced from domestic sources. After that, industrial development in the United States resulted in increased industrial wastewater discharge into municipal collection systems. This led to establish in 1952 a government public policy about the Natural Resources: The Constitution of Commonwealth, cited in article VI section 19 as “This will be The Commonwealth’s the most efficient Conservation Law of its Natural Resources as the most beneficial and development that will benefit the whole community”.

In 1970 three government agencies were created. The Environmental Protection Agency (EPA), the Department of Natural Resources, and the Solid Waste Authority; all of this watch and regulate the conservation of our environment. In the same decade of 1970 was when most of the Federal Environment Protection laws were created. For example, the Environment Public

Policy Law (1970), The Clean Water Law (1972), and the Potable Water Law (1974). The principal federal agency, that regulates all these environment laws, is the EPA.

Human, domestic and industrial waste has to be treated before being release into water bodies. Otherwise this waste would contaminate the receiving environments. The EPA administrates, both the Clean Water Act (1972) and the Federal Water Pollution Control Act (1987). These Acts are to restore and to maintain the chemical, physical and biological integrity of the nation's waters and to regulate the toxics and non-toxics discharges into the waters. These Acts dictate that all the federal agencies must work together to develop a plan with all the states agencies for proper attention and improvement of water quality to protect the aquatic life, fish, wildlife, and for others uses, like agricultural, industrial and other use of potable water.

There is an EPA program called "The National Pollutant Discharge Elimination Systems (NPDES) and it has been designed to regulate the sources that discharge their wastewater into U.S. waters and its territories. The EPA also regulates NPDES permits for discharge of rain waters from industries and municipalities. This program has two components. First, limits the amount of contaminants that can be discharged based on an existent quid. Second, it requires a high standard technology for the water treatment that the industry must comply with. For example, there are three types of technology: Best Practicable Control Technology, Best Conventional Technology, and Best Available Technology Economically Achievable.

The most important characteristics of wastewater are: the total solid content, particle size distribution, turbidity, color, temperature, conductivity, density, and odor. To design a treatment process properly, characterization of the wastewater is probably the most important step in the process (Tchobanoglous et al., 2003).

Municipal wastewater is mainly comprised of water (99.9%) together with relatively small concentrations of suspended and dissolved organic and inorganic solids. Among the organic substances present in sewage are carbohydrates, lignin, fats, soaps, synthetic detergents, proteins and their decomposition products, as well as various natural and synthetic organic chemicals from the industrial processes. Table 1 shows the levels of the major constituents of strong, medium and weak domestic wastewaters (Tchobanoglous et al., 2003).

Table 1: MAJOR CONSTITUENTS OF TYPICAL DOMESTIC WASTEWATER.

Parameter	Strong	Medium	Weak
Total solids	1200	700	350
Dissolved solids	850	500	250
Suspended solids	350	200	100
Nitrogen	85	40	20
Phosphorus	20	10	6
Chloride	100	50	30
Alkalinity	200	100	50
Grease	150	100	50
5-day Biological oxygen demand	300	200	100

3.1.1. WASTEWATER TREATMENT

Nature has an amazing ability to cope with small amounts of wastewater and pollution, but it would be overwhelmed if we didn't treat the billions of gallons of wastewater and sewage produced every day before releasing it back to the environment. Treatment plants reduce pollutants in wastewater to a level nature can handle. Wastewater includes substances such as

human waste, food scraps, oils, soaps and chemicals. In homes, this includes water from sinks, showers, bathtubs, toilets, washing machines and dishwashers. Businesses and industries also contribute their share of used water that must be cleaned. Wastewater may also include storm runoff. Although some people assume that the rain that runs down the street during a storm is fairly clean, it isn't. Harmful substances that wash off roads, parking lots, and rooftops can harm our rivers and lakes.

The major aim of wastewater treatment is to remove as much of the suspended solids as possible before the remaining water, called effluent, is discharged back to the environment. As solid material decays, it uses up oxygen, which is needed by the plants and animals living in the water.

The primary treatment removes about 60 percent of suspended solids from wastewater. This treatment also involves aerating (stirring up) the wastewater, to put oxygen back in. Secondary treatment removes more than 90 percent of suspended solids.

Wastewater entering the treatment plants may include items like wood, rocks, and even dead animals. Unless they are removed, they could cause problems later in the treatment process. Most of these materials are sent to a landfill. For that the first step of the process is screening. In the pumping stage the wastewater system relies on the force of gravity to move sewage from your home to the treatment plant. So wastewater treatment plants are located on low ground, often near a river into which treated water can be released. If the plant is built above the ground level, the wastewater has to be pumped up to the aeration tanks. From here on, gravity takes over to move the wastewater through the treatment process.

One of the first steps that a wastewater treatment facility can do is to just shake up the sewage and expose it to air. This causes some of the dissolved gases that taste and smell bad to

be released from the water. Wastewater enters a series of long, parallel concrete tanks. Each tank is divided into two sections. In the first section, air is pumped through the water.

As organic matter decays, it uses up oxygen. Aeration replenishes the oxygen. Bubbling oxygen through the water also keeps the organic material suspended while it forces 'grit' (coffee grounds, sand and other small, dense particles) to settle out. Grit is pumped out of the tanks and taken to landfills. Wastewater then enters the second section or sedimentation tanks. Here, the sludge (the organic portion of the sewage) settles out of the wastewater and is pumped out of the tanks. Some of the water is removed in a step called thickening and then the sludge is processed in large tanks called digesters. As sludge is settling to the bottom of the sedimentation tanks, lighter materials are floating to the surface.

Many cities also use filtration in sewage treatment. After the solids are removed, the liquid sewage is filtered through a substance, usually sand, by the action of gravity. This method gets rid of almost all bacteria, reduces turbidity and color, removes odors, reduces the amount of iron, and removes most other solid particles that remained in the water (USGS, 2012).

Finally, the wastewater flows into a disinfection tank, where the chemical chlorine is added to kill bacteria, which could otherwise pose a health risk. The chlorine is mostly eliminated as the bacteria are destroyed, but sometimes it must be neutralized by adding other chemicals. This protects fish and other marine organisms, which can be harmed by the smallest amounts of chlorine. The effluent is then discharged to a local river or the ocean

3.1.2. BIOLOGICAL WASTEWATER TREATMENT

Biological wastewater treatment is an engineering process where the microorganisms (mostly bacteria and protozoa) decompose the organic materials in the wastewater, thereby reducing its BOD content. Wastewater collection systems, such as the separated or the combined with the storm sewer systems, play an important role in protecting human and animal health and local water quality (EPA, 2010a). The Clean Water Act (1977) established a minimum performance level that all wastewater treatment plants must meet and required that the Environmental Protection Agency (EPA) to develop standards for the secondary treatment plants. They identified the minimum level of effluent quality to be obtained by secondary treatments in terms of BOD, SS, and pH (EPA, 2010b). For example, typical domestic wastewater enters the treatment plant with a BOD higher than 200 mg/L, but primary settling reduces this by 30%. Therefore, influent BOD concentration entering the main biological wastewater (i.e., activated sludge system) is approximately ~140 mg/L (Boyle, 1974). To comply with the current regulation, effluent BOD should be lower than 30 mg/L (30-day average).

The core of biological wastewater treatment is the aeration tank and secondary sedimentation tank (see Figure 1). A key factor in the operation of any biological wastewater treatment system is an adequate supply of oxygen to the aeration tank. Aerobic bacteria need not only organic materials as substrates (carbon and energy source) but also oxygen as an electron acceptor. Without an adequate supply of oxygen, the extent and rate of aerobic biological degradation of the waste is diminished, resulting in poor effluent water quality and operating problems such as septicity and sludge bulking.

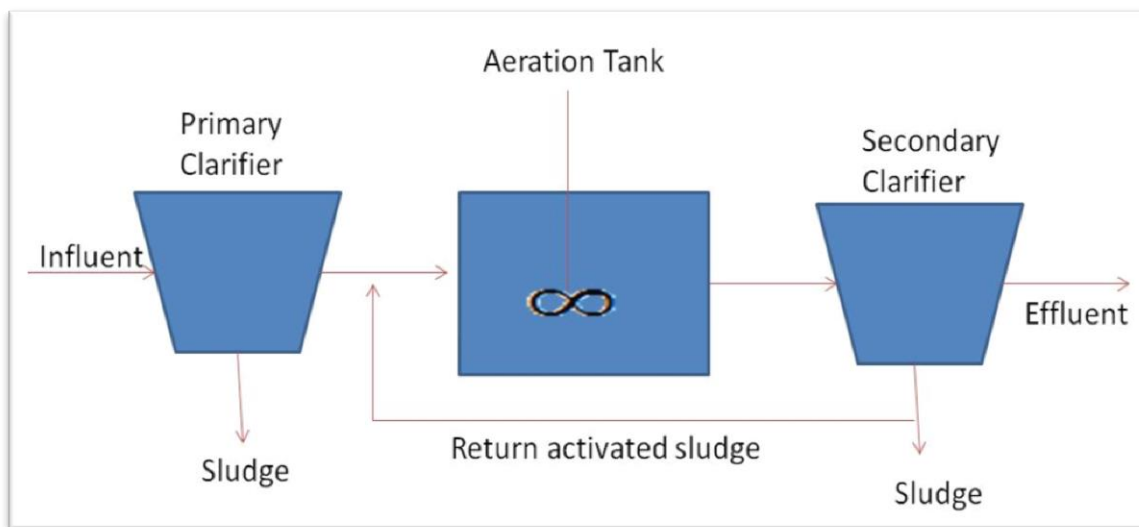


Figure 1: Illustration of activated sludge system.

3.1.2.1. ACTIVATED SLUDGE

The activated sludge system is the most commonly used process in biological wastewater treatment (municipal and industrial). The activated sludge process includes three basic components: an aeration tank in which the microorganisms are kept in suspension and aerated, liquid-solids separation, and a recycle system (Tchobanoglous et al., 2003). Then the activated sludge effluent with flocs enter the secondary sedimentation tank where they typically settle down gravitationally in a settling time of about 1.5-2.5 hrs. Some of the concentrated sludge is sent back to the aeration tank (i.e., return activated sludge) to keep the microbial concentration constant. Other fractions are discarded and subject to further stabilization before final disposal or utilization.

3.1.2.2. DISINFECTION

Effluent disinfection is done to reduce the numbers of pathogenic microorganisms in the water effluent discharged to the waterways. The effectiveness of disinfection depends on the quality of the water being treated (e.g., turbidity, pH, etc.), the type of disinfection being used, the disinfectant dosage (concentration and time), and other environmental variables. Common disinfectants include ozone, chlorine, ultraviolet light, or sodium hypochlorite. The ideal requirements for a good disinfectant are shown in Table 2.

Table 2: REQUIREMENTS OF DISINFECTANTS. (Tchobanoglous et al., 2003)

Characteristics	Properties
Availability	Must be available in big amounts and at a reasonable price
Deodorizing ability	Should deodorize while disinfecting
Homogeneity	Uniform in composition
Noncorrosive	Should not disfigure metals
Nontoxic	Should be toxic for microorganism and nontoxic to humans and other animals
Penetration	Should penetrate through surface
Safety	Safe to transport, store, handle and use
Toxicity at ambient temperatures	Should work in ambient temperatures range
Stability	Should have low loss of germicidal action

Over recent history, chlorine has been the disinfectant of choice (Tchobanoglous et al., 2003). Chlorination remains the most common form of wastewater disinfection in North America due to its low cost and long history of effectiveness. The principal forms of chlorine used at wastewater treatment plants are chlorine, sodium hypochlorite, calcium hypochlorite and chlorine dioxide. When chlorine in the form of gas is added to water, two reactions take place

and should be taken in consideration: hydrolysis, which is the reaction in which chlorine gas combines with water to form hypochlorous acid, and ionization which is how hypochlorous acid becomes a hypochlorite ion (Tchobanoglous et al., 2003).

3.1.3. MAYAGÜEZ WASTEWATER TREATMENT PLANT PROCESS

In Puerto Rico, the government agency responsible for the typical wastewater treatment is the Puerto Rico Aqueduct and Sewage Authority (Autoridad de Acueductos y Alcantarillados de Puerto Rico, AAA, in Spanish). Wastewater is typically treated via pretreatment, primary treatment and secondary treatment.

In Mayagüez wastewater treatment plant as the first step in the pretreatment, bar-racks with approximately 4.5-cm openings remove large solids such as fats, wood particles 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 sand, gravel and rocks are dried and are typically taken to the municipal landfill.

Regarding the primary treatment, approximately 60% and 30% of SS and BOD, respectively, are removed in the sedimentation tank. The supernatants are then sent to the aeration tanks. These tanks have a capacity of 1.136 million of liters each.

The 4 reactors are the same in dimensions and have a capacity of approximately 2.788 million gallons each. The anaerobic and anoxic part has a combined capacity of 230,503 gallons, the aeration tank has a capacity of approximately 1.258 million gallons and the secondary clarifier tank has a capacity of 1.3 million gallons. The process of removal of nutrients (phosphorous and nitrogen) occurs in the anoxic part after having gone through the aeration tank.

First the mixed liquor enters the anaerobic, then passes by anoxic and then to the aeration tank. From the aeration tank, where nitrification occurs, about half of the flow is recirculated directly to the anoxic tank and the other part goes to the secondary clarifier for the effluent.

This recirculation allows the release of nitrite and nitrate (denitrification) in gas and the same happens with phosphate. This is because the right environment is provided to microorganisms with dissolved oxygen concentrations of 1-3 ppm or mg /L and then sent to one with 0.1 mg /L, forcing them to consume oxygen molecules of nitrate and phosphate. Part of the sludge accumulating on the bottom of the clarifier is recycled back and mixed with the raw water. Other parts are wasted and are treated in sludge management processes. Disposing amount is determined based on the ratio of food (COD and BOD) to microorganisms (MLVSS) in the advanced process control.

The next step is the secondary sedimentation, and from here now is that the secondary treatment begins, the sedimentation of the sludge takes place in a 227,100-liter tank (3.66 meters deep and 7.62 meters in diameter). The last step in the AAA is the disinfection, where they inject chlorine to the water to kill or make inactive the pathogenic microorganisms, prior to discharge to the bay.

3.2.WATER QUALITY

Surface and groundwater quality assessment and management are major issues these days due to contamination caused mostly by agricultural, municipal and industrial activities. Serious efforts have been made to combat surface and groundwater contamination worldwide, through the implementation of national and international policies. Despite such efforts, water resources continue suffering from detrimental effects.

Several contaminants are currently threatening aquatic systems on a worldwide scale. The impact and behavior of contaminants in an aquatic ecosystem are complex and involve natural processes such as adsorption, desorption, precipitation-solubilization, filtration, biological uptake, excretion, and sedimentation.

3.2.1. WASTEWATER QUALITY

Prior to 1940, the principal components of wastewaters were from domestic sources. After this the industrial development grew significantly, increasing the amount of industrial wastewater and the amount of heavy metals and synthesized organic materials have increased. As technology changes so does the compounds discharged and that way the characteristics of wastewater. Some of the most important physical characteristics of wastewater are its total solids content, particle size distribution, turbidity, color, temperature, conductivity, density, specific weight, among others.

Wastewater contains a variety of solids materials. When characterizing the wastewater usually the coarse materials are removed before the analysis. The sizes of the particles are important because the effectiveness of chlorine and UV disinfection is dependent on particle size. Turbidity, that measures the light transmitted in water, is another test used that indicates the water quality. The results of turbidity are reported in nephelometric turbidity units (NTU). Colloidal matter will scatter or absorb light and prevent its transmission. Historically, the word condition referred to the age of wastewater, which is determined qualitatively by its color and odor. Fresh wastewater is normally light brownish but as the time in the collection system increases and more anaerobic conditions develop, the color of wastewater changes from brown to gray to dark gray and at the end to black. When the color of wastewater is dark the water is

described as septic. Five-day Biochemical oxygen demand (BOD₅) is the most used parameter to assess the potential presence of organic pollution. This test measures the dissolved oxygen used by microorganisms in the biochemical oxidation of organic matter and it is too affected by the particles sizes (Tchobanoglous et al., 2003).

3.3.NANOSCIENCE

Nanoscience is the study of phenomena and the manipulation of materials at the nanoscale (1-100 nm). Nanotechnologies got started in the early 1980s with the appearance of a new type of microscope (atomic force microscope), which allowed not only the observation of units of atoms and molecules, but also their physical manipulation and the relative scale of comparison (Brar et al., 2010).

This technology involves the production and application of substances and structures at the nanoscale. Within this size range substances can have very different properties when compared to their larger counterparts, reflecting surface properties that become important. Different experiments have shown that nanoparticles can change their behavior (agglomeration and sedimentation) depending on the surrounding conditions (Limbciah et al., 2008; Rezwan et al., 2005; Rezwan et al., 2004). The mayor advantage that has come from this nanotechnology is the large increase in surface area to volume ratio. With this new technology also comes the risks that it can impose on the human health and the environment.

In nanotechnology, a particle is defined as a small object that behaves as a whole unit in terms of its transport and properties. Particles are classified according to size, for example fine particle diameters cover a range between 100 and 2500 nm. Nanoparticles can have amorphous or crystalline form, and their surfaces can act as carriers for liquid droplets or

gases. Nanoparticles may or may not exhibit size-related properties that differ significantly from those observed in the bulk (Buzea et al., 2007).

Nanotechnology is a multibillion dollar global industry with large increases in the production of ENPs predicted over the next decade (Royal Commission on Environmental Pollution, 2008) and, as a result, ENPs are being increasingly released to the environment. One problem in investigating the environmental fates and roles of nanoparticles is detecting and quantifying them in real environment surroundings (Jarvie et al., 2009). According to Zhang et al. (2008), one of the biggest concerns is that when nanoparticles are in an aquatic environmental they can accumulate in fish and enter into the food chain.

3.3.1. NANOPARTICLES

Nanotechnology has widespread application in agricultural, environmental and industrial sectors, ranging from fabrication of molecular assemblies to microbial array chips (Brar and Verma, 2011). Nanotechnology has touched many spheres of utility services, including consumer products, health care, transportation, energy, and agriculture (Taton et al., 2000). Nanoparticles are also used in the production of ceramics, catalysts, pigments, optoelectronics, and fuel additives, foods, cleaning and personal care products, cosmetics, and pharmaceutical formulations (Ju-Nam and Lead, 2008). Nanoparticles are added to bulk materials to improve their physical properties. Since the 1990s there has been a rapid increase in the implementation of nanotechnologies. Achievements in this area find practical applications in many fields of industry and daily life, e.g. in medicine. In 2004, the annual production of nanomaterials amounted to about 1,000 tons (Maynard, 2006). Estimates are that, by 2014, approximately 15% of all products on the market will have some kind of nanotechnology incorporated into their

manufacturing process (AIBS, 2011). Examples of the use of the most common ENPs are shown in Table 3.

Table 3: EXAMPLES OF NANOPARTICLES AND THEIR USES.
(Bystrzejewska-Piotrowska et al., 2009)

Nanoparticles	Nanoproducts
Ag	Antimicrobial agent in domestic appliances (e.g. fridges, vacuum cleaners, air conditioning), paints, textiles, plastics, varnish
TiO ₂ , ZnO	Paints, cements, sunscreen, car cosmetics, catalysts, UV-protection, batteries
Ferrofluid	Magnetic resonance imaging, drug delivery, biochemical assays
Fe ₂ O ₃	Concrete additive
Fe ₃ O ₄	Biochemical assays, biomanipulation, removal of contaminants
Fe	Purification of ground waters from PCBs, organochlorine pesticides and chlorinated organic solvents, degradation of PAH-based contaminants

3.3.2. IRON NANOPARTICLES

Iron oxide nanoparticles are very small particles (from 1-100 nm) which the two main forms are magnetite (Fe₃O₄) and oxidized form maghemite (γ-Fe₂O₃). Iron oxide has superparamagnetic properties which allow them to have potential applications in many fields, especially in bimolecular activities, because they are the only clinically approved nanoparticle, these nanoparticles are used for MRI, medical diagnosis and therapeutics (Figuerola et al., 2010). Also they are used in commercial products such as cosmetics and sunscreens, cancer diagnosis as magnetic resonance imaging agents.

The side effects of these nanoparticles in humans are urticaria, diarrhea and nausea, but very mild and short duration. On the other hand, high concentrations of iron in the body may be toxic and it is related with some carcinogenic developments and protein, polysaccharide, lipids, and DNA damage; this is caused by the degradation to iron ions, forming hydroxyl radicals (Singh et al., 2010).

Besides affecting the human being, it affects the environment, contaminating water and soils. The presence of these nanoparticles in water and soil may cause deformation and abnormalities in the growth of plants and development of bio-organism. The iron oxide nanoparticles are proven to be the less toxic of a couple of metallic oxides studied, this is why is used in biomedical procedures (Garcia et al., 2011).

Because of the great use in biomedical field, the production of iron oxide nanoparticles has increased during the years. Health and environmental effects of common iron is well-known. However, when we look at the nanoscale, the story is different and consequential hazards based on shape and size are yet to be explored. The nanoparticles released from different uses, used in our household and industrial commodities find their way through waste disposal routes into the wastewater treatment facilities and end up in wastewater sludge. A future escape of nanoparticles into the effluent contaminates waters and soils with the contact. Another way of the water to get contaminated is by air pollution, because air may contain these iron nanoparticles, caused by volcano eruptions and fires, the pollutant is mixed with precipitation and ends up in the different water bodies. With time it could become very harmful to the environment and hence to the human health because metal particles bioaccumulates.

The understanding of the presence, behavior and impact of these nanoparticles in wastewater and wastewater sludge is necessary. The contacts between plants and these particles

may be toxic depending on the concentration and properties of the different nanoparticles present. Plants absorb their nutrients from the soil where these nanoparticles may be present and they accumulate in the plant. This accumulation may cause changes in plant development; changes in their growth and reproduction (Ma et al., 2010).

3.3.3. ENVIROMENTAL IMPACT OF NANOPARTICLES

The major route of nanoparticles release into the natural environment is through sewage and industrial wastewater discharges (Jarvie et al., 2009). Therefore, wastewater treatment plants act as the gateways controlling release of ENPs from domestic and industrial sources to aquatic or terrestrial environments: either via treated effluent which is discharged into surface waters or, via sewage sludge disposal to land (Colvin, 2003).

Further escape of these nanoparticles into the effluent will contaminate the aquatic and soil environment. Hence, an understanding of the presence, behavior and impact of nanoparticles in wastewater and wastewater sludge is necessary (Brar et al., 2010).

Nowack and Bucheli (2007) determined that particle size is relevant for particle toxicity. The stability of nanoparticles in aquatic environment plays an important role in determining their environmental implication and potential risk to human health. These nanoparticles can remain in the environment for long periods and can be potentially toxic to aquatic life (Oberdörster, 2004). Blaser et al. (2008) performed an analysis to assess the emission and fate of the released silver to wastewater and they discovered that it is incorporated into sewage sludge and may spread further on agricultural fields. The impact that nanomaterials have on wastewater treatment is largely unknown. Moreover, questions remain regarding efficient ways to remove these nanoparticles from industrial wastewaters and sewage treatment plants. When thinking about ENP impacts on

the environment, the following issues should be taken into account: identification and quantification of sources, determination of the environmental release pattern, establishment of concentrations in the environment, and examination of bioaccumulation.

3.3.4. HETEROAGGREGATION

Colloidal heteroaggregation processes have received significant interest in recent years because of their potential use in many technological processes. Aggregation of binary colloids with different composition, charge or size has been shown to be important in industrial applications, waste water treatment, and stability of emulsions (López-López et al., 2009).

Aggregation of one-component colloidal dispersions is usually described in terms of the cluster-size distribution. These processes can be modeled using the Smoluchowski coagulation equation (Levine, 2002):

$$\frac{dC_i}{dt} = \frac{1}{2} \sum_{j=1}^{i-1} k_{j,i-j} C_j C_{i-j} - C_i \sum_{j=1}^{\infty} k_{ij} C_j \quad (1)$$

where C is the cluster-size distribution, i.e., the concentration of clusters composed by i particles, and k_{ij} is the absolute aggregation rate constant controlling the reaction.

In two-component systems, the reactivity of two clusters depends not only on the aggregate size but also on the composition of each. The effective aggregation rate constant is given by,

$$K_{eff} = x^2 k_{AA} + (1-x)^2 k_{BB} + 2x(1-x) \quad (2)$$

where K_{eff} is the effective dimer formation rate constant, x is the relative concentration of monomers of type A, k_{AA} and k_{BB} are the absolute homoaggregation rate constants, and K_{AB} is the absolute heteroaggregation rate constant. However, this is not a real constant because every reaction between two different monomers reduces the relative concentration of minority particles (López-López et al., 2009). The interaction energy between two colloidal particles is due to two contributions: the Van der Waals dispersion energy term and an electrostatic interaction term that is caused by the overlap of the electric double layers surrounding the particles (Levine, 2002).

4. MATERIALS, EQUIPMENT, AND METHODOLOGY

A series of lab-scale experiments were performed to assess the impact of the $\text{ENP}_{\text{Fe-Surf}}$ in the wastewater treatment. For this, actual wastewater from the Mayagüez wastewater treatment plant was collected. This wastewater treatment plant is located in the west coast of Puerto Rico.

