Treatability study of an intermittent biosand filter for reduction of *Escherichia coli* and *Enterococcus*

by

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ABSTRACT

According to the United Nations, although access to safe drinking water is improving worldwide, still over 1 billion people lack access to such. The intermittent biosand filter (IBSF), which is an adaptation of a slow sand filter, is an appropriate technology for removal of pathogens and suspended solids from water at the household level in developing communities due to its low cost and operational simplicity. In particular, pathogens are removed through a combination of adsorption, microbial activity, mechanical trapping, and natural death due to deprivation of oxygen and nutrients. However, the details of these mechanisms are not completely understood. This study measured particularly the *Escherichia coli*, *Enterococcus* populations and dissolved oxygen (DO) concentration in the water throughout the IBSF sand bed depth over time in order to understand the impact and contribution of the microbial and oxygen deprivation mechanisms in the *Escherichia coli* and *Enterococcus* removal by the IBSF. In addition the phosphate removal by the IBSF and a visualization of the biofilm developed over the sand grains over time were accessed.

During this study, the *E. coli* population removal percent by the IBSF was up to 98%. According to the obtained results this high removal percent could not be only an effect of the biolayer, it could be also caused by other mechanisms, such as the lack of DO concentration inside the IBSF. After 30 days of filter use, the removal percent of *E. coli* increased with time and depth. In terms of the *Enterococcus* population, the IBSF remove

up to 98.7 %, having the higher reduction at the biolayer. It seems that there was no difference between the *Enterococcus* reductions at the middle and the end of the IBSF sand bed depth and it was not influenced by the lack of DO concentration.

Since the first week of the IBSF use, the development of the biofilm over the sand grains was observed until 29.7 cm of the sand bed depth and had a high diversity that increase over time. The results demonstrated the IBSF could remove up to 86.4 % of the phosphate present in the feeding water. One of the possible capture mechanisms for the phosphate removal in the IBSF could be adsorption to the iron particles that were present in the sand bed.

RESUMEN

Según un las Naciones Unidas, a pesar de los esfuerzos concentrados a mejorar el acceso a agua potable a nivel mundial, todavía cerca de un billón de personas carecen de acceso a agua segura para el consumo. El biofiltro intermitente de arena (IBSF), el cual es una adaptación del tradicional filtro lento de arena, es considerado una tecnología apropiada para la reducción de patógenos y solidos suspendidos en el agua cruda a nivel de los hogares en comunidades en desarrollo, debido a su bajo costo y su simple modo de operación. La reducción en la concentración de patógenos puede deberse a una combinación de mecanismos como adsorción, actividad microbiana, atrapamiento mecánico y a la muerte natural debido a la escases de oxígeno disuelto y de nutrientes.

Sin embargo, la interacción de estos mecanismos y su impacto en la efectividad del IBSF para mejorar la calidad del agua no han podido ser explicadas con certeza y claridad anteriormente. Este estudio tuvo como objetivo principal monitorear las concentraciones de *Escherichia coli*, *Enterococcus* y oxígeno disuelto en el agua a través de la profundidad del lecho de arena del IBSF y a través del tiempo, con el propósito de entender el impacto y contribución de los mecanismos de oxigeno disuelto y actividad microbiana en la remoción de *Escherichia coli* y *Enterococcus.* En adición, la remoción de fosfato por el IBSF y una visualización de la biocapa desarrollada sobre los granos de arena a través del tiempo fue estudiado.

Durante este estudio, el porcentaje de remoción de *E. coli* por el IBSF fue de hasta un 98%. De acuerdo a los resultados obtenidos, este alto porciento de remoción puede ser no solo por el efecto de la biocapa, sino también por otros mecanismos de remoción como la disminución en la concentración de oxigeno disuelto dentro del IBSF. Luego de usar el filtro por un periodo de tiempo mayor a 30 días, la remoción de *E. coli* aumenta a través del tiempo y de la profundidad del lecho de arena del filtro. En términos de la concentración de *Enterococcus*, durante este estudio se logro obtener un porciento de remoción de hasta 98.7%, ocurriendo la mayor remoción en la capa de arena donde se localiza el mayor desarrollo de la biocapa. Según los resultados, la profundidad del lecho de arena luego de los 29.7 cm y la disminución en la concentración del oxígeno disuelto no parecen influenciar la remoción de *Enterococcus.*

Desde la primera semana de uso del IBSF, el desarrollo de una biocapa sobre los granos de arena fue observado hasta los 29.7 cm de profundidad del lecho de arena del IBSF y su diversidad parecía aumentar con el tiempo. Los resultados demuestran que el IBSF puede remover hasta un 86.4 % del fosfato presente en el agua. Uno de los posibles mecanismos para la remoción de fosfato durante este estudio puede ser su posible adsorción a partículas de hierro que estaban presentes en el lecho de arena.

"Never doubt that a small group

of committed people can change the world.

Indeed, it is the only thing that ever has"

- Margaret mead

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LIST OF ABBREVIATIONS

AT = Appropriate Technologies

CFU = Colony Forming Units

DO = Dissolved Oxygen

EPA = Environmental Protection Agency

FA = Filter A

FB = Filter B

HWTT = Household Water Treatment Technology

IBSF = Intermittent Bio Sand Filter

SEM = Scanning Electron Microscope

SSF = Slow Sand Filter

WHO = World Health Organization

XRD = X-Ray Diffraction

1 INTRODUCTION

"Thousands have lived without love,
not one without water."
-W.H. Auden

Water is an indispensable resource to human life and wellbeing. In 2010, the United Nations General Assembly under the Resolution 64/292 recognized that access to clean water is "*essential to the realization of all human rights*". Although access to safe drinking water is improving worldwide, "*in 2012, 748 million people did not have access to safe water",* the progress percent increased only 17% in a time period of twenty-two years (WHO/UNICEF, 2014). The lack of access to safe drinking water have been affecting the least developed regions in Latin America, principally in rural areas of Dominican Republic, Ecuador, Peru, Nicaragua and Haiti (WHO/UNICEF, 2014). For this reason, researchers, governmental and nongovernmental organizations have been concentrating efforts in the development and implementation of appropriate technologies (AT) accessible for communities with poor water quality as part of the solution to reduce waterborne diseases and hence the wellbeing of their people.

Water treatment technologies, focused mainly on pathogen removal, have been developed and implemented in communities lacking of safe water sources. Among them are boiling, chlorination, solar disinfection, settling and various filtration methods. One of the most promising filtration methods is the intermittent biosand filter (IBSF). The IBSF, which is an adaptation of a slow sand filter (SSF), emerges as an appropriate technology for the removal of turbidity and pathogens from raw water, due to its low cost, operational

simplicity, and local availability of raw materials. The IBSF has been implemented in more than 70 countries worldwide (CAWST, 2009). Typical IBSF's are cast with concrete, metal or plastic, having cross-sectional areas of 30 cm by 30 cm, and can filter approximately 20 L of raw water per batch feed.

Although it is considered an AT to treat unsafe water in developing countries, there have been problems with usage rate, transportation (the filter weight is approximately 150 kg) and maintenance. For this reason, the IBSF demands design improvements to satisfy the users needs, while keeping or increasing its efficiency. To identify potential improvements to the IBSF design it is important to understand how the filter works. It is believed that pathogens are removed in the IBSF through a combination of mechanisms such as adsorption, biological activity, mechanical trapping, and natural death. However, the details on how these mechanisms interact and relate through the filter are not well understood. One approach to address and understand this correlation is to monitor pathogen and dissolved oxygen (DO) levels throughout the filter in time, to evaluate the contribution of microbial activity and mechanical mechanisms in pathogen reduction.

1.1 RESEARCH SCOPE AND OBJECTIVES

1.1.1 SCOPE

The main goal of this research is to evaluate the pathogen reduction through a bench-scale IBSF by monitoring *Escherichia coli* (*E. coli*) and *Enterococcus* levels as function of the DO concentrations, filter depth and time. The results from this study will contribute to better understanding of the removal mechanisms within the filter for future improvements, optimization and modifications in the filter design.

1.1.2 OBJECTIVES

The specific objectives of this research are to:

1. design and construct a suitable bench scale experimental apparatus, keeping the hydraulic and filtration properties of a typical biosand filter, to enable water quality sampling at several points within the filter sand bed;
2. monitor pathogen population as a function of the ripening period, DO concentration, and during the idle time through the sand bed;
3. visualize the biofilm developed over the sand grains with respect to the sand bed depth and time, and
4. evaluate the potential removal of phosphate by the IBSF.

2 LITERATURE REVIEW

"No water, no life.
No blue, no green"

-Sylvia Earle

Advanced water treatment technologies have been installed worldwide as part of water treatment plants to distribute safe drinking water at an affordable cost. However, in some low and medium income countries, mainly in communities located in rural areas, conventional water treatment plants are not feasible because of capital costs, operating costs and geographic location. Various household water treatment technologies (HWTT) have been developed and implemented in low-income communities to eradicate the lack of access of safe water of this population. The main target of those HWTT's is to remove water turbidity and pathogens population. Some of the most used HWTT alternatives are based on thermal, chemical, photolytic or filtration methods (Smieja, 2011).

Boiling water is one of the most common methods used for raw water disinfection (destruct or eliminate the pathogenic microorganism present). This thermal method has been proved by several studies to be effective for the pathogens inactivation (Clasen et al., 2008; Sobsey, 2002). In places where the fuel, electricity or the materials needed to heat the water are expensive and scarce, it may result in an inappropriate and expensive method.

Chlorination, first used in the 1900's, is a widely used chemical disinfection method because of its effectiveness, low cost and residual effect, which prevents re-contamination of the treated water. The dose of chlorine necessary to disinfect a specific volume of raw

water will depend in the quality of the water to be treated and the temperature (Reynolds & Richards, 1996). In some communities, lack of the equipment and facilities to conduct reliable quality tests of the water source, make it difficult to determine the right dose of chlorine. Moreover, availability of chlorine is also limited in many cases.

When a transparent container for water storage is available, solar disinfection (photolytic method) can be an alternative to treat water. Through the exposure of water in solar radiation, the pathogens and the water can absorb UV-B (220 - 315 nm) and UV-A (315 – 400 nm) radiation, which stops the cellular reproduction by protein denaturation, the prevention of DNA replication and the changes in DO concentration (Smieja, 2011). The solar disinfection method is considered easy to use and studies have confirmed reduction in diarrheal dieses after using this method to treat the water (Preez & Conroy, 2010). Some of the disadvantages are the necessary and continuous disinfection of the storage container, the high probability of water recontamination, the inappropriate application of this method to treat water with high turbidity levels and the long time of exposure needed to guarantee the desired disinfection (Loo et al, 2012).

2.1 FILTRATION

Filtration is a physical process on which many HWTT's are based, and have been used for more than 200 years ago. It allows water to flow through a bed of granular media, usually sand. As the water passes through the medium, suspended and colloidal particles become trapped due to several mechanisms: interception, flocculation, straining, and

sedimentation. The densest particles are strained. Particles flowing at sufficient low velocity are intercepted and attach by weak electrostatic forces to the filter medium. If the water is chemically treated prior filtration, additional flocculation can occur, allowing particle size to grow so that these larger particles can be removed by other mechanisms. Heavy particles settle out on the filter medium.

The filtration process is an essential step in water treatment. It is crucial to achieve water quality parameters, such as turbidity, which is caused by the presence of suspended materials like clay, silt, finely divided organic material, plankton, and other particulate material in water. Although turbidity may not adversely affect health, these particles may harbor microbiological contaminants that are harmful to human health or decrease the effectiveness of disinfectants. With the promulgation of more stringent regulations for the removal of *Giardia* cysts and *Cryptosporidium* oocysts, filtration has become broadly used in the United States.

Filters are classified according to the types of media used or the water filtration rate. They can be design and constructed with a single, dual or multimedia layers of medium. Anthracite, sand and garnet are the commonly used filtration media. According to the water filtration rate, filters are classified as rapid filters or slow filters. The filtration rate is the flow rate of water applied per unit cross sectional area of the filter. It is the velocity of the water approaching the face of the filter and is defined by the following equation:

$$v_a = \frac{Q}{A_s} \qquad (2\text{-}1)$$

where v_a is the filtration rate; Q is the flow rate onto filter surface; and A_S is the cross sectional area of filter. Sand filters are characterized by the effective size (sieve size that will allow the passing of the 10% of the total weight of the sand grains) and the uniformity coefficient, which represents the sieve size passing 60% of the sand divided by the effective size (Reynolds & Richards, 1996).

2.1.1 RAPID SAND FILTRATION

The rapid sand filtration was first design by George W. Fuller in 1920. Rapid sand filtration is the filter type most commonly installed in a water treatment plant and its water inflow is always treated first by chemical coagulation, flocculation and sedimentation. This type of filter has a uniformity coefficient of 1.7 and an effective size of 0.35 to 0.70 mm. Although rapid sand filtration can filter high water volumes per time unit, some of this technology disadvantages are the sand pores clogging with large suspended particles and the complicated and expensive maintenance and operation (Reynolds & Richards, 1996).

2.1.2 SLOW SAND FILTRATION

John Gibb invented the slow sand filtration technology in 1804. The process of slow sand filtration (SSF) consists of continuous raw water percolation through the pores of a 1 to 2 m fine sand layer at filtration rates between 0.1 and 0.4 $m^3/h/m^2$. The effective size of the sand grains in slow sand filters is from 0.2 to 0.4 mm (Reynolds & Richards, 1996).

While the raw water is treated, a biofilm layer, also known as *schmutzdecke,* will establish on the surface of the sand grains causing the presence of biological activity inside the filter allowing the biodegradation of a portion of the naturally occurring organic matter (Huisman & Wood, 1974). Figure 2.1 shows the schematic of a traditional slow sand filter.

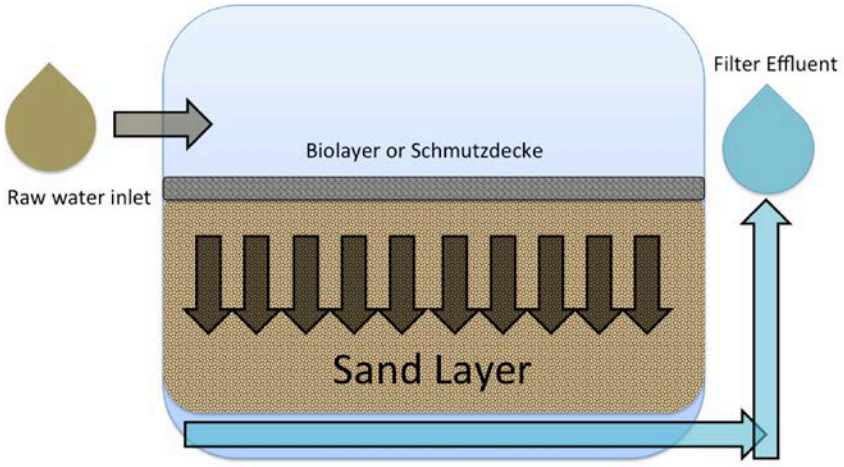


Figure 2.1 Traditional slow sand filter.

SSF is a water filtration system used for the removal of both suspended and dissolved organic matter in the water, by a combination of biological, mechanical and chemical mechanisms (USEPA, 2014). SSF's are called by Haig et al. (2007), "black boxes", because they have been used to remove suspended particles in the water by biological mechanisms (e.g. predation and natural depth) and mechanical mechanisms (e.g. absorption, screening) without an specific and directly corroboration.

Although the SSF was described by the World Health Organization (WHO) as *"a simple, inexpensive and reliable method of water purification*" (Huisman & Wood, 1974), and

is a technology capable to treat raw water without the use of chemicals and electricity, it requires an intensive land use due to its low filtration rate and continuous raw water feeding, making it inappropriate for some communities with poor resources (e.g. no availability of chemicals and electricity). Table 2.1 summarizes the maximum common contaminants removal percent reported for a SSF.

Table 2.1 SSF removal efficiency. Adapted from (Gimbel & Collins, 2006).

Contaminant	Maximum Removal Percent	Reference
Turbidity	<1 NTU	(`Buzunis, 1995)
Bacteria		
Enteric bacteria	90 – 99.99%	(Hijnen et al., 2007)
Giardia Cysts	99 – 99.99%	(Bellamy et al., 1985)(Stauber et al., 2006)
Crystosporidium oocysts	>99.9%	(Hijnen et al., 2007)
Virus		
Enteric viruses	99 – 99.99%	(Poynter and Slade, 1997)
Nitrate	95%	(Aslan, 2008)
Iron Manganese	30 – 90%	(Jenkins, Tiwari, & Darby, 2011)(Ellis & Wood, 1985)
Pesticides	0 - 100%	(Lambert and Graham, 1995)
TOC and COD	<15 – 25%	(Haarhoff and Cleasby, 1991)
True Color	25 – 40%	(Ellis & Wood, 1985)

2.2 INTERMITTENT BIOSAND FILTER (IBSF)

The IBSF is a household level adaptation of the traditional SSF, designed in the 1990's by Dr. David Manz at the University of Calgary at Canada. This water treatment technology was first implemented in 1996 in Nicaragua, and nowadays it is estimated that over 200,000 IBSF's have been installed around the world (CAWST, 2009). The IBSF emerges from the extensively applied SSF knowledge, being the main difference the filtration mode. In SSF the water is filtered continuously while in the IBSF the filtration occurs in batch mode. In the SSF the water feeding and flow rate are constants throughout the operation, while in the IBSF water is fed intermittently causing variable flow rates.

Recently, Young–Rojanschi et al., (2014) compared the effect of both filtration approaches (i.e. continuous vs. intermittent) within the IBSF design in the removal of *E. coli*, MS2 bacteriophage, and turbidity. The study concluded that continuous operation was significantly better than intermittent operation for bacterial, virus and turbidity removal. However, the filtration rates employed in their experiments could have influenced the results as the filtration rates were out of the typical SSF ranges (0.1 to 0.4 m/h). For instance, the continuous mode was operated at rates of 0.01 m/h, which are too slow, while the intermittent or batch mode was carried out at 0.69 m/h (which is too high for the intended filtration rate in an IBSF). Low filtration rates for the continuous mode may have provided longer contact time for the filtered water with the biofilm layer, hence, causing higher bacterial and virus removal. Likewise, high filtration rates in the batch mode could

have increased the velocity of the water through the medium, causing some of the particles to be sheared off the medium reducing the turbidity removal efficiency.

2.2.1 BIOFILM LAYER

The IBFS is a biological treatment. A biofilm layer in the IBSF, as in the SSF, is formed due to the filter's pores clogging caused by the removal of suspended particles (e.g. organic and inorganic matter) in the first 50 mm of the sand layer. The biolayer established in the first 50 mm of sand bed area, does not extend more deeply likely due to depletion in the dissolved oxygen concentration through the filter depth. This zone has a high diversity of microorganism, such as algae, rotifers, protozoa and bacteria that are attached to the sand grains and covered by extracellular polymeric substance, which is nearly 85% of the biofilm composition. The microorganism species established in the biofilm layer are strongly related with the ecological biodiversity of the feeding water. A microbial community of *Acidovirax, Halomonas*, *Sphinobium*, and *Spingomonas* has been identified in SSF biofilm (Haig, 2014), meaning that they could be found in the adaptation of the SSF, the IBSF. The complete development of the biofilm layer usually requires 15 to 30 days of IBSF feeding with raw water at least once per day. Higher biological productive raw water can reduce the time for the complete biofilm layer development (Palmateer et al., 1999).

The biological processes that probably take place in the biolayer zone are predation, scavenging and metabolic breakdown. Protozoa play the main role in the predation process.

According to Lloyd (1996), the protozoa are capable of ingesting bacteria, which occurs when protozoa, which can be suspended in the standing water or attached to the sand grains, consume particles and bacteria that are in the water. The microorganisms need energy for metabolic functions. The microbial population established in the *schmutzdecke* oxidizes the organic matter in the water, to satisfy and complete metabolic functions, which will cause a reduction in the water organic matter content (Huisman & Wood, 1974) .

Many studies emphasize that the IBSF should reach higher removal efficiency with media aging and the complete development of the biofilm. Stauber et al. (2006), conducted controlled experiments in the laboratory and in the field, showing the impact of the biofilm establishment for the E. coli removal. Were observed improvements in the removal rate over the filter use from 94 to up to 99%. However, Palmeteer et al. (1999) obtained a significant removal of heterotrophic bacteria (83%) without the complete biofilm development.

2.2.2 COMPONENTS AND DESIGN PARAMETERS

The most important design parameter in sizing filters is the loading or filtration rate. For slow sand filters, water is applied to the sand at a loading rate of 0.1 to 0.4 $m^3/h/m^2$, as mentioned earlier. For an IBSF, at the maximum hydraulic head, the filtration rate should not exceed 0.4 $m^3/h/m^2$ (Buzunis, 1995).

The IBSF consists of a 1.0 m height and 0.3 m width basin. The basin design is available in concrete, metal or plastic. The concrete version, which weighs approximately 150 kg, is constructed using a durable concrete mix of cement, fine sand and gravel. The filter basin is filled with one sand layer of 543 mm and two gravel layers of 50 mm each one. The sand grain size, which is the most important parameter in the IBSF design, is between 0.1 to 0.7 mm (Jenkins et al., 2011; Stevik et al., 2004). Although it has been highly recommended by the literature the use of crushed rock sand for the sand bed, IBSFs built with river sand have demonstrated comparable contaminants removal levels to those installed with crushed rock sand (Jenkins et al., 2011). The gravel layers are located at the bottom of the basin for sand support and have been identified as the separation layer and drainage layer. The grain size of the separation layer is from 0.7 to 6 mm, while the grain size of the drainage layer is from 6 to 12 mm.

Both sand and gravel needs to be rinsed first (before the filter is packed) with the cleanest water available to remove all the impurities that could add turbidity to the treated water (CAWST, 2009). However, it has been recommended that exposure of the river sand to a disinfection technique, like sunlight, be used to eliminate the organic content that the river sand could have (CAWST, 2009). To minimize the sand disturbance during water feeding to the filter, a diffusor plate, consisting of 3 mm diameter holes in a 2.5 cm by 2.5 cm grid pattern, sits 70 mm above the sand layer. Several materials such as galvanized metal, corrugated acrylic and polyethylene plastic have been used to build the diffusor plate. Figure 2.2 presents a schematic of a typical concrete IBSF.