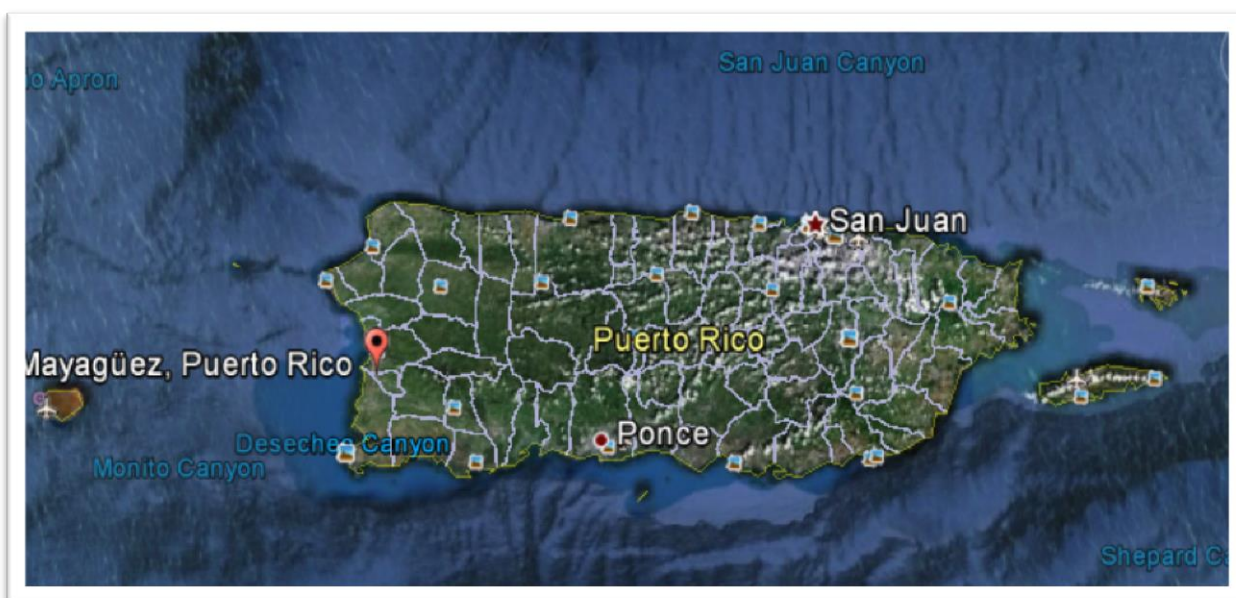


Figure 2: Map of Puerto Rico where the municipality of Mayagüez is marked. (Google Maps, 2012)

A general description of principal materials and methodology employed in this research is discussed including laboratory feasibility test with physical models, water quality measurements and data analysis.

4.1.MATERIALS

This section is comprised of description of the nanoparticles, wastewater and some other materials needed to accomplish the objectives of the research. Furthermore, relevant information about instruments utilized to perform water quality analysis is included.

4.1.1. WASTEWATER

Wastewater was collected from the Mayagüez wastewater treatment plant. It was used upon sampling, if not, it was stored in Nalgene 5 gallon bottles for no more than 24 hours prior to use and was aerated constantly to maintain aseptic.

Figure 3 presents a scheme of the Mayagüez Regional Wastewater Treatment Plant where the wastewater samples were collected from, and Figure 4 shows an aerial picture of that treatment plant (Google Maps, 2012).

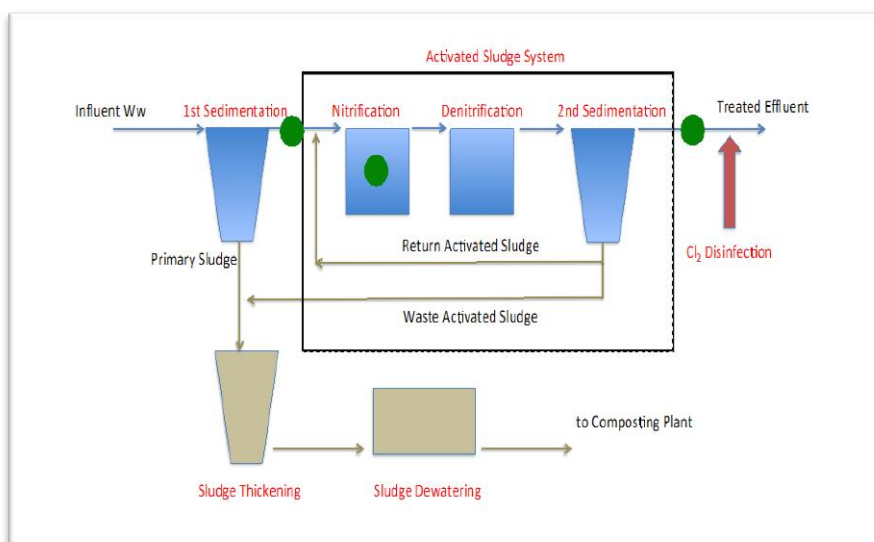


Figure 3: Process scheme of Mayagüez wastewater treatment plant. Circles indicate the sampling locations.



Figure 4: Aerial view of the Mayagüez wastewater treatment plant.

Samples for experimentation were collected from:

- Wastewater that came into the main biological treatment system after the first sedimentation tank (hereby called influent),
- Wastewater exiting the main settling tank of the biological treatment system, before the Chlorine injection (hereby called effluent)
- Mixed liquor suspended solids (MLSS) from the aeration tank.

4.1.2. NANOPARTICLES

Commercially available iron oxide nanoparticles (Ferrotec MSG W11) were used as the model ENP. These nanoparticles are hematite (Fe_2O_3), iron oxide nanoparticles coated with

surfactant has been bought from Ferrotec (USA) Corporation, and hereafter they will be called ENP_{Fe-surf}. Table 4 shows key characteristics of these nanoparticles. Total soluble iron (Fe) content was determined to be 59.3 mg Fe per mL of ENP_{Fe-surf} according to the Phenanthroline Method (APHA, AWWA, WEF, 2006).

Table 4: CHARACTERISTICS OF IRON OXIDE NANOPARTICLES TO BE USED IN THIS STUDY. (Ferrotec, 2010)

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

4.1.3. OTHER MATERIALS

Other materials for the proposed research included Petri dishes, deionized water (DI), m-FC Broth, filtration funnels, bacteriological membranes, burner/lighter, air pump, Erlenmeyer flasks (different sizes), graduated cylinder (100 mL), and pipets.

4.2. EQUIPMENTS

Collected water samples were then analyzed in order to determine their physical, chemical and biological properties. Table 5 summarizes the analysis for the samples and includes the method and equipment used for each analysis.

Table 5: EQUIPMENT FOR BIO-CHEMICAL ANALYSIS.

Parameter to be analyzed	Instrument	Method
pH	pH meter model ORION720A+	
COD	HACH DR/2010	HACH Method 8000 Low range (0 to 150 mg/L) High range (20 to 1500 mg/L)
Color	HACH DR/2010	HACH Method 8025
Turbidity	HACH 2100P Turbidimeter	
Suspended Solids	Filtration	Standard Methods 2540 Dried at 103-105°C
Iron Concentration	HACH DR/2010	Modified Phenanthroline Method (EPA Standard Methods 3500)
Nitrate	HACH DR/2010	HACH Method 10071 NitraVer 5 Nitrate Reagent Powder Pillow
Phosphate	HACH DR/2010	HACH Method 8190
BOD	HQ440d LBOD101	EPA Standard Methods 5210
Chlorine Concentration	HACH Pocket Colorimeter II, Chlorine	HACH Method 8021, DPD Free Chlorine Reagent
Fecal coliforms	Membrane Filtration	0.45 µm membrane, followed by incubation at 44.5°C on m-FC growth medium
Hydrodynamic diameter of particles	Brookhaven Instruments BI-90 Plus	Particle Size Analyzer
Point of zero charge	Brookhaven Instruments BI-90 Plus	Zeta Potential Analyzer in solution of KNO ₃

4.3.METHODOLOGY

The effect of nanoparticles in the wastewater treatment plant has been studied by simulating the treatment process in an SBR. This section consists of a general description of the procedure, the system components of this simulation and other supplementary experiments done in the present study.

4.3.1. SEQUENCING BATCH REACTOR

4.3.1.1. EXPERIMENT DESCRIPTION

In order to visualize the effect of the ENP in the wastewater, a lab-scale SBR consisting of two Phipps & Bird 2000 mL B-Ker²® Lab Jars equipped with aeration device was used. Aeration was provided to maintain a dissolved oxygen concentration of ~5 mg/L during the reaction mode. Initially, each jar has received 200 mL MLSS and 1800 mL influent wastewater. The SBR's were run at room temperature (25 ± 1 °C). One reactor labeled “reactor A” functioned as a control where no $\text{ENP}_{\text{Fe-surf}}$ was added. The second reactor labeled “reactor B” is where $\text{ENP}_{\text{Fe-surf}}$ was added. Prior to adding the $\text{ENP}_{\text{Fe-surf}}$ both reactors have being put through six, four hour cycles. Experiments showed that the SBRs run at a 4-hr cycle (3-hr reaction, 0.5-hr settling, and 0.5-hr decant and refill) were stabilized after the 6th cycle, resulting in constant effluent for COD and SS concentrations. At the onset from the 7th to 10th cycles, the treatments SBRs were loaded with $\text{ENP}_{\text{Fe-surf}}$.

After settling time of each sequence, 67 percent of the supernatant has been decanted through the side hole (decant mode). Different analyses were made to the water decanted. The

newly formed sludge was left in the reactor and then the reactor was refilled with untreated influent (refill mode), for a new cycle.

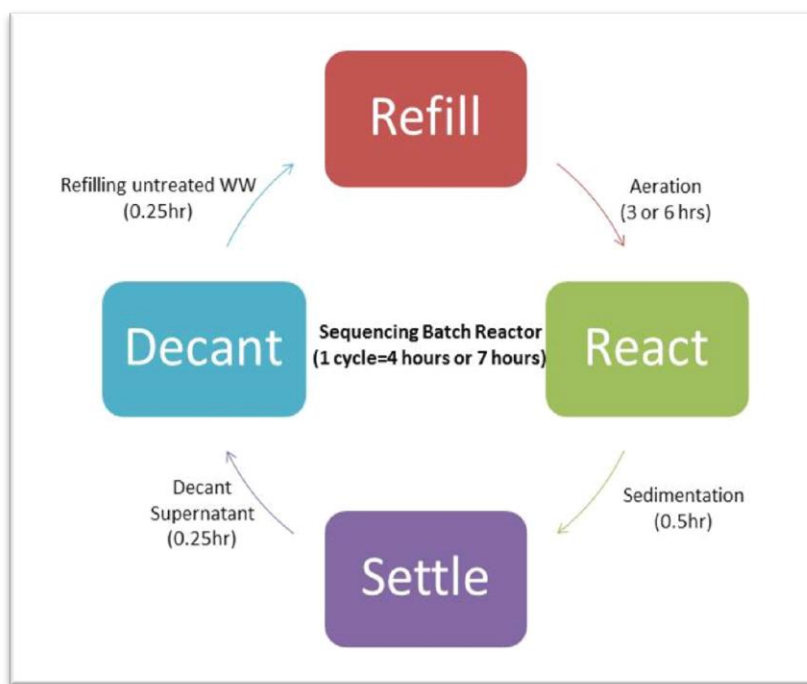


Figure 5: Cycle of SBR.

4.3.1.2. WATER QUALITY AND DATA ANALYSIS

SBR effluents were analyzed for soluble COD, soluble BOD, SS, pH, hydrodynamic particle diameter, soluble Fe, turbidity, and apparent color. The filtrate collected during the SS analysis with a 1.2- μm glass fiber filter was used for analysis of soluble COD and soluble Fe by the HACH Method 8000 and Method 8008, respectively. SS and soluble BOD were analyzed in accordance to the Standard Methods (APHA, AWWA, WEF, 2006). pH values were measured with an ion selective electrode connected to the Orion Model 720A pH meter. A HACH 2100P Turbidimeter was used for turbidity measurement. Apparent color was determined according to

the HACH Method 8025. Hydrodynamic particle diameters were determined by dynamic light scattering (Brookhaven Instruments BI-90 Plus Particle Size Analyzer) with the autocorrelation function of the intensity fluctuation of the scattered light.

Exposure of ENPs in terms of the product of concentration and contact time may produce dissimilar fate and impacts of ENPs in SBRs. In this regard, different amount of $\text{ENP}_{\text{Fe-surf}}$ has been injected to the SBRs running at the different contact times. Results (see Table 6) were compared with each of them.

Table 6: DIFFERENT SBR EXPERIMENTS.

ENP Injection	Contact Time (Hours)
0.5 mL/L	3
0.5 mL/L	6
1.5 mL/L	3
1.5 mL/L	6

4.3.2. SLUDGE VOLUME INDEX

To determine potential effect of $\text{ENP}_{\text{Fe-surf}}$ on sludge settling, the sludge volume index (SVI) was measured in accordance to the Standard Methods (APHA, AWWA, WEF, 2006). Mixed liquor samples collected from the treatment plant were separated and put in six 1 L graduated cylinders. In each cylinder a certain amount of $\text{ENP}_{\text{Fe-surf}}$ were added (see Table 7) to observe its effect on sediment quantity. The $\text{ENP}_{\text{Fe-surf}}$ were injected from cylinder 1 through cylinder 6, shaking each one of them, with a 10 minute interval between each injection. After

loading, the cylinders were allowed 30 minutes of settling time. Afterwards sludge volume, turbidity, color, and pH of supernatant were analyzed. For the color analysis, the supernatant samples were filtered first.

Table 7: ENP_{Fe-surf} SPIKE FOR SLUDGE VOLUME INDEX TESTS

Cylinder	ENP spike (mL/L)
1	0.0
2	0.5
3	1.5
4	3.0
5	4.0
6	5.0

The SVI was determined in accordance to the Standard Methods (APHA, AWWA, WEF, 2006) as follows:

$$\text{SVI (mL/g)} = \frac{\text{Settled sludge volume after a 30-min settling (in mL/L)} \times 1000}{\text{MLSS (in mg/L)}} \quad (3)$$

4.3.3. SPECIFIC OXYGEN UPTAKE RATE

Oxygen uptake rate (OUR) tests were conducted to assess the impact of ENP_{Fe-surf} on the respiration of mixed liquors. Pre-aerated mixed liquor samples were placed in BOD bottles where the different concentrations of ENP_{Fe-surf} (0 - 110.6 mg soluble Fe/L mixed liquor) were loaded. To ensure that cell respiration was not substrate-limited, 100 mg/L of sCOD (in the form of glucose) was added. DO concentrations were read for a minimum of 20 min or until DO levels

dropped below 1 mg/L. Specific oxygen uptake rate (SOUR) was calculated by dividing the OUR by the mixed liquor volatile suspended solids (MLVSS) concentration as follows:

$$SOUR \text{ (mg/g/hr)} = \frac{OUR \text{ (mg/L/min)} \times 60 \text{ min/hr} \times 1000 \text{ mg/g}}{MLVSS \text{ (mg/L)}} \quad (4)$$

The percentage SOUR inhibition was calculated by the following equation:

$$SOUR_{inhibition}(\%) = \frac{SOUR_{control} - SOUR_{ENP}}{SOUR_{control}} \times 100 \quad (5)$$

where $SOUR_{control}$ is the average SOUR without any $ENP_{Fe-surf}$ loadings and $SOUR_{ENP}$ is the average SOUR at a given $ENP_{Fe-surf}$ loading.

4.3.4. ENP IN DISINFECTION

Effects of $ENP_{Fe-surf}$ present in the treated effluent on disinfection were tested for the removal and inactivation of fecal coliforms. For this, real wastewater effluents were collected and spiked with chlorine (Cl_2 , in the form of sodium hypochlorite) and $ENP_{Fe-surf}$. A solution of 1 percent concentration of chlorine were prepared by diluting the commercial Clorox (5.25% Sodium Hypochlorite) with DI water. The water used in this experiment was the effluent water from the Mayagüez wastewater treatment plant before disinfection. Six test tubes of 100 mL were filled with the effluent and injected with different amount of the chlorine solution and $ENP_{Fe-surf}$ (see Table 8). The initial Cl_2 concentrations were targeted at 5 and 10 mg Cl_2 /L that was in the range of typical chlorine dosages to the effluent from biological wastewater treatments

(Tchobanoglous et al., 2003). $\text{ENP}_{\text{Fe-surf}}$ was loaded in the range of 1.8 – 88.9 mg/L as soluble Fe. Low $\text{ENP}_{\text{Fe-surf}}$ concentrations (<10 mg/L as soluble Fe) were tested to include the $\text{ENP}_{\text{Fe-surf}}$ concentrations found in the SBR effluents.

Table 8: $\text{ENP}_{\text{Fe-surf}}$ SPIKE FOR DISINFECTION TEST.

Test tubes	ENP spike mL/100mL
1	0
2	0.05
3	0.15
4	0.30
5	0.40
6	0.50

After adding ENP, the test tubes will be gently shaken and let stand for 30 minutes. After a contact time of 30 mins, free residual Cl_2 and fecal coliforms concentrations were determined. The HACH Pocket Colorimeter II Test Kit (Figure 6) was used to measure free Cl_2 concentrations.

Bacteriological enumeration was done with membrane filtration technique with a 0.45- μm cellulose ester membrane. For fecal coliforms, the Petri dishes containing the filtered membrane were placed on the HACH m-FC broth and incubated for 24 hours at 44.5 °C.

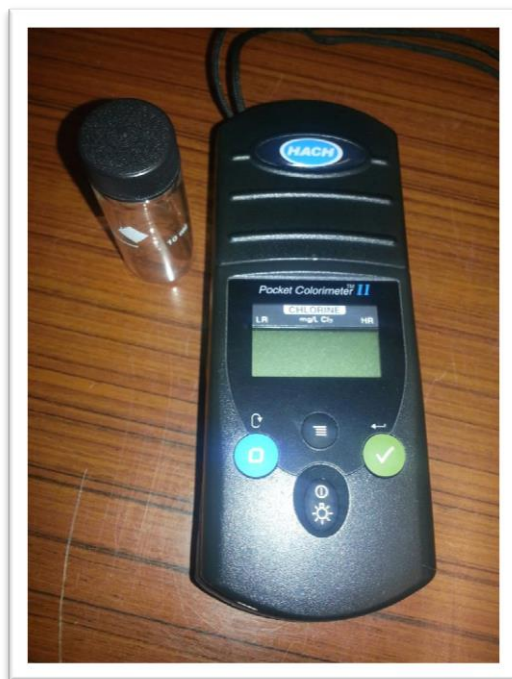


Figure 6: HACH Pocket Colorimeter II Test Kit used to determine free chlorine.

4.3.5. ENP INTERACTIONS WITH CLAY AND HUMIC ACID

Solutions of clay or humic acid at 250 mg/L were prepared with montmorillonites (or humic acid). Montmorillonites (Na-SWy-2) were obtained from the Clay Minerals Society Source Clays Repository (West Lafayette, IN). Their characteristics are available in Gao and Pedersen (2005). Humic acid (CAS #1415-93-6) was purchased from Alfa Aesar. The solution was sonicated for 10 minutes (200 W/L and 50 KHz). 10 μ L of ENP_{Fe-surf} solution was spiked to 15 mL of clay solution, or humic acid solution. Hydrodynamic particle diameter was determined using dynamic light scattering (DLS) method in the course of 24 hrs. Zeta potential of the same solution was analyzed too for the electronegativity of each component and the interaction between them.

4.3.6. STATITISTICAL DATA ANALYSIS

Student's t-test was used to determine any significant differences among the effluent water quality data between the treatment and control SBRs. Differences between means at a confidence level of 5% ($P < 0.05$) were considered to be statistically significant.

5. RESULTS AND DISCUSSION

The results from the experimental studies that aimed to understand the effect of $\text{ENP}_{\text{Fe-surf}}$ on a wastewater treatment plant are discussed in this chapter. A series of lab scale experiments were conducted to characterize water quality parameters.

5.1. MATERIALS CHARACTERIZATION

The main materials of this experiment were the wastewater collected from the Mayagüez wastewater treatment plant and the iron oxide nanoparticles purchased from Ferrotec. The following two subchapters show the main characteristics of wastewater and iron oxide nanoparticles.

5.1.1. WASTEWATER

Whenever wastewater was collected, analyses were made. In Table 9, the main characteristics of the wastewater used in the main experiment are shown, in which the values presented correspond to the average with the standard deviations, see Appendix A for more detailed data. All experiments were conducted on sampled wastewater with the following range of values, unless otherwise stated, and the numbers of tested samples are shown in parenthesis.

Table 9: MAIN CHARACTERISTICS OF WASTEWATER. (Mean±Standard Deviation)

Parameter	Influent	Mixed Liquor Suspended Solids (MLSS)
sCOD (mg/L)	143.6±23.5 (n=7)	-----
BOD (mg/L)	83.8±31.2 (n=12)	-----
SS (mg/L)	48.9±13.7 (n=10)	2251±849 (n=8)
pH	6.6±0.1 (n=5)	6.4±0.2 (n=5)
Sludge Volume Index (SVI)	-----	183±19 (n=2)

5.1.2. IRON OXIDE NANOPARTICLES

The iron oxide nanoparticles used in the study are commercially available iron oxide nanoparticles (Ferrotec MSGW11). The solution is mostly water and magnetite; see Table 4 for the main characteristics.

To quantify the amount of iron, a thermo gravitational analysis (TGA) was made. In a TGA analysis, the sample was exposed to high temperatures to evaporate all the components (water and surfactants) except for iron. It was assumed that the mass obtained after 800 °C was iron. As shown in Figure 7, approximately 24 % of the initial mass was still present after a temperature of 800 °C in the first run. In the second run, about 18 % was present. Therefore, it is construed that approximately 22% (by wt.) of iron oxide nanoparticle solution was iron. The four runs made in this analysis are shown in Appendix B.

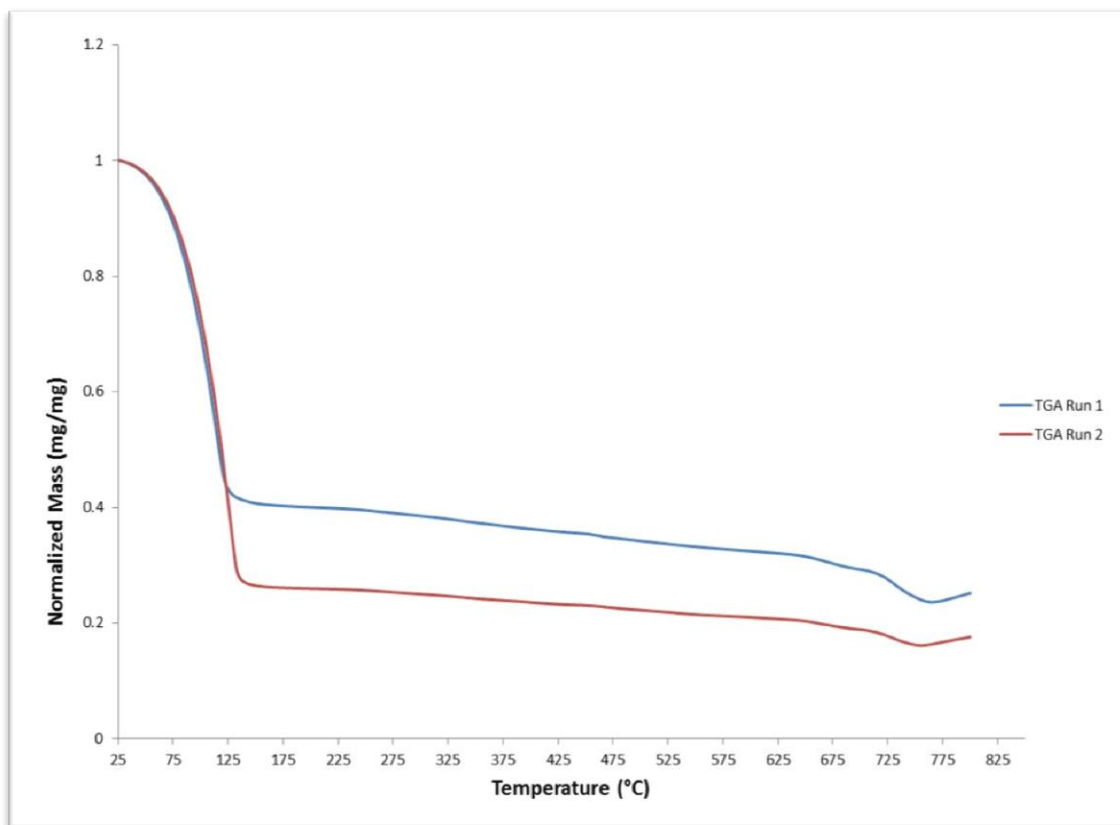


Figure 7: TGA Analysis for the ENP_{Fe-surf}.

5.2. OPERATION OF SEQUENCING BATCH REACTOR (SBR)

Both the control and treatment SBRs were run in the same manner up to the sixth cycle at which a pseudo stabilization in terms of effluent quality was attained. Then, only the treatment SBR received $\text{ENP}_{\text{Fe-surf}}$ at an application rate of 1.5 mL/L from the 7th to 10th cycles. The control SBR was run in the same manner but without the $\text{ENP}_{\text{Fe-surf}}$ addition. The aeration time was three hours with a 30 minutes settling time. After each sequence the supernatant from both reactors was collected and analyzed. All SBRs experiment results are shown in Appendix C.

5.2.1. WATER QUALITY PARAMETERS

The supernatant from each sequence was collected for water quality analysis. Water characterization in terms of pH, turbidity, COD, BOD, SS, iron concentration, among others, was carried out. Sludge volume index was also measured in the reactors after each run.

5.2.1.1. CHEMICAL OXYGEN DEMAND (COD)

With the addition of $\text{ENP}_{\text{Fe-surf}}$, statistically higher ($p < 0.05$) sCOD concentrations were found in the treatment SBR effluent (see Figure 8). In a separate experiment, $\text{ENP}_{\text{Fe-surf}}$ was added at different concentrations to DI water and measured for COD. Results showed a proportional increase of COD to the amount of $\text{ENP}_{\text{Fe-surf}}$ added (see Figure 9). Therefore, the increased sCOD concentrations in the treatment SBR effluent were attributed to COD exerted from the oxidation of both inorganic iron and organic surfactants on $\text{ENP}_{\text{Fe-surf}}$.