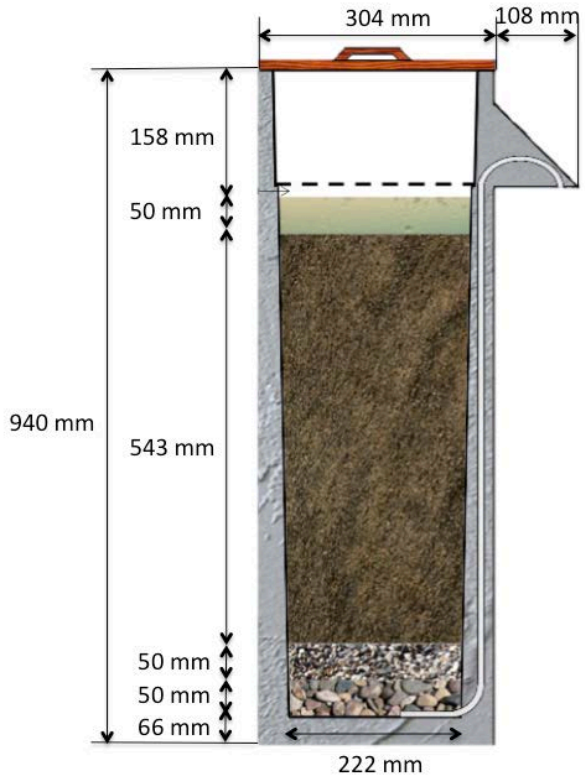


Figure 2.2 IBSF concrete design (CAWST, 2009).

2.2.3 FILTRATION PROCESS

The IBSF intermittent operation starts with the addition of approximately 20 liters of raw water under close to plug flow hydraulics (Elliott et al., 2011). The raw water dosing volume (i.e. batch) depends on the filter pore volume. It must be a maximum 1:1 ratio (pore: batch volume), or otherwise the IBSF contaminant removal efficiencies can decrease (Elliott et al., 2011; Jenkins et al., 2011). The hydraulic head forces the water to percolate through the sand and gravel layers and flows from the outlet tube, making the use of

electrical power unnecessary for the IBSF operation. When the head difference between the water level in the filter reservoir and the outlet tube is zero, the water stops flowing and the pause period start (idle time). It is recommended an idle time from 1 to 48 hours (CAWST, 2009). The pause period has been identified as one of the most important factors in contaminants removal, especially for virus removal (Elliott et al., 2011; Jenkins et al., 2011). Figure 2.3 shows the water path through the IBSF until the pause period is achieved.

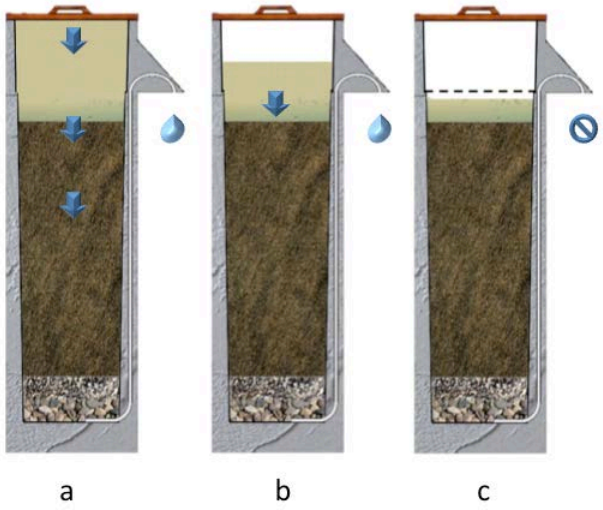


Figure 2.3 (a,b,c) IBSF water treatment process (Mofifed from CAWST, 2009).
When the raw water is added, it enters the IBSF reservoir (2.3a), percolates through IBSF filtration media (2.3b) and stops flowing from the outlet tube when there is no head difference between the water reservoir level and the outlet tube (2.3c).

The pause period and the slow filtration rate of the IBSF allow the development of a biofilm over the sand grains surface, mainly at the top of the IBSF sand layer. During the pause period time, the oxygen concentration in the biofilm layer is being depleted. To maintain the necessary aerobic conditions for the biological activity in this sand layer section, the outlet tube is located at a specific height, leaving a standing water zone of 5 cm

over the sand layer top surface. This standing water zone is essential for the oxygen diffusion from the air into the water during the pause period, helping the survival of the microorganism already established in the biofilm layer (Buzunis, 1995). Another factor that could impact the optimum conditions of the biofilm layer is the disturbance caused by the raw water addition. Although a diffusor plate is part of the IBSF design, the standing water height contributes to minimize the sand layer disturbance. The IBSF designer recommends a standing water zone of 5 cm for the optimum diffusion of oxygen but according to Palmateer et al. (1999), 2 cm to 3 cm could be a suitable level. However, Young–Rojanschi & Madramootoo (2014) stated that even 5 cm of standing water zone could disturb the sand layer when the raw water was fed to the IBSF.

With the IBSF usage, the removed particles from the raw water and the microbial extracellular polymeric substances can clog the filter pores and decrease the water flow rate to less than 30 L/h (Mauclaire et al., 2004). To restore the water flow rate a wet harrowing procedure, as described by the Biosand filter manual: Design, Construction, Installation, Operation and Maintenance by CAWST, is performed by the user. Although the IBSF maintenance does not require an extensive physical and economical effort, the wet harrowing can affect significantly the bacteria and turbidity removal, at least the next seven days after conducting the maintenance procedure. However no adverse effect in virus removal has been observed (Jenkins et al., 2011) .

2.2.4 CONTAMINANT REMOVAL MECHANISMS

It is believed that pathogens and turbidity are removed through a combination of physical and biological mechanisms such as adsorption, predation, mechanical trapping, and natural death. One of the physical removal mechanisms related with the filter media size is the straining. When the IBSF is packed, the sand grains form filtration pores between them, causing the entrapment of suspended particles that are larger in size than the pores. According to Stevik et al. (2004), the particles could be removed by straining if they are 0.2 times the size of the filtration grain size. Under this statement suspended solids with particle size above 20 μm should be removed by straining while particles with a diameter less than 20 μm could be removed by other filtration mechanisms, such as adsorption. The adsorption mechanism could results from an interaction between the particles in water and the sand grains surface by a chemical bonding or electrostatic forces. Bacteria could be removed by adsorption, which can be affected by water pH, temperature, filtration rate and the presence of organic matter (Stevik et al., 2004).

The biological mechanisms in the IBSF and in the slow sand filtration are not well understood (Elliott et al., 2011; Jenkins et al., 2011; Mauclaire et al., 2004). One of the reasons is the microbial diversity of the biofilm developed over filtration media surface (Devadhanam & Pillay, 2008). Although the literature suggests that process such as food competition, predation and natural death occur in the biofilm layer, several studies have indicated that the biofilm microorganism type plays a major role in the pathogens removal.

Several laboratory and field studies have been focused in the evaluation of the IBSF effectiveness to improve water quality parameters. Table 2.2 summarizes the maximum common contaminants removal percent reported for studies conducted in the laboratory and in the field.

Table 2.2 IBSF removal efficiency.

Contaminant	Maximum Removal Percent		Reference
	Laboratory	**Field**	
Turbidity	95 %	85 %	(Buzunis, 1995)
Bacteria	> 96.5%	87.9 – 98.5 %	(Buzunis, 1995)
Fecal coliforms	96%	-	(Buzunis, 1995)
Escherichia coli	94%	99%	(Stauber et al., 2006)
Giardia Cysts	> 99%	-	(Palmateer et al., 1999)
Crystosporidium oocysts	99.98%	-	(Palmateer et al., 1999)
Heterotrophic	>83%		(Palmateer et al., 1999)
Virus			
MS2 bacteriophage	71%	-	(Jenkins et al., 2011)

2.3 PATHOGEN INDICATORS

According to the Environmental Protection Agency, indicators are *"physical, chemical or other parameters whose presence at a level outside the limits may reflect a problem in the water quality"*. Most pathogens are of enteric origin. Testing a water sample for the presence of a specific pathogen could result in an expensive and long process. For

this reason, indicators are used to identify possible fecal contamination of a water source with fecal contamination. Total coliforms, fecal coliforms (i.e. thermotolerant coliforms), *Escherichia coli* and *Enterococcus* are indicators of a fecal contamination and hence, of pathogens. High population of the indicators of fecal contamination indicates a potential risk of contracting a waterborne disease.

Escherichia coli is the most common fecal coliform in the digestive track of the warm-blooded animals. Hence it is considered an indicator of fecal contamination in a water source. This bacteria is a bacillus, non- spore forming, Gram negative, anaerobic or facultative anaerobic (Rompré, et al., 2002). Figure 2.4 (a) shows the *E. coli* enumeration and identification method using MI Agar (USEPA, 2002b).

Enterococcus are non-spore forming, facultative anaerobic, fermentative, Gram positive bacteria that inhabits the intestines. The *Enterococcus* cells occur singly, in pairs or in chains and with a 0.5 to 1 μm in diameter. This bacteria group can survive in a hostile environment and they are stains resistant to most of the antibiotics available nowadays (Suchitra & Kundabala, 1937). Visual appearance of *Enterococcus* cultivated in MeI Agar is presented in Figure 2.4 (b).

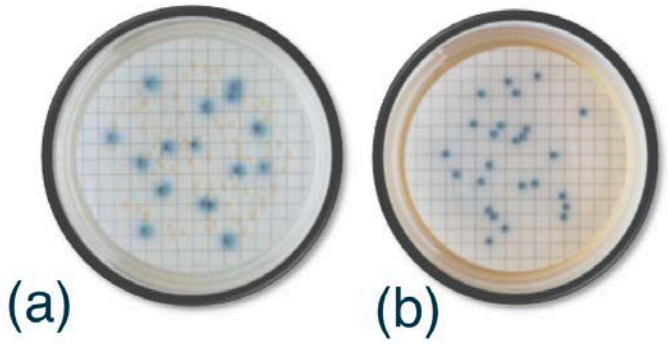


Figure 2.4 (a, b) *E. coli* and total coliforms cultivated over MI Agar.
(a) The light cream – clear colonies are total coliforms; the blue colonies are identified as *E. coli*. Adapted from (USEPA, 2002b) (b) The blue colonies are *Enterococcus* colonies cultivated over MeI Agar. Adapted from (USEPA, 2002a).

3 METHODOLOGY

"For the things we have to learn
before we can do them, we learn by doing them"

-Aristotle

Detailed descriptions of the experimental methods performed to achieve the objectives of this research are presented in this section. The materials used to conduct the experiments are also mentioned and described.

3.1 BENCH SCALE EXPERIMENTAL APPARATUS

Under the hypothesis that hydraulic characteristics such as filtration rate and equilibrium height of standing water influence the pathogen reduction mechanisms in the IBSF, an experimental apparatus was built to have the same operational height and filtration rate of a conventional IBSF. The bench scale model consisted of a clear acrylic tube with operational height of 0.8 m, but a reduced cross sectional area from 0.0383 m^2 to 0.0071 m^2. The smaller cross sectional area provided convenience in the experimental procedure reducing the volume of water needed to feed the filter in 1:1 pore volume ratio (including the volume of the standing water zone). The water outlet consisted of a clear vinyl tube housed by a PVC pipe with a diameter of 1.91 cm. Figure 3.1 shows a sketch of the bench scale filter design and a real photo of the bench scale IBSF after completing the installation. Detailed dimensions of the experimental IBSF bench scale are provided in Table 3.1 The installation of the bench scale experimental apparatus was made using as a reference the Center for Affordable Water Sanitation Technology manual (CAWST, 2009).

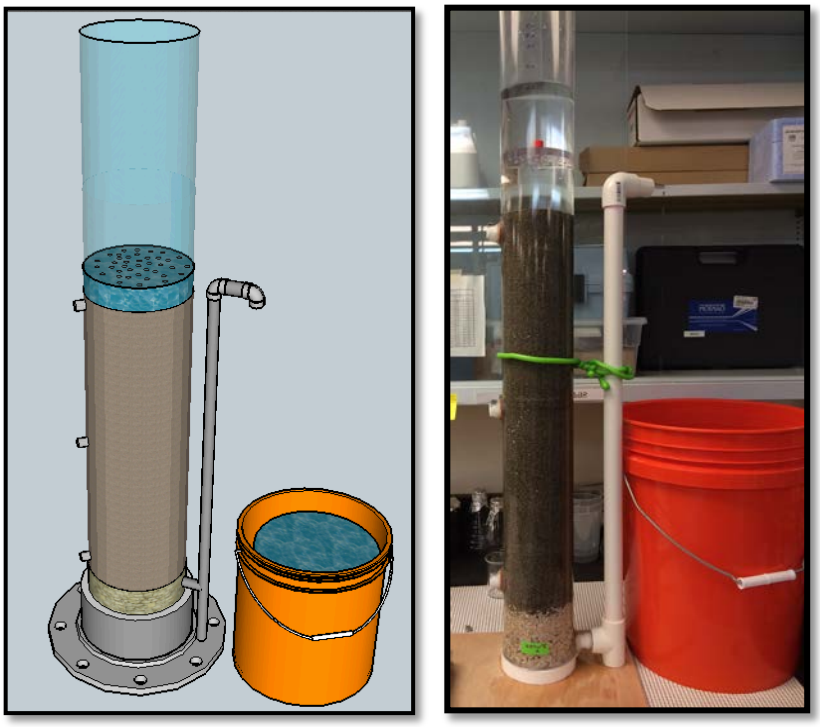


Figure 3.1 IBSF experimental apparatus sketch and real photo after completing the installation.

Table 3.1 Dimensions of the IBSF bench scale.

Column		Outlet vinyl tube	
Diameter	9.5 cm	Inside diameter	6.35 mm
Total height	1.0 m	Outside diameter	9.53 mm
		Tube height	695 mm

3.1.1 FILTRATION MEDIA

The experimental IBSF was packed with crushed rock sand and with two different layers of white gravel. Figure 3.2 shows the filtration media layers location and the IBSF experimental apparatus dimensions. Once packed, the effective volume of the bench-scale IBSF was 2.5 L.

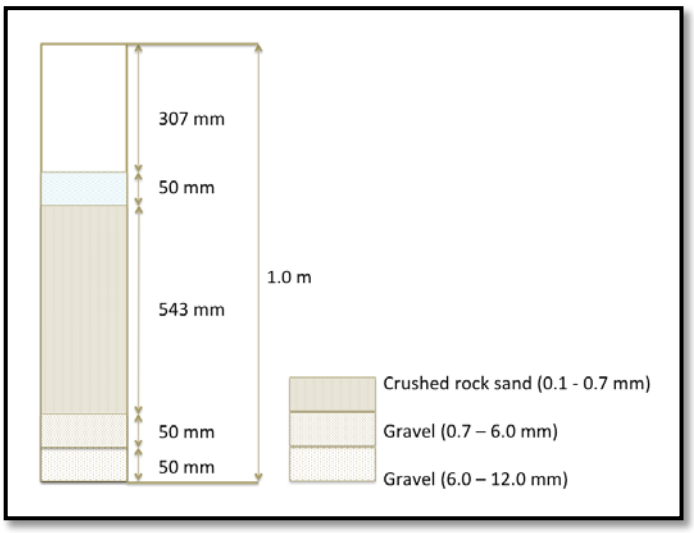


Figure 3.2 Filtration media layer location.

Crushed rock sand was obtained from a local filter manufacturer in Haiti, while white gravel was obtained from a local hardware store. The gravel was sieved to find two ranges of particle size, 6.0 – 12.0 mm and 0.7 – 6.0 mm. The gravel was rinsed with tap water until no turbidity was visually observed and then dried in the oven at 105 °C. Crushed rock sand (particle size from 0.1 – 0.7 mm) was rinsed several times with distilled water until the desired cleaning level using as a reference the jar test as established by the

CAWST IBSF installation manual (CAWST, 2009). The sand was then dried in the oven at 105 °C for approximately 48 hours. To characterize the crushed rock sand, a sieve analysis and mineralogical X-Ray diffraction analysis (XRD) were performed.

For the sand sieve analysis a sand sample of 153.7 g was used. Six screens and a catch pan were used. The sieves numbers and theirs opening size were #25 (0.710 mm), #35 (0.500 mm), #40 (0.425 mm), #50 (0.355 mm), #60 (.250 mm) and #140 (0.105 mm) and they were staked in descendent order (sieve #25 at the top). The sand sample was poured to the sieve with the larger opening size and shakes it for 5 minutes. After shaking the sieve set, the mass of the retained sand in each sieve was weighted. The effective size and the uniformity coefficient of the coarse rock sand were determined. To prepare the sand sample for the XRD analysis, a sand sample of about 1 gram was crushed manually with a mortar until the sand grains turned to dust. The sample was taken to the UPR-NSF Earth X-ray Analysis Center (EXACt) where the sample was analyzed.

3.1.2 SAMPLING PORTS

To measure physical, chemical and biological water quality parameters throughout the filter, the bench scale model included three side ports at specified heights that allowed small water samples to be taken. The sampling ports were located at distances of 2.54, 29.7, and 51.8 cm below the top sand layer. The side ports were connected to circular perforated vinyl tubes that allowed the collection of representative water samples from the specified

port location (Refer to Table 3.2 and Figure 3.3). All the perforations were covered with polypropylene Spectra/Mesh Screen with a pore size of 105-µm to prevent sand particles to gain access to the water samples, but still allowing the passage of the microorganism of interest.

Table 3.2 Sampling ports dimensions

Ring Diameter	50 mm
Wall distance	22.2 mm
Tube DO	9.53 mm
Tube ID	6.35 mm
Perforations quantity	5
Mesh opening size	105 µm

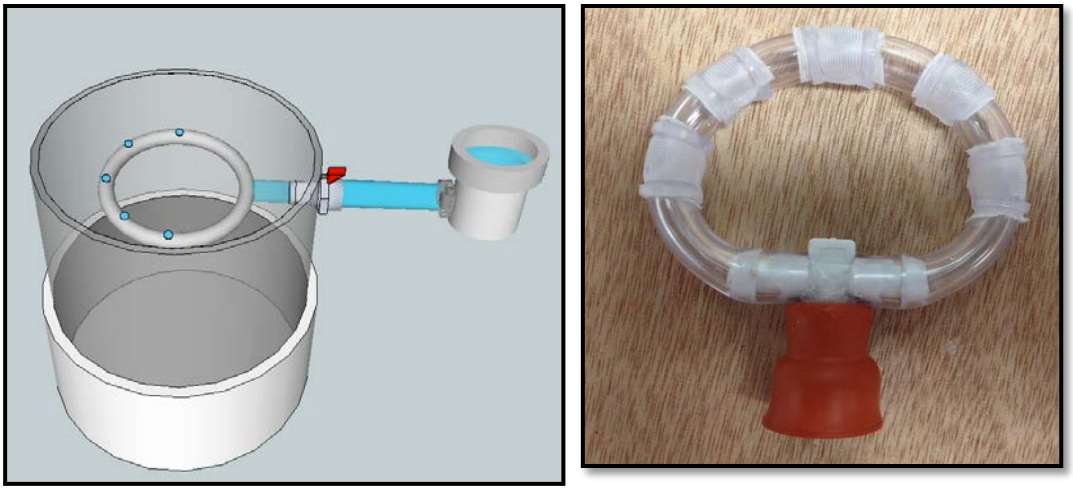


Figure 3.3 Sampling ports and IBSF experimental bench scale connection diagram and a real photo of the sampling ports prior to the ports installation.

3.1.3 FEED WATER

The IBSF is intended to operate in batch mode (i.e. intermittent feed). Each water volume fed into the IBSF should be allowed to remain within the granular medium for a certain amount of time (usually 1 - 48 h). The water is then expelled away by adding a new batch of raw water, which replaces the previous batch. The cycle repeats over and over. For this experimental design, the water was replaced every 24 hours. Raw surface water from a local creek (Oro Creek) that crosses the Mayagüez campus was used as the water source to feed the bench-scale IBSF. This water source has similar *E. coli* population than a sampled water source in Haiti (approximately 1,650 CFU/100 mL). The feed water was collected weekly and stored at 4°C in the laboratory to prevent drastic biological population changes during the storage time. The water volume to feed the bench-scale IBSF was allowed to reach room temperature (approximately 21°C) before the addition to the IBSF.

3.2 TRACER STUDY

The bench-scale IBSF was initially tested with semi-continuous, step-input tracer studies to determine the hydraulic behavior of the filter. The tracer was performed using sodium chloride solution with a concentration of 796 mg/L. This solution is an appropriate tracer because it is a conservative reactant and non-biodegradable. The tracer tests were conducted individually for each of the bench scale model sampling ports. The sodium chloride solution was introduced to the bench-scale model as a batch inflow of 2.3 L and the filter response was monitored continuously until the 90 % of the tracer concentration

was reached. Electrical conductivity was used as the tracer concentration indicator and was measured using the Multi-parameter PCSTestr35, Oakton meter.

3.3 PATHOGEN POPULATION REDUCTION IN THE IBSF

3.3.1 EXPERIMENTAL PROCEDURES

Two bench-scale IBSF apparatus were operated in parallel for ten consecutives weeks. The parallel operation was used for reproducibility of results. Each IBSF apparatus was fed with raw, untreated water with a maximum loading head of 10 cm, once every 24 hours. This maximum loading head was established to operate the bench-scale IBSF at the same filtration rate of a conventional IBSF. Because the reservoir of the experimental bench-scale apparatus could not accommodate the full batch volume, a peristaltic pump at a nominal rate of 50 mL/min was used to keep a constant water head above the sand bed as the water percolates the filter. However, continual use of the IBSF clogs the filter and decreases the filtered water flow rate, so the peristaltic pump was adjusted to the appropriate raw water-feeding rate every time needed. To prevent algae growth the experimental bench-scale apparatus was covered with aluminum foil.

As suggested by Elliot et al. (2011) and Young-Rojanschi & Madramootoo (2014), a control for the microorganism natural survival under the 24-hour idle time was used. The control consisted in keeping a separate raw water sample next to the filters of the same

filter batch volume in an aluminum-covered flask at room temperature (at the same conditions of the IBSF bench scale).

Water samples were collected every three days after the idle time (i.e. before a new batch of water was added) from the points identified in Figure 3.4. From each sampling port, approximately 50 mL of water was drawn. This quantity was small enough to prevent the unsaturation of the sand layer. The water samples were analyzed for physical, bacteriological and chemical parameters in duplicate. The water sampling through the sand bed depth facilitated the development of a profile relating the parameters concentration in function of the IBSF depth.

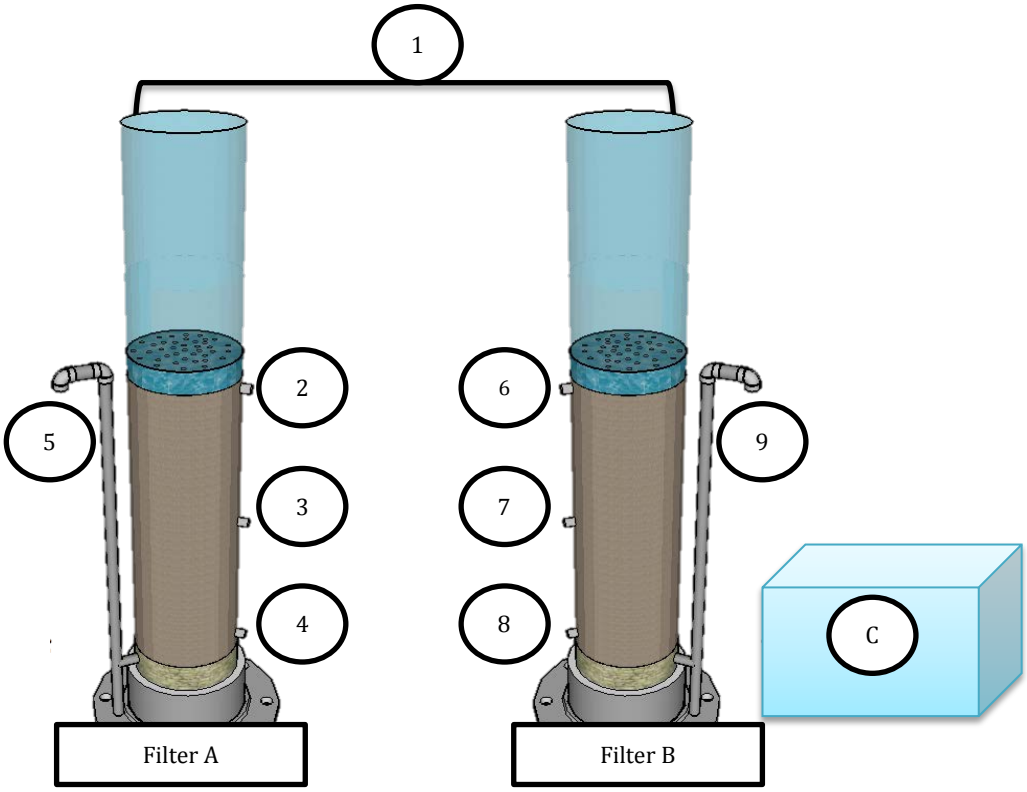


Figure 3.4 Water sampling points.

3.3.2 WATER QUALITY PARAMETERS

Basic water quality parameters such as pH, turbidity, temperature, and conductivity were measured. The pH and the temperature were monitored using the Oakton pH 5+ portable meter. The turbidity and the conductivity were monitored with the Oakton T-100 handheld turbidity meter and the Accumet XL500 meter, respectively. The filtration rate was also measured for each batch. As part of the research objectives, the potential of phosphate removal by an IBSF treatment was evaluated. The phosphate concentration of the filtered water from Filter A was measured every other day using the Hach Pocket Colorimeter II, phosphate UV.

3.3.3 DISSOLVED OXYGEN

Under the hypothesis that DO concentration could influence the pathogens presence, DO concentration was measured in each sampling point along with pathogens levels. The measurement was performed using the Oakton DO 110 portable meter and a flow throw cell apparatus built in the laboratory to minimize the exposure of the water sample to ambient conditions. The DO concentration measurements with sand bed depth were used to analyze the relation between pathogens levels and available DO concentration.

3.3.4 BACTERIOLOGICAL TEST

In order to quantify the pathogen population present on each water sample, two commonly indicators of water fecal contamination were used. Those indicators were *Escherichia coli* and *Enterococcus*. For the *Escherichia coli* quantification, the EPA Method 1604 with MI medium was performed (USEPA, 2002) . The *Enterococcus* population in each sample was quantified using the EPA Method 1600 with *Enterococcus* Indoxyl-β-D-Glucoside Agar (mEI) (USEPA, 2002a). Both methods of quantification use the membrane filtration technique.

3.3.5 PATHOGEN POPULATION REDUCTION DURING THE IDLE TIME

In order to evaluate and analyze the effect of the filter maintenance in the pathogen levels during the idle time, an additional sampling phase was schedule after the ten-week trial period and after conducting the filter maintenance procedure. To prevent unsaturation conditions within the sand bed, 20 mL was drawn from each sampling port and the control every 8 hours during the 24 hours idle time. The collected water samples were tested for *Escherichia coli* and *Enterococcus* population using the methods mentioned in section 3.3.4. The study was conducted in both filters.

3.4 VISUALIZATION OF THE BIOFILM OVER THE IBSF MEDIUM

3.4.1 VISUALIZATION OF THE BIOFILM THROUGH THE IBSF DEPH

At the completion of the 10-week filtration period, a sample of the sand grains and gravel surface from Filter A were examined and visualized by means of a Scanning Electron Microscope (SEM) and organic matter (OM) content. Using a Pasteur pipette, sand samples of approximately 1 gram were collected at depths of 2.54 cm, cm, and 51.8 cm below the sand top surface (corresponding to the depth of the sampling ports). The gravel layer was also sampled.

The SEM was used to identify any surface modification over the sand and gravel grains due to the filter ripening (e.g. heterotrophic bacteria establishment). A sample for each depth was observed and compared to a control sample. The samples were fixed at the time of sampling with 5% glutaraldehyde (v/v 0.1 M phosphate buffer) for 3 hours. After the fixation time, the sample was rinsed twice in phosphate buffer and dehydrated for 20 minutes (serial steps) in 70%, 90% and 100% acetone v/v in 0.1 M phosphate buffer (Law et al., 2001). The samples were coated with gold using a Denton Vacuum Desk IV sputter-coater and then observed under the SEM. The OM content in each collected sample was quantified by the gravimetrical method of the loss on ignition (Young-Rojanschi & Madramootoo, 2014).

3.4.2 VISUALIZATION OF THE BIOLAYER DEVELOPMENT IN THE IBSF

According to the literature, the biolayer is located mainly in the first two centimeters of the top IBSF sand bed. To visualize the development of the biolayer a third, smaller IBSF prototype was built. This filter consisted of a PVC column with a diameter of 7.62 cm and an operational height of 38.1 cm. The water outlet consisted of a clear vinyl tube housed by a PVC pipe with a diameter of 1.91 cm. Figure 3.5 shows a real photo of the apparatus. Detailed specifications of the IBSF prototype are provided in Table 3.3. The installation of this IBSF was performed following the same guidelines described by CAWST in the IBSF installation manual (CAWST, 2009).

Figure 3.5 Photo of the IBSF prototype for the biolayer development visualization.

Table 3.3 IBSF prototype specifications for biolayer visualization.

Layer	Thickness
Sand	5. 2 cm
Gravel for separation	1.3 cm
Drainage Gravel	2.54 cm

Basic water quality parameters such as pH, turbidity and temperature were measured daily for 29 days. The pH and the temperature were monitored using the Oakton pH 5+ portable meter. The turbidity was monitored with the Oakton T-100 handheld turbidity meter. Using a Pasteur pipette a sand sample from the biolayer was collected every ten days, for a total of four sand samples for the biofilm visualization using the SEM. All samples were fixed and dehydrated, using the same method mentioned in the section 3.4.1.

4 RESULTS AND DISCUSSION

"No amount of experimentation
can ever prove me right;
a single experiment
can prove me wrong"

-Albert Einstein

4.1 SAND CHARACTERIZATION

The IBSF needs a certain sand particle size to treat the raw water effectively. This section shows a sieve and a mineralogical X-Ray Diffraction analysis performed to the crushed rock sand employed in the IBSF bench scale apparatus.

4.1.1 SAND SIEVE ANALYSIS

The sieve analysis results are presented in Figure 4.1. From this analysis the effective size (d_{10}) and the uniformity coefficient (d_{60}/d_{10}) was determined.

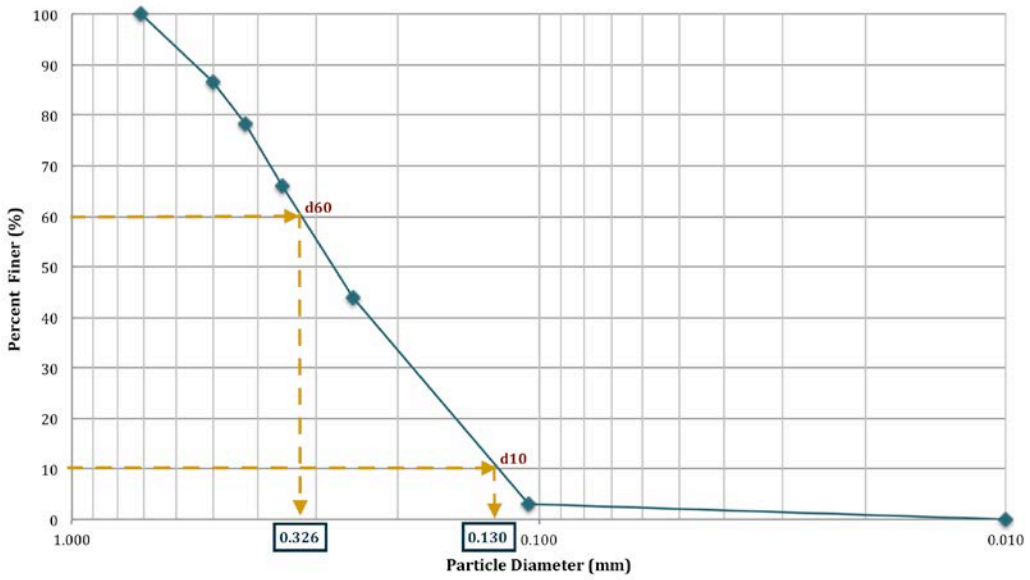


Figure 4.1 Particle size distribution of the crushed rock sand.

The effective size from the sand sample analyzed, resulted in 0.130 mm, which according to the CAWST IBSF installation manual (CAWST, 2009), is near the recommended range (0.15 to 0.20 mm). In terms of the uniformity coefficient of the sand sample, the obtained value was 2.51, which is just 0.01 higher than the maximum recommended value, 1.5 to 2.5 (CAWST, 2009).

The data obtained from the sieve analysis demonstrated the sand was usable for the intended purpose in this study. The sand grain size used for the IBSF yields a significant impact in the IBSF efficiency. According to Jenkins et al. (2011) the use of finer sand increases the bacteria removal. However, other researchers have used sand grains with lower uniformity coefficient and higher effective size than the ones recommended by the CAWST. For instance, Elliot et al. (2011) used the Accusand silica sand with $d_{10} = 0.27$ and $d_{60}/d_{10} = 1.4$ and reported higher bacteria and virus removals.

4.1.2 SAND MINERALOGICAL X-RAY DIFRACTION ANALYSIS

XRD analysis was used to gain information about the mineralogical characteristics of the filter medium (i.e., crushed rock sand). Results from this analysis are presented in Figure 4.2 and 4. 3. Some of the minerals identified in the spectrum were quartz and calcite. Quartz (SiO_2) is one of the most common minerals found worldwide and calcite ($CaCO_3$) has been use previously as a filtration medium in slow sand filters.

From the sand sample, it was easy to visually identify dark gray grains that exhibited a magnetic charge. The XRD analysis in the sand sample identified peaks that have a similar peak pattern with augite and briartite. Augite is a meta-silicate, which have iron in the ferrous and ferric states. It also contains calcium, magnesium and aluminum (Sigamony, 1944). Briartite is a sulfide mineral, which contains copper and germanium. Both minerals are dark gray.

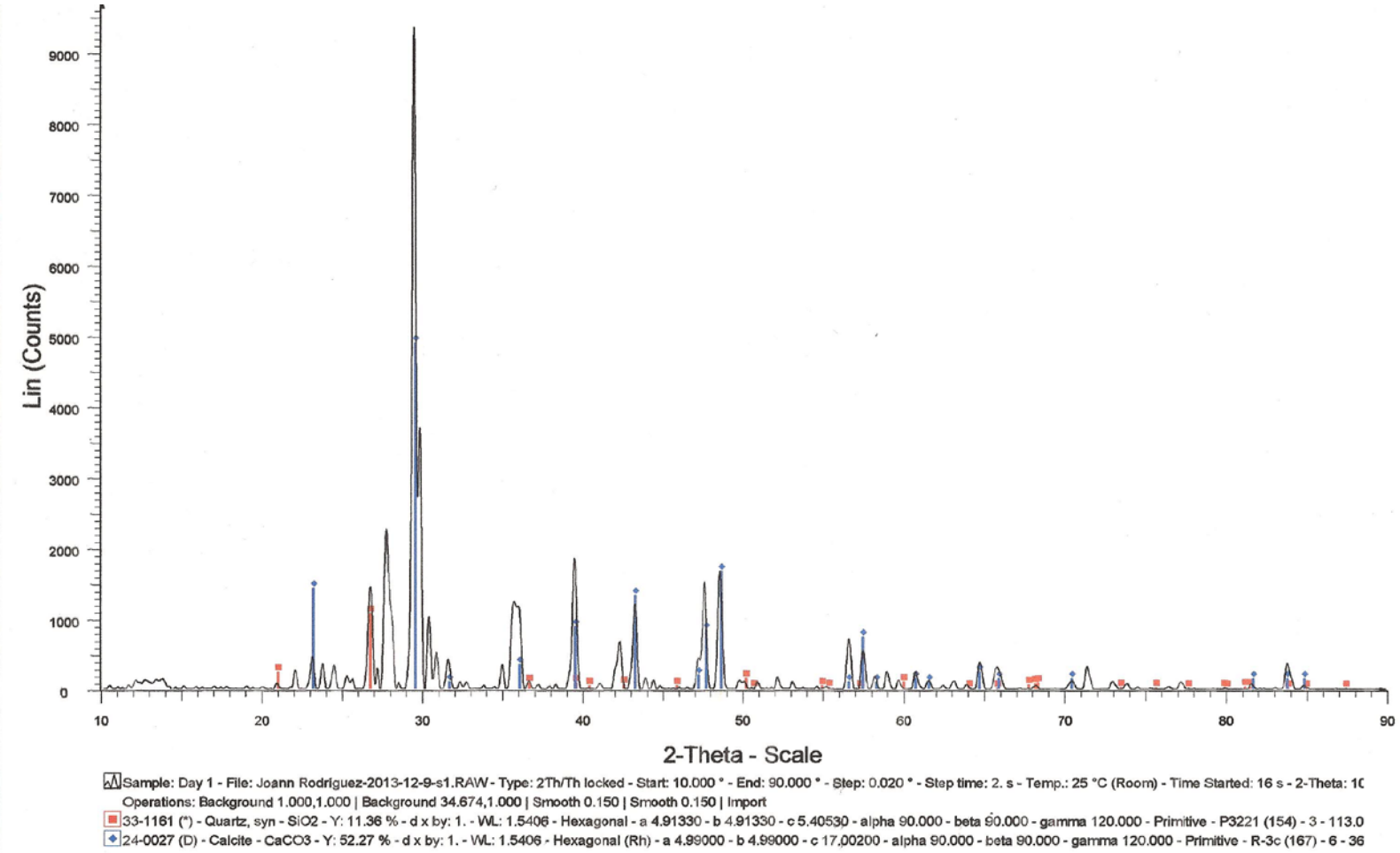


Figure 4.2 XRD spectrum for the crushed rock sand sample.

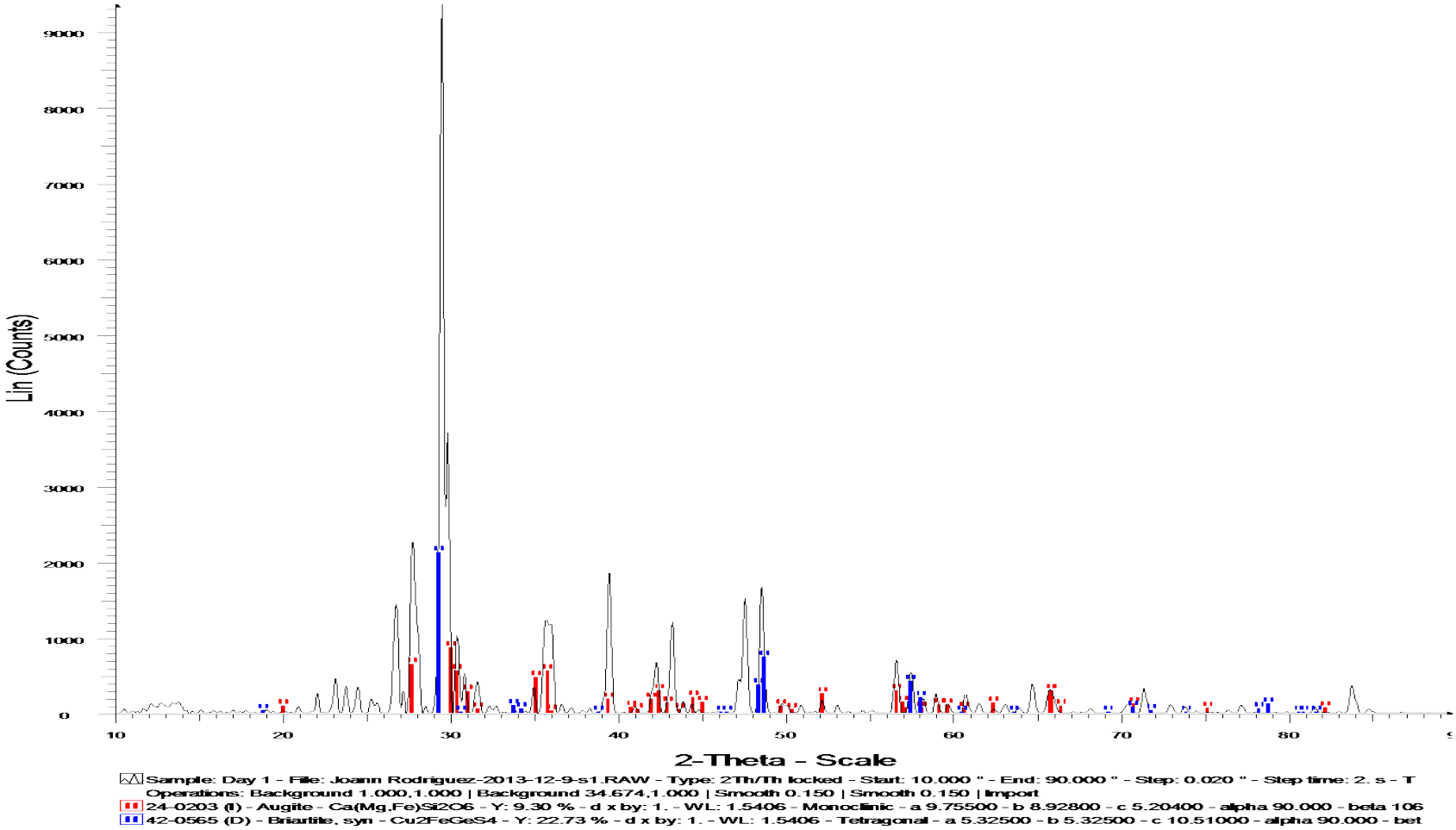


Figure 4.3 XRD analysis results, spectrum with iron compounds identified.

4.2 IBSF BENCH SCALE SYSTEM CHARACTERIZATION

4.2.1 HYDRAULIC FLOWRATE AND HEAD CORRELATION

One of the major advantages of the IBSF is its capacity to operate without electrical power, making it an appropriate technology for developing countries or for communities were electrical power resources are limited. The IBSF operation without electricity is possible due to the water driving force obtained from the water head in the IBSF water reservoir. An equation to describe the filtration flow rate in function of the water head for the bench scale IBSF was developed. Figure 4.4 shows a 4^{th} order polynomial trend for both the hydraulic flow rate and head with time in a filtered batch. Both parameters have a sharp decrease during the first 15 to 20 minutes, but after 20 minutes of water filtration, the decrease became smoother. The water flow from the filter stops when the water head reach the 0 cm, which occurs approximately 60 minutes after the IBSF filtration run.

Figure 4.5 shows the hydraulic flow rate against the water head in the IBSF reservoir. It is observed that it has a linear correlation described by $y=0.223x + 0.0477$. This linear equation allows the calculation of the water flow rate knowing a specific water reservoir level. It is important to emphasize that this equation is valid only for a clean IBSF, having a sand bed with the same characteristics (in grain size) as the one used for this study. Once the IBSF is put in operation, the equation coefficients are expected to change over time because of the sand bed clogging, which may cause an increment in the head losses.

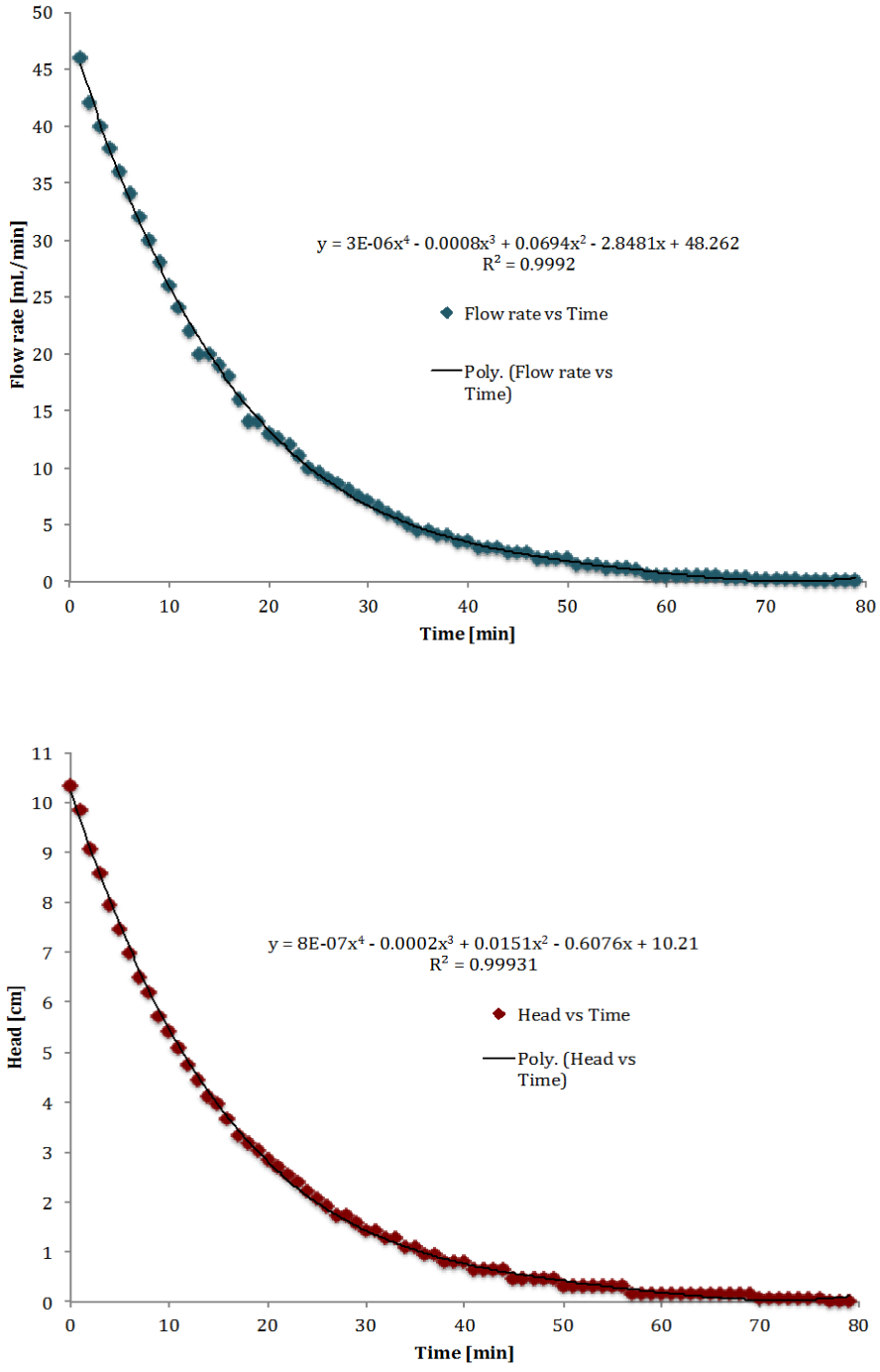


Figure 4.4 IBSF hydraulic flow rate and water reservoir level change over time during an IBSF run.