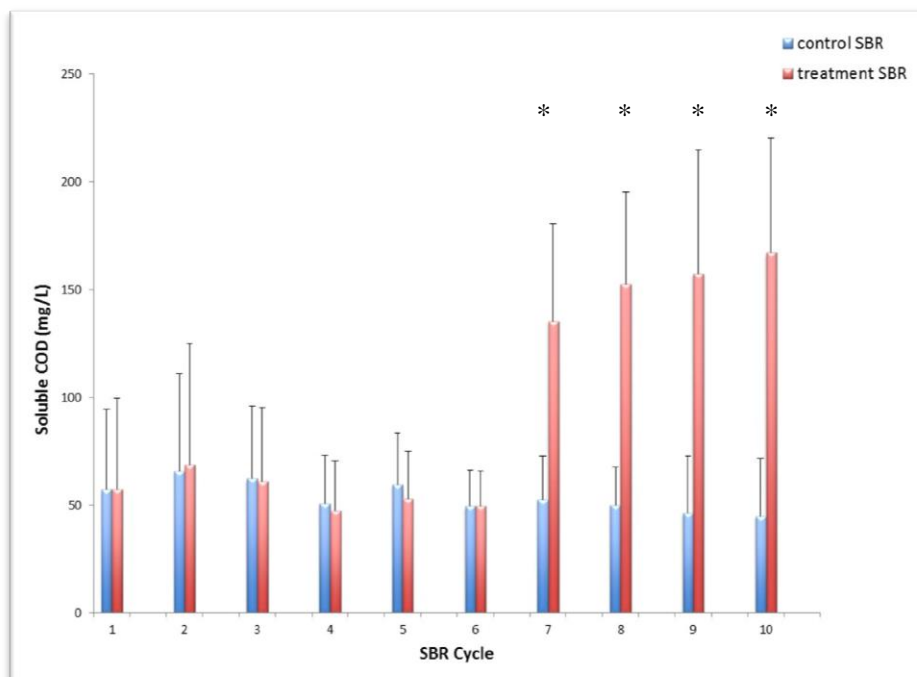


Figure 8: Concentration of COD in the SBR effluents. Error bars are standard deviations of four replicate runs. A significant difference ($p < 0.05$) of the data between the control and treatment SBRs is indicated with the (*) symbol.

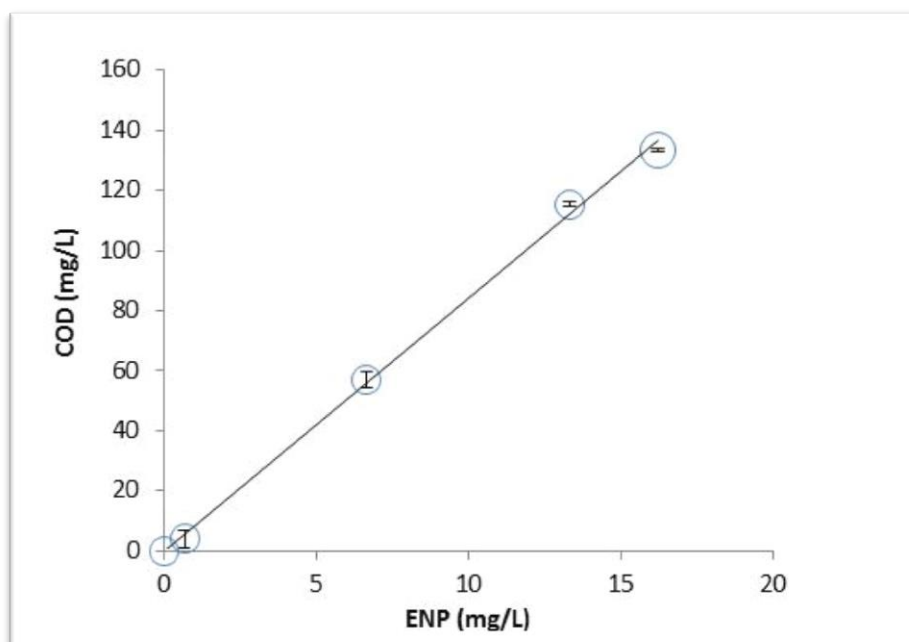


Figure 9: Relation between COD concentration and $\text{ENP}_{\text{Fe-surf}}$ addition to DI water. Error bars are standard deviations of three replicate runs.

5.2.1.2. SUSPENDED SOLIDS (SS)

SS concentrations were apparently higher with $\text{ENP}_{\text{Fe-surf}}$ addition in the treatment SBR effluent than the effluent of the control SBR, but the differences were not statistically significant ($p > 0.05$) (see Figure 10). It can be concluded that particle aggregation occurred to some extent after $\text{ENP}_{\text{Fe-surf}}$ addition, resulting in higher SS concentrations. Further investigation of the sludge is recommended to explain this phenomenon.

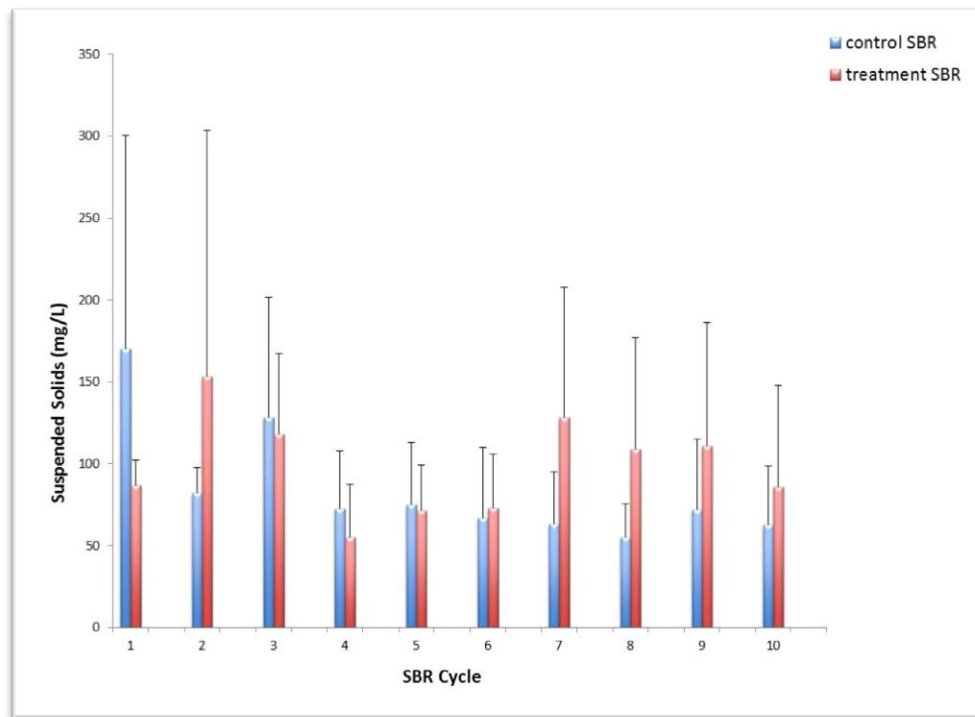


Figure 10: Suspended Solids in the SBR effluents. Error bars are standard deviations of four replicate runs.

5.2.1.3. pH

Slightly higher pH was found in the samples from the treatment SBR (see Figure 11). A pH of 7.80 ± 0.04 ($n=8$) at the $\text{ENP}_{\text{Fe-surf}}$ loading of 88.9 mg/L as soluble Fe in comparison with the control SBR effluent of 7.69 ± 0.11 ($n=8$).

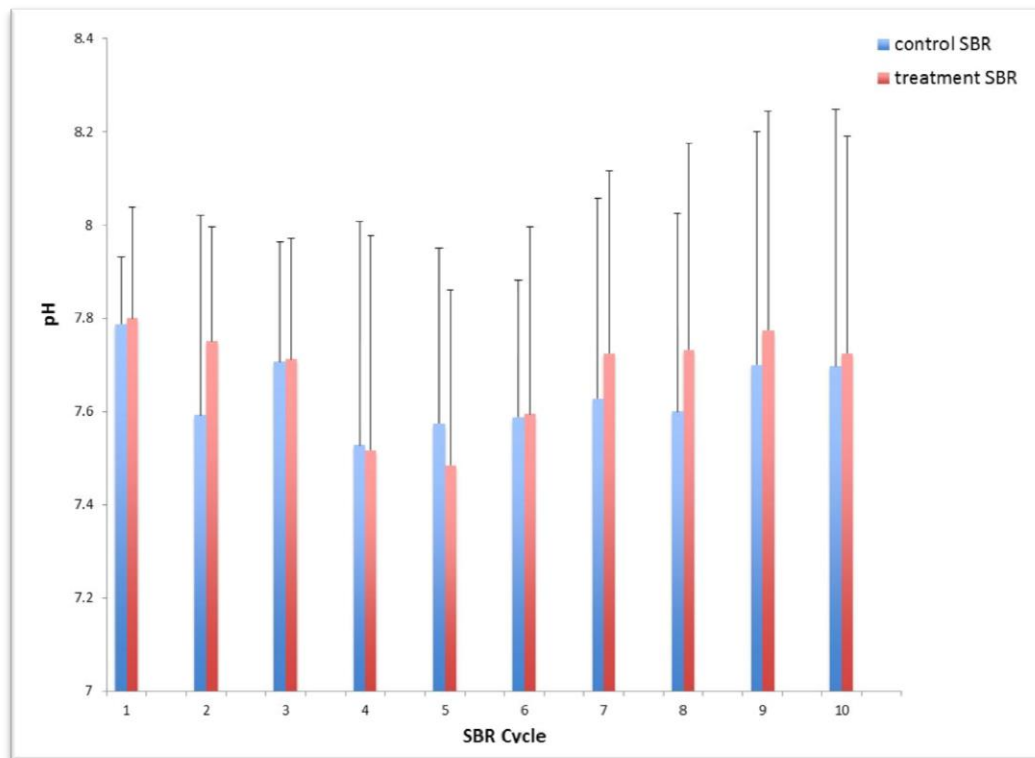


Figure 11: pH values in SBR effluents. Error bars are standard deviations of four replicate runs.

5.2.1.4. TURBIDITY

The trend of turbidity was found very similar to that of sCOD concentrations, showing a statistically higher turbidity after $\text{ENP}_{\text{Fe-surf}}$ addition in the treatment SBR effluent than in the control SBR effluent (see Figure 12). The color of the treatment SBR was very dark black

because of the presence of iron oxide nanoparticle. Hydrodynamic particle diameter analysis, which is shown later in this chapter, also supported the presence of the applied nanoparticles in the effluent.

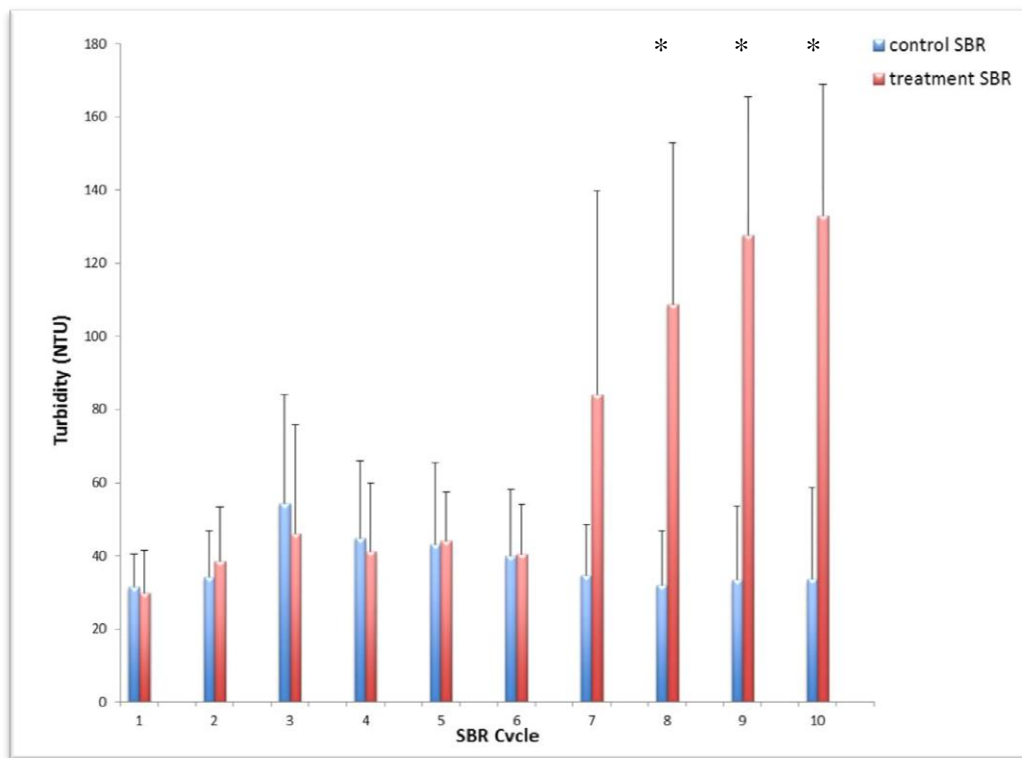


Figure 12: Turbidity in SBR effluents. Error bars are standard deviations of four replicate runs. A significant difference ($p<0.05$) of the data between the control and treatment SBRs is indicated with the (*) symbol.

5.2.1.5. BIOLOGICAL OXYGEN DEMAND (BOD)

The difference in BOD concentrations after $\text{ENP}_{\text{Fe-surf}}$ addition from the seventh to tenth sequences to the treatment SBR was not significant, compared to those from the control SBRs. However, as previously shown in Figure 8, sCOD concentrations were significantly greater in the effluent of the treatment SBR after $\text{ENP}_{\text{Fe-surf}}$ addition from the 7th cycle than in the control SBR. This resulted in much lower BOD/sCOD ratios (ranges between 9.6-19.0%) in the

treatment SBR than in the control SBR (33.9-53.1%). Such phenomenon, lower BOD/sCOD ratio in the treatment SBR effluent was due likely to the presence of $\text{ENP}_{\text{Fe-surf}}$. Having a low BOD/sCOD ratio means greater non-biodegradable fraction.

This assumption was verified with a separate BOD experiment with $\text{ENP}_{\text{Fe-surf}}$ solutions at concentrations ranging from 0.67 to 6.67 mg/L in DI water. The results revealed that $\text{ENP}_{\text{Fe-surf}}$ did not exert noticeable BOD, indicative of the stability of the surfactants coated on the ENP surface in biological activity. However, as mentioned previously, COD concentrations were proportionally increased to the amount of $\text{ENP}_{\text{Fe-surf}}$ added due probably to COD exerted from the oxidation of both inorganic Fe and organic surfactants on $\text{ENP}_{\text{Fe-surf}}$.

5.2.1.6. SOLUBLE IRON CONCENTRATION

The results from total soluble Fe measurement in the SBR's effluent (see Figure 13) supported the presence of significant fraction of $\text{ENP}_{\text{Fe-surf}}$ added. $\text{ENP}_{\text{Fe-surf}}$ application at 1.5 mL/L was equivalent to 88.9 mg/L total soluble Fe according to the Phenanthroline Method (APHA, AWWA, WEF, 2006). At the different $\text{ENP}_{\text{Fe-surf}}$ concentrations, the absorbance was measured in accordance to the Method. Figure 14 shows a calibration curve of soluble iron concentration measured with the Phenanthlorine Method.

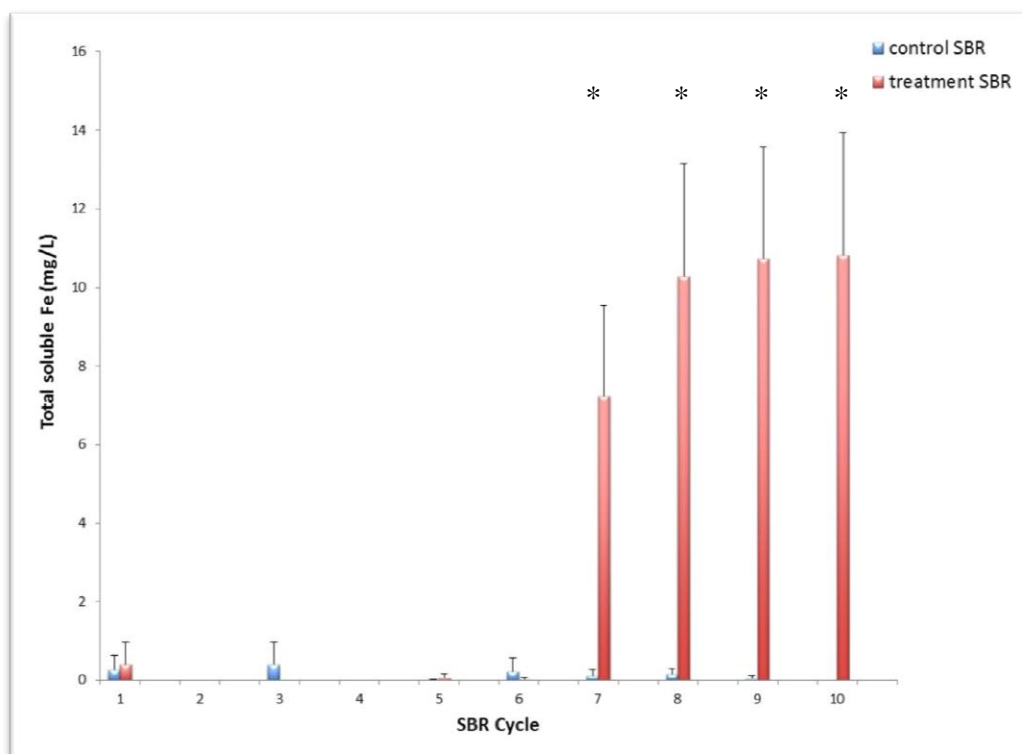


Figure 13: Total soluble Fe concentration in the SBR effluents. Error bars are standard deviations of two replicate runs. A significant difference ($p < 0.05$) of the data between the control and treatment SBRs is indicated with the (*) symbol.

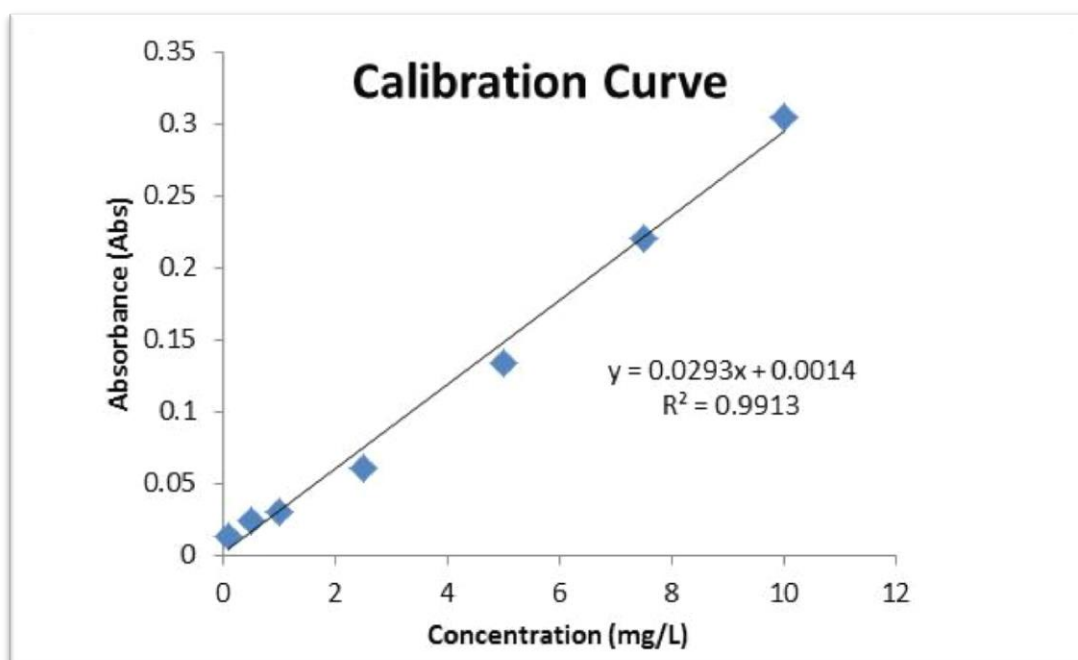


Figure 14: Calibration curve used to convert absorbance to iron concentration.

Soluble Fe concentration of approximately 7.6 mg/L in the effluent with the ENP_{Fe-surf} loading of 88.9 mg/L was found. Therefore, mass balance on total soluble Fe concentrations in the treatment SBR effluent revealed that up to 8.7% weight of the applied ENP_{Fe-surf} were present in the effluent. This implies that ENP_{Fe-surf} will be introduced into environmental receptors through the treated effluent and potentially could adversely impact them. The ENPs used in this study had surface coatings with proprietary surfactants. Surface coatings are typically required for intended application of ENPs. These coatings can impart surface charge to the nanoparticles, either positive or negative, and can make the ENPs stable against aggregation (Limbach et al., 2008).

5.2.1.7. HYDRODYNAMIC PARTICLE DIAMETER

Mean particle diameters in the treatment SBR effluent after ENP_{Fe-surf} addition was 200 nm or less and those in the control SBR were in the 700 nm range (Figure 15), hence statistically they were much smaller ($p < 0.05$) than those in the control SBR effluent. The mean diameter was in term of number of particles. As for sCOD results, this was due to the presence of ENP_{Fe-surf}, not incorporated to sludge, in the treatment SBR effluent.

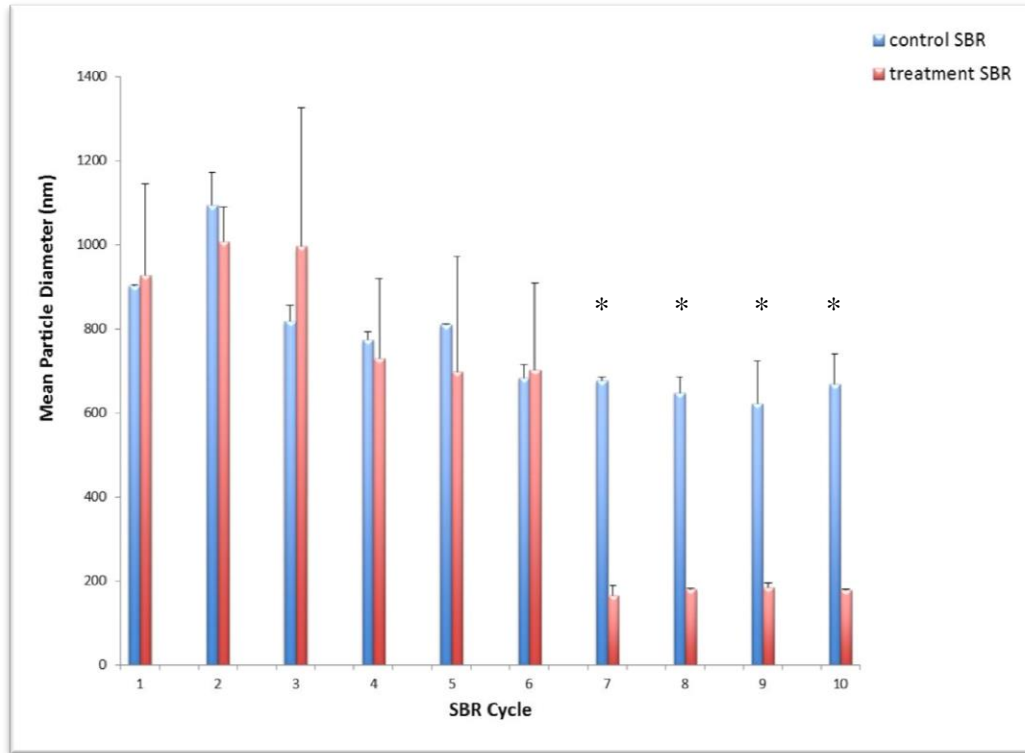


Figure 15: Hydrodynamic particle diameter in the SBR effluents. Error bars are standard deviations of two replicate runs. A significant difference ($p < 0.05$) of the data between the control and treatment SBRs is indicated with the (*) symbol.

5.2.1.8. SLUDGE VOLUME INDEX (SVI)

No significant differences in sludge volume index were found regardless of the $ENP_{Fe-surf}$ application rates (see Table 10) and they were in the range of 150-200. Therefore, the $ENP_{Fe-surf}$ addition to MLSS did not play an important role in sludge settling. In the current study this could be attributed to the poor settling characteristics (sludge volume index >180) of the wastewater sludge tested for analysis of sludge volume index. Sludge volume index (SVI) values below 100 are generally desired for good settling because values greater than 150 are typically associated with filamentous growth leading to sludge bulking (Tchobanoglous et al., 2003).

Table 10: SLUDGE VOLUME INDEX AT DIFFERENT ENP_{Fe-surf} APPLICATION RATE.

ENP _{Fe-surf} injection (mg/L)	Sludge Volume Index (SVI)
0	183±19
44.5	167±24
88.9	186±25
266.7	196±7
355.6	166±40
444.5	153±50

Resulting supernatants from the SVI study were analyzed for turbidity, apparent color, and hydrodynamic particle diameter. With ENP_{Fe-surf} addition, turbidity and apparent color were increased proportional to the amount of ENP_{Fe-surf} added. However, mean particle diameters were very similar at ~160 nm regardless of ENP_{Fe-surf} application rates. Further study is needed to clarify this behavior.