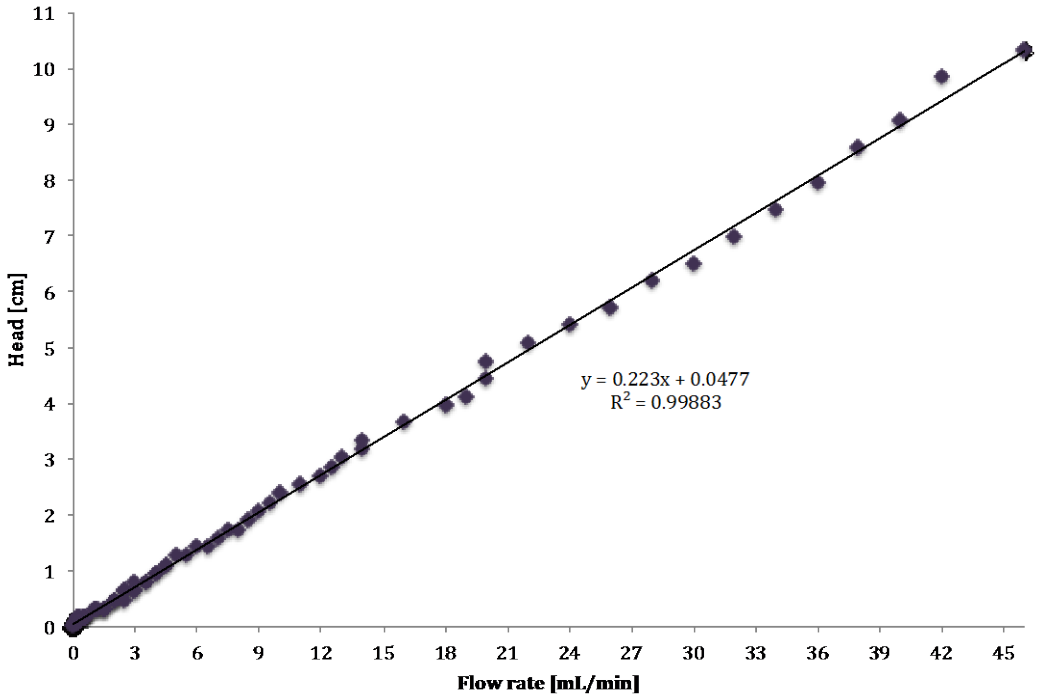


Figure 4.5 Flow rate and water reservoir head relationship.

4.2.2 IBSF BENCH SCALE TRACER TEST

The IBSF bench scale was tested with semi-continuous, step-input tracer studies with sodium chloride (796 mg/L and 1,600 µS/cm), to determine the hydraulic behavior of the filter bench scale as measured by the Morrill Dispersion Index (MDI). The residence time distribution curve (RTD) is presented in Figure 4.6.

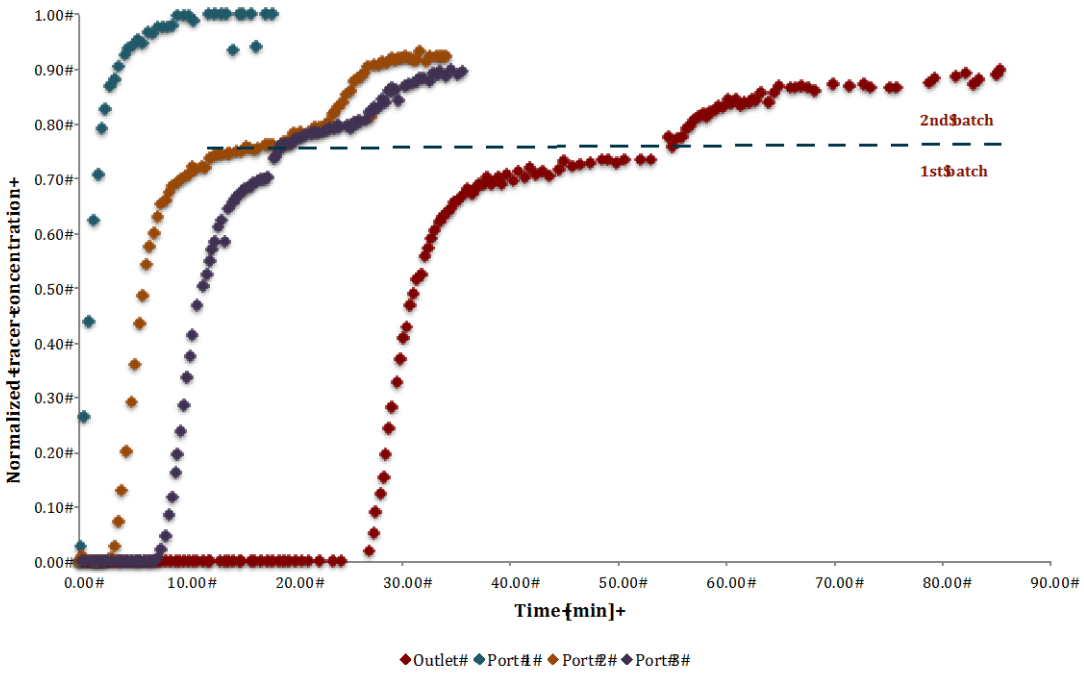


Figure 4.6 Residence time distribution curve.

This data shows that the filter has an MDI of 16 at the first sampling port located at 2.54 cm from the top of the sand bed surface, 7 at the second sampling port (29.7 cm) and 4 at the third sampling port (58.1 cm). The MDI decreased to 3 at the final effluent port. This implies that the filter behaved overall as a plug flow reactor with some dispersion. These

data suggest that the ports toward the top of the filter correspond to more of a mixing behavior, while those toward the bottom correspond to more of a plug flow-type behavior with some dispersion.

The near plug flow reactor behavior of the IBSF was reported previously by Elliot et al. (2008). One of the most important factors affecting the results in this tracer study is the intermittent feeding. This type of feeding, which simulates the actual operation of a biosand filter, causes a change in the head difference between the filter water reservoir level and the outlet tube, resulting in continuously slowing the outflow rate. This decrease in flow rate could be influencing diffusive mechanisms and affecting the filter behavior.

4.2.3 INITIAL CONDITIONS ON THE BENCH SCALE IBSF

The whole study was conducted in two bench-scale IBSFs, named Filter A (FA) and Filter B (FB), for reproducibility purposes. The parameters that were monitored and analyzed during the study were temperature, turbidity, conductivity, DO, pH, E. coli and Enterococcus. Samples were taken from the filter at each sample port depth before adding a new batch (i.e. before replacing the raw water batch previously added on each filter). Initial conditions for each parameter in both filters were monitored and are presented in Table 4.1. At this stage, the water fed into the filters was deionized (DI) water. Although DI water had very low values in turbidity and conductivity with neutral pH before its addition

to the IBSF, the sampled points resulted with higher values probably because of the DI water contact with the sand grains.

Table 4.1 Initial levels on the monitored parameters within the IBSF before adding the 1st raw water batch.

Filter	Depth [cm]	Temperature [°C]	Turbidity [NTU]	Conductivity [µS/cm]	DO [mg/L]	pH	*E. coli* [CFU/100mL]	*Enterococcus* [CFU/100mL]
Filter A								
	**2.54**	23.7	0.89	32.10	6.45	8.80	0	0
	29.7	20.8	0.28	50.41	6.34	8.59	0	0
	51.8	20.5	0.24	59.33	6.25	8.34	0	0
	Out	22.4	0.05	53.45	7.05	7.85	0	0
Filter B								
	**2.54**	21.0	0.95	37.34	6.91	8.94	0	18
	29.7	20.8	0.23	60.42	6.39	8.58	0	3
	51.8	20.6	0.23	75.96	6.34	8.34	0	4
	Out	22.2	0.02	66.71	7.12	7.83	0	6

4.3 IBSF PERFORMANCE

As previously stated in Chapter 3, section 3.3, FA and FB where operated in parallel and monitored for ten consecutive weeks. The monitored parameters were effluent flow rate, turbidity, temperature, pH, conductivity, DO, *E. coli* and *Enterococcus* population.

4.3.1 HYDRAULIC FLOWRATE

The hydraulic flow rate was measured at the IBSF exit, every 24 hours, immediately after the addition of a new raw water batch (at the maximum operational head). As Figure 4.7 shows, both filters FA and FB behaved similarly in overall during the ten weeks experimental time period, which indicates replication of both filters in terms of the filtration rate parameter. It started with the maximum hydraulic flow rate recommended for IBSF, 50 mL/min, and decreased since the first week. From the 2nd to 7th week the flow rate of both FA and FB were almost constant, but after the 7th week it started to have a continuous decrease (about 25 % decrease by last week).

Several studies have pointed out the filter clogging as the main reason for the decrease in the hydraulic flow rate. IBSF clogging can be influenced by frequently feeding the IBSF with raw water that have high concentrations of organic matter and turbidity. The high concentrations of organic matter could induce the continuous biofilm development over the sand grains, reducing the pore spaces between the sand grains where the water percolates (Leverenz et al., 2009). Additionally, high concentration of suspended particles

(i.e. turbidity) could reduce the time period to get the IBSF clogged. To facilitate the experimental procedure and to reduce the amount of raw water, FA and FB were fed once every 24 hours. But, in developing countries, one batch of treated water (approx. 20 L) will not be enough to satisfy the daily water demand for an average family, demanding an increase in the filtration frequency in a 24 hour period (i.e., adding more than one raw water batch daily). This increase in the filter feeding frequency could cause a shift to the left in Figure 4.7, corresponding to earlier decrease in the IBSF hydraulic flow rate.

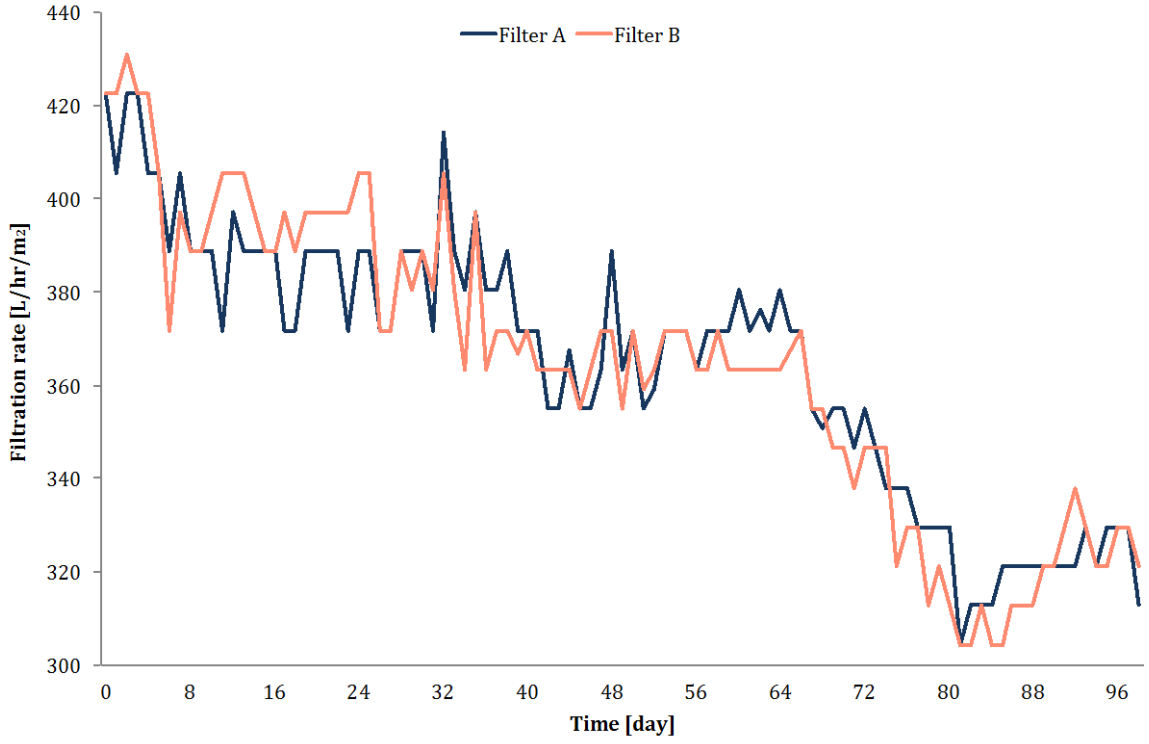


Figure 4.7 FA and FB filtration rate during the ten weeks experimental time period.

Further studies need to be performed in order to evaluate the impact of the filter feeding frequency in the hydraulic flow rate.

4.3.2 TURBIDITY REMOVAL BY IBSF

Figure 4.8 presents the turbidity values in the feeding water (inlet) and effluent water samples, in both FA and FB, and in the control water sample. The turbidity removal was over 90% (up to 99.77%) since the first week of the experiment, demonstrating the IBSF effectiveness in water turbidity removal.

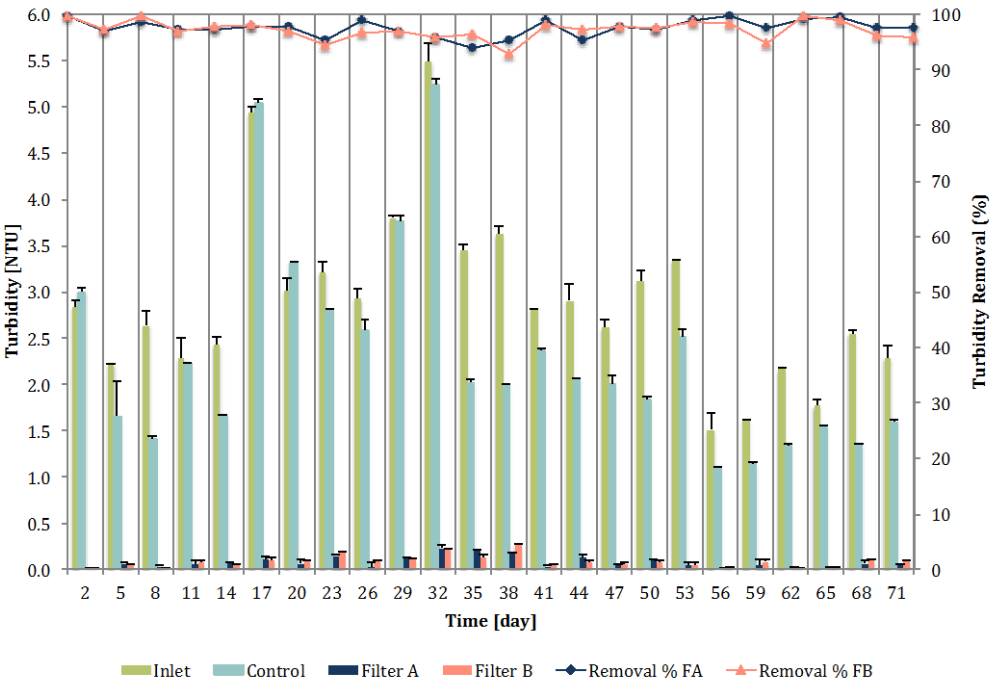


Figure 4.8 Turbidity removal by filters FA and FB.

Similar removal rates were obtained in previous IBSF laboratory studies, although lower turbidity percent (85%) has been obtained in field studies (Buzunis, 1995; Elliott et.

al, 2011; Jenkins et. al, 2011; Stauber et al., 2006; Young-Rojanschi & Madramootoo, 2014). A statistical analysis to compare the performance of FA and FB for turbidity removal revealed that there was no significant difference between both filters (*p=0.734*).

The turbidity values in the feeding water (inlet) vary in time due to the uncontrolled variations in the natural surface water source (Oro Creek) from where the raw water was obtained on a weekly basis. This turbidity variations in the raw water source impacted FA and FB turbidity removal in some of the testing days, as shown in day 32 (Refer to Figure 4.8), confirming the relationship between the feeding water quality and the resulting IBSF removal efficiency. One of the possible mechanisms responsible for the turbidity removal, in addition to the mechanical trapping, could be sedimentation and straining of heavy particles on top of the filter medium.

Figure 4.9 presents a comparison between the turbidity levels in the feeding water (at the filter's inlet) and the control sample after a 24-hour period. The control sample consisted in a specific volume of raw water maintained at rest in a separate container next to the filters. As seen in Figure 4.9, the control sample had a reduction in turbidity. The possible mechanism causing this reduction in turbidity is the settling of suspended particles due to gravity. This result suggests that up to 30% (in most of the tested days) of the suspended particles in the feeding water are coarse dispersions heavily enough to be removed by sedimentation or by straining over the sand bed surface.

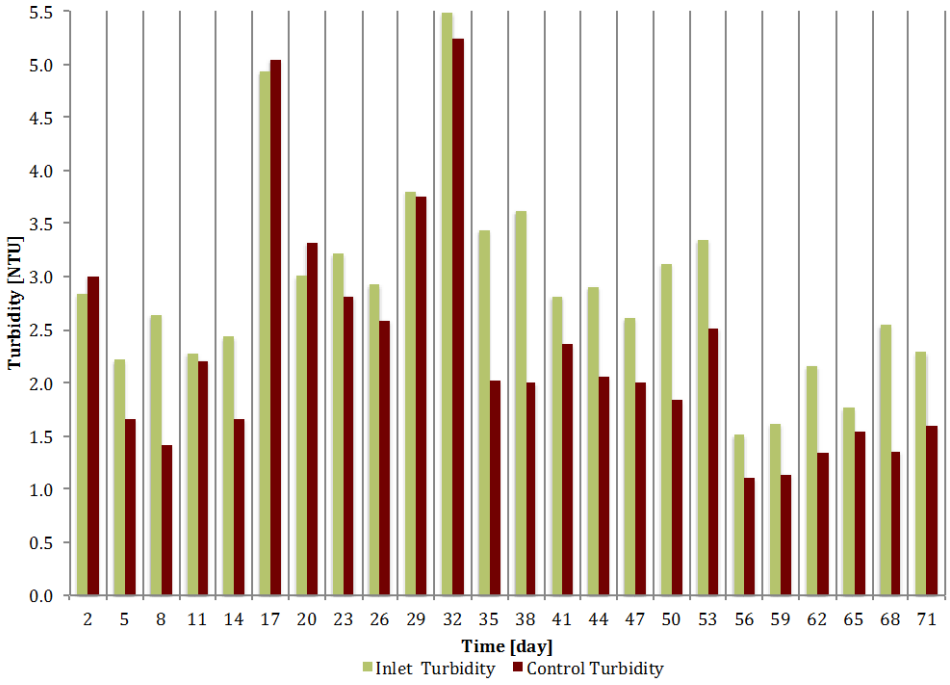


Figure 4.9 Turbidity decrease in the raw water by sedimentation**.**

Although assumptions about the size of the particle causing turbidity can be made with the obtained results, the inequality in constituents that can cause turbidity in the water (inorganic and organic matter) makes difficult the establishment of reliable conclusions about the possible action of the absorption mechanism in the IBSF. Comparing the hydraulic flow rate decrease with time (Figure 4.7) and the turbidity removal percent in Figure 4.8 across the entire study, lower IBSF hydraulic flow rates do not necessarily determine turbidity removal, because more than 90% of turbidity removal was detected since the first testing day when the hydraulic flow rates were the maximum values observed. This result contrasts with the results obtained in another IBSF study, where higher turbidity removals were related with lower hydraulic flow rates (Baig et. al, 2011).

Turbidity measurements were also performed through FA and FB sand bed depth. Figure 4.10 shows the turbidity measurements recorded from FA and FB water samples respectively. Referring to Figure 4.10, the IBSF sand bed depth were a crucial factor to obtain the low turbidity measurements recorded at the outflow water sample. Analyzing FA and FB in terms of the turbidity removal through the sand bed depth, the behavior of both filters seems to be similar, having just a few variations in some of the tested days (i.e., day 5^{th}, 14^{th}, 29^{th}) mainly on the first sampling port located at 2.54 cm of the sand bed depth. Although, according to the overall behavior, those discrepancies appear to be just random variations.

The turbidity removal in the sampling point located at 2.54 cm of the FA and FB sand bed depth, where the higher biofilm development was expected, had slight changes over time. The lower sampling ports located at 29.7 and 51.8 cm decreased their turbidity removal capacity through time, although the turbidity measurements in those sampling points never exceeded the 0.63 NTU. Comparing the 29.7 and 51.8 cm sampling ports, despite the fact that the 51.8 cm sampling port is located in a nearest end point of the sand bed, there appears to be no difference between the two sampling ports efficiency in the turbidity removal.

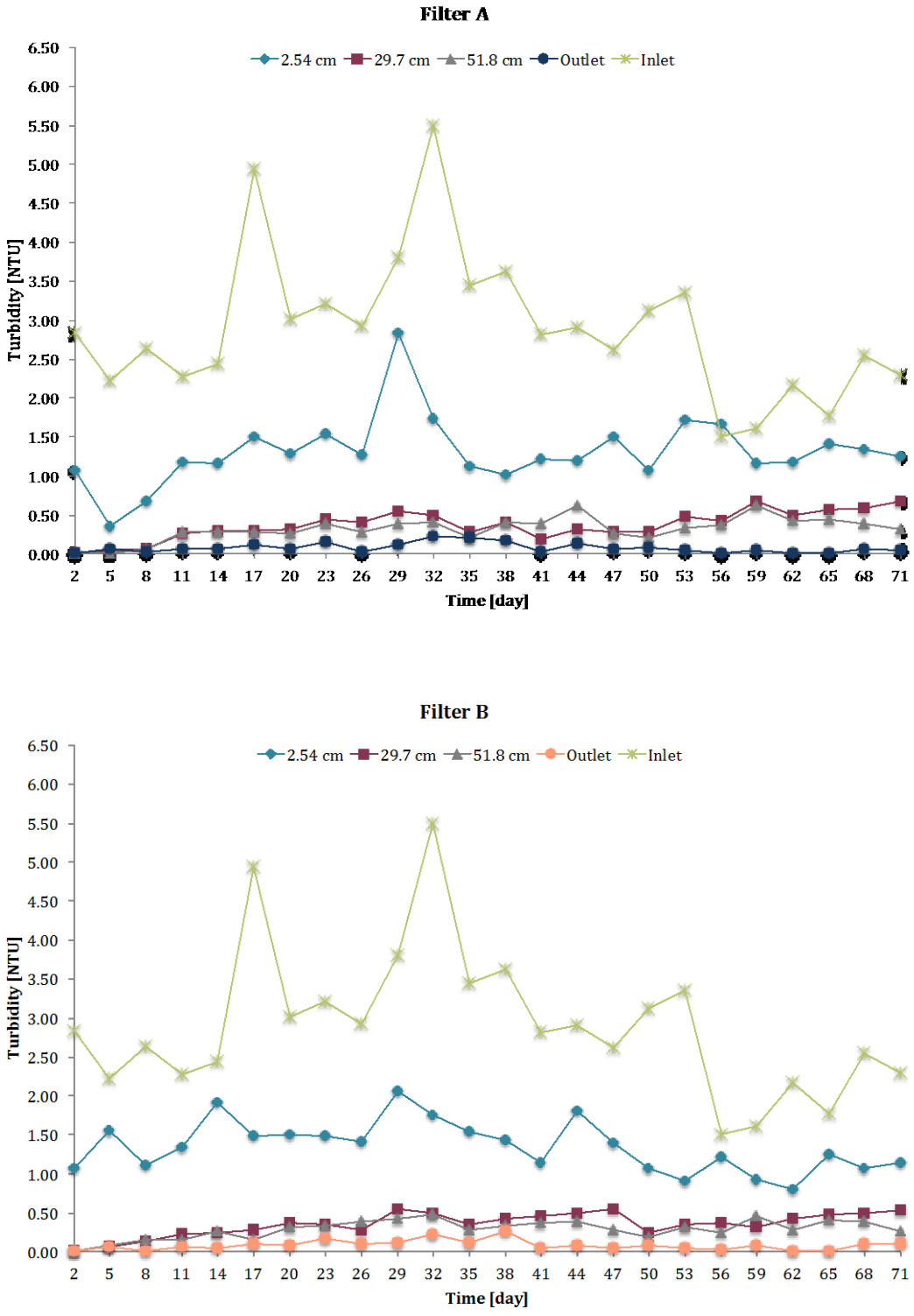


Figure 4.10 Turbidity levels through FA and FB sand bed depth.

This result could lead future research to evaluate the filter efficiency when the sand bed depth is reduced to 29.7 cm instead of 51.8 cm and optimize the use of materials. The last sampling point, which is located at the filter outlet, represents the turbidity of the complete batch volume after the IBSF treatment. The turbidity values at this location were lower than the ones recorded at the 51.8 cm sampling point. Because there was little difference between the 29.7 cm and the 51.8 cm sampling point in the turbidity values, the final layer of sand could not be the responsible for that additional turbidity reduction. The possible explanation to the lower turbidity levels in the outflow point is the height of the outlet pipe, which could be preventing the exit of some of the particles that could pass through the sand bed. Although this could be one of the logical explanations, the particles that could be capable of passing through the entire sand bed are the finest particles, which because of their lightweight could be dragged out of the filter.

4.3.3 TEMPERATURE

Temperature was monitored in the feed water and in each sampling point at the end of the idle period of each treated batch. Both filters (FA and FB) had similar temperature trend over time. The feed water temperature was maintained at an average 20.9±0.49 °C. The variations in temperature in each sampling point through time are presented in Figure 4.11. The water samples drawn from the first sampling port, located at a depth of 2.54 cm below the sand bed surface, exhibit an increase in temperature of 1.3 ± 0.8 °C when

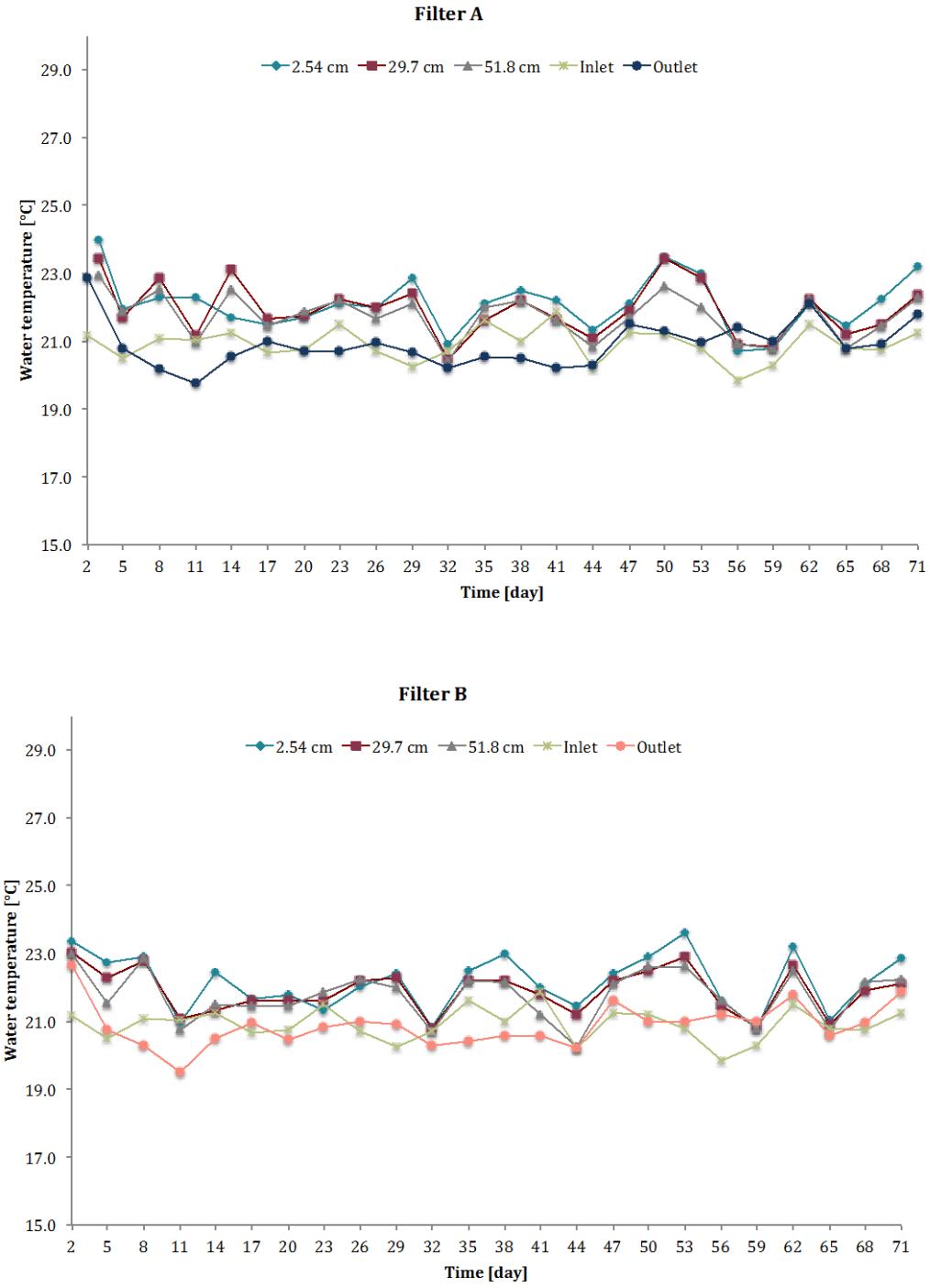


Figure 4.11 Temperature fluctuations over time inside FA and FB.

compared to the feeding water for both filters (*p <0.0001*). In the rest of the sampling points, at 29.7 cm and 51.8 cm depth, the temperature was slightly lower than in the first sampling point.

The temperature decreased on average 0.2 ± 0.5 °C and 0.3 ± 0.3 °C respectively. In overall the temperatures inside the filter were higher than the feeding water temperature through all the experimental time period. The feeding water and the effluent water from FA and FB do not have a statistical significance difference (*p= 0.8390*) in terms of temperature. Previous studies have demonstrated that the temperature of the water inside the filter can impact the IBSF removal efficiency, specially the microorganism removal. Schijven et al. (2013) studied the pathogenic viruses and bacteria removal (bacteriophage MS2 and *E. coli* WR1 were used as model microorganisms) at various filter temperatures (4 – 19 °C) and young *schmutzdecke* ages (4 -1105 days old). The results showed that at higher temperatures, higher removals were obtained for both model microorganism, even in filters with young *schmutzdecke* (Schijven et al., 2013).

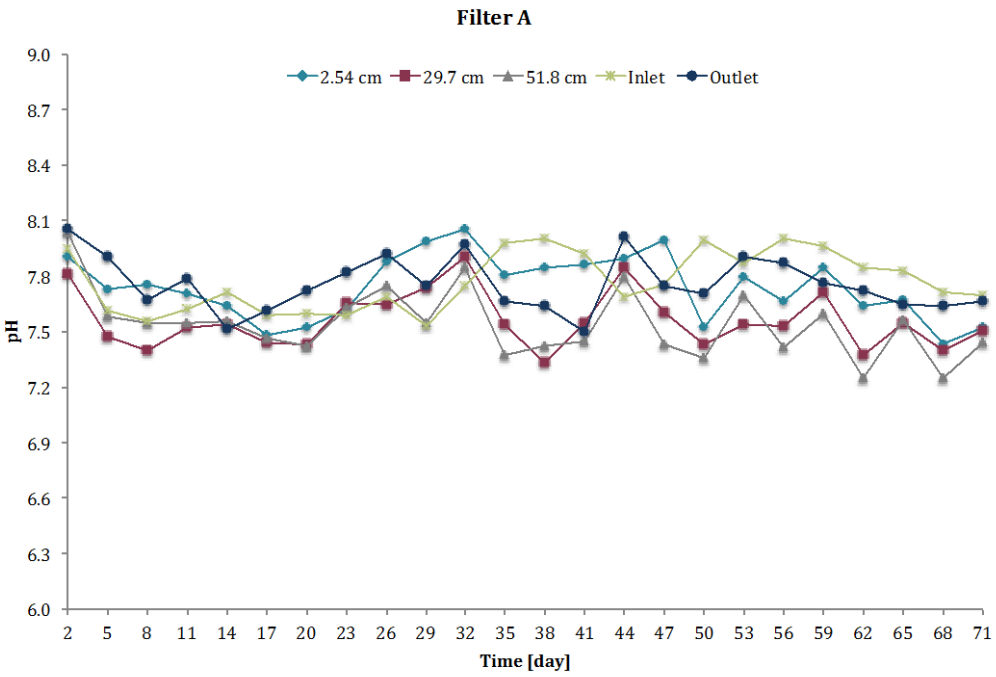
In addition, higher temperatures could influence the reaction velocity inside the filter and could accelerate the metabolism rate of the microorganism established inside the filter (Huisman & Wood, 1974). Adversely, it had been found that high filter temperatures (20-30 °C) could increase the reproduction of heterotrophic bacteria inside the filter, leading an increase in the population of heterotrophic bacteria in the effluent compared to the feeding water population (World Health Organization, 2003).

4.3.4 pH

pH values in each sampling point through the experimental time period are presented on Figure 4.12. Overall, both FA and FB seem to have the same behavior over time. However, a different pH trend can be observed between the outlet and inlet sampling points, before and after the 32th day. Before day 32 the effluent had slightly higher pH values than in the feeding water, having an average of 7.79 in the effluent, while in the feeding water the average pH was 7.65. Although the observed changes in the pH values do not have a statistical significance difference, an increase in pH can be attributed to biological denitrification. According to Aslan & Cakici (2007) and Murphy et al. (2010), which studied nitrification, denitrification and ammonification in slow sand filters and in IBSF, respectively, concluded that such processes (specifically denitrification) may increase the alkalinity and thus, the pH.

The first sampling point (2.54 cm) has a pH up to 8.06, indicating the possible denitrification process by microorganism in the biolayer. In deeper sand layers (29.7 cm and 51.8 cm) lower pH values, down to 7.40, were found. The pH values inside the IBSF sand bed conserved the same tendency during all the experiment, having a decrease in pH values with respect to the sand bed depth (i.e. the deeper the sand bed, the lower the pH).

As mentioned before, after the 32th day of experiment, the filters effluents started to have lower pH values than the feeding water. Also, referring to Figure 4.12, the deeper sand bed layers show a slightly decrease in the pH value compared to the days before the 32th day. This pH decrease could be caused as a result of simultaneous denitrification and



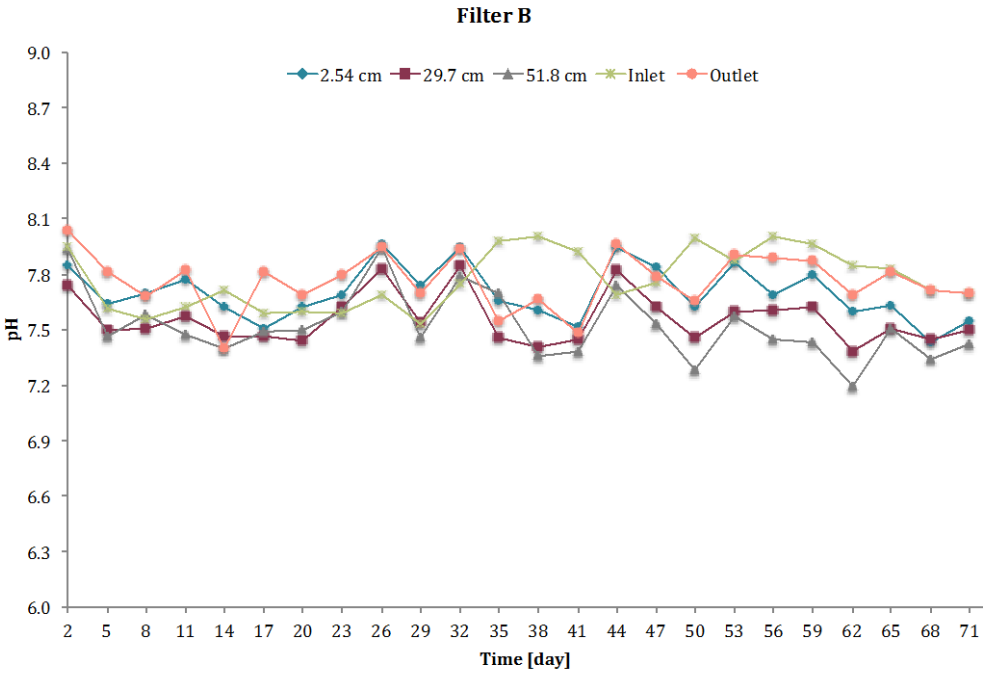


Figure 4.12 pH values recorded in each sampling point over time.

nitrification inside the filter (Nakhla & Farooq, 2003) (Murphy et al., 2010). The microorganisms established in the biolayer, which could require up to 30 days to develop depending on the feeding water biological activity and which is located mainly in the first sand bed layers, could break down the organic matter (OM) present in the feeding water. When the microorganism uses the organic matter as a source of energy for their metabolism, the products from this process (i.e. amino acids) are used for biochemical reactions that could take place in the deeper layers of the IBSF sand bed. Some of the biochemical process that has been identified in this area of an IBSF is nitrification, which increases nitrite and nitrate levels and reduces the water pH by the H^+ ion formation (Huisman & Wood, 1974).

Both nitrification and denitrification are two processes that were beyond the scope of this study. Hence, they were not monitored during the study. Further research is needed to confirm the nitrification and denitrification process in the IBSF by measuring the NH_3, NO_2^- and NO_3^- concentrations through the sand bed depth and conducting experiments in order to identify the presence of ammonia-oxidizing bacteria (*Nitrosomonas*, *Nitrococcus*, Nitrobacter, *Nitrosospira* and *Nitrospina*) (Watson et al., 1981) inside the IBSF.

4.3.5 CONDUCTIVITY

The conductivity of the feeding water was between 439.1 – 363.3 μS/cm while the average effluent (in both filters) was 402.2 – 250.5 μS/cm. The comparison between the

feeding water and the effluent from FA and FB conductivity does not follow a specific pattern over the time (Figure 4.13). During the last experimental days (after the 59th day), the conductivity values detected in the feeding water and the IBSF effluent are very similar, resulting in no significant removal or addition of conductivity by the IBSF (*p= 0.0842*).

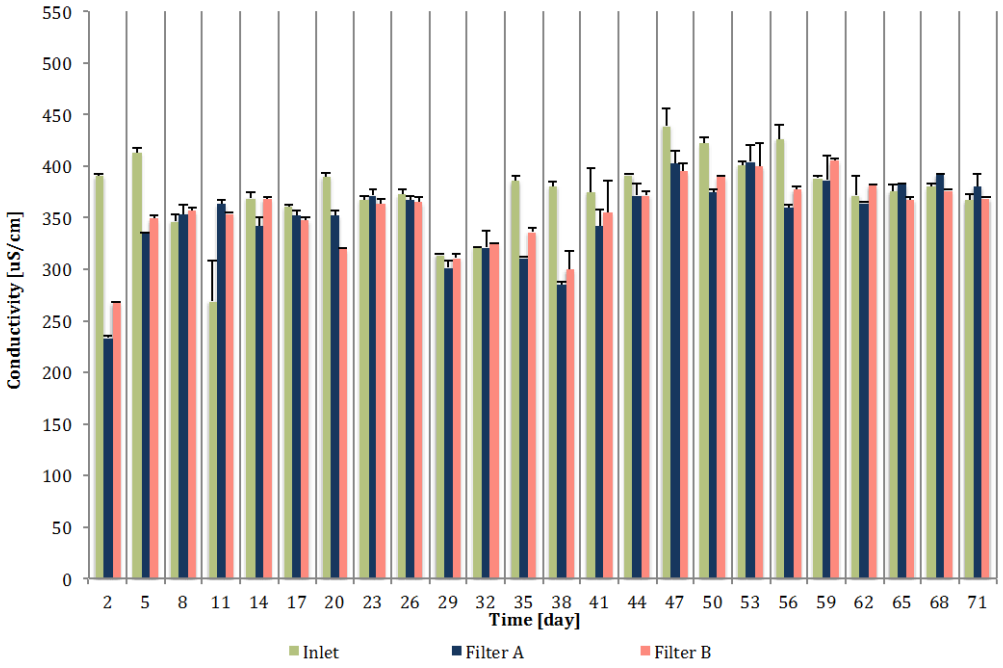


Figure 4.13 Conductivity levels in the feeding water and IBSF effluent over time.

Figure 4.14 shows the conductivity trend within the filters with time. As seen, the conductivity values have slightly increases and decreases through the sand bed depth until about the third week. After that week, the conductivity values get uniform through all the filters sand beds. Both IBSF bench scales columns seem to have the same conductivity trend through the sand bed depth over time. In general no significant changes in the

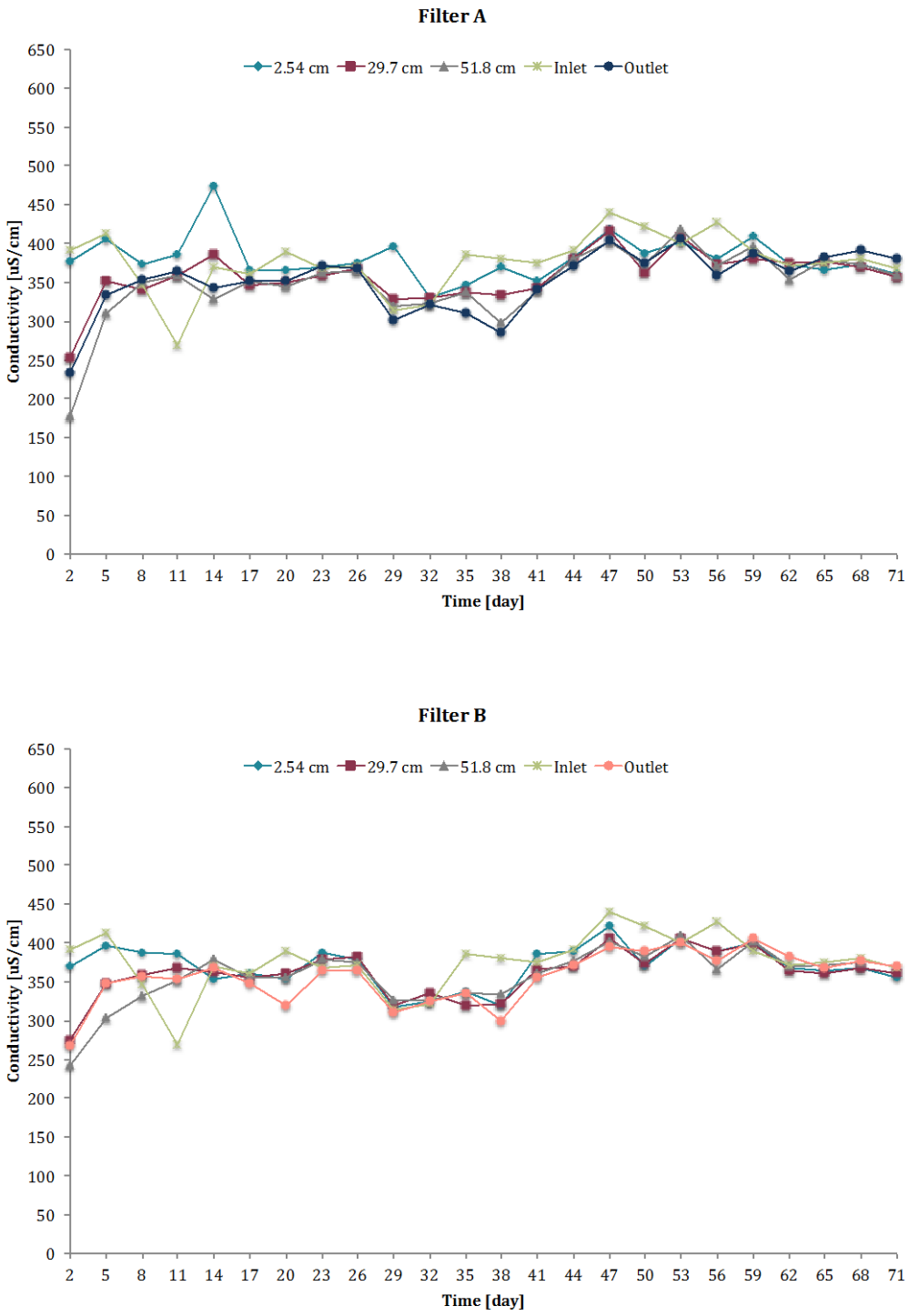


Figure 4.14 Conductivity changes in FA and FB through sand bed depth and time.

conductivity values through the filter sand bed depth and in the IBSF effluent were identified after the first month of the filter use, although, slight changes inside the filter were detected during the first week.