5.2.1.9. INTERACTIONS WITH CLAY AND HUMIC ACID

DLS analysis was conducted to check the potential of heteroaggregation of ENP_{Fe-surf} with clays and humic acid that are present abundant in wastewaters. When ENP_{Fe-surf} were present alone in DI water, mean particle diameter was decreased by approximately 15% at the end of 24-h experiment (see Figure 16), probably due to self-aggregation and that settlement of ENP_{Fe-surf} may have resulted from magnetic attractive forces between particles (Wiesner et al., 2006). Also, the density of ENP_{Fe-surf} was higher (1.17 g/mL) than that of water (as previously shown in Table 4). Humic acid also showed a reduction in mean particle diameter similar to that of ENP_{Fe-surf} (approximately 15% reduction). Larger-sized clay particles settled down to a greater extent than

smaller-sized humic acid or ENP_{Fe-surf}, resulting in a greater decrease of mean particle diameter by, more or less, 30%. Mean particle diameters in both (ENP_{Fe-surf}-clay) and (ENP_{Fe-surf}-humic acid) solutions remained fairly constant and decreased only by 7% or less after 24 h. This implies the stability of ENP_{Fe-surf}, clay, and humic acid in the binary mixtures, not making aggregation and settlement but remaining in the aqueous phase. Zhang et al. (2009) reported stabilization or reduction of metal oxide nanoparticle aggregation due to natural organic matter adsorption to nanoparticles and subsequently high probability to remain in aquatic environment. Each DLS analysis is shown in Appendix D.

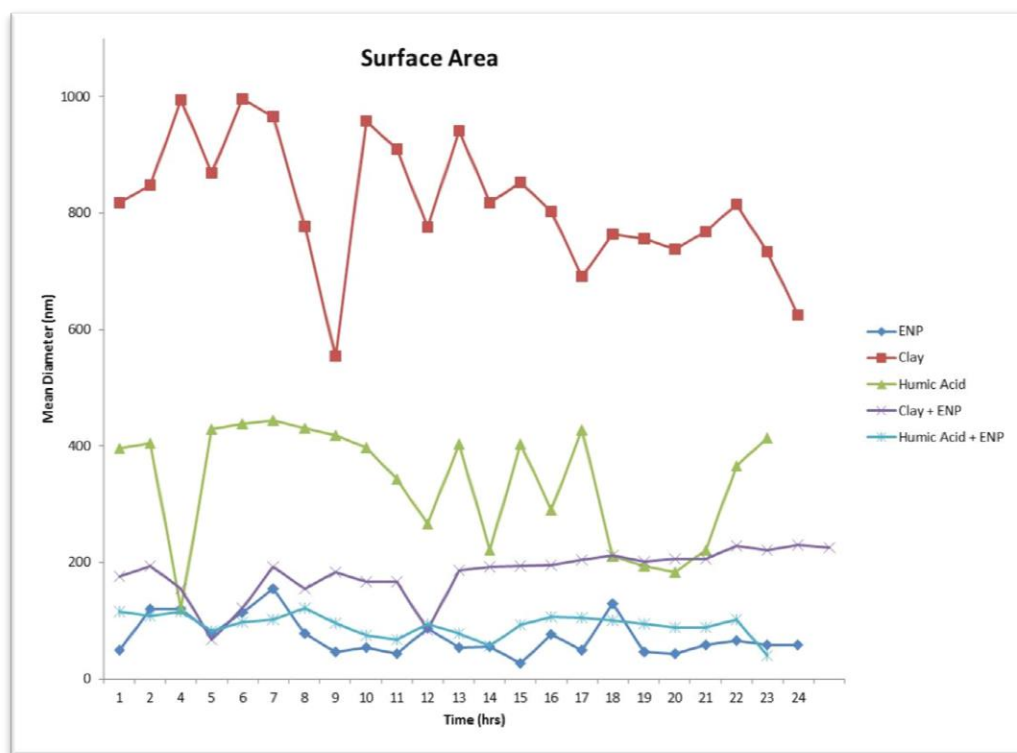


Figure 16: Mean particle diameter in the solutions of ENP_{Fe-surf}, clay, humic acid, ENP_{Fe-surf} + clay, and ENP_{Fe-surf} + humic acid as function of time.

5.3. RESPONSE OF SBR TO DIFFERENT HYDRAULIC RETENTION TIMES

Findings from the studies with different hydraulic retention times (HRT) on effluent water quality are presented and discussed in this chapter. The testing of these experimental and analytical procedures were similar as shown in the previous chapter. The $\text{ENP}_{\text{Fe-surf}}$ loading was of 29.6 mg/L as Fe. Two different SBR cycles were tested: 4-hr cycle (3-hr reaction, 0.5-hr settling, and 0.5-hr decant/refill) and 7-hr cycle (6-hr reaction, 0.5-hr settling, and 0.5-hr decant/refill). From now each SBR is going to be called 3-hr or 6-hr. At the onset from the 7th to 10th cycles, the treatment SBRs were loaded at 0.5 mL $\text{ENP}_{\text{Fe-surf}}$ per L of mixed liquor, which was equivalent to 29.6 mg as Fe per L of mixed liquor. Control SBRs without $\text{ENP}_{\text{Fe-surf}}$ loadings were also run in parallel.

5.3.1. WATER QUALITY PARAMETERS

The supernatant from each sequence was collected for water quality analysis. Water characterization in terms of pH, turbidity, COD, BOD, SS, iron concentration, among others, was carried out. Sludge volume index was also measured in the reactors after each run.

5.3.1.1. TURBIDITY, sCOD, sBOD, SS CONCENTRATIONS AND pH VALUES

Despite alkaline characteristics of $\text{ENP}_{\text{Fe-surf}}$ ($\text{pH} > 10$), the treatment SBR effluents had almost the same pH values that the control SBR effluents had (7.6 ± 0.1 ($n=4$)) as the low $\text{ENP}_{\text{Fe-surf}}$ loading of 29.6 mg/L as Fe was applied to the treatment SBRs.

The following figures show the concentrations of turbidity, sCOD, sBOD, and SS in the effluents from the 7th to 10th SBR cycles. In each figure the average values of the cycles from 7th and 10th were plotted. The effluents from the ENP_{Fe-surf} loaded treatment SBRs had significantly ($p < 0.05$) increased concentrations of turbidity and sCOD than the corresponding control SBR effluents (see Figures 17 and 18). The effluent sBOD and SS concentrations were apparently higher for the ENP_{Fe-surf}-loaded treatment SBRs than the corresponding control SBRs but the differences were insignificant ($p > 0.05$) (see Figures 19 and 20).

As such, effluent water quality was not improved with a longer aeration time of 6 hours. Deterioration in effluent water quality in terms of turbidity, sCOD, sBOD and SS was found due to the ENP_{Fe-surf} loading. As shown in section 5.2.1, when ENP_{Fe-surf} was added at the different concentrations to deionized water, turbidity and COD concentration increased proportional to the amount of ENP_{Fe-surf} added, but BOD concentration remained the same. Therefore, the substantial increases of turbidity in the treatment SBR effluent were attributed to the physical presence of ENP_{Fe-surf}, and likely, the oxidation of both inorganic Fe and organic surfactants on ENP_{Fe-surf} was responsible for the increases of sCOD concentrations in the ENP_{Fe-surf} loaded SBR effluents.

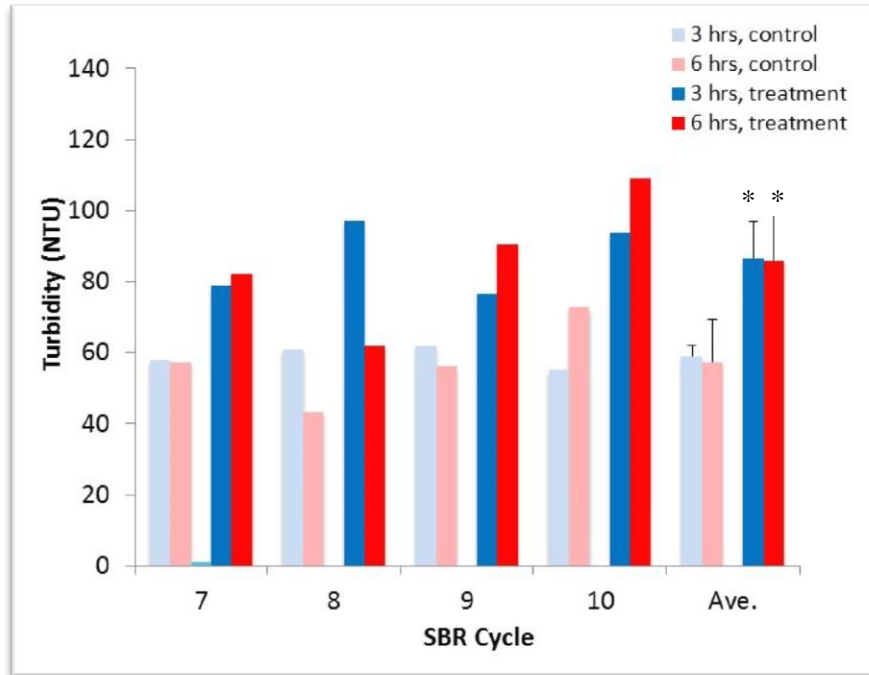


Figure 17: Concentrations of effluent turbidity of the control and treatment SBRs at different hydraulic retention times. Data shows values measured after the 7th to 10th SBR cycles. Error bars represent the standard deviations (n = 4). A significant difference ($p < 0.05$) of the averaged data between the control and corresponding treatment SBRs is indicated with symbol (*).

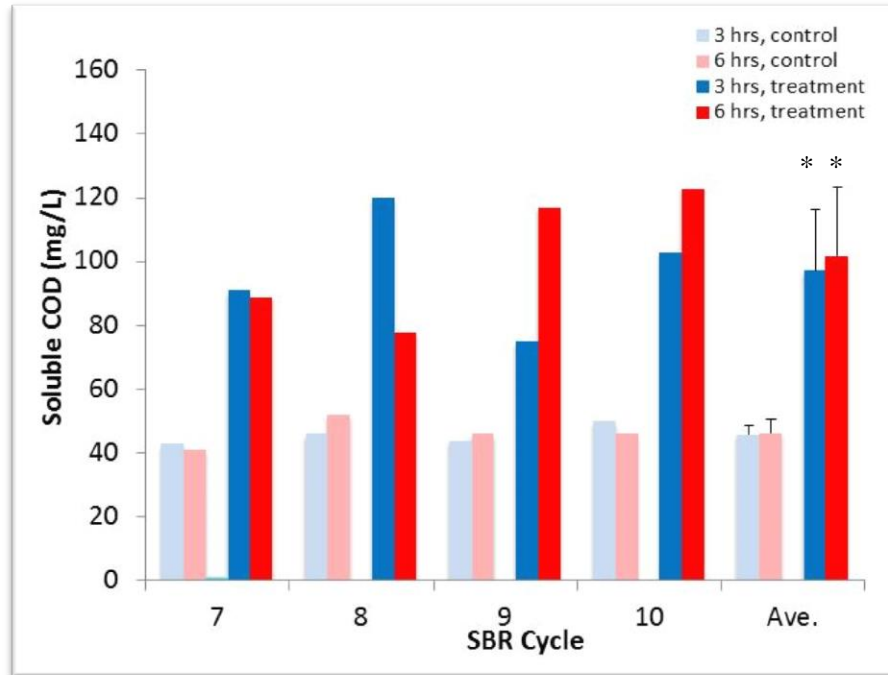


Figure 18: Concentrations of effluent soluble COD of the control and treatment SBRs at different hydraulic retention times. Data shows values measured after the 7th to 10th SBR cycles. Error bars represent the standard deviations (n = 4). A significant difference ($p < 0.05$) of the averaged data between the control and corresponding treatment SBRs is indicated with symbol (*).

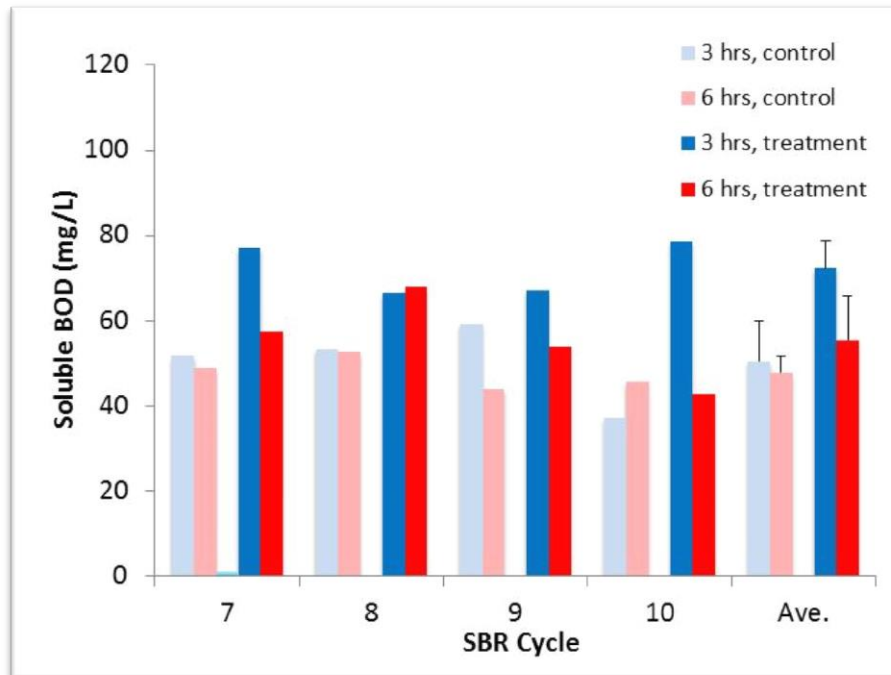


Figure 19: Concentrations of effluent soluble BOD of the control and treatment SBRs at different hydraulic retention times. Data shows values measured after the 7th to 10th SBR cycles. Error bars represent the standard deviations (n = 4).

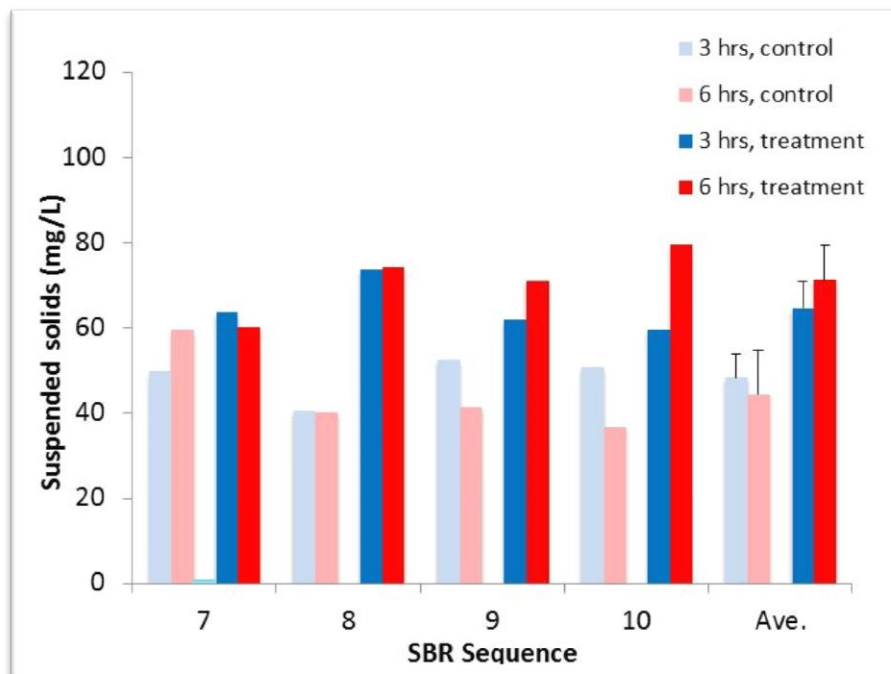


Figure 20: Concentrations of effluent suspended solids of the control and treatment SBRs at different hydraulic retention times. Data shows values measured after the 7th to 10th SBR cycles. Error bars represent the standard deviations (n = 4).

5.3.1.2. IRON CONCENTRATION AND HYDRODYNAMIC PARTICLE DIAMETERS IN SBR EFFLUENT

Substantial increases in Fe concentrations were found in the SBR effluents due to the $ENP_{Fe-surf}$ loadings (see Figure 21). Between the two $ENP_{Fe-surf}$ loaded treatment SBRs, the differences in Fe concentrations were insignificant ($p > 0.05$), although SBRs run at 6-hr HRT had a slightly greater Fe concentrations (2.7 ± 0.5 mg/L as Fe) than the other run at 3-hr HRT (2.3 ± 0.3 mg/L as Fe). As the initial $ENP_{Fe-surf}$ loading was at 29.6 mg/L as Fe. The effluent $ENP_{Fe-surf}$ concentrations were equivalent to 7.8 ± 1.1 % of weight for the SBRs run at 3- hr HRT and 9.2 ± 1.7 % of weight. for the SBRs run at 6- hr HRT.

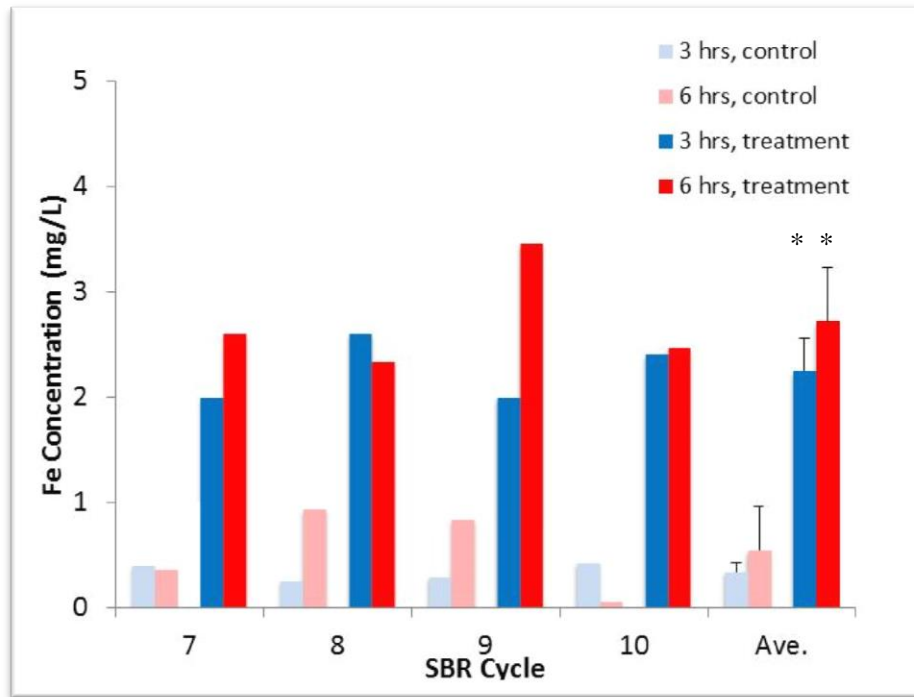


Figure 21: Effluent iron concentration of the control and treatment SBRs at different hydraulic retention times. Data shows values measured after the 7th to 10th SBR cycles. Error bars represent the standard deviations ($n = 4$). A significant difference ($p < 0.05$) of the averaged data between the control and corresponding treatment SBRs is indicated with symbol (*).

In a previous section, it was shown that 8.7% weight (on average) of the loaded ENP_{Fe-surf} were present in the effluent from the SBRs that were run with a 4-hr cycle and an ENP_{Fe-surf} loading rate of 88.9 mg/L as Fe. The effluent mean hydrodynamic particle diameters were significantly reduced from 750-910 nm to 190-240 nm due to the ENP_{Fe-surf} loading (see Figure 22). This supports the findings of the ENP_{Fe-surf} concentrations in the SBR effluents as aforementioned. This implies that ENP_{Fe-surf} will be introduced into aquatic environmental receptors through the treated effluent and potentially could adversely impact them, whereas ENP_{Fe-surf} incorporated in wastewater sludge, assuming that the rest of the ENP_{Fe-surf} are going to be found on the sludge (from mass balance), may affect terrestrial environment once disposed of.

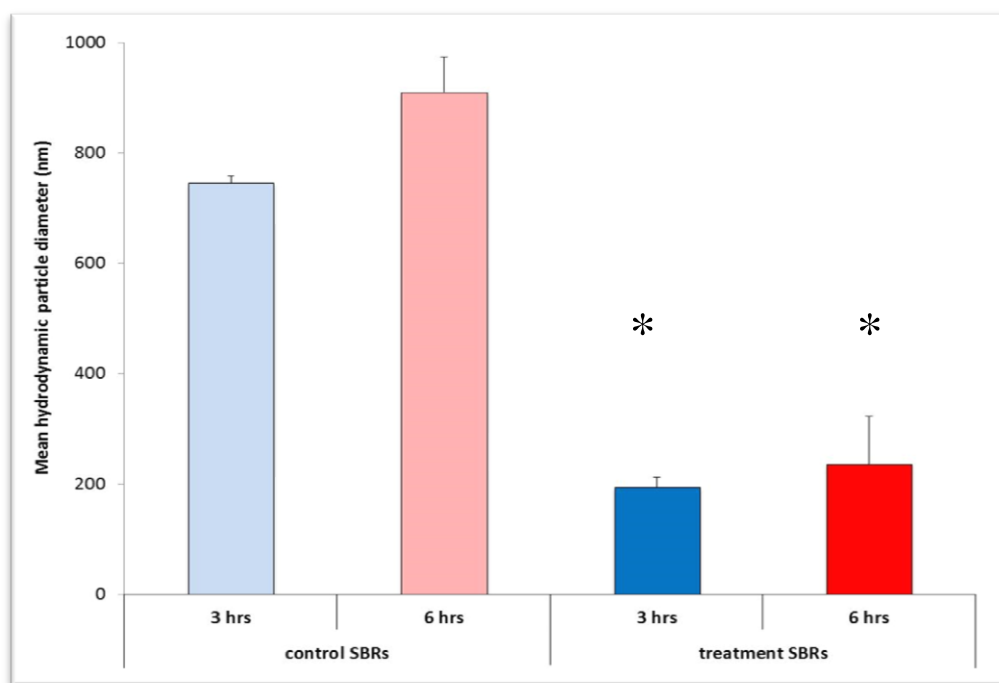


Figure 22: Mean hydrodynamic particle diameters of the control and treatment SBRs at different hydraulic retention times. Data shows the average of the values measured after the 7th to 10th SBR cycles. Error bars represent the standard deviations (n = 4). A significant difference ($p < 0.05$) of the data between the control and corresponding treatment SBRs is indicated with symbol (*).

5.4.RESPONSE OF SBR TO DIFFERENT LOADINGS OF ENP_{Fe-surf}

Findings from the studies with different ENP_{Fe-surf} loadings on effluent water quality are presented and discussed in this chapter. Experimental and analytical procedures were the same as shown in the previous chapter. Exceptions were in that SBRs were run with a 7 hr cycle consisting of 6 hr aeration, 0.5 hr settling, and 0.5 hr decant/refill and at the onset from the 7th to 10th cycles. The treatment SBRs were loaded with ENP_{Fe-surf} at either 0.5 or 1.5 mL per L of mixed liquor, which was equivalent to 29.6 or 88.9 mg/L of mixed liquor, respectively.

5.4.1. WATER QUALITY PARAMETERS

The supernatant from each sequence was collected for water quality analysis. Water characterization in terms of pH, turbidity, COD, BOD, SS, iron concentration, among others, was carried out. Sludge volume index was also measured in the reactors after each run.

5.4.1.1. TURBIDITY, sCOD, sBOD, SS CONCENTRATIONS AND pH VALUES

The effluents from the ENP_{Fe-surf} loaded treatment SBRs showed significant increases ($p < 0.05$) in sCOD (see Figure 23) and turbidity (see Figure 24) concentrations than the control SBR effluents. Between the ENP_{Fe-surf} loaded treatment SBRs, statistically higher turbidity and sCOD concentrations were observed for the SBRs with greater ENP_{Fe-surf} loadings (i.e., 88.9 vs. 29.6 mg/L as Fe).

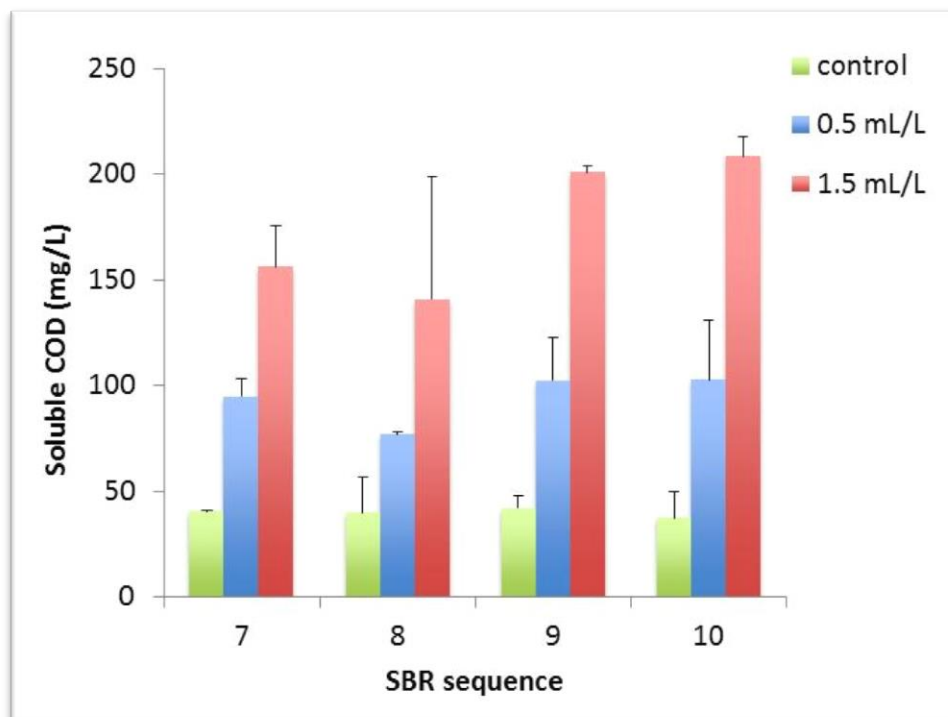


Figure 23: Concentrations of sCOD in the SBR effluents as a function of $\text{ENP}_{\text{Fe-surf}}$ loadings. Error bars are standard deviations of four replicate runs.

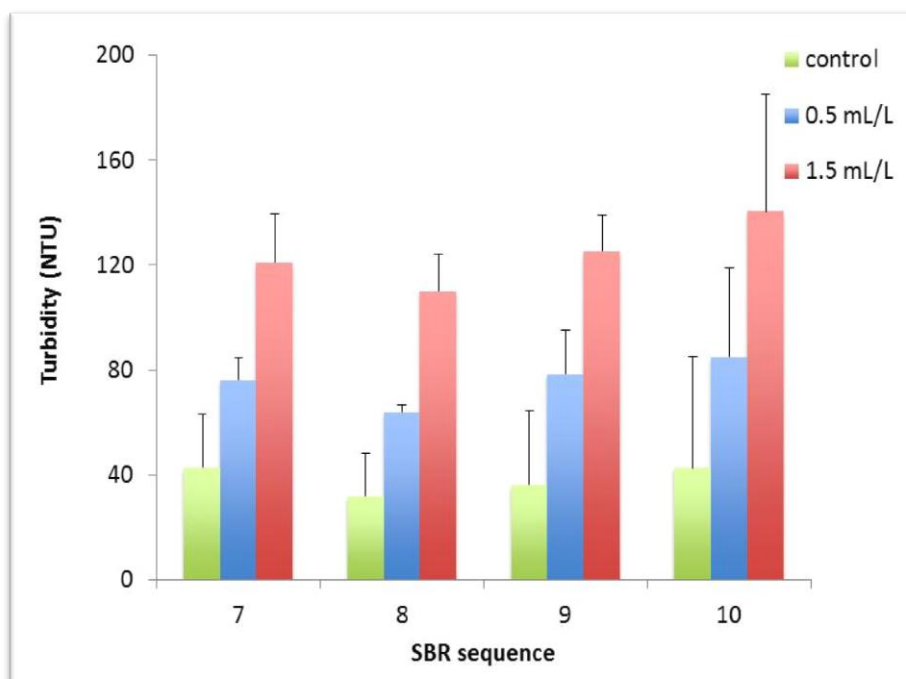


Figure 24: Turbidity in the SBR effluents as a function of $\text{ENP}_{\text{Fe-surf}}$ loadings. Error bars are standard deviations of four replicate runs.

The effluent sBOD concentrations were higher from the ENP_{Fe-surf} loaded treatment SBRs than from the control SBRs ($p < 0.05$). However, as seen in Figure 25 there were no differences in the effluent sBOD concentrations between two ENP_{Fe-surf} loaded treatment SBRs ($p > 0.05$). On the other hand, the effluent SS concentrations (see Figure 26) were generally higher for the ENP_{Fe-surf} loaded SBRs than the control SBRs, but they were not statistically different among the SBRs ($p > 0.05$).

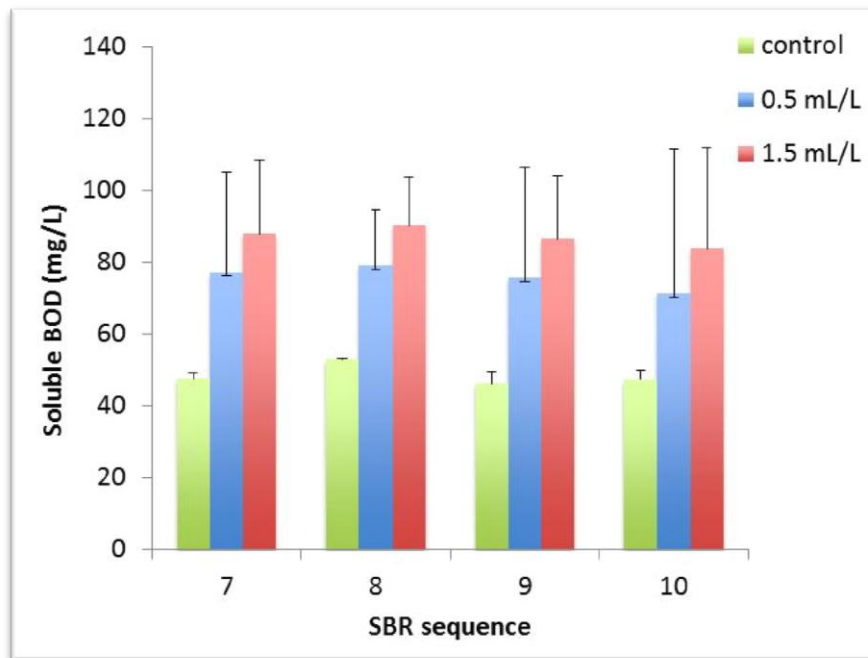


Figure 25: Concentrations of sBOD in the SBR effluents as a function of ENP_{Fe-surf} loadings. Error bars are standard deviations of four replicate runs.

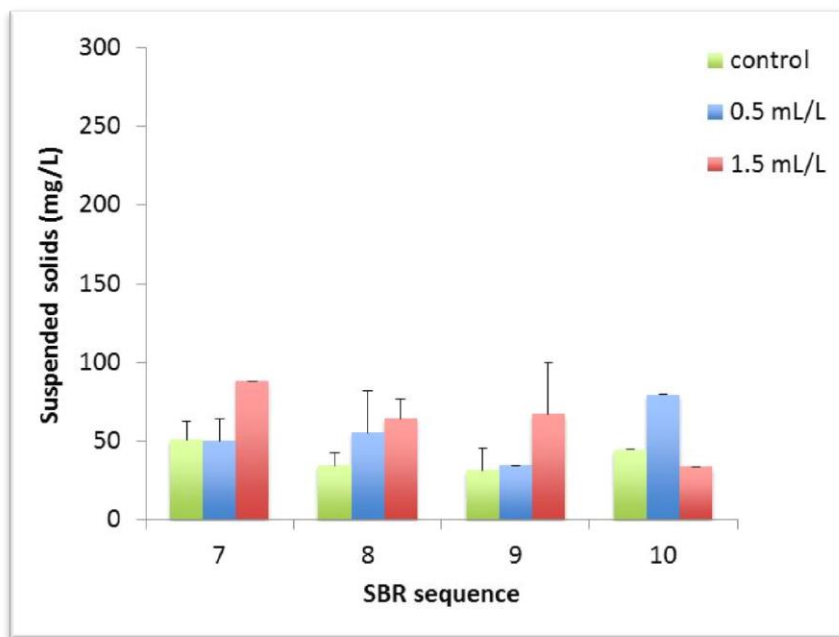


Figure 26: Suspended Solids in the SBR effluents as a function of ENP_{Fe-surf} loadings. Error bars are standard deviations of four replicate runs.

Due to the alkaline characteristics of ENP_{Fe-surf} (shown in Table 1), the treatment SBR effluents had slightly higher pH values of 7.76 ± 0.05 (n=8) and 7.80 ± 0.04 (n=8) at the ENP_{Fe-surf} loadings of 29.6 and 88.9 mg/L as Fe, respectively, than the control SBR effluent of 7.69 ± 0.11 (n=8).

5.4.1.2. IRON CONCENTRATIONS AND HYDRODYNAMIC PARTICLE DIAMETERS

Substantial increases in the Fe concentrations were found in the SBR effluents due to the ENP_{Fe-surf} loadings (Figure 27). Between two ENP_{Fe-surf}-loaded treatment SBRs, increases in the Fe concentrations were proportional to the ENP_{Fe-surf} loadings. The concentration increases from ~2.7 to ~7.6 mg/L in the effluent with the increase of the ENP_{Fe-surf} loadings from 29.6 to 88.9 mg/L as Fe. The trend was that similar fractions of the initially loaded ENP_{Fe-surf} were found in

the effluent from the ENP_{Fe-surf} SBRs loadings as shown in Figure 28 (9.3% at 29.6 mg/L loading vs. 8.7% at 88.9 mg/L loading).

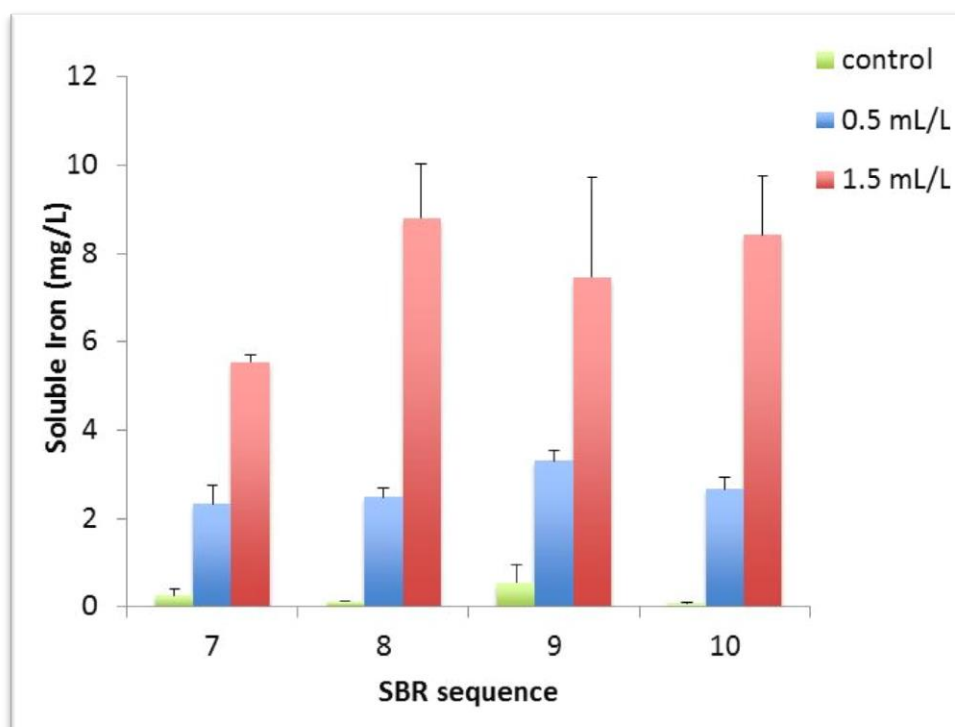


Figure 27: Soluble iron concentration in the SBR effluents as a function of ENP_{Fe-surf} loadings. Error bars are standard deviations of four replicate runs.

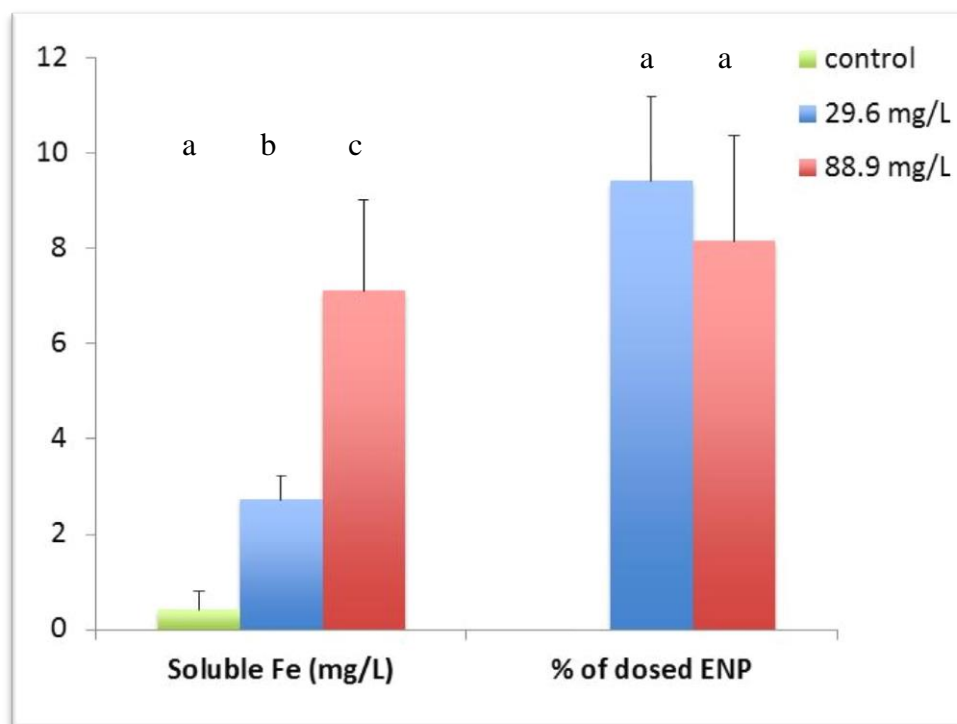


Figure 28: Fraction of soluble iron found in the effluent in terms of initial loadings. A significant difference ($p < 0.05$) of the data among the SBRs for each water quality parameter is indicated with different letters (a, b, c).

The effluent mean hydrodynamic particle diameters were significantly reduced from 760-910 nm to 150-210 nm (see Figure 29). This supports the findings of the presence of $\text{ENP}_{\text{Fe-surf}}$ (~9%) in the SBR effluents as aforementioned. As shown in Figure 30 the mean hydrodynamic particle diameters found during a period of 24 hrs in the DI water where $\text{ENP}_{\text{Fe-surf}}$ was spiked at 59.3 mg/L as Fe was 160-190 nm (170 nm on average), which are very similar to 150-210 nm in the SBR effluents. Therefore, it is unlikely that heteroaggregation of $\text{ENP}_{\text{Fe-surf}}$ with wastewater colloidal components occurred during the SBR operation.

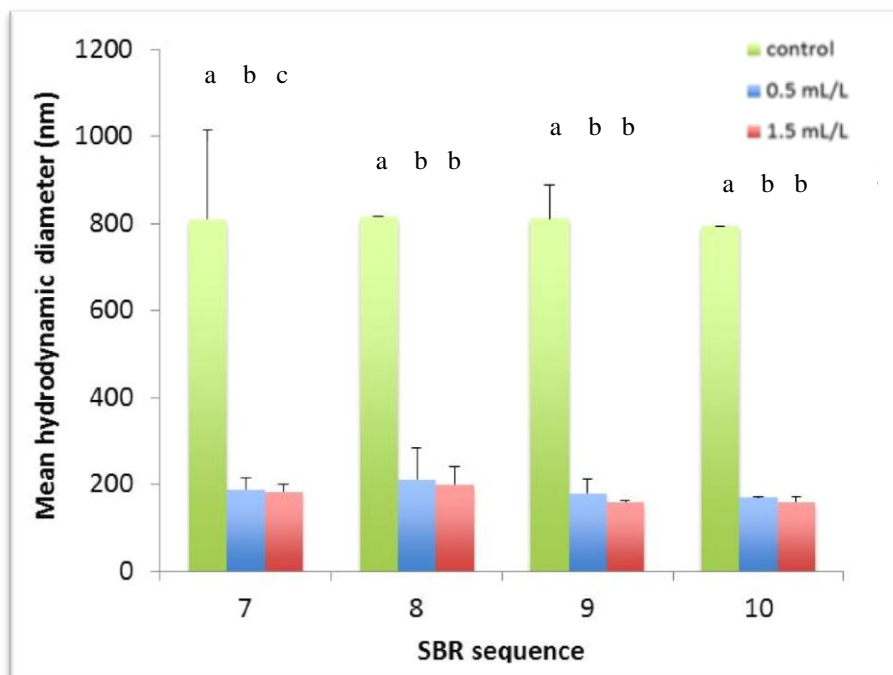


Figure 29: Mean hydrodynamic particle diameters in the SBR effluents as a function of $\text{ENP}_{\text{Fe-surf}}$ loadings. Error bars are the standard deviations of four replicate runs

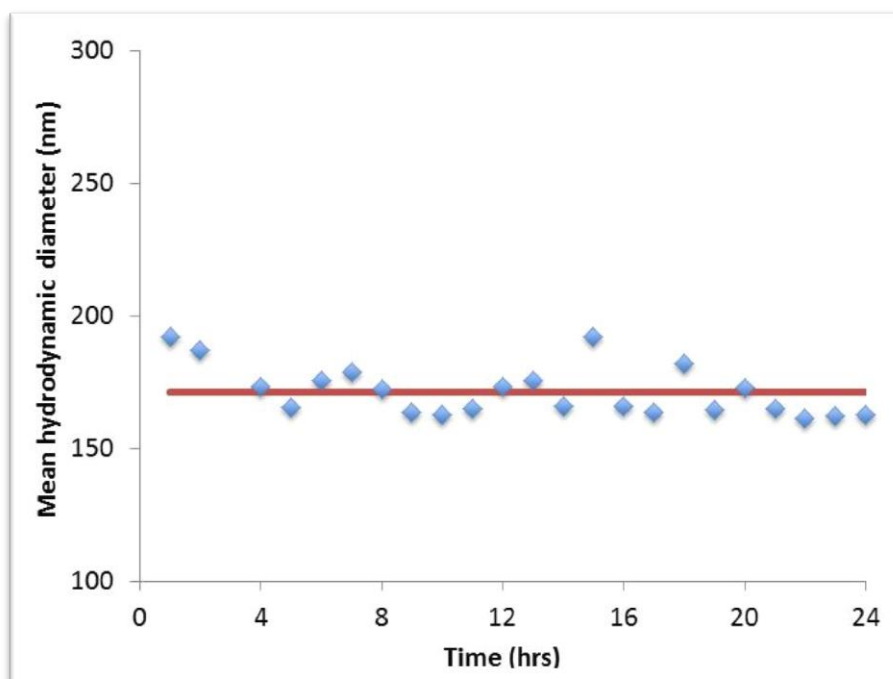


Figure 30: Mean hydrodynamic particle diameters in DI water where $\text{ENP}_{\text{Fe-surf}}$ was added at 59.3 mg/L as Fe.

5.5. RESPONSE OF ACTIVATED SLUDGE TO DIFFERENT ENP_{Fe-surf} LOADINGS

5.5.1. OXYGEN UPTAKE RATES WITH ENP_{Fe-surf} LOADINGS

The greater ENP_{Fe-surf} loading was applied to the mixed liquor, the more inhibitory respiration the system had (see Figure 31). This resulted in less OUR at a greater ENP_{Fe-surf} loading.

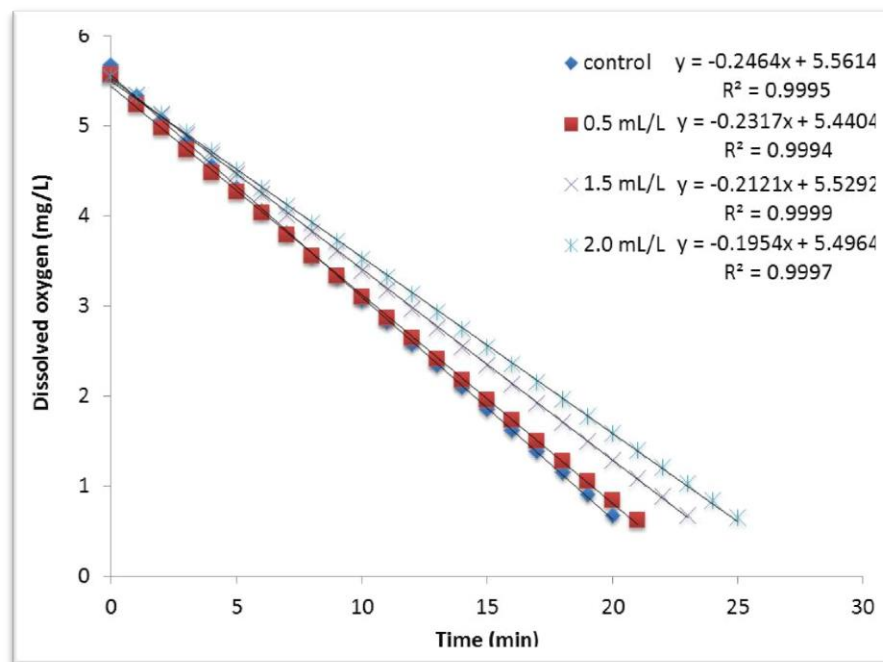


Figure 31: Reduction of oxygen uptake rate (OUR) and due to the ENP_{Fe-surf} loadings to mixed liquors.

Consequently, decreases in SOUR were observed at 0.0091 mg O₂/g MLVSS/hr per mg/L of ENP_{Fe-surf} (as Fe) loading, but the differences were not statistically significant ($p > 0.05$) (see Figure 32). SOUR inhibition was more pronounced with an increase of ENP_{Fe-surf} loadings:

on average, 10.4, 15.8 and 22.7% SOUR inhibition with $ENP_{Fe-surf}$ for loadings of 29.6, 88.9 and 110.6 mg/L as Fe, respectively. Experiments run for this information are shown in Appendix E.

It is, therefore, believed that the $ENP_{Fe-surf}$ loadings would not produce significant inhibitory effect on the biological respiration of the mixed liquor biomass, but they might have exerted inhibition to some extent. Therefore, those increases in the concentrations of effluent turbidity, sCOD, and sBOD could have also been linked to potential inhibitory respiration of activated sludge due to the $ENP_{Fe-surf}$ loadings to the SBR.

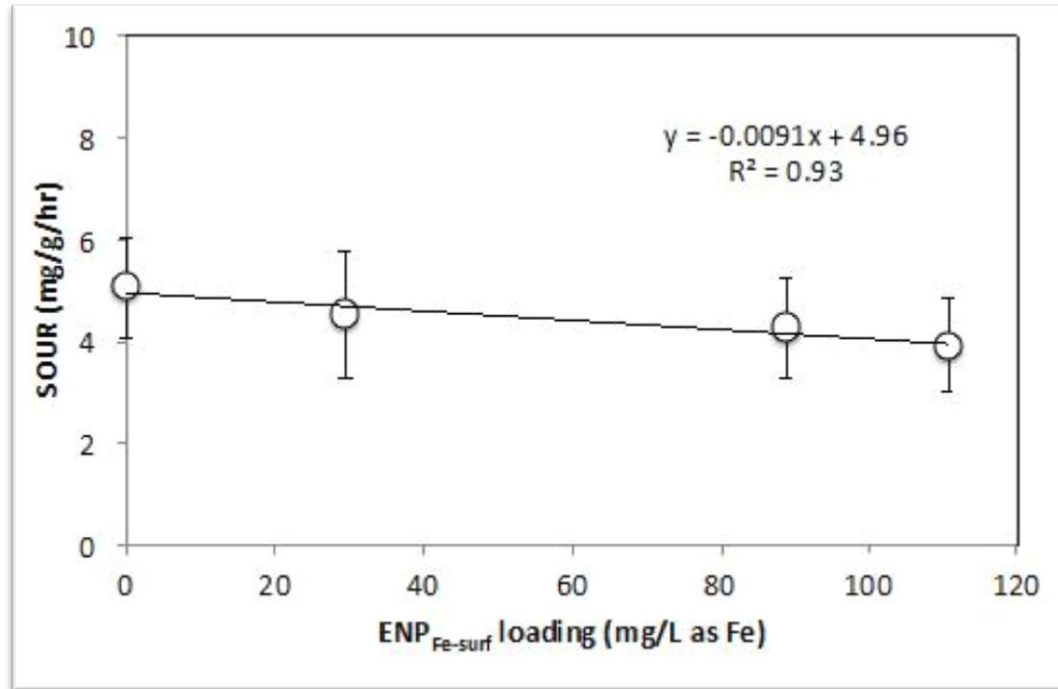


Figure 32: Reduction of specific oxygen uptake rate (SOUR) due to the $ENP_{Fe-surf}$ loadings to mixed liquors. A significant difference ($p < 0.05$) of the data was not found.

5.5.2. SLUDGE VOLUME INDEX AT DIFFERENT $ENP_{Fe-surf}$ LOADINGS

No significant changes in SVI were resulted from the $ENP_{Fe-surf}$ loadings (see Figure 33) to either good ($SVI < 100$) or bad ($SVI > 100$) settling sludge. Therefore, it is believed that the $ENP_{Fe-surf}$ loadings to MLSS did not play an important role in sludge settling. The Fe concentrations were increased proportional to the increase of $ENP_{Fe-surf}$ loadings. 96-118% of the initial $ENP_{Fe-surf}$ was present in the supernatants after 30-min SVI experiments, with the bad settling sludge having slightly lower Fe concentrations (i.e., 96%).

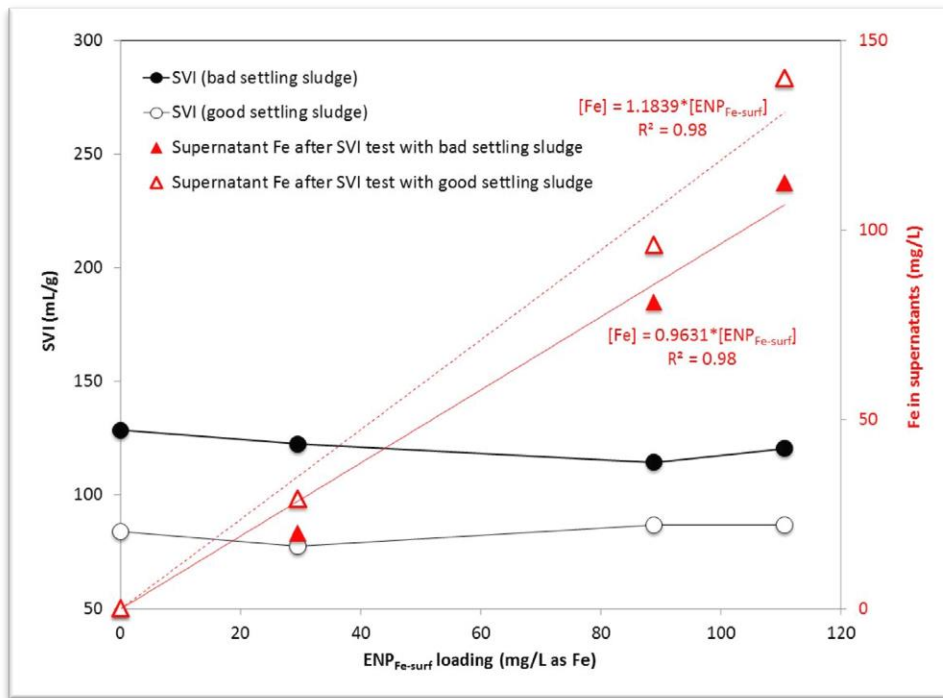


Figure 33: Sludge Volume Index (SVI) and Fe concentrations in the SVI supernatants of bad ($SVI > 100$) and good ($SVI < 100$) settling sludge.