4.3.6 DISSOLVED OXYGEN

The DO concentration was measured in all the sampling points to develop the DO concentration profile through the IBSF sand bed. Figure 4.15 shows the DO profile through the sand bed depth of FA and FB. The DO concentration seems stable in each sampling point located inside the sand bed over time, after the first week. Comparing the sand bed monitored depths, there is a significant decrease in the concentration of DO between the feed water and the first sampling point located at 2.54 cm from the sand bed top, with $p < 0.0001$. This decrease in the DO concentration in this sand bed layer could be related to the oxygen consumption by biochemical oxidation reactions (Murphy et al., 2010). However, according to Buzanis (1995), a standing zone of 5 cm is necessary for the appropriate diffusion of oxygen into the biolayer. During this experiment anoxic conditions were reached in the biolayer with DO concentrations down to 4.45 mg/L. Similar results were obtained in previous studies (Young-Rojanschi & Madramootoo, 2014).

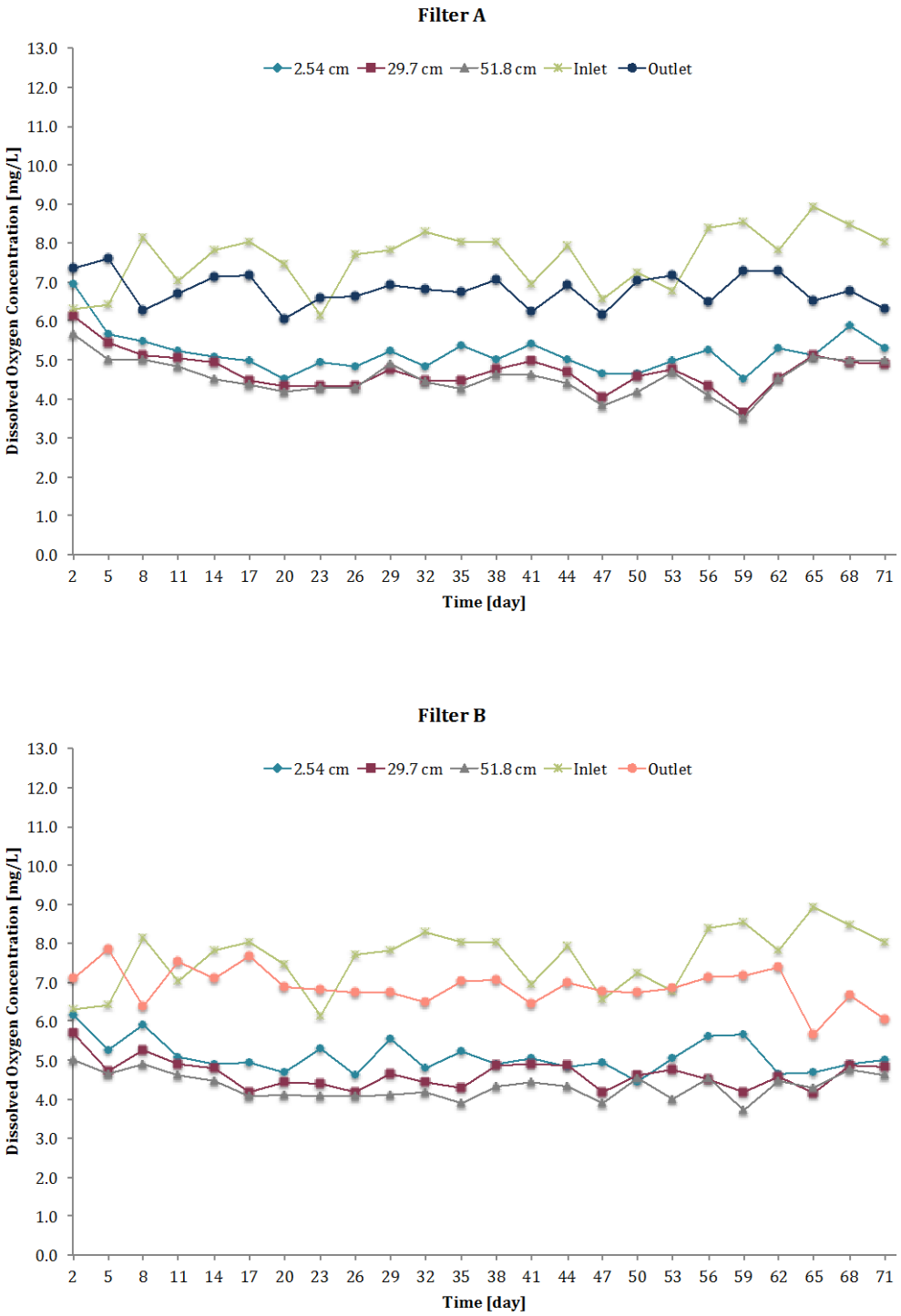


Figure 4.15 Dissolved oxygen concentrations in all sampling points from FA and FB over time.

Between the 2.54 cm, 29.7 cm and 51.8 cm sampling points, there is an additional decrease in the DO concentration, however the difference between them is lower in magnitude than the DO difference between the feeding water and the 2.54 cm sampling point. It seems that DO concentrations are lower at deeper sand layers, but there is no significant difference between the DO concentration in the 29.7 cm and 51.8 cm sampling points (*p=0.3206*). The lowest DO concentration measured inside the IBSF sand was 3.44 mg/L in FA and 3.66 mg/L in FB indicating anoxic conditions.

Both IBSF (FA and FB) had a very similar DO profile. The DO concentration at the FA and FB effluent was higher that the DO concentration measured in the 51.8 cm sampling point. This increase in the DO concentration could be caused by the aeration of the water at the outlet pipe (Young-Rojanschi & Madramootoo, 2014).

The DO deficit through the ten weeks study was calculated taking into account the saturation value of oxygen in water, which depends on temperature. According to Figure 4.16, the deeper the sand bed, the higher the DO deficit. It is also clear that the deficit in all the sampling points inside the sand bed increase over time. The DO deficit has a noticeable increase from sampling point to sampling point during the first and second week. However, the DO deficit between the 29.7 cm point and the 51.8 cm point seems to be very similar after the second week, indicating very similar oxygen consumptions from the 29.7 cm to the end of sand bed.

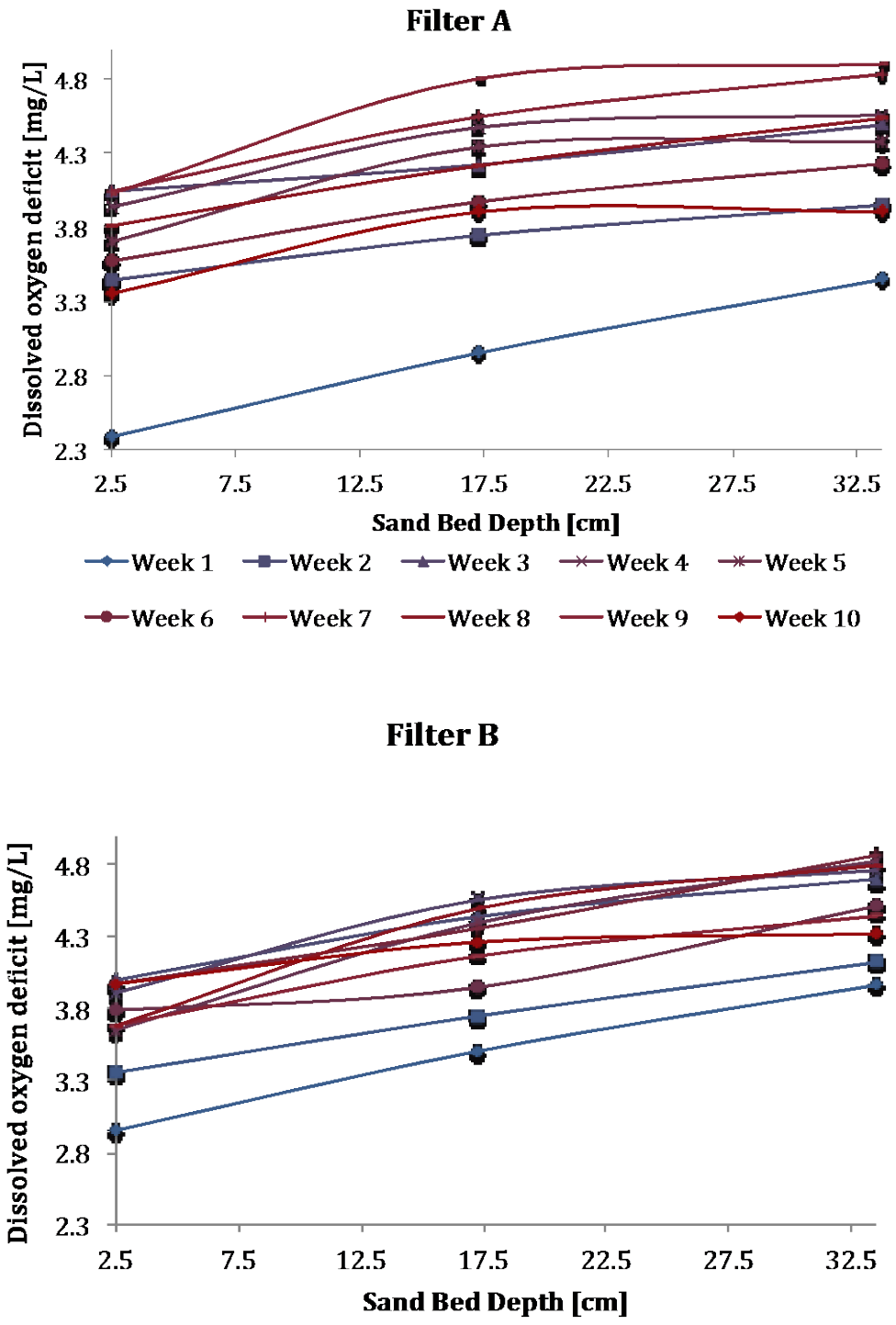


Figure 4.16 Weekly DO deficit through the sand bed depth.

4.3.7 *E.COLI* REMOVAL

E. coli levels were also monitored in the influent, effluent and through the IBSF during the ten weeks period. Figure 4.17 shows the *E. coli* population in the influent (feeding water) and effluent (from FA and FB) as well as the *E. coli* population difference between filter's inlet and outlet, denoted by removal percent. As it can be observed, there was a considerable decrease in *E. coli* population in the effluent when compared to the E. coli levels in the influent. The IBSF decreased the *E. coli* population in the water down to 10 CFU/100mL, and achieve a removal rate up to 98%.

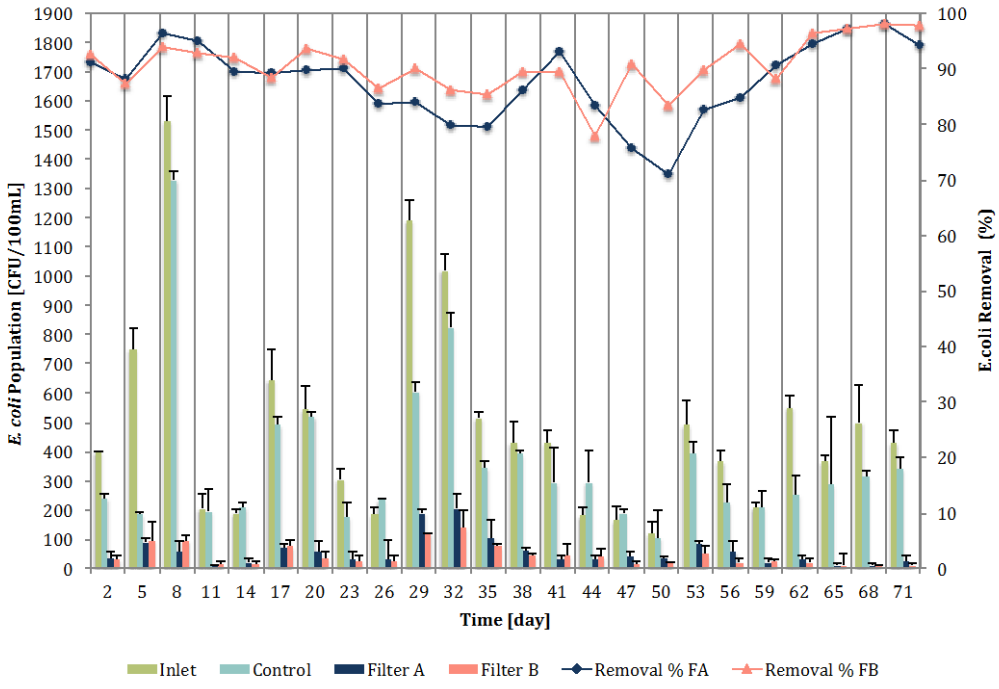


Figure 4.17 *E. coli* levels in the filter's influent and effluent, and removal percent by the IBSF.

The removal of *E. coli* was significant since the very first week, suggesting that the removal does not necessarily depend in the biolayer, but by other mechanisms, such as adsorption or trapping. The *E. coli* population in the effluent seems to have a dependence on the feed water *E. coli* population.

Figure 4.17 also depicts *E. coli* levels measured in the control sample. A slight decrease in *E. coli* population is observed, which could mean that the amount of nutrients present in the feed water aren't sufficient for the *E. coli* survival after the 24 hours of idle time. The *E. coli* population fluctuations in feed water could be one of the reasons for the variations in the *E. coli* removal percent over the first 4 weeks. At higher population in the feed water, higher population of *E. coli* was found in the IBSF effluent (Figure 4.17). However after the 38th day, this *E. coli* removal dependence on the feeding water population seems to have less importance, because, even though the *E. coli* population in the feed water decreased, the effluent seems to have the same removal rate than the one when high *E. coli* population are present in the feed water. A clear example of this behavior is the removal rate during the sixth week.

Figure 4.18 presents the *E. coli* levels monitored within the IBSF (A and B) through the sampling points at different filter depths. The *E. coli* population had the most noticeable decrease in the first sampling point (at 2.54 cm), where the biolayer was expected to develop.

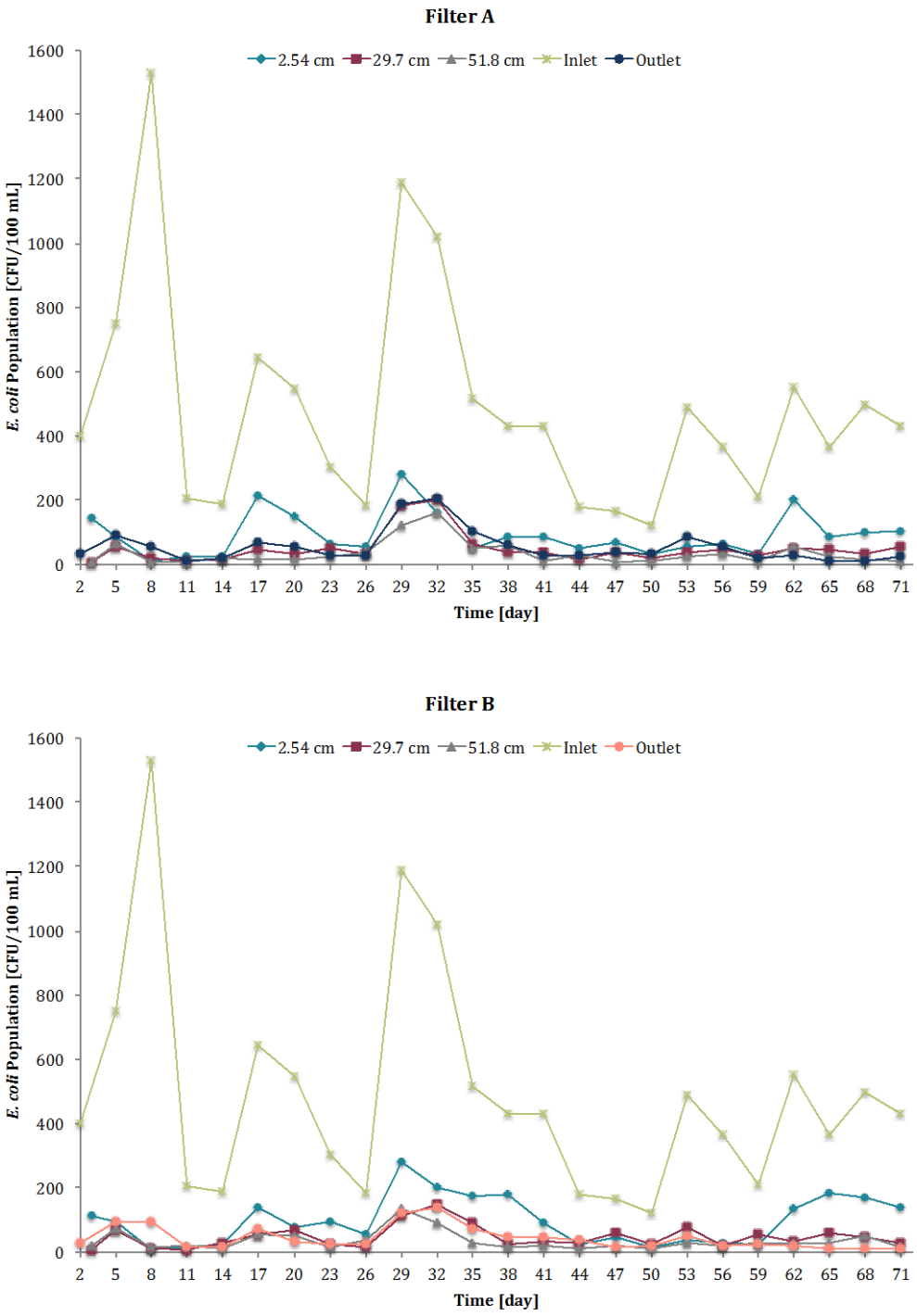


Figure 4.18 *E. coli* population through the sand bed.

An R Data Analysis was performed to determine the impact of the DO concentration, the sand bed depth and filter ripening time on the *E. coli* removal. In addition, similar *E. coli* removals in two similar filters were accessed (between FA and FB). A logistic regression, also called a logit model was used. Figure 4.19 shows the *E. coli* population in each sampling point including the feed water, as an average of the population values obtained from FA and FB over time (b-splines degree 3 and 3 knots). In the right graph the *E. coli* population in the feeding water was not included to amplify the scale and have a better visualization of the *E. coli* population at the other sampling points b- splines.

The abbreviations in the graph legend means the *E. coli* population in CFU/100 mL at each sampling point located at different sand bed depth and the one at the filter effluent (P1 = 2.54 cm, P2 = 29.7 cm, P3 = 51.8 cm and P4 = filter's effluent). It seems that the behavior of the sampling points in terms of the *E. coli* population change over time. During the first 60 days the effluent had higher population of *E. coli* than the ones found in the deeper layer of the sand bed depth (at P3 =51.8 cm). After this day the effluent started to have lower population of *E. coli* than any other point inside the sand bed.

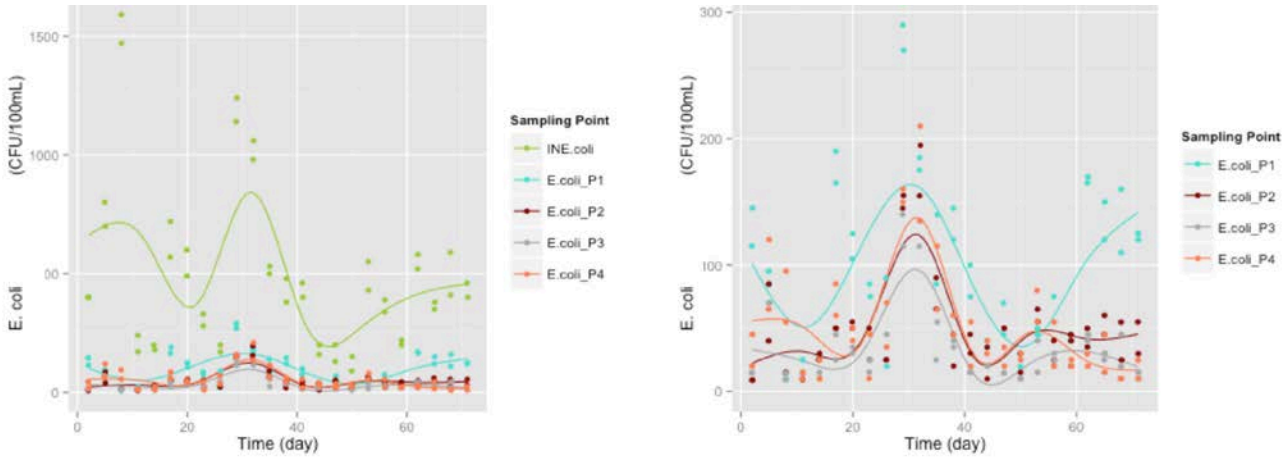


Figure 4.19 *E. coli* population as an average of FA and FB vs. time (b-splines degree 3, 3 knots).

Figure 4.20 shows the b-splines of the *E. coli* removal rate in each sampling point vs. time. The behavior of the sampling points seems to change over time making it difficult to make overall conclusions about the sampling points efficiencies in terms of the *E. coli* removal rate. Comparing the *E. coli* removal behavior at P2 and at P3, the removals seems to be very similar until near the day 30. After day 30, the removal percent increased over time at the P3.

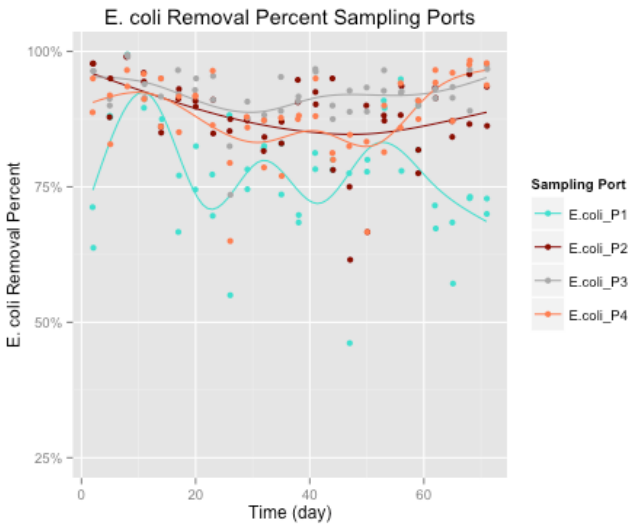


Figure 4.20 Average *E. coli* removal percent in each sampling point vs. time (b-spline degree 3, 3 knots)

The statistical model with the co-variables of Filter, sampling point, time, dissolved oxygen at the inlet water and dissolved oxygen at sampled point was:

$$y^*_{ijk} = \left(\frac{y_{ijk}}{1-y_{ijk}}\right) = X^t_{ij}\alpha + f_j(t) + u_{ij} + \epsilon_{ijk} \qquad (4.1)$$

where the model output is:

$$y_{ijk} = \frac{(in\ E.coli - E.coli)}{(in\ E.coli)} \qquad (4.2)$$

where:

$$f_j(t) \approx \sum_{r=1}^{R} \gamma_r B_{rj}(t) \qquad (4.3)$$

and:

i= 1,2 Filter (FA=1, FB=2),

j= 1,2,3,4 (Sampling point),

k= 2,5,8,11,…65,68.71,

Time = 8 times per filter for a total of 384,

y^*_{ijk}: logit transformation of the model output,

X^t_{ij}: transpose matrix of the model co-variables,

α: coefficient vectors of the model co-variables,

$f_j(t)$: model approximation curves of the *E. coli* reduction percentage,

B_{rj}: base matrix for the B-splines (to construct the $f_j(t)$ curves), and

Y: coefficient vectors of the B-splines.