In a separate experiment (four runs of SVI analysis are shown in Appendix F), the bad settling sludge was physically diluted 1.25 – 2 times and subjected to the SVI experiment at an $ENP_{Fe-surf}$ loading of 88.9 mg/L as Fe. Regardless of the dilutions, similar SVIs in the range of

87.4 ± 5.1 mL/g ($n=4$) were found, which was insignificantly different ($p>0.05$) from the SVIs of the good settling sludge (87 mL/g).

On the other hand, slightly increased Fe concentrations of 89.5 ± 3.4 mg/L were measured in the supernatants, compared to 81 mg/L for the undiluted sludge. Despite the different $\text{ENP}_{\text{Fe-surf}}$ loadings, the mean hydrodynamic particle diameters in the SVI supernatants were found in the range of 250 - 260 nm. In addition, the supernatants of the diluted SVI tests had slightly smaller particle diameters ranging from 230 to 250 nm due probably to the physical dilution of the MLSS.

Sludge settling was not affected by $\text{ENP}_{\text{Fe-surf}}$ loadings if it is been judged by the SVI experiment with two different sludges with $\text{SVI}>100$ (bad settling) and $\text{SVI}<100$ (good settling). SVI values below 100 are generally desired for good settling, and the values greater than 150 are typically associated with filamentous growth leading to sludge bulking (Tchobanoglous et al., 2003). As mentioned previously, this indirectly implies that partitioning/biosorption of the untreated $\text{ENP}_{\text{Fe-surf}}$ to the MLSS did not play a critical role in settling of $\text{ENP}_{\text{Fe-surf}}$. More study is, however, recommended to better understand relationship between the $\text{ENP}_{\text{Fe-surf}}$ stability and sludge settleability.

5.6. ENP_{Fe-surf} IN THE DISINFECTION

Effects of ENP_{Fe-surf} present in the treated effluent on disinfection were tested for the removal and inactivation of fecal coliforms and THB. For this, real wastewater effluent collected from the Mayagüez biological treatment plant was spiked with chlorine (Cl₂, in the form of sodium hypochlorite) and ENP_{Fe-surf}. The initial Cl₂ concentrations were targeted at 5 and 10 mg Cl₂/L, in the range of typical chlorine dosages to the effluent from biological wastewater treatments (Tchobanoglous et al., 2003). ENP_{Fe-surf} was loaded at 3.0 mg/L as Fe that was similar to those of the effluent Fe concentrations from the ENP_{Fe-surf}-loaded SBRs found in the current study. For comparison purpose, lower and higher ENP_{Fe-surf} loadings than 3.0 mg/L as Fe were also tested. After a contact time of 30 mins, the concentrations of free residual chlorine and fecal coliforms were determined. Data and pictures of the petri dishes where bacteria were counted can be found in Appendix G.

5.6.1. DISINFECTION OF ENP_{Fe-surf} CONTAINED EFFLUENT

An increase in Cl₂ consumption (i.e., Cl₂ demand) was observed as the ENP_{Fe-surf} loadings were increased when injected in wastewater (see Figure 34). The same behavior was observed when the nanoparticles were loaded in pure water. As mentioned in the previous sections, ~9% of the loaded ENP_{Fe-surf} were present in the SBR effluents. Having this under consideration, experiments with this concentration, and between those concentrations, were conducted.

When the disinfection was conducted with a lower initial Cl₂ concentration of 4.4 mg/L and greater ENP_{Fe-surf} loadings from 5.9 to 88.9 mg/L as Fe, no residual Cl₂ concentrations were determined after a 30-min contact time. On the contrary, when a higher initial Cl₂ concentration

of 11.1 mg/L and smaller $\text{ENP}_{\text{Fe-surf}}$ loadings from 1.8 to 8.9 mg/L as Fe were applied, residual Cl_2 concentrations in the range of 0.48 – 0.67 mg/L were found after a 30-min contact time with a lower $\text{ENP}_{\text{Fe-surf}}$ loading having a higher residual Cl_2 concentration.

$\text{ENP}_{\text{Fe-surf}}$ present in the effluent, as was found in this study, increased Cl_2 consumption (or Cl_2 demand). This can be attributed to the chemical reactions of Cl_2 species, such as hypochlorous acid and hypochlorite ion, with iron (Fe). Complete Cl_2 consumption was achieved at the $\text{ENP}_{\text{Fe-surf}}$ loadings of 177.6 mg/L as Fe or greater after a 30-min contact time in either pure water or real wastewater effluent. Therefore, it can be hypothesized that removal and inactivation of fecal coliforms in the disinfection of wastewater effluent would be reduced due to the Cl_2 being scavenged by the $\text{ENP}_{\text{Fe-surf}}$.

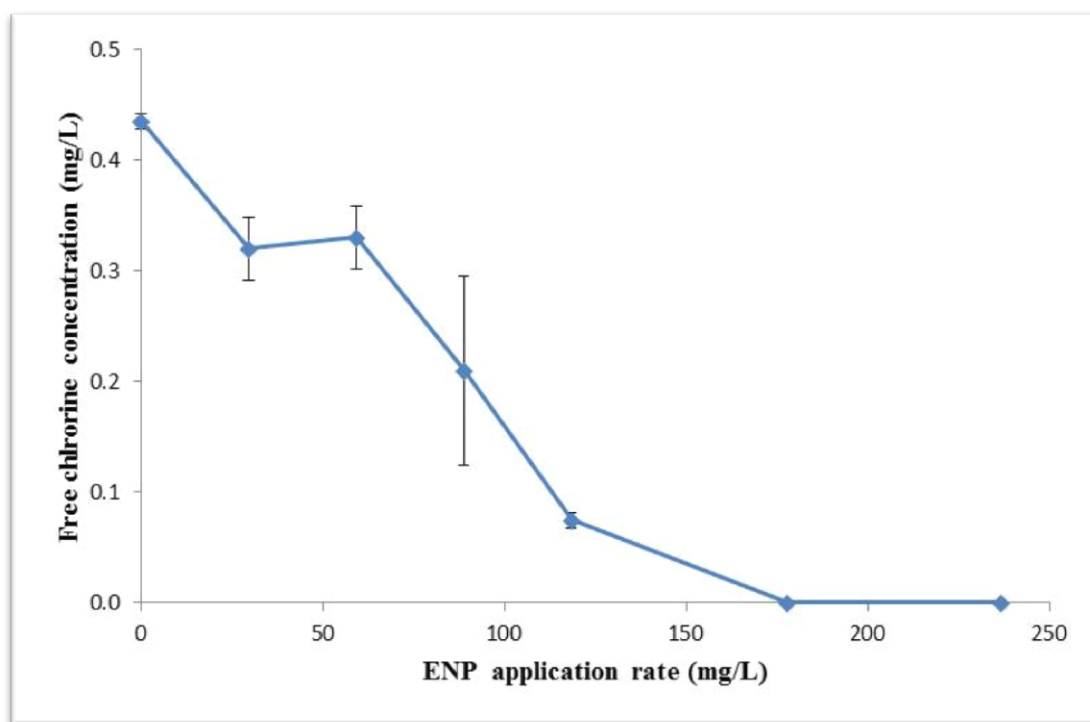


Figure 34: Concentrations of Residual Cl_2 (free) depending of ENP injection. Cl_2 was measured after a 30-min contact time. The initial chlorine concentration was at 4.12 mg/L. Number of runs for standard deviation = 4.

5.6.2. ENHANCED FECAL COLIFORM SURVIVAL IN THE PRESENCE OF $ENP_{Fe-surf}$

When $ENP_{Fe-surf}$ was loaded alone at 3.0 and 8.9 mg/L as Fe, the concentrations of fecal coliforms were not decreased but slightly increased ($p>0.05$) by 6.4 and 4.6%, respectively. A further comparison was made with fecal coliform removals at lower (1.8 mg/L as Fe) and higher (44.5 and 88.9 mg/L as Fe) $ENP_{Fe-surf}$ loadings. Results showed no changes in fecal coliform removal when $ENP_{Fe-surf}$ was loaded at 1.8 mg/L as Fe. But, when $ENP_{Fe-surf}$ was loaded at elevated concentrations of 44.5 and 88.9 mg/L as Fe, 14.7% and 36.2%, respectively, of fecal coliform removals were achieved.

Bacterial requirement for growth includes the sources of carbon and energy, growth factors, and nutrients (Tchobanoglous et al., 2003). Iron can be required as a macronutrient among other nutrients, such as nitrogen, phosphorus, sulfur and other metal ions. Therefore, it is suspected that low $ENP_{Fe-surf}$ loadings at 3.0 and 8.9 mg/L as Fe could supplement the growth requirement of fecal coliforms, resulting in a slight increase of their growth. But, $ENP_{Fe-surf}$ overload (44.5 mg/L as Fe or higher) would exhibit toxic effects on fecal coliform survival, leading to removal of fecal coliforms.

As shown in Table 11, when Cl_2 was dosed initially at 5.7 and then 11.4 mg/L in the absence of $ENP_{Fe-surf}$, the removal of fecal coliforms was achieved at >99.9 and 100%, respectively. The removal of fecal coliforms was unlikely affected in the disinfection with $ENP_{Fe-surf}$ loadings up to 8.9 mg/L as Fe. More Cl_2 was consumed with an increase of $ENP_{Fe-surf}$ loading. Therefore, the overall removal of fecal coliforms varied depending on both the concentrations of initial Cl_2 and the extent of $ENP_{Fe-surf}$ loadings.

The removal and inactivation of fecal coliforms in the disinfection process was affected negatively in the presence of the $\text{ENP}_{\text{Fe-surf}}$ in the wastewater effluent. However, such an adverse effect occurred only at the extremely elevated $\text{ENP}_{\text{Fe-surf}}$ loadings of 44.5 mg/L as Fe or greater. Therefore, it is unlikely that the $\text{ENP}_{\text{Fe-surf}}$ present in the SBR effluent at a low concentration (<10 mg/L as Fe) will cause significantly negative impact on the disinfection of fecal coliforms in the wastewater effluent as long as a disinfection takes place with the initial Cl_2 concentrations of 5.7-11.4 mg/L. For this reason it is critical to apply disinfection of wastewater effluent because low effluent $\text{ENP}_{\text{Fe-surf}}$ concentrations (<7.5 mg/L as Fe) may enhance the growth and activities of fecal coliforms, deteriorating bacteriological effluent quality.

Table 11: CHLORINE (Cl_2) DEMAND AND FECAL COLIFORM CONCENTRATIONS AS A FUNCTION OF THE INITIAL Cl_2 AND $\text{ENP}_{\text{Fe-surf}}$ CONCENTRATIONS DURING DISINFECTION.

Experimental set-up			Results		
Reactor	Initial Cl_2 (mg/L)	$\text{ENP}_{\text{Fe-surf}}$ (mg/L as Fe)	Residual Cl_2 (mg/L)	Cl_2 demand (mg/L)	Fecal coliforms* (#/100mL)
1	0	0	0	0	$(55 \pm 14) \times 10^3$
2	0	3.0	0	0	$(59 \pm 2) \times 10^3$
3	0	8.9	0	0	$(58 \pm 4) \times 10^3$
4	5.7	0	0.4	5.31	10 ± 14
5	5.7	3.0	0.4	5.33	0**
6	5.7	8.9	0.4	5.34	5 ± 7
7	11.4	0	0.7	10.67	0**
8	11.4	3.0	0.7	10.70	0**
9	11.4	8.9	0.7	10.71	0**
* Data is the average of two measurements.					
** No colonies were found at 10^1 dilutions.					

6. CONCLUSIONS AND RECOMMENDATIONS

6.1. CONCLUSIONS

Based on the results obtained, it is construed that a significant fraction (~8.7%) of ENP_{Fe-surf} applied were present in the effluent stream. The stable presence of ENP_{Fe-surf} was confirmed with the analyses of mean particle diameters and Fe concentrations in the effluent from the treatment SBR which received an ENP_{Fe-surf} application of 1.5 mL/L. Consequently, aqueous ENP_{Fe-surf} deteriorated the effluent water quality at a statistically significant level ($p < 0.05$) with respect to sCOD, turbidity, and apparent color. This implies that ENP_{Fe-surf} will be introduced into environmental receptors through the treated effluent and potentially could adversely impact them.

Deterioration in effluent water quality in terms of turbidity, soluble COD, soluble BOD and SS was found due to the ENP_{Fe-surf} loading, regardless of the SBR reaction (i.e., aeration) times. Effluent water quality was not improved with a longer SBR HRT of 6 hrs. ~7.8 and ~9.2% weight of the initially loaded ENP_{Fe-surf} were present in the effluents of SBRs run at 3- and 6- hr HRT, respectively. Sludge settling was slightly improved by 4.8-7.4% with ENP_{Fe-surf} loading of 29.6 mg/L as Fe depending on the intrinsic SVI and MLSS concentrations.

Despite the different ENP_{Fe-surf} loadings of 29.6 and 88.9 mg/L as soluble Fe, deterioration in effluent water quality was not changed. In fact, regardless of the extent of ENP_{Fe-surf} loadings and SBR hydraulic residence time, ~9% weight of the loaded ENP_{Fe-surf} was present in the SBR effluent. Deteriorated wastewater effluent quality, i.e., higher turbidity ($p < 0.05$), sCOD ($p < 0.05$), sBOD ($p > 0.05$) and SS ($p > 0.05$), was attributed mainly to the physical presence

of the stable fractions of ENP_{Fe-surf} and, to some extent, to inhibitory respiration of activated sludge produced by the ENP_{Fe-surf}.

It was unlikely that the ENP_{Fe-surf} present in the SBR effluent at a low concentration (<3 mg/L as Fe) will cause significantly negative impact on the removal fecal coliforms in the wastewater effluent as long as a disinfection takes place with the initial Cl₂ concentrations of 5.7-11.4 mg/L.

6.2. RECOMMENDATIONS

In order to gather better understanding of the influence of the presence of ENP_{Fe-surf} on effluent water quality, the following recommendations can be made:

- Run the experiment with other types of nanoparticles. Different ENPs such as Ag and TiO₂ are expected to exert dissimilar effects on biological wastewater treatment processes and resulting effluent water quality. However, it has not been quantified in a process level.
- Analyze the sludge left in the reactors with respect to ENP_{Fe-surf} since only approximately 9% of the ENP_{Fe-surf} loaded was found in the effluent. It is unknown how the ENP_{Fe-surf} incorporated to the sludge will influence sludge management processes such as anaerobic digestion, dewatering, and composting.
- Investigate potential impact of the presence of ENP_{Fe-surf} on wastewater disinfection process at different levels of disinfectants and ENP_{Fe-surf} with respect to the inactivation of pathogens.

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8. APPENDIX

A. Wastewater Characterization

Whenever wastewater was collected, analyses were made. The samples were taken during a 5 day period, Monday at 3:00 pm, Tuesday at 10:00 am, Thursday at 1:00 pm, Friday at 6:30 am, and again Monday 8:00 am. The samples were also taken at different times to be able to obtain an estimate of wastewater characteristics. During the first two days the analysis were done in duplicate, Day 1a, Day 1b, to evaluate the possible fluctuation in the values.

A.1. Nitrogen Analysis

Table 12: NITROGEN ANALYSIS FOR THE EFFLUENT WASTEWATER FOR FIVE DIFFERENT DAYS.

	Equipment Reading	Dilution	Value (mg/L)
Day 1a	OR	2	
Day 1b	6	2	12
Day 2a	0	3	0
Day 2b	0	3	0
Day 3	1	2	2
Day 4	1	2	2
Day 5	1	2	2

(Note) OR: over range

Table 13: NITROGEN ANALYSIS FOR THE INFLUENT WASTEWATER FOR FIVE DIFFERENT DAYS.

	Euipment Reading	Dilution	Value (mg/L)
Day 1a	6	3	18
Day 1b	19	3	57
Day 2a	4	3	12
Day 2b	5	3	15
Day 3	6	2	12
Day 4	4	2	8
Day 5	6	2	12

A.2. Phosphate Analysis

Table 14: PHOSPHATE ANALYSIS FOR THE EFFLUENT WASTEWATER FOR FIVE DIFFERENT DAYS.

	Equipment Reading	Dilution	Value (mg/L PO_4^{-3})
Blank	0.2	1	0.2
Day 1a	3.46	2	6.9
Day 1b	3.3	2	6.6
Day 2a	1.54	5	7.7
Day 2b	1.46	5	7.3
Day 3	1.68	5	8.4
Day 4	1.86	5	9.3
Day 5	1.78	5	8.9

Table 15: PHOSPHATE ANALYSIS FOR THE INFLUENT WASTEWATER FOR FIVE DIFFERENT DAYS.

	Equipment Reading	Dilution	Value (mg/L PO_4^{-3})
Day 1a	3.68	3	11.0
Day 1b	3.66	3	11.0
Day 2a	2.97	5	14.9
Day 2b	3.01	5	15.1
Day 3	2.66	5	13.3
Day 4	2.69	5	13.5
Day 5	2.35	5	11.8

A.3. COD Analysis

Table 16: COD ANALYSIS FOR THE EFFLUENT WASTEWATER FOR FIVE DIFFERENT DAYS.

	Equipment Reading	Dilution	Value (mg/L)
Day 1a	6	2	12
Day 1b	8	2	16
Day 2a	8	2	16
Day 2b	6	2	12
Day 3	24	2	48
Day 4	17	2	34
Day 5	6	2	12

Table 17: COD ANALYSIS FOR THE INFLUENT WASTEWATER FOR FIVE DIFFERENT DAYS.

	Equipment Reading	Dilution	Value (mg/L)
Day 1a	40	3	120
Day 1b	49	3	147
Day 2a	50	3	150
Day 2b	48	3	144
Day 3	63	3	189
Day 4	40	3	120
Day 5	45	3	135

Table 18: SOLUBLE COD ANALYSIS FOR THE EFFLUENT WASTEWATER FOR FIVE DIFFERENT DAYS.

	Equipment Reading	Dilution	Value (mg/L)
Day 1a	18	1	18
Day 1b	17	1	17
Day 2a	18	1	18
Day 2b	17	1	17
Day 3	32	1	32
Day 4	27	1	27
Day 5	23	1	23

Table 19: SOLUBLE COD ANALYSIS FOR THE INFLUENT WASTEWATER FOR FIVE DIFFERENT DAYS.

	Equipment Reading	Dilution	Value (mg/L)
Day 1a	78	1	78
Day 1b	80	1	80
Day 2a	81	1	81
Day 2b	78	1	78
Day 3	81	1	81
Day 4	60	1	60
Day 5	88	1	88

A.4. Suspended Solid

Table 20: SUSPENDED SOLID ANALYSIS FOR THE EFFLUENT WASTEWATER FOR FIVE DIFFERENT DAYS.

Label	Paper (g)	Liquid (g)	Paper+Solid (g)	Diff (g)	SS (mg/L)
Day 1a	0.1060	121.6	0.1151	0.0091	74.84
Day 1b	0.1065	124.9	0.1070	0.0005	4.00
Day 2a	0.1076	163.5	0.1080	0.0004	2.45
Day 2b	0.1071	165.7	0.1073	0.0002	1.21
Day 3a	0.1078	152.0	0.1092	0.0014	9.21
Day 3b	0.1153	151.7	0.1163	0.0010	6.59
Day 4a	0.1136	165.3	0.1124	0.0012	7.26
Day 4b	0.1084	172.5	0.1073	0.0011	6.38
Day 5a	0.1077	144.6	0.1151	0.0074	51.18
Day 5b	0.1075	134.7	0.1088	0.0013	9.65

Table 21: SUSPENDED SOLID ANALYSIS FOR THE INFLUENT WASTEWATER FOR FIVE DIFFERENT DAYS.

Label	Paper (g)	Liquid (g)	Paper+Solid (g)	Diff (g)	SS (mg/L)
Day 1a	0.1057	71.3	0.1096	0.0039	54.70
Day 1b	0.1126	104.3	0.1174	0.0048	46.02
Day 2a	0.1063	153.7	0.1125	0.0062	40.34
Day 2b	0.1067	158.1	0.1137	0.007	44.28
Day 3a	0.1083	152.4	0.1205	0.0122	80.05
Day 3b	0.1140	151.0	0.123	0.009	59.60
Day 4a	0.1084	155.9	0.1134	0.005	32.07
Day 4b	0.1087	155.4	0.1142	0.0055	35.39
Day 5a	0.1067	81.3	0.1106	0.0039	47.97
Day 5b	0.1129	114.3	0.1184	0.0055	48.12

Table 22: SUSPENDED SOLID ANALYSIS FOR THE MLSS FOR FIVE DIFFERENT DAYS.

Label	Paper (g)	Liquid (g)	Paper+Solid (g)	Diff (g)	SS (mg/L)
Day 1a	0.1164	5	0.1292	0.0128	2560
Day 1b	0.1220	10	0.1352	0.0132	1320
Day 2a	0.1154	5	0.1481	0.0327	6540
Day 2b	0.1133	5	0.146	0.0327	6540
Day 3a	0.1170	10	0.1478	0.0308	3080
Day 3b	0.1081	9	0.1388	0.0307	3411
Day 4a	0.1135	8	0.1354	0.0219	2737
Day 4b	0.1080	9	0.1286	0.0206	2288
Day 5a	0.1171	10	0.1292	0.0121	1210
Day 5b	0.1232	10	0.1372	0.0140	1400

A.5 pH

Table 23: pH ANALYSIS FOR THE EFFLUENT, INFLUENT AND MLSS FOR FIVE DIFFERENT DAYS.

Day	Effluent	Influent	MLSS
Day 1	6.41	6.47	6.5
Day 2	6.51	6.57	6.2
Day 3	6.48	6.58	6.2
Day 4	6.54	6.59	6.4
Day 5	6.51	6.57	6.6

B. Thermogravimetric Analysis (TGA) for the ENP_{Fe-surf}

To quantify the amount of iron, a TGA was performed. In a TGA, the sample was exposed to high temperatures to evaporate all the components except for iron. The following graphs were resulted from the TGA.

B.1. First and Second Run

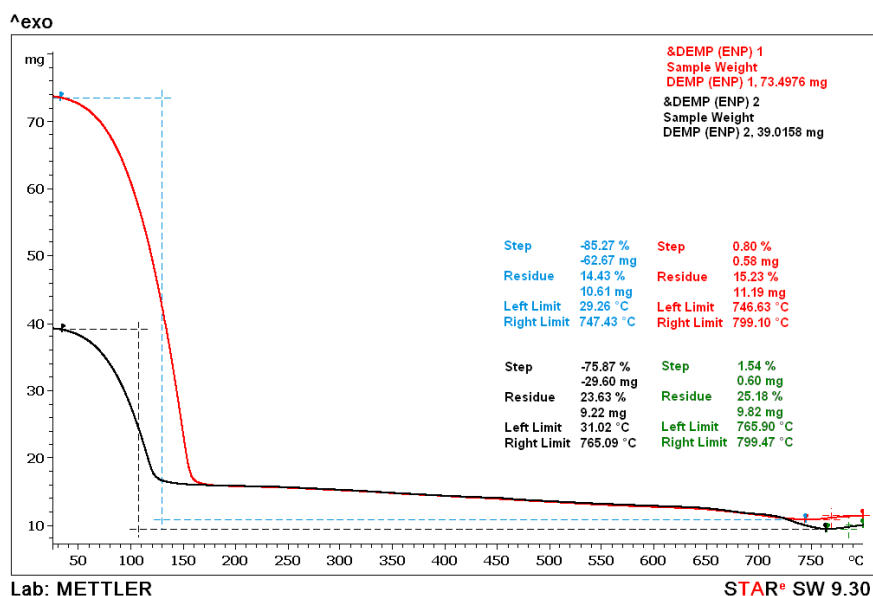


Figure 35: TGA graph generated by the equipment when ENP_{Fe-surf} were heated until 800°C for the first and Second Run.

B.2. Third and Fourth Run

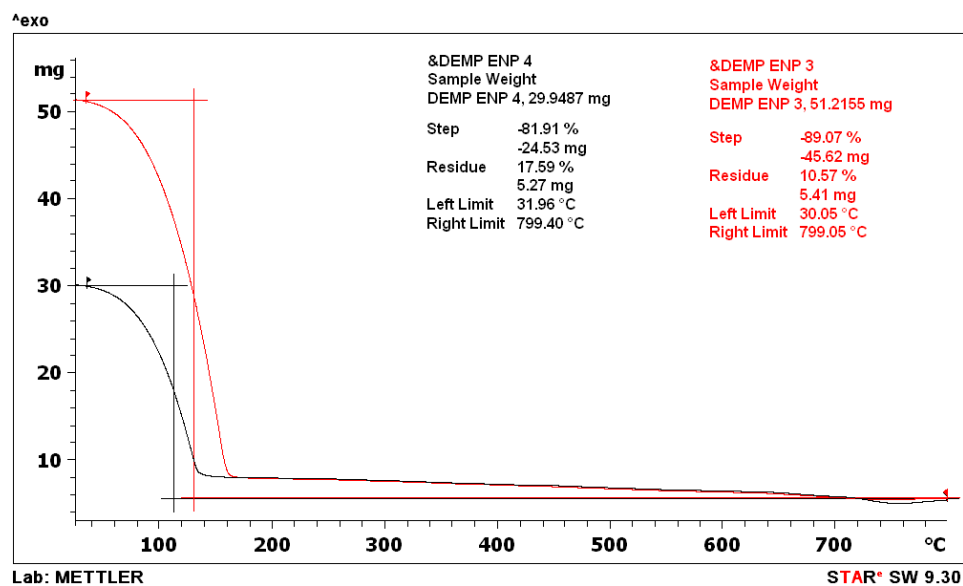


Figure 36: TGA graph generated by the equipment when ENP_{Fe-surf} were heated until 800°C for the third and Fourth Run.