The model assumed the intercept u and the ε errors independent (i.e, $u_{ij} \perp \varepsilon_{ijk}$) and normal distributions with the following characteristics:

$$u_{ij} \approx N(0,\sigma^2_u) \quad (4.4)$$

$$\varepsilon_{ijk} \approx N(0,\sigma^2) \quad (4.5)$$

In this model, "in *E. coli*" refers to the population at the feeding water and "*E. coli*" the population at the sampled point. Figure 4.21 shows the experimental raw data and the approximations made by the model. The results show the well fitting of the model approximations b-splines with the experimental data b-splines.

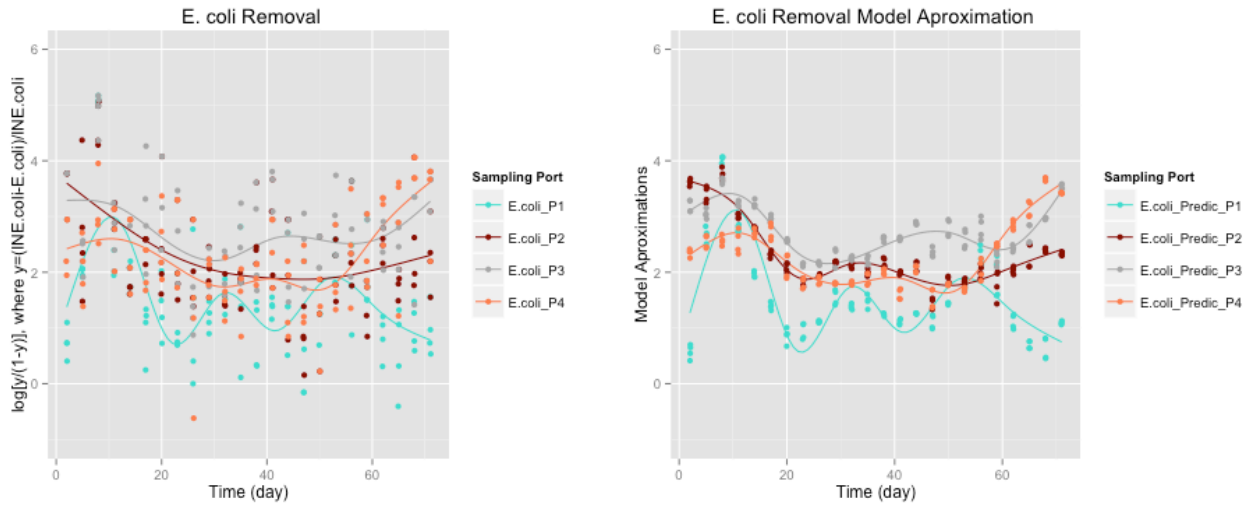


Figure 4.21 *E. coli* removal in each sampling point vs. time where y=(in *E. coli- E. coli)/in E. coli.*

A Shapiro-Wilk normality test was applied, which results with a p-value = 0.2424, indicating that with a 95% of probability the data follow a normal distribution. The model results are summarized in Table 4.2.

Table 4.2 Summarized results from the statistical model, *E. coli* removal.

Factor	Df	Sum Sq	Mean Sq	F value	p- value
Filter	1	0.23	0.227	0.401	0.5268
Sampling point	3	72.53	24.176	42.699	<2e-16
bs (Time, df = 7, degree = 3)	7	67.20	9.600	16.955	<2e-16
log (in oxygen)	1	5.73	5.731	10.122	0.00160
log (oxygen)	1	6.00	6.003	10.602	0.00124
Depth: Time	21	62.93	2.997	5.293	4.26e-12
Residuals	349	197.60	0.566		

According to the obtained results, there is no difference between FA and FB in terms of the *E. coli* removal, which means, if two IBSF that where installed following the same procedure and using the same filtration medium, operated in the same manner (including the idle time period) and using the feeding water from the same source, they should have the same efficiency in *E. coli* removal. Those results could mean that the variable efficiency of the IBSF in the field could be caused by external factors of the IBSF design (i.e., different operation manners, different feed water sources, etc.)

Referring to Table 4.2, the sand bed depth has a significant impact in the *E. coli* removal, which matches with the results presented in Figure 4.18. The DO concentration in the feed water and in the sample location is a factor that influence in the *E. coli* removal. Those results affirm the lack of DO as a *E. coli* removal mechanism pointed out in previous IBSF studies (Buzunis, 1995).

4.3.8 *ENTEROCOCCUS* BACTERIA REMOVAL

Besides *E. coli*, *Enterococcus* levels were also monitored during the ten weeks study in the IBSF. The *Enterococcus* removal percent and the respective in the feeding water, the control sample and the FA and FB effluent are presented in Figure 4.22. The IBSF was capable to remove up to 98.7 %, having significant reductions in FA and FB effluent from the first week of filters operation. The *Enterococcus* population in the effluent seems to have a dependence on the feeding water population.

In the control samples, after the 24 hours of idle time, the results show a decrease in the *Enterococcus* population, nevertheless, the concentration decrease is less than the one found in the *E. coli* population,, which could indicate the *Enterococcus* has a higher capacity to survive in aquatic environments than *E. coli* (Suchitra & Kundabala, 1937).

The *Enterococcus* removal through the sand bed depth was also accessed. Figure 4.23 shows the Enterococcus population through the filter's sand bed with time. Overall, both filters (FA and FB) allowed similar *Enterococcus* removal.

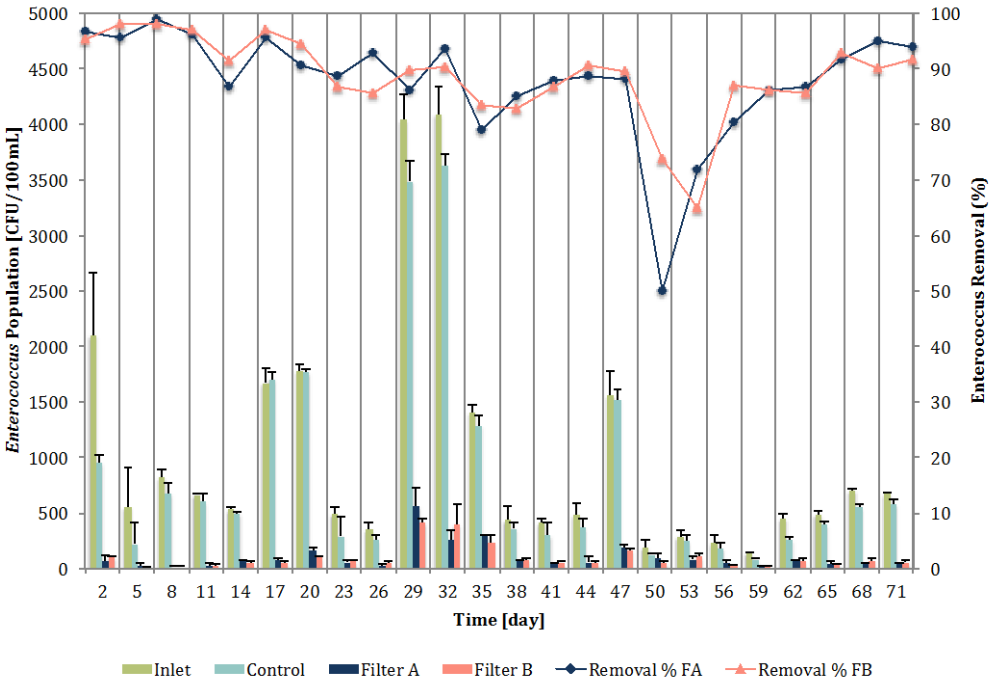


Figure 4.22 *Enterococcus* levels and removal percent by the IBSF.

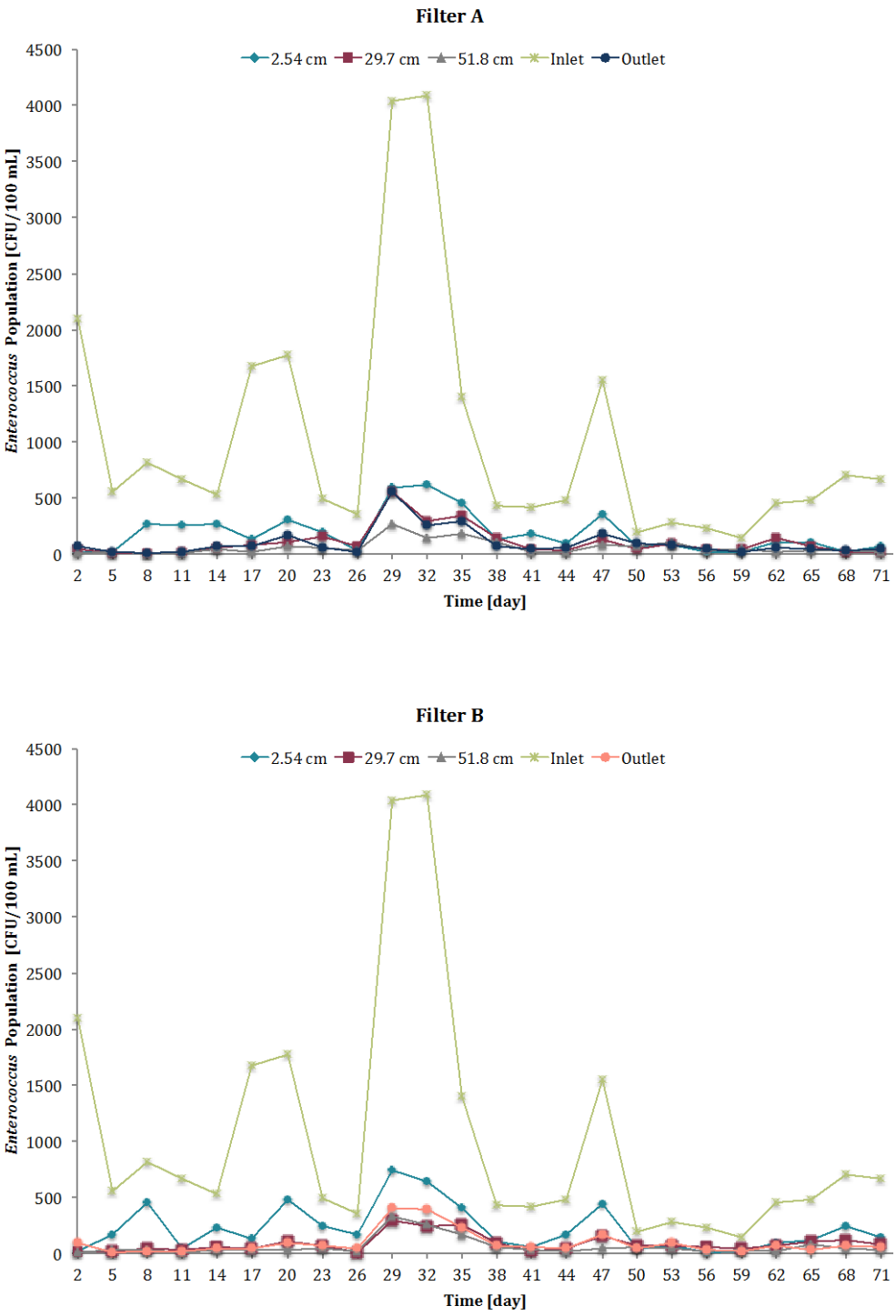


Figure 4.23 *Enterococcus* population through the sand bed.

The higher reduction, according to the data, takes place in the biolayer (at 2.54 cm). It seems that there is little difference between the *Enterococcus* reductions at 29.7 cm compared to the removal at 51.8 cm.

Before the 50th day, the lower sand layers seem to have an impact in the overall reduction inside the IBSF, but after that day, the deeper sand layers do not seem to add a significant reduction. Essentially, the *Enterococcus* population in the effluent is the same than those registered in the two sampling points succeeding the biolayer.

An R Data Analysis, similar to the one applied for the *E. coli* removal, was performed to the *Enterococcus* removal between two similar filters (between FA and FB). In addition the impact of the DO concentration (at the feeding water and at all the sampling points), the sand bed depth and filter ripening time on the *Enterococcus* removal. A logistic regression was used. Figure 4.24 shows the b-splines (degree 3 and 3 knots) of the *Enterococcus* population in each sampling point versus time. This graph includes the feeding water population. Is important to mention that the values used are an average of the population values obtained from FA and FB over time (b-splines degree 3 and 8 knots). The graph located at the right is the same values plotted in the graph at the left but without the feeding water b-spline. The graph legend has the same point representation explained in the previous section, but referring to the *Enterococcus* population.

The trends of the sampling points in terms of the *Enterococcus* population have variations over time. After the 50th day the effluent had lower population of *Enterococcus* than the ones found in the sampling point at 17.5 cm (P2). But suddenly after this day the

effluent started to have higher population of *Enterococcus* than any other point inside the sand bed. However, at the end of the tested days, the effluent has the lowest *Enterococcus* population of all the points sampled. Figure 4.25 shows the same *Enterococcus* population profile through the sand bed over time, including the population at the effluent, but in terms of the removal percentage.

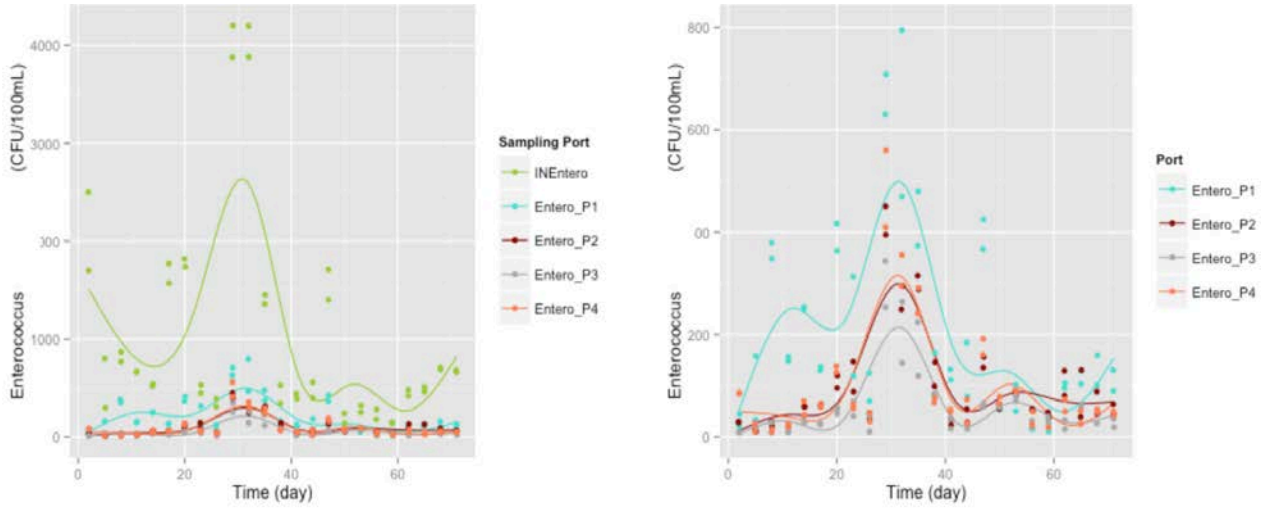


Figure 4.24 *Enterococcus* population as an average of FA and FB vs. time (b-splines degree 3, 3 knots).

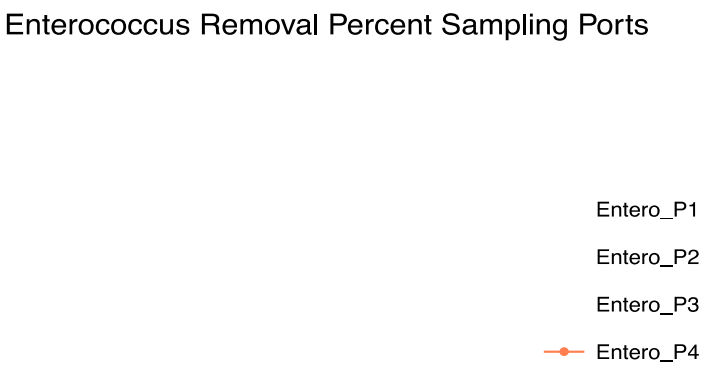


Figure 4.25 *Enterococcus* removal percent in each sampling point vs. time (b-spline degree 3, 3 knots)

The statistical model used to analyze the statistical significance of the sand bed depth, the ripening period and DO concentration on the *Enterococcus* removal was similar to the one used for the *E. coli* analysis but been the model output:

$$y_{ijk} = \frac{(in\ Enterococcus - Enterococcus)}{(in\ Enterococcus)} \qquad (4.6)$$

"in *Enterococcus*" represents the population at the feeding water and "*Enterococcus*" the population at the sampled point. Figure 4.26 presents the experimental raw data and the approximations made by the model b-splines.

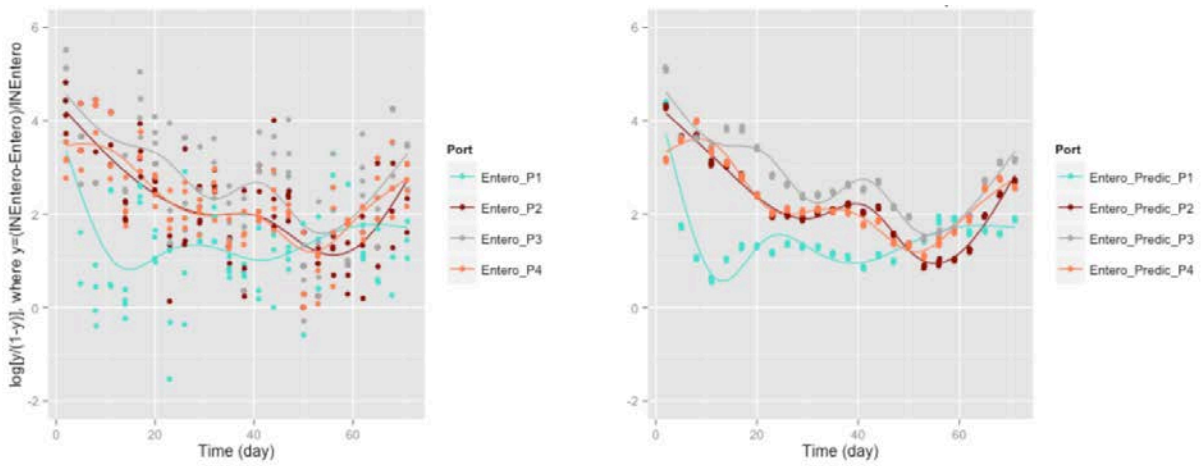


Figure 4.26 *Enterococcus* removal in each sampling point vs. time where y=(in *Enterococcus* - *Enterococcus)/in Enterococcus*

The results confirm the well fitting of the model b-splines with the experimental data b-splines. The normality test applied was the Shapiro-Wilk test, which results with a p-value = 0.1144, meaning the data follows a normal distribution. The model results are tabulated in Table 4.3. Referring to Table 4.3, there is no difference between FA and FB in terms of the *Enterococcus* removal, which is similar to the results obtained from the *E. coli* removal analysis. The sand bed depth, the DO concentration in the feed water and the filter maturation (use time) are factors that influence the *Enterococcus* removal by the IBSF. But in terms of the DO concentration through the sand bed depth, this factor, according to the results, do not influence in the *Enterococcus* removal.

Table 4.3 Summarized results from the statistical model, *Enterococcus* removal.

Factor	Df	Sum Sq.	Mean Sq.	F value	p- value
Filter	1	0.26	0.256	0.373	0.5418
Sampling point	3	88.13	29.375	42.787	<2e-16
bs (Time, df = 7, degree = 3)	7	163.62	23.374	34.046	<2e-16
log (in oxygen)	1	4.64	4.640	6.758	0.00973
log (oxygen)	1	0.89	0.894	1.302	0.25464
Port: Time	21	68.01	3.239	4.717	1.96e-10
Residuals	349	239.60	0.687		

A possible mechanism responsible for the Enterococcus removal could be adsorption. As mentioned before in section 4.1.2, in the filtration medium, iron compounds were found in the XRD analysis (Figure 4.3). To evaluate the effect that iron particles may have in the monitored bacteria (*E. coli* and *Enterococcus*), a separate set of batch experiments were conducted by filtering raw water through two different sand bed layers: one in its original state and the other with no iron particles (the iron was removed with the aid of a magnet). Simultaneously, another trial was conducted using the removed iron particles as a filter medium. The procedure for treating and filtering the water remained the same, allowing the raw water to be contact with the medium for 24 hours and then filtrated The results are presented in Figure 4.27.

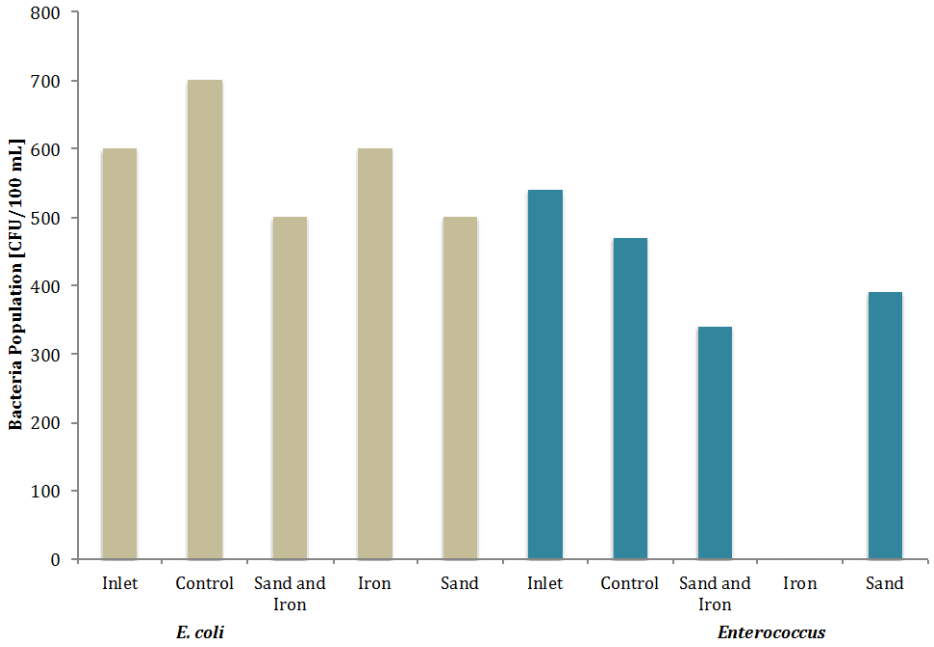


Figure 4.27 *E. coli* and *Enterococcus* removal by different filtration media (sand having iron particles, sand with no iron, and iron particles).