C. Sequencing Batch Reactors runs

Both the control and treatment SBRs were run in the same manner up to the sixth cycle at which a pseudo stabilization in terms of effluent quality was attained. Then, only the treatment SBR received $\text{ENP}_{\text{Fe-surf}}$ from the 7th to 10th cycles. After each sequence the supernatant from the reactors were monitored for the water quality parameters.

C.1. Three hours retention time (aeration) with 1.5 mL/L $\text{ENP}_{\text{Fe-surf}}$ injection.

The treatment SBR received $\text{ENP}_{\text{Fe-surf}}$ at an application rate of 1.5 mL/L from the 7th to 10th cycles. The aeration time was three hours. After each sequence the supernatant from both reactors was collected and analyzed. Four experiments were run at these specified conditions.

C.1.1. First Experiment

Table 24: DATA OF THE DIFFERENT WATER QUALITY ANALYSIS MADE FOR THE CONTROL REACTOR

Sequence	Turbidity (NTU)	pH	Sludge Height (in)	COD (mg/L)	SS (mg/L)
7	33.0	8.14	0.125	71	49.66
8	37.0	8.12	0.125	70	62.11
9	32.0	8.25	0.125	40	53.67
10	32.0	8.30	0.063	38	49.41

Table 25: DATA OF THE DIFFERENT WATER QUALITY ANALYSIS MADE FOR THE TREATED REACTOR

Sequence	Turbidity (NTU)	pH	Sludge Height (in)	COD (mg/L)	SS (mg/L)
7	47.0	8.22	0.125	162	72.15
8	105.0	8.22	0.125	137	80.35
9	125.0	8.25	0.125	57	59.24
10	129.0	8.23	0.063	71	50.58

C.1.2. Second Experiment

Table 26: DATA OF THE DIFFERENT WATER QUALITY ANALYSIS MADE FOR THE CONTROL REACTOR

Sequence	Turbidity (NTU)	pH	Sludge Height (in)	COD (mg/L)	SS (mg/L)
7	24.7	7.65	0.313	25	41.58
8	18.8	7.61	0.313	27	32.59
9	20.9	7.65	0.313	22	41.47
10	15.1	7.69	0.313	23	35.27

Table 27: DATA OF THE DIFFERENT WATER QUALITY ANALYSIS MADE FOR THE TREATED REACTOR

Sequence	Turbidity (NTU)	pH	Sludge Height (in)	COD (mg/L)	SS (mg/L)
7	86.3	7.71	0.250	71	17.20
8	90.8	7.73	0.313	101	59.92
9	99.1	7.82	0.250	104	75.98
10	92.5	7.78	0.250	108	49.90

C.1.3. Third Experiment

Table 28: DATA OF THE DIFFERENT WATER QUALITY ANALYSIS MADE FOR THE CONTROL REACTOR

Sequence	Turbidity (NTU)	pH	Sludge Height (in)	COD (mg/L)	SS (mg/L)
7	54.8	7.09	0.375	63	99.50
8	50.5	7.08	0.375	54	71.12
9	62.5	7.05	0.375	84	121.21
10	69.7	6.97	0.375	84	103.37

Table 29: DATA OF THE DIFFERENT WATER QUALITY ANALYSIS MADE FOR THE TREATED REACTOR

Sequence	Turbidity (NTU)	pH	Sludge Height (in)	COD (mg/L)	SS (mg/L)
7	162.0	7.26	0.375	85	184.62
8	171.0	7.15	0.375	99	186.60
9	182.0	7.13	0.375	110	197.47
10	180.0	7.10	0.375	114	157.22

C.1.4. Fourth Experiment

Table 30: DATA OF THE DIFFERENT WATER QUALITY ANALYSIS MADE FOR THE CONTROL REACTOR

Sequence	Turbidity (NTU)	pH	Sludge Height (in)	sCOD (mg/L)	SS (mg/L)	Iron (Abs)	Iron (mg/L)
7	26.3	7.63	0.375	52	122.03	0.008	0.23
8	21.8	7.59	0.313	49	121.15	0.003	0.06
9	18.3	7.85	0.375	40	113.56	0.004	0.09
10	17.6	7.83	0.375	35	77.28	0.001	<0.02

Table 31: DATA OF THE DIFFERENT WATER QUALITY ANALYSIS MADE FOR THE TREATED REACTOR

Sequence	Turbidity (NTU)	pH	Sludge Height (in)	sCOD (mg/L)	SS (mg/L)	Iron (Abs)	Iron (mg/L)
7	41.0	7.71	0.375	139	60.34	0.166	5.62
8	68.9	7.83	0.313	175	40.46	0.243	8.25
9	105.0	7.90	0.375	192	55.89	0.257	8.72
10	131.0	7.79	0.313	191	73.37	0.254	8.62

C.2. Six-hour retention time with 0.5mL/L and 1.5mL/L ENP injections.

The treatment SBRs received $\text{ENP}_{\text{Fe-surf}}$ at an application rate of 1.5 mL/L or 0.5mL/L from the 7th to 10th cycles. The aeration time was six hours. After each sequence the supernatant from the reactors was collected and analyzed. Two experiments were run at these specified conditions.

C.2.1. First Experiment

Table 32: DATA OF THE DIFFERENT WATER QUALITY ANALYSIS MADE FOR THE CONTROL REACTOR

Sequence	Turbidity (NTU)	pH	Sludge Height (in)	sCOD (mg/L)	SS (mg/L)	SBOD (mg/L)	Iron (Abs)	Iron (mg/L)
7	57.4	7.68	0.500	41	59.45	56.1	0.012	0.36
8	43.3	7.51	0.563	52	40.26	51.9	0.049	1.63
9	56.2	7.73	0.375	46	74.29	50.4	0.026	0.84
10	72.8	7.82	0.375	46	101.96	58.0	0.003	0.06

Table 33: DATA OF THE DIFFERENT WATER QUALITY ANALYSIS MADE FOR THE TREATED (1.5 mL/L) REACTOR

Sequence	Turbidity (NTU)	pH	Sludge Height (in)	sCOD (mg/L)	SS (mg/L)	SBOD (mg/L)	Iron (Abs)	Iron (mg/L)
7	134.0	7.75	0.375	170	88.05	73.3	0.161	5.45
8	100.0	7.72	0.500	100	73.12	80.8	0.085	2.85
9	135.0	7.64	0.563	199	90.15	74.0	0.173	5.86
10	172.0	7.83	0.563	215	216.25	64.0	0.220	7.46

Table 34: DATA OF THE DIFFERENT WATER QUALITY ANALYSIS MADE FOR THE TREATED (0.5 mL/L) REACTOR

Sequence	Turbidity (NTU)	pH	Sludge Height (in)	sCOD (mg/L)	SS (mg/L)	SBOD (mg/L)	Iron (Abs)	Iron (mg/L)
7	82.1	7.70	0.375	89	60.09	75.3	0.078	2.61
8	61.8	7.71	0.438	78	74.18	73.3	0.020	0.64
9	90.4	7.58	0.500	117	170.88	70.0	0.103	3.47
10	109.0	7.79	0.500	123	79.58	61.7	0.174	5.89

C.2.2. Second Experiment

Table 35: DATA OF THE DIFFERENT WATER QUALITY ANALYSIS MADE FOR THE CONTROL REACTOR

Sequence	Turbidity (NTU)	pH	Sludge Height (in)	sCOD (mg/L)	SS (mg/L)	SBOD (mg/L)	Iron (Abs)	Iron (mg/L)
7	28.7	7.56	0.500	40	42.31	46.5	0.006	0.16
8	20.3	7.64	0.563	28	28.83	53.3	0.005	0.12
9	16.7	7.77	0.375	38	22.24	48.6	0.009	0.26
10	12.7	7.77	0.375	29	44.88	49.1	0.004	0.09

Table 36: DATA OF THE DIFFERENT WATER QUALITY ANALYSIS MADE FOR THE TREATED (1.5 mL/L) REACTOR

Sequence	Turbidity (NTU)	pH	Sludge Height (in)	sCOD (mg/L)	SS (mg/L)	SBOD (mg/L)	Iron (Abs)	Iron (mg/L)
7	108.0	7.83	0.375	143	123.69	102.5	0.167	5.65
8	120.0	7.85	0.500	182	55.91	99.8	0.234	7.94
9	116.0	7.86	0.563	203	44.78	99.0	0.267	9.07
10	109.0	7.88	0.563	202	34.05	103.5	0.276	9.37

Table 37: DATA OF THE DIFFERENT WATER QUALITY ANALYSIS MADE FOR THE TREATED (0.5 mL/L) REACTOR

Sequence	Turbidity (NTU)	pH	Sludge Height (in)	sCOD (mg/L)	SS (mg/L)	SBOD (mg/L)	Iron (Abs)	Iron (mg/L)
7	70.7	7.83	0.375	101	40.34	96.8	0.061	2.03
8	65.9	7.74	0.438	76	37.20	90.0	0.078	2.61
9	66.3	7.88	0.500	88	34.63	97.3	0.093	3.13
10	61.3	7.88	0.500	83	13.02	99.7	0.085	2.85

C.3. Three-hour retention time (aeration) with 0.5 mL/L ENP injection.

The treatment SBR received $ENP_{Fe-surf}$ at an application rate of 0.5 mL/L from the 7th to 10th cycles. The aeration time was three hours. After each sequence the supernatant from both reactors was collected and analyzed. Two experiments were run at these specified conditions.

C.3.1. First Experiment

Table 38: DATA OF THE DIFFERENT WATER QUALITY ANALYSIS MADE FOR THE CONTROL REACTOR

Sequence	Turbidity (NTU)	pH	Sludge Height (in)	sCOD (mg/L)	SS (mg/L)	SBOD (mg/L)	Iron (Abs)	Iron (mg/L)
7	58.0	7.56	0.375	43	49.82	54.5	0.013	0.40
8	61.0	7.67	0.313	46	40.58	58.6	0.009	0.26
9	61.8	7.61	0.375	44	52.63	52.2	0.010	0.29
10	55.2	7.70	0.375	50	50.65	33.6	0.014	0.43

Table 39: DATA OF THE DIFFERENT WATER QUALITY ANALYSIS MADE FOR THE TREATED (0.5 mL/L) REACTOR

Sequence	Turbidity (NTU)	pH	Sludge Height (in)	sCOD (mg/L)	SS (mg/L)	SBOD (mg/L)	Iron (Abs)	Iron (mg/L)
7	78.9	7.59	0.438	91	63.60	100.5	0.060	2.00
8	97.3	7.61	0.500	120	73.72	74.3	0.078	2.61
9	76.7	7.51	0.438	75	61.81	71.8	0.060	2.00
10	93.7	7.67	0.500	103	59.50	105.5	0.072	2.41

C.3.2. Second Experiment

Table 40: DATA OF THE DIFFERENT WATER QUALITY ANALYSIS MADE FOR THE CONTROL REACTOR

Sequence	Turbidity (NTU)	pH	Sludge Height (in)	sCOD (mg/L)	SS (mg/L)	SBOD (mg/L)	Iron (Abs)	Iron (mg/L)
7	8.9	7.40	0.438	24	0.89	10.1	0.002	0.02
8	11.5	8.34	0.531	21	7.69	6.8	0.001	<0.02
9	9.0	8.31	0.625	25	7.13	8.3	0.009	0.26
10	9.6	8.26	0.750	28	13.07	6.9	0.009	0.26

Table 41: DATA OF THE DIFFERENT WATER QUALITY ANALYSIS MADE FOR THE TREATED (0.5 mL/L) REACTOR

Sequence	Turbidity (NTU)	pH	Sludge Height (in)	sCOD (mg/L)	SS (mg/L)	SBOD (mg/L)	Iron (Abs)	Iron (mg/L)
7	66.8	8.20	0.375	52	21.90	4.3	0.056	1.85
8	77.0	8.34	0.563	72	29.65	14.6	0.080	2.68
9	74.0	8.36	0.719	82	23.46	16.5	0.088	2.96
10	79.7	8.37	0.813	83	28.89	17.9	0.091	3.06

D. DLS Analysis

DLS analysis was conducted to check the potential of heteroaggregation of ENP_{Fe-surf} with clays and humic acid that are present abundant in wastewaters. “Intensity” and “surf. area” appeared in Tables 42-46 means the diameters of the particles were measured by the DLS with an autocorrelation of the intensity and surface area functions, respectively.

Table 42: DYNAMIC LIGHT SCATTERING ANALYSIS OF ENP_{Fe-surf} IN A PERIOD OF 24 HOUR.

ENP		
Time (hr)	Intensity (nm)	Surf. Area (nm)
1	191.9	49.9
2	186.9	120.4
3	149.7	0.9
4	173.0	120.3
5	165.2	77.3
6	175.3	113.5
7	178.6	155.1
8	172.1	78.0
9	163.7	45.8
10	162.8	53.8
11	165.0	44.0
12	173.0	86.7
13	175.4	53.2
14	165.9	55.7
15	191.8	26.5
16	165.7	77.1
17	163.7	48.7
18	181.8	129.0
19	164.4	46.8
20	172.9	43.9
21	164.9	57.9
22	161.1	66.1
23	162.4	58.3
24	162.8	58.2

Table 43: DYNAMIC LIGHT SCATTERING ANALYSIS OF CLAY IN A PERIOD OF 24 HOUR.

Clay		
Time (hr)	Intensity (nm)	Surf. Area (nm)
1	869.9	818.5
2	1177.8	848.3
3	1034.1	994.0
4	870.7	870.0
5	967.4	996.7
6	961.1	965.2
7	901.3	777.3
8	1083.1	554.5
9	955.9	958.4
10	879.6	910.3
11	889.3	776.3
12	0.1	0.1
13	914.3	942.2
14	889.0	817.5
15	815.2	852.9
16	804.2	803.2
17	1067.2	691.5
18	852.1	763.9
19	802.8	756.9
20	843.4	738.4
21	811.2	768.0
22	828.7	815.3
23	714.7	733.5
24	1019.4	625.1

Table 44: DYNAMIC LIGHT SCATTERING ANALYSIS OF HUMIC ACID IN A PERIOD OF 24 HOUR.

Humic Acid		
Time (hr)	Intensity (nm)	Surf. Area (nm)
1	517.5	395.8
2	461.2	405.1
3	538.5	119.5
4	500.7	429.4
5	493.3	438.0
6	457.3	444.2
7	459.1	430.2
8	476.1	419.4
9	447.5	397.0
10	404.5	342.7
11	414.0	266.3
12	441.2	403.0
13	0.1	0.1
14	377.6	221.3
15	415.1	404.1
16	375.0	290.4
17	425.0	427.0
18	412.6	211.2
19	446.5	194.1
20	0.1	0.1
21	371.8	184.2
22	392.0	221.3
23	406.7	366.3
24	434.4	414.1

Table 45: DYNAMIC LIGHT SCATTERING ANALYSIS OF THE ENP_{Fe-surf} AND CLAY IN A PERIOD OF 24 HOUR.

Clay + ENP		
Time (hr)	Intensity (nm)	Surf. Area (nm)
1	354.9	175.4
2	371.6	194.7
3	393.6	154.7
4	390.0	67.0
5	385.6	121.2
6	370.8	192.0
7	388.0	154.2
8	371.5	183.3
9	377.6	167.4
10	370.6	167.3
11	350.2	84.4
12	361.0	186.9
13	357.2	192.6
14	354.9	194.4
15	359.6	196.0
16	343.4	204.2
17	339.7	212.1
18	341.7	200.9
19	328.5	206.5
20	328.2	205.8
21	323.7	229.4
22	328.2	221.5
23	325.5	230.9
24	330.3	225.2

Table 46: DYNAMIC LIGHT SCATTERING ANALYSIS OF THE ENP_{Fe-surf} AND CLAY IN A PERIOD OF 24 HOUR.

Clay + ENP		
Time (hr)	Intensity (nm)	Surf. Area (nm)
1	354.9	175.4
2	371.6	194.7
3	393.6	154.7
4	390.0	67.0
5	385.6	121.2
6	370.8	192.0
7	388.0	154.2
8	371.5	183.3
9	377.6	167.4
10	370.6	167.3
11	350.2	84.4
12	361.0	186.9
13	357.2	192.6
14	354.9	194.4
15	359.6	196.0
16	343.4	204.2
17	339.7	212.1
18	341.7	200.9
19	328.5	206.5
20	328.2	205.8
21	323.7	229.4
22	328.2	221.5
23	325.5	230.9
24	330.3	225.2

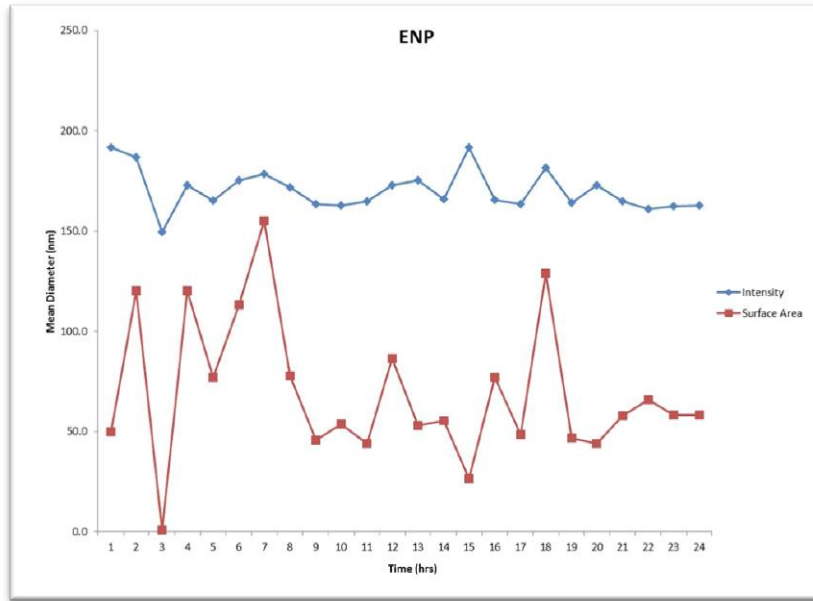


Figure 37: Mean Diameter of the ENP_{Fe-surf} in a 24 hour period in terms of Intensity and Surface area.

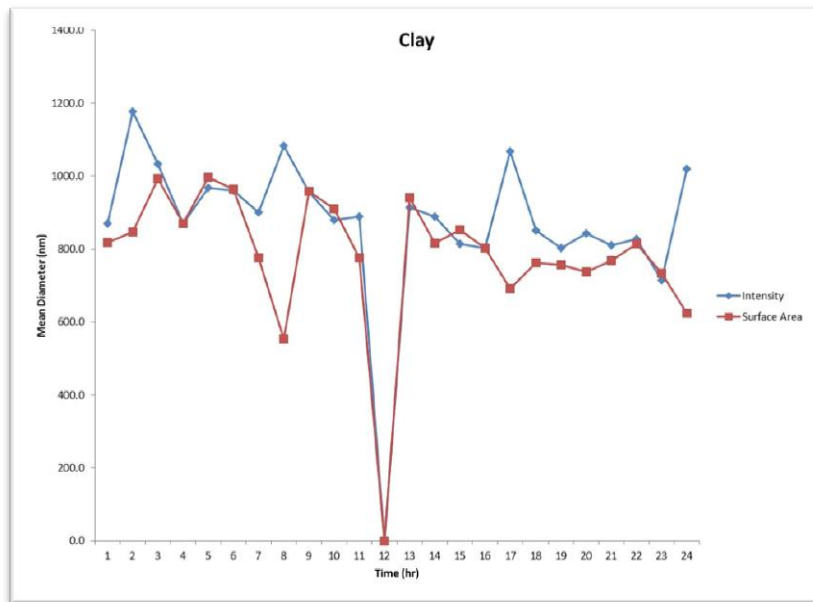


Figure 38: Mean Diameter of the ENP_{Fe-surf} in a 24 hour period in terms of Intensity and Surface area.

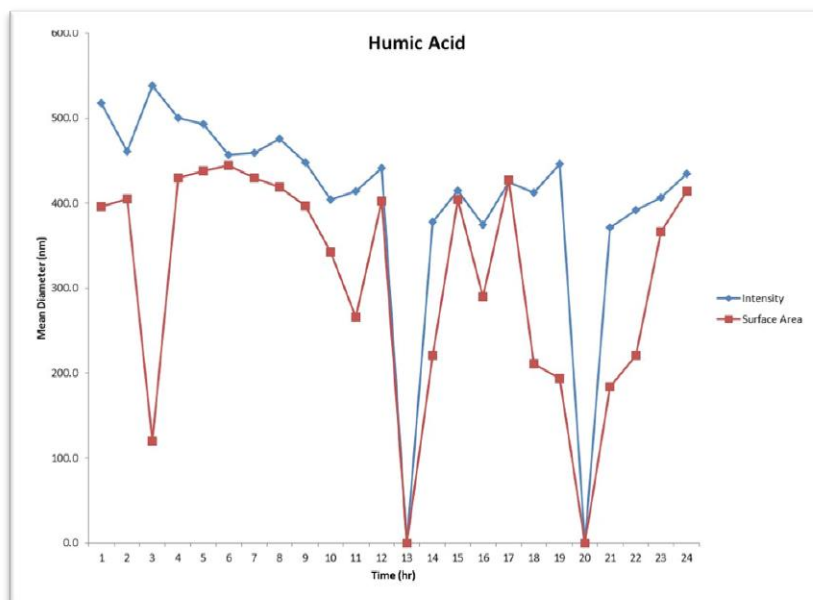


Figure 39: Mean Diameter of the ENP_{Fe-surf} in a 24 hour period in terms of Intensity and Surface area.

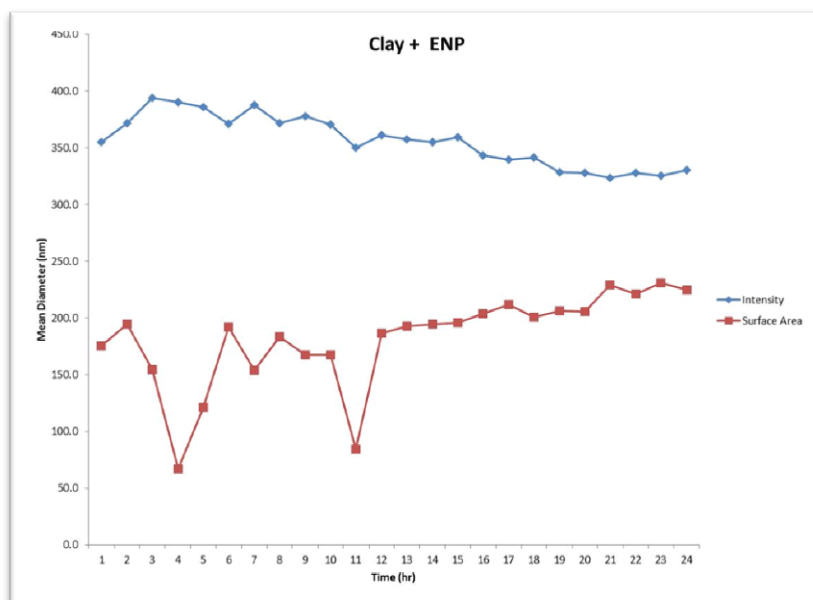


Figure 40: Mean Diameter of the ENP_{Fe-surf} in a 24 hour period in terms of Intensity and Surface area.

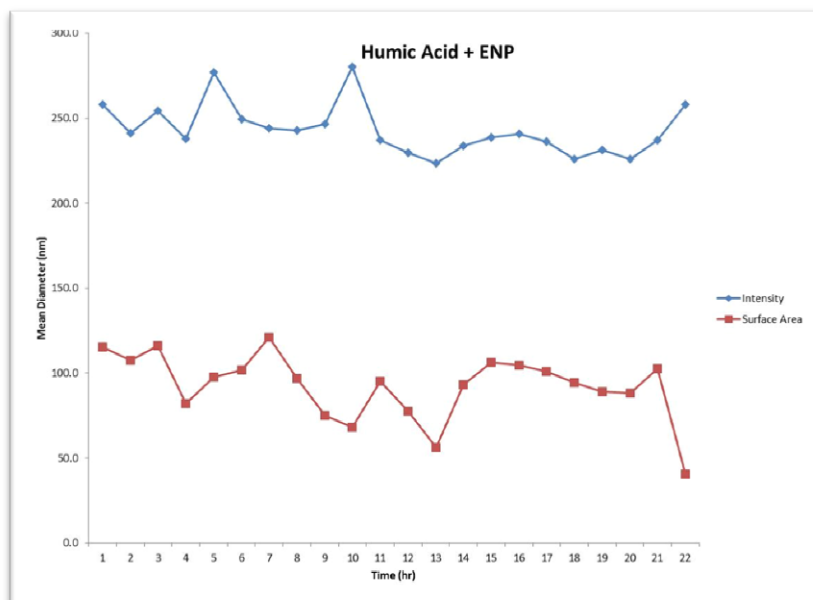


Figure 41: Mean Diameter of the ENP_{Fe-surf} in a 24 hour period in terms of Intensity and Surface area.

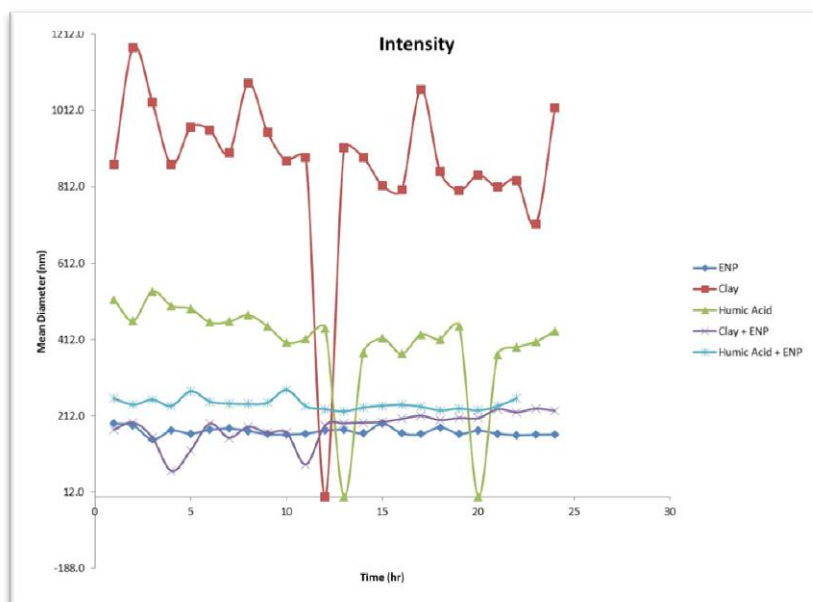


Figure 42: Comparison of all Mean Diameters in a 24 hour period in terms of Intensity.

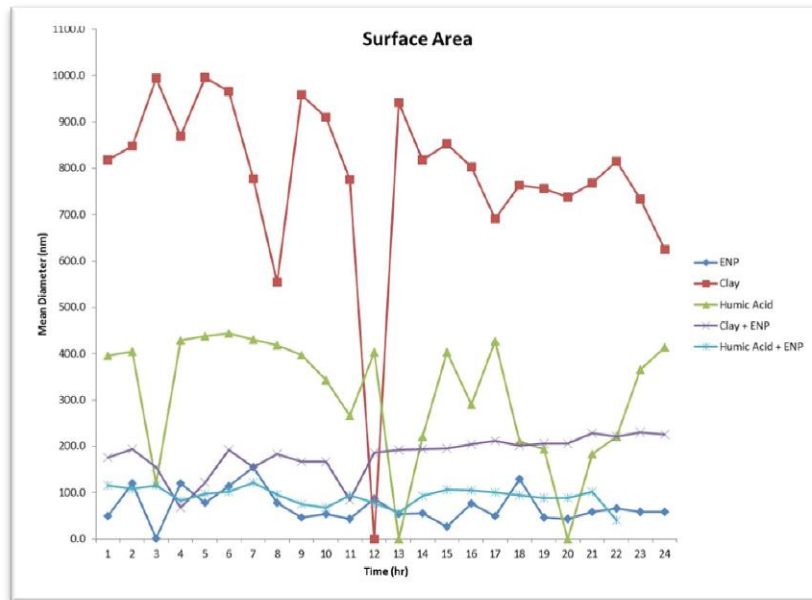


Figure 43: Comparison of all Mean Diameters in a 24 hour period in terms of Surface Area.

E. OUR Experiment

Oxygen uptake rate (OUR) tests were conducted to assess the impact of $\text{ENP}_{\text{Fe-surf}}$ on the respiration of mixed liquors microorganisms. Pre-aerated mixed liquor samples were placed in BOD bottles where the different concentrations of $\text{ENP}_{\text{Fe-surf}}$ were loaded.

Table 47: OUR AND SOUR CONCENTRATION DUE TO THE $\text{ENP}_{\text{Fe-surf}}$ LOADINGS. FIRST TRIAL

ENP	OUR	SOUR at 24 °C	SOUR at 20 °C
mL/L	mg/L/min	mg/g/h	mg/g/h
0	0.044	1.13	0.93
0.25	0.040	1.03	0.85
0.5	0.039	1.01	0.83
1	0.038	0.97	0.80
2	0.035	0.89	0.73

Table 48: OUR AND SOUR CONCENTRATION DUE TO THE $\text{ENP}_{\text{Fe-surf}}$ LOADINGS. SECOND TRIAL

ENP	OUR	SOUR at 24 °C	SOUR at 20 °C
mL/L	mg/L/min	mg/g/h	mg/g/h
0	0.035	0.90	0.74
0.25	0.030	0.77	0.64
0.5	0.029	0.75	0.61
1	0.028	0.73	0.60
2	0.026	0.67	0.55

F. Sludge Volume Index at Different $\text{ENP}_{\text{Fe-surf}}$ Application Rate.

To determine potential effect of $\text{ENP}_{\text{Fe-surf}}$ on sludge settling, the sludge volume index (SVI) was measured in accordance to the Standard Methods. Each cylinder was loaded with a certain amount of $\text{ENP}_{\text{Fe-surf}}$.

F.1. Water Quality Analysis

Afterwards sludge volume, turbidity, color, and pH of supernatant were analyzed. For the color analysis, the supernatant were filtered first. Four run were made.

F.1.1. First and Second Run

Table 49: RESULTS FOR FIRST AND SECOND SVI EXPERIMENT

ENP(mL/L)	Sludge Volume(mL)			Turbidity (NTU)			pH			Color (units of ptCo APHA)		
	1	2	Avg	1	2	Avg	1	2	Avg	1	2	Avg
0	785	675	730	2.24	3.89	3.065	7.06	6.7	6.88	17	8	12.5
0.5	738	600	669	25.4	30.9	28.15	7.05	6.7	6.875	396	226	311
1.5	815	673	744	134	78.1	106.05	7.23	6.8	7.015	4710	634	2672
3	805	765	785	167	125	146	7.25	6.8	7.025	6500	1258	3879
4	778	550	664	211	149	180	7.35	6.9	7.125	8600	1633	5116.5
5	755	470	612.5	213	244	228.5	7.33	6.9	7.115	10300	2002	6151

F.1.2. Third Run

Table 50: RESULTS FOR THIRD SVI EXPERIMENT

ENP (mL/L)	Sludge V (mL)	Turbidity(NTU)	pH	Color (units of ptCo APHA)
0	50	23.6	6.63	4
0.5	115	117	6.54	1980
1.5	80	134	6.54	920
3	15	175	6.63	1560
4	20	172	6.61	1500
5	12	206	6.55	1680

F.1.3. Fourth Run

Table 51: RESULTS FOR FOURHT SVI EXPERIMENT

ENP(mL/L)	Sludge Vol. (mL)	Turbidity (NTU)	pH	Color (units of ptCo APHA)
0	310	5.75	6.49	23
0.5	370	32.7	6.68	810
1.5	390	71.7	6.58	540
3	440	116	6.66	8100
4	390	138	6.59	4600
5	410	153	6.69	11800

G. Bacteriological Analysis

Effects of $\text{ENP}_{\text{Fe-surf}}$ present in the treated effluent on disinfection were tested for the removal and inactivation of fecal coliforms and THB. For this, real wastewater effluent collected from the Mayagüez biological wastewater treatment plant was spiked with chlorine. Different scenarios were analyzed.

G.1. ENP and CHLORINE to EFFLUENT

Table 52: CHLORINE DEMAND AND FECAL COLIFORM CONCENTRATIONS AS A FUNCTION OF THE INITIAL Cl_2 AND $\text{ENP}_{\text{Fe-surf}}$ INJECTION. THE ADDITIONS WERE ADDED TO EFFLUENT FROM THE WASTEWATER TREATMENT PLANT.

Reactor	ENP (mL)	ENP (mg/L)	pH	Initial Cl_2 (mg/L)	Residual Cl_2 (mg/L)	Dilution	Bacteria counted	Bacteria Amount*
1	0.00	0.00	8.17	20.0	0.57	100	1	1×10^2
2	0.10	5.92	8.17	20.0	0.27	100	0	0
3	0.30	17.76	8.20	20.0	0.00	100	4	4×10^2
4	0.50	29.60	8.40	20.0	0.00	100	139	139×10^2
5	0.75	44.40	8.67	20.0	0.00	100	197	197×10^2

* Colony counted per 100 mL (i.e., CFU/100 mL)

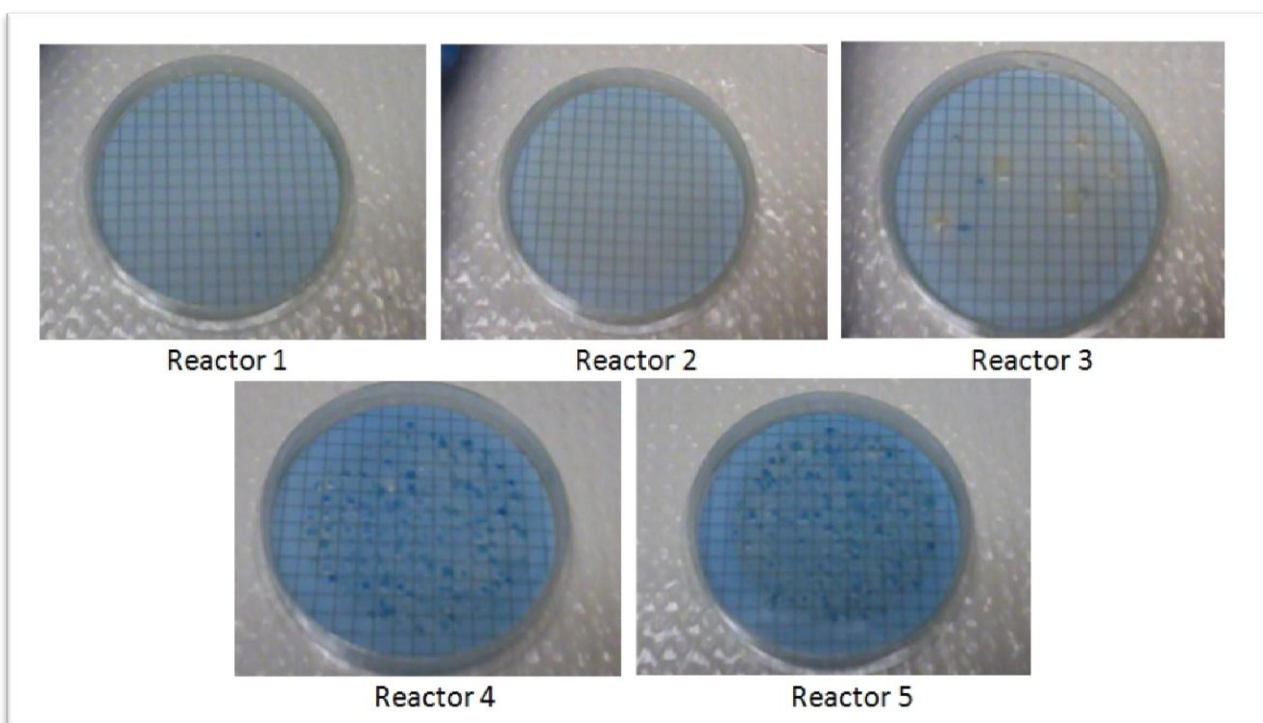


Figure 44: Bacterial colonies developed from Reactor 1 to 5. ENP_{Fe-surf} and chlorine added to effluent.

G.2. ENP to Effluent First Run

Table 53: CHLORINE DEMAND AND FECAL COLIFORM CONCENTRATIONS AS A FUNCTION OF THE INITIAL Cl₂ AND ENP_{Fe-surf} INJECTION WHEN NANOPARTICLES WERE ADDED TO EFFLUENT FROM THE WASTEWATER TREATMENT PLANT.

Reactor	ENP (mL)	pH	Dilution	Bacteria counted	Bacteria Amount
1	0.00	8.12	100	Blue circle around brown ones	TMTC
2	0.10	8.18	100	Brown (TMTC)	TMTC
3	0.30	8.28	100	Brown (TMTC)	TMTC
4	0.50	8.18	100	Brown (TMTC)	TMTC
5	0.75	8.16	100	Brown (TMTC)	TMTC
6	0.03	8.24	100	Brown (TMTC)	TMTC
7	0.05	8.17	100	Brown (TMTC)	TMTC
8	0.08	8.18	100	Brown (TMTC)	TMTC
Blank	0.00	0.00	1	0	0

(Note) TMTC: too many to count

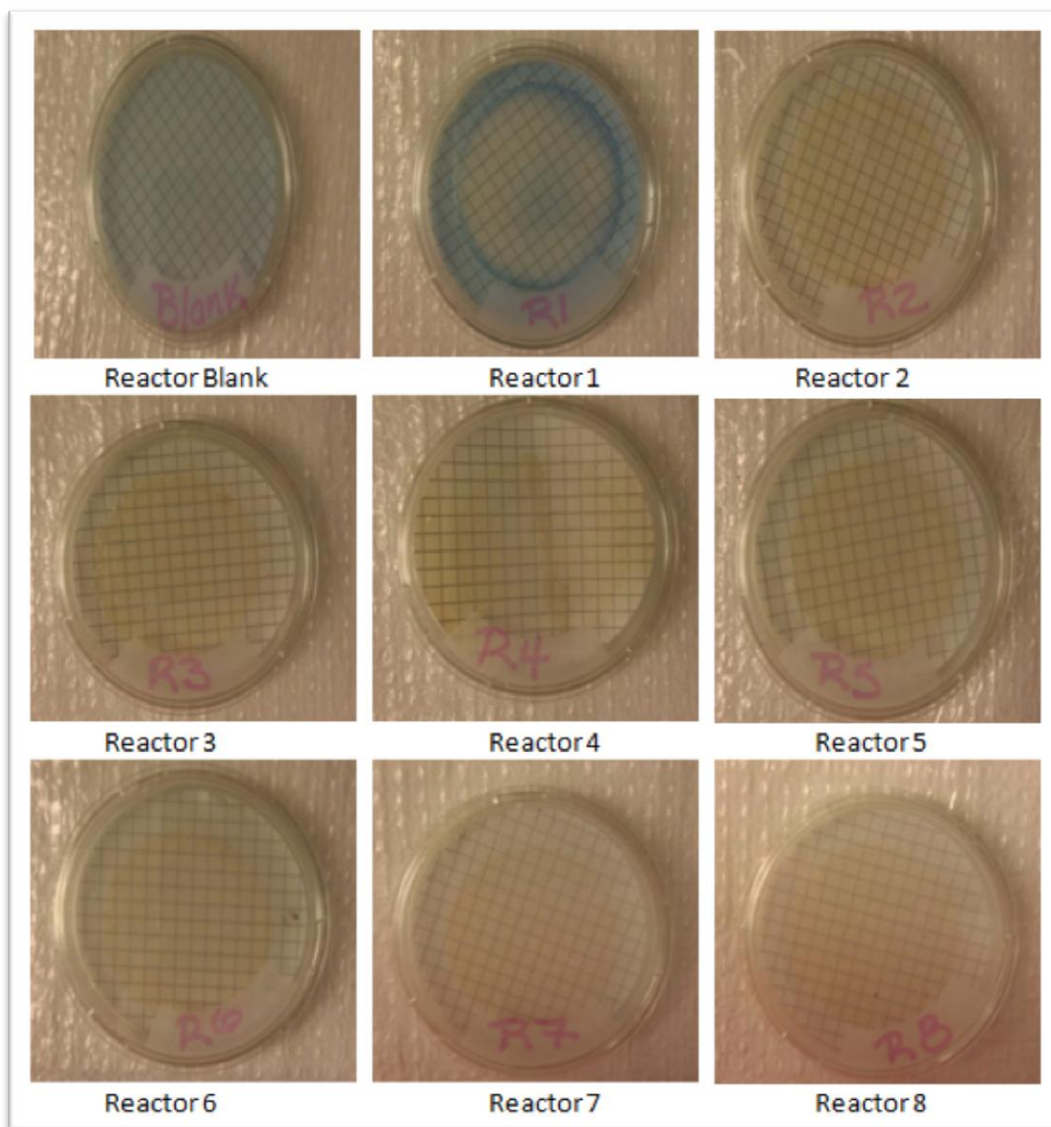


Figure 45: Amount of bacteria counted from Reactor 1 to 8 and on Blank reactor. $ENP_{Fe-surf}$ added to effluent.

G.3. ENP to Effluent Second Run

Table 54: CHLORINE DEMAND AND FECAL COLIFORM CONCENTRATIONS AS A FUNCTION OF THE INITIAL Cl_2 AND $\text{ENP}_{\text{Fe-surf}}$ INJECTION WHEN NANOPARTICLES WERE ADDED TO EFFLUENT FROM THE WASTEWATER TREATMENT PLANT.

Reactor	ENP (mL)	pH	Dilution	Bacteria counted	Bacteria Amount*
1	0.00	8.12	1000	95	95×10^3
2	0.10	8.17	1000	110	110×10^3
3	0.30	8.26	1000	131	131×10^3
4	0.50	8.17	1000	60	60×10^3
5	0.75	8.22	1000	78	78×10^3

* Colony counted per 100 mL (i.e., CFU/100 mL)

G.4. ENP in PBS

Table 55: CHLORINE DEMAND AND FECAL COLIFORM CONCENTRATIONS AS A FUNCTION OF THE INITIAL Cl_2 AND $\text{ENP}_{\text{Fe-surf}}$ INJECTION WHEN NANOPARTICLES WERE ADDED TO A PHOSPATE BUFFERED SOLUTION.

Reactor	ENP (mL)	pH	Dilution	Bacteria counted	Bacteria Amount*
1	0.00	7.59	1×10^7	72	72×10^7
2	0.10	7.81	1×10^7	117	117×10^7
3	0.30	7.96	1×10^7	141	141×10^7
4	0.50	8.08	1×10^7	105	105×10^7
5	0.75	8.20	1×10^7	177	177×10^7

* Colony counted per 100 mL (i.e., CFU/100 mL)

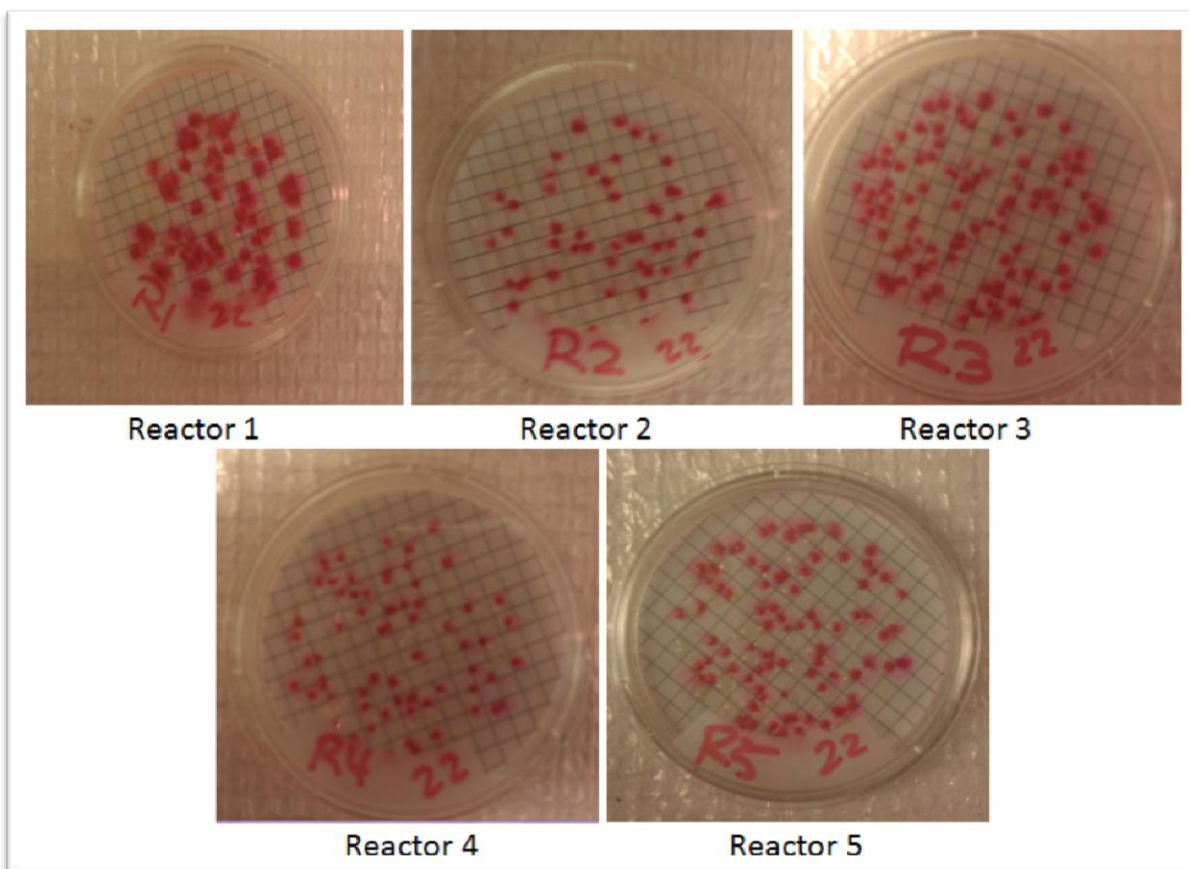


Figure 46: Amount of bacteria counted from Reactor 1 to 5. ENP_{Fe-surf} added to a Phosphate Buffered Solution.

G.5. ENP and Cl₂ in PBS

Table 56: CHLORINE DEMAND AND FECAL COLIFORM CONCENTRATIONS AS A FUNCTION OF THE INITIAL Cl₂ AND ENP_{Fe-surf} INJECTION WHEN INJECTIONS WERE MADE TO A PHOSPHATE BUFFERED SOLUTION

Reactor	ENP (mL)	ENP mg/L	pH	Initial Cl ₂ (mg/L)	Residual Cl ₂ (mg/L)	Dilution	Bacteria counted	Bacteria Amount*
1	0.00	0.00	7.65	20.0	1.15	1x10 ⁷	88	88x10 ⁷
2	0.10	5.92	7.90	20.0	0.26	1x10 ⁷	137	137x10 ⁷
3	0.30	17.76	7.98	20.0	0.04	1x10 ⁷	1	1x10 ⁷
4	0.50	29.60	8.03	20.0	0.00	1x10 ⁷	212	212x10 ⁷
5	0.75	44.40	8.28	20.0	0.00	1x10 ⁷	91	91x10 ⁷
6	0.00	0.00	7.97	0.00	N/A	1x10 ⁷	TMTC	TMTC

(Note) TMTC: too many to count

* Colony counted per 100 mL (i.e., CFU/100 mL)

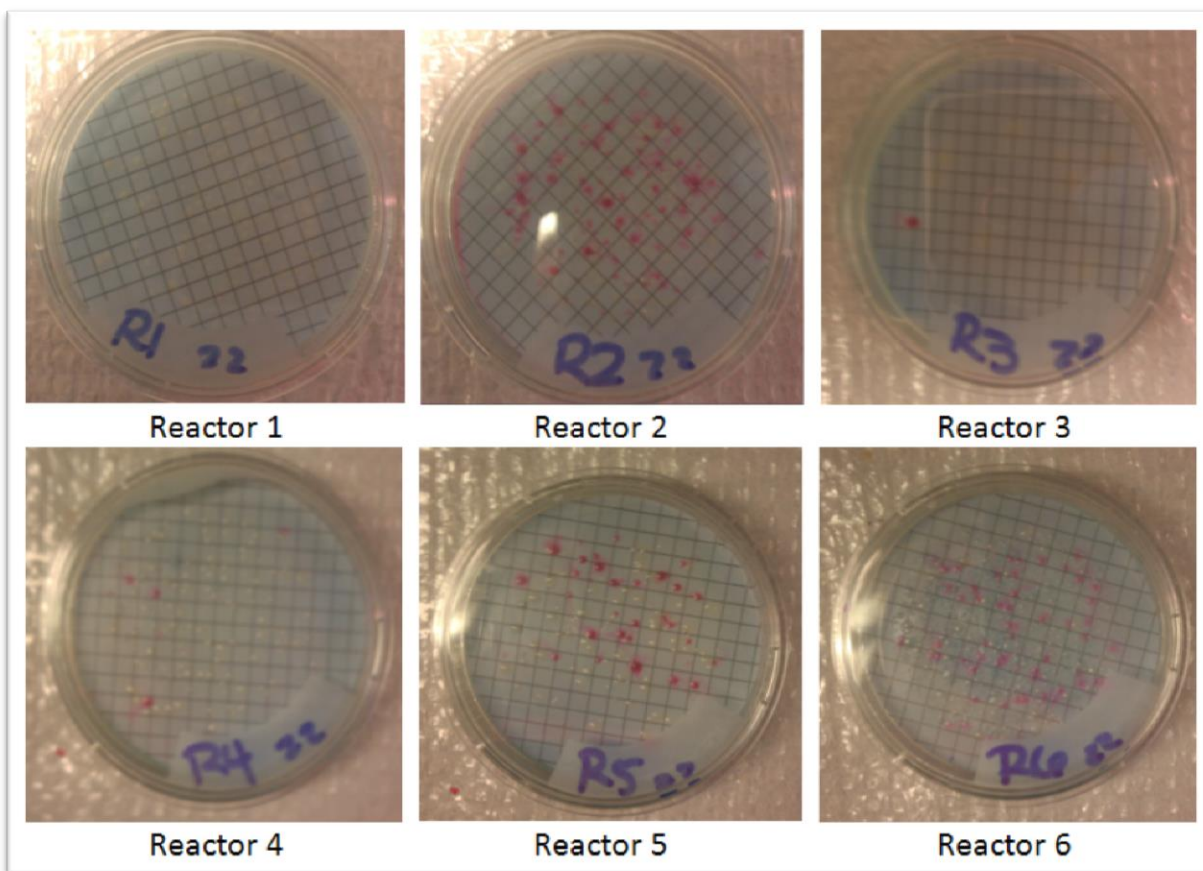


Figure 47: Amount of bacteria counted from Reactor 1 to 6. $\text{ENP}_{\text{Fe-surf}}$ and chlorine added to a Phosphate Buffered Solution.