According to the results observed in Figure 4.27, the iron particles present in the sand used as the filtration medium in FA and FB seems to have a high impact in the *Enterococcus* removal (no detection levels). However, for the *E. coli* the behavior is not the same. It seems that the *E. coli* reduction is the same whether the sand does or does not have iron particles. This may confirm that the *E. coli* removed in the filter's lower sand layers is due mostly by the lack of DO than by adsorption. Opposed to this are the Enterococcus reduction levels, which is apparently well influenced by the presence of iron particles in the sand (probably by adsorption).

4.3.9 PHOSPHATE REMOVAL

The monitored phosphate concentrations over time are presented in Figure 4.28. According to the results, the IBSF removed up to 86.4 % of the phosphate present in the feeding water. Similar results were obtained by Pell and Nyberg, (1989), who monitored the phosphorous (phosphate constitutes approximately the 44% of dissolved phosphorous) reduction in a SSF and obtained a total removal of 83 %. Pell and Nyberg, (1989) demonstrated that phosphorous removal does not occurred in a specific sand layer of the SSF.

The phosphate removal in the IBSF does not seem to be related with the filter maturation, because high removal percent were obtained since the first week of experimentation. No linear correlation between the *E. coli* removal and the phosphate removal in the IBSF (FA) was found (Pearson correlation = -0.1776, *p=0.6295*). Similarly, no linear correlation between the *Enterococcus* removal and the phosphate removal in the IBSF (FA) was identified (Pearson correlation = 0.1377, *p=0.7045*). The low DO concentrations found inside the IBSF bed also does not have a linear correlation with the phosphate removal (Pearson correlation = 0.0084, *p=0.9829*).

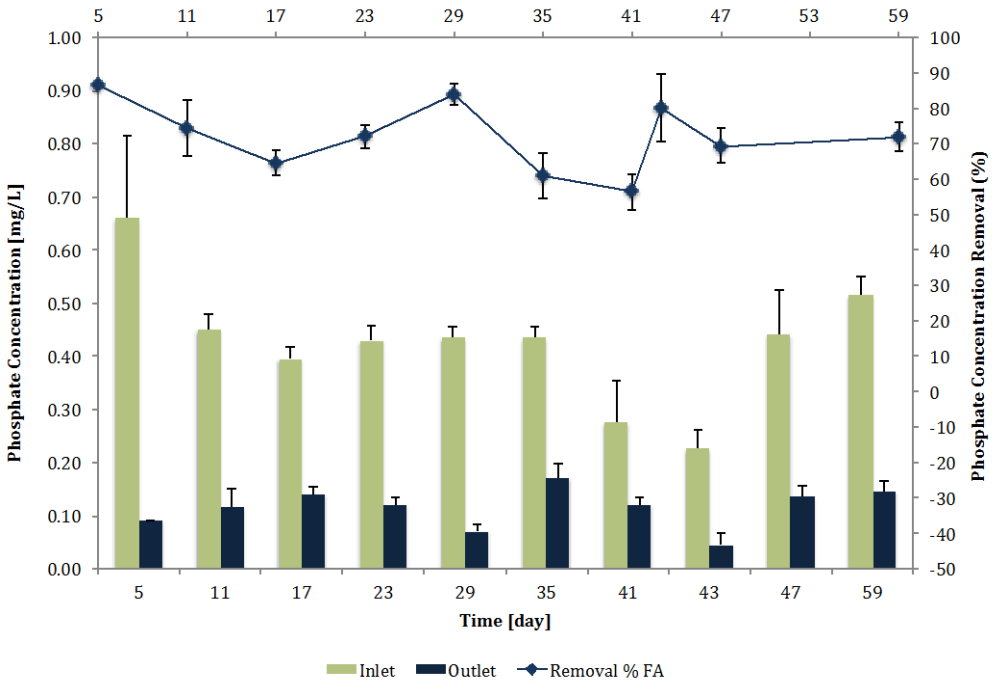


Figure 4.28 Phosphate removal by the IBSF treatment.

These results could indicate that the phosphate removal could not be related with the biological activity mechanism or with the anoxic conditions inside the IBSF. One of the possible capture mechanisms for the phosphate removal in the IBSF could be adsorption. According to the XRD analysis for the crushed rock sand used as the filtration medium in FA and FB, different forms of iron were detected in the sample (Figure 4.3). Erickson et. al, (2007), recognized that phosphate can form a strong bond with iron by surface adsorption.

4.4 ILDE TIME BACTERIA REMOVAL

The *E. coli* and *Enterococcus* removal rate during the idle time was studied measuring the bacteria population changes every 8 hours during the 24 hours of idle time. Figure 4.29 presents the results obtained for *E. coli* and *Enterococcus* after 72 days of FA and FB operation. Because there is no statistical difference between FA and FB, the results from this experiment are presented as an average of the values obtained in both filters. The *E. coli* population in the control sample seems to start decreasing after the 8 hours from 92 to 75% of the initial population. This could indicate that there is no change in the reduction of *E. coli* population due to natural death after 8 hours of stagnant conditions during an idle time of 24 hours.

The reduction in the *E. coli* population in the first centimeters of the sand bed (at 2.54 cm), where the biolayer is expect to be developed, have no substantial changes in the *E. coli* population over time (from 52% at time 0 to 47% at time 24 hours). According to the reduction pattern in Figure 4.29, the reductions obtained in this sand layer occurs at the beginning of the raw water batch addition when the water is flowing through the sand pores.

Although there is a substantial decrease in the *E. coli* population at this point compared to the concentration in the feed water (52% of the initial population), the *E. coli* population seem to be almost steady until the 16th hour of idle time, when it started to have an increase. This could be possible because of the probable availability of nutrients and organic matter in this area, making it possible the reproduction of this bacterium.

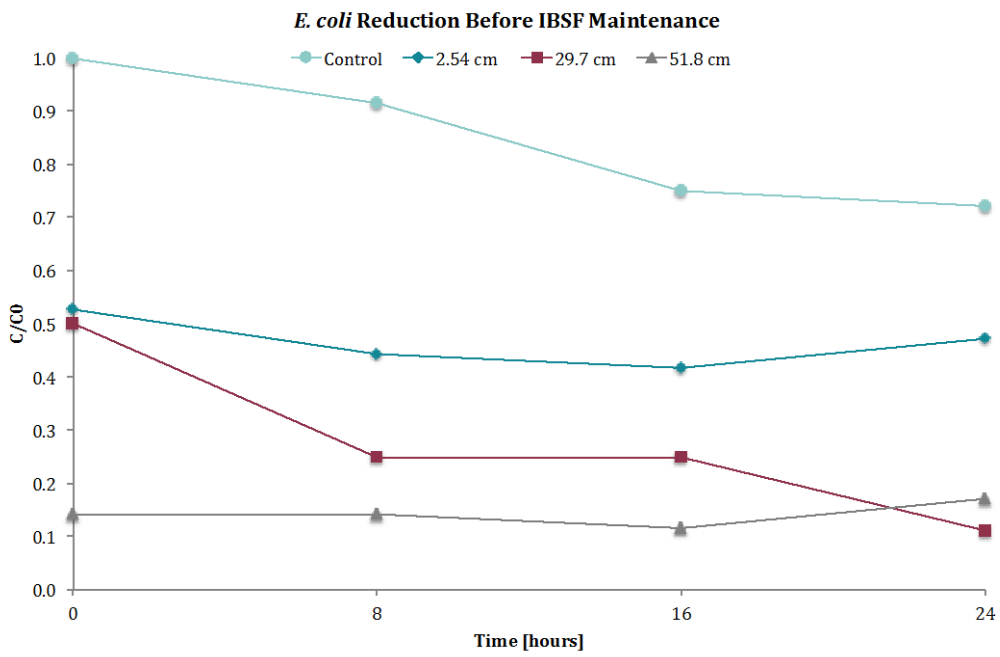


Figure 4.29 *E. coli* removal during the idle time (24 hours period through the sand bed).

In the deeper layers of the sand bed, at the 29.7 cm and 51.8 cm points, the removal pattern seems to be different, even though the population at the 24 hours are very similar in both points, 11% of the initial population at the 29.7 cm point and 17% in the 51.8 cm point. In the 17.5 cm sampling point, at the beginning of the idle time the concentration of the *E. coli* bacteria is almost the same value than the one present in the 2.54 cm. But, after 8 hours the population decreases to half, and then started to decrease after 16 hours down to 11% of the initial population. It seems that the *E. coli* bacteria do not reach the deeper layers of the sand bed, because of the low population found in this area just after the batch addition.

The same measurements were done to analyze the *Enterococcus* removal during the idle time. The behavior of the *Enterococcus* bacteria, according to the results presented in Figure 4.30, differs from the *E. coli* behavior (Figure 4.29). The *Enterococcus* bacterium seems to have the capacity of adapting to the hostile environment that could become in stagnant water after a few hours, it only decreased its population just 3% after 24 hours in the control sample.

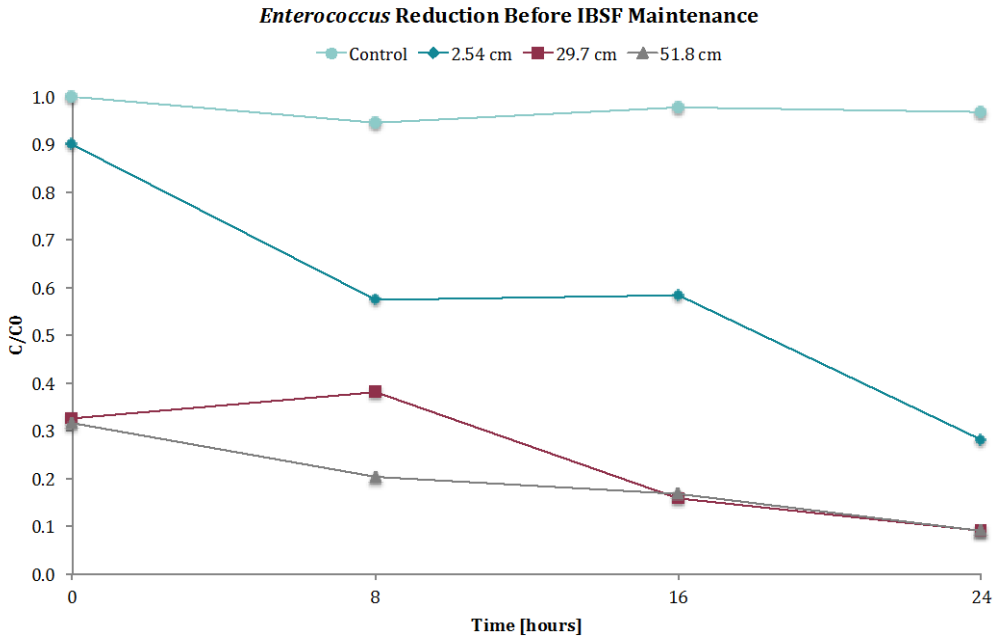


Figure 4.30 Enterococcus removal during the idle time of 24 hours through the sand bed.

In the first point located at 2.54 cm in depth from the sand bed surface, the decrease in population needed up to the 24 hours of idle time to decrease to 28% of the initial population. Immediately after the raw water batch addition, the population decreases only

to 10%, and then after completing the idle time there was present only 28% of the fed population. This could indicate the *Enterococcus* could be removed by predation by other microorganism established in the biolfim or by their death or inactivation resulted from the competition for organic matter needed for their metabolism process. According to the results presented in Figure 4.29 and Figure 4.30, it appears that the *E. coli* is removed in the biolayer due to mechanical trapping or by their attachment to the extra polymeric substance because there is no significant change in their population over time. While the *Enterococcus* removal in this sand layer seems to be more related with biological processes due to other microorganisms, because the results show a significant decrease in their population over time.

In the deeper sand layers, at 29.7 cm and 51.8 cm of sand bed depth, the results suggest that there could be taking place very similar removal processes for the *Enterococcus* removal after the 16 hours of idle time. Those results indicate that an IBSF with a total sand bed depth of 29.7 cm could be equally effective that one IBSF with 51.8 cm if the minimum idle time is 16 hours. In the case of *E. coli*, the sand bed reduction could be effective with a minimum idle time of 24 hours.

4.4.1 IBSF MAINTENANCE IMPACT IN THE BACTERIA REDUCTION

The impact of the IBSF maintenance was evaluated doing a similar experiment than the one presented in the previous section after conducting the recommended IBSF

maintenance. The maintenance process consisted in a wet harrowing, which caused a disturbance just in the first 2.54 cm of the sand bed (CAWST, 2009). The obtained results with respect to the *E. coli* population are presented in Figure 4.31. According to the results, the maintenance seems to affect mainly the first two sampling points located in the sand bed (at 2.54 cm and 29.7 cm from the top).

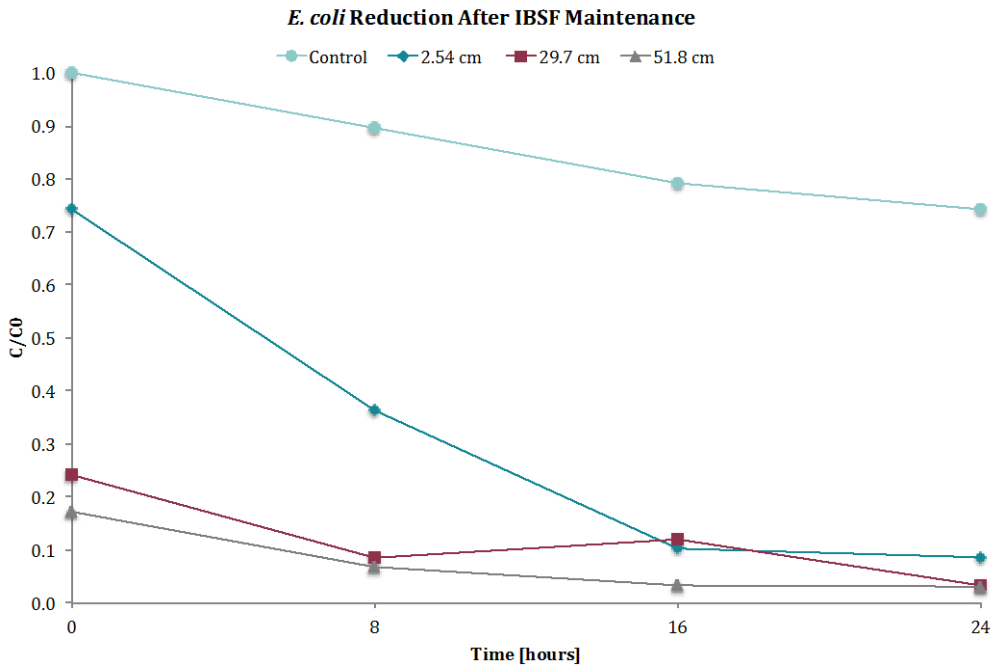


Figure 4.31 *E. coli* removal during the idle time of 24 hours through the sand bed after the IBSF maintenance.

The *E. coli* population in the control had the same behavior compared to the results obtained previously (samples taken before the maintenance), which suggest that similar external conditions were present. In the 2.54 cm the *E. coli* removal seems to be affected by the maintenance process. Before the maintenance, according Figure 4.29, the *E. coli* could

survive in the biolayer conditions through the 24 hours of idle experiment, but after the maintenance, although the removal immediately after the batch addition decreased (from 47 to 26%), the E. coli seems not be capable to survive during the 24 hours of idle time. One of the possible reasons for this change could be the removal during the maintenance process of organic matter and nutrients that was available in that sand layer, causing a conditions of higher competition between other microorganism, and the *E. coli* could been used for the metabolism process by other microorganism.

In deeper sand layers (at 29.7 cm and 51.8 cm) the *E. coli* reductions are higher than the ones found before the maintenance. This could suggest that the deeper sand layers are not affected by the maintenance process and the increased in the reduction of *E. coli* could be a respond of the filter continuous maturation in that area.

The *Enterococcus* removal by the IBSF after the maintenance process was also evaluated and the results are presented in Figure 4.32. In this case, the sampling points located at 2.54 and 29.7 cm from the top sand surface present a change, while the conditions in the deeper sand layers do not appear to change according to the results obtained in the 51.8 cm sampling point.

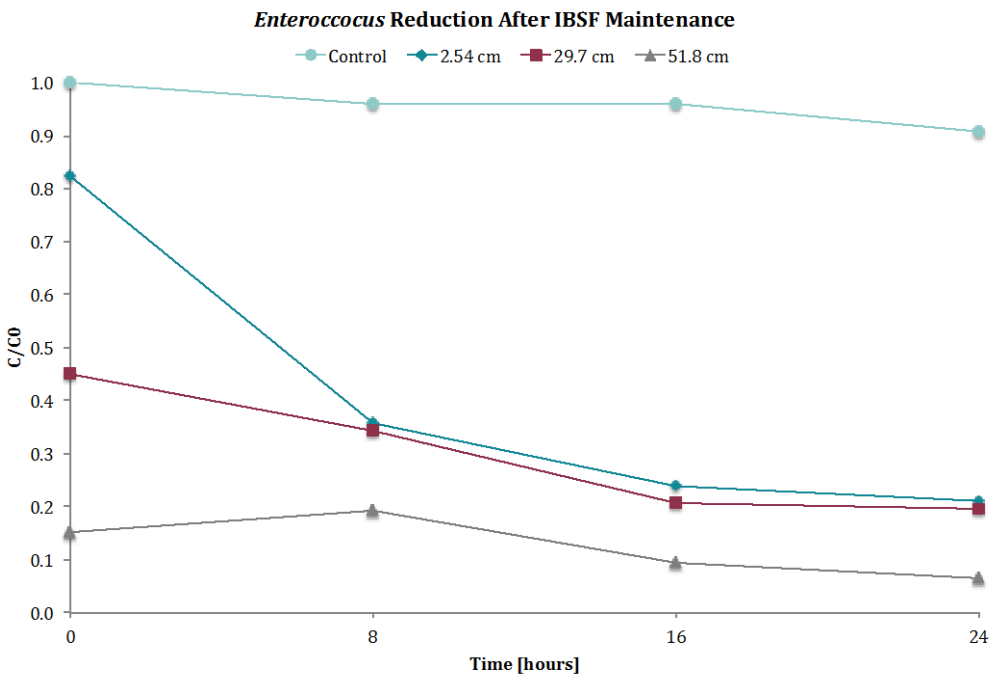


Figure 4.32 *Enterococcus* removal during the idle time of 24 hours through the sand bed after the IBSF maintenance.

The *Enterococcus* removal at the sampling point located at 2.54 cm, increased after the maintenance process from a 43 to 64 % after 8 hours of idle time, which appears to be similar results than the one obtained in analysis of *E. coli* removal. However, the results obtained in the sampling point at 29.7 cm differ from the results obtained with the *E. coli* removal. At this point the Enterococcus removal obtained immediately after the raw water addition decreased from 69 to 55 %. The last sampling point (at 51.8 cm), according to the results presented in Figure 4.32, was not affected by the maintenance process.

The obtained results suggest that the filter maintenance increase in overall the *E. coli* and *Enterococcus* removal, especially at the biolayer. However, this increase depends

on the idle time period. Those obtained results contrast with results obtained in previous studies were the maintenance process had a modest reductive effect in the bacterial removal (Jenkins et al., 2011). Further investigations are needed to monitor the impact of the maintenance process in other water quality parameters like the turbidity, pH, dissolved oxygen and phosphate removal, to have results that support the explanations to the changes in bacteria removals after the IBSF maintenance.

4.5 BIOFILM VISUALIZATION

Several sand samples were observed through the SEM, in order to visualize the biofilm development over the sand grains over time. Additionally, the visualization of the sand surface variations through the sand bed depth was accessed.

4.5.1 BIOLAYER DEVELOPMENT VISUALIZATION

Before starting the addition of the first raw water batch to the filter, a sand sample from the sand bed was obtained. The result from the SEM observations of this sample is presented in Figure 4.33. It is clear in the left image that the surface of the sand grains is very diverse, some grains have a very smooth surface, while other sand grains have a more rough and irregular surface. The majority of the sand particles have a particle size bigger that 100 μm and have irregular shapes. In the right image, a magnified visualization of one of the grains with an irregular surface is presented. The surface of this specific grain seems

to have some smooth sections, but also evidence of some particles over the surface with a variety of shapes.

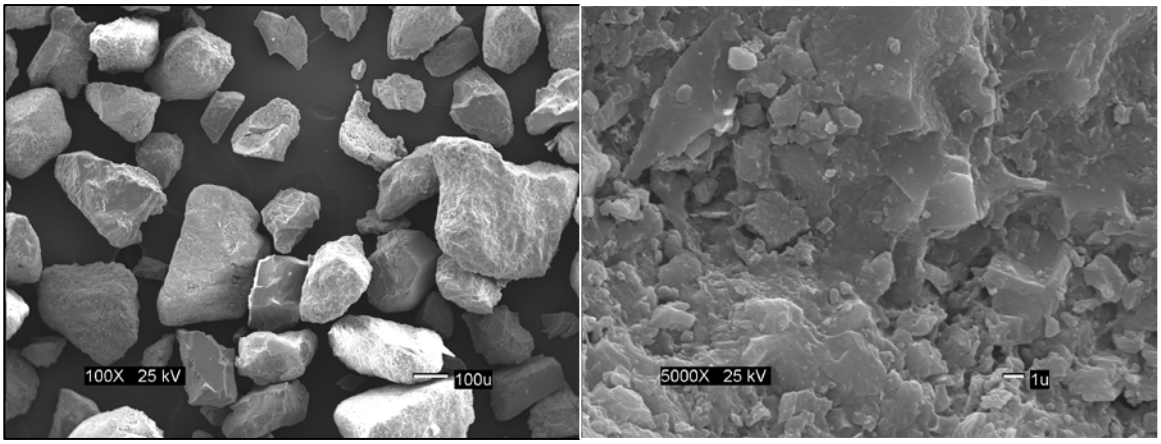


Figure 4.33 Visualization of a control sand sample under the SEM.

After the first batch addition, sand samples from the first 2.54 cm of the sand bed depth (where the biolayer is expected to develop) were obtained weekly during one month. Figure 4.34 shows the images obtained from the SEM visualization of those samples. During the first week (a, b) an increase in particles over the sand grain surface is observed. The particles have different shapes and the identification of a specific microorganism is difficult due to the large variety of shapes. A thin substance is present; this could indicate the presence of extra polymeric substance, which is the first indication of a biofilm development (Law et al., 2001) .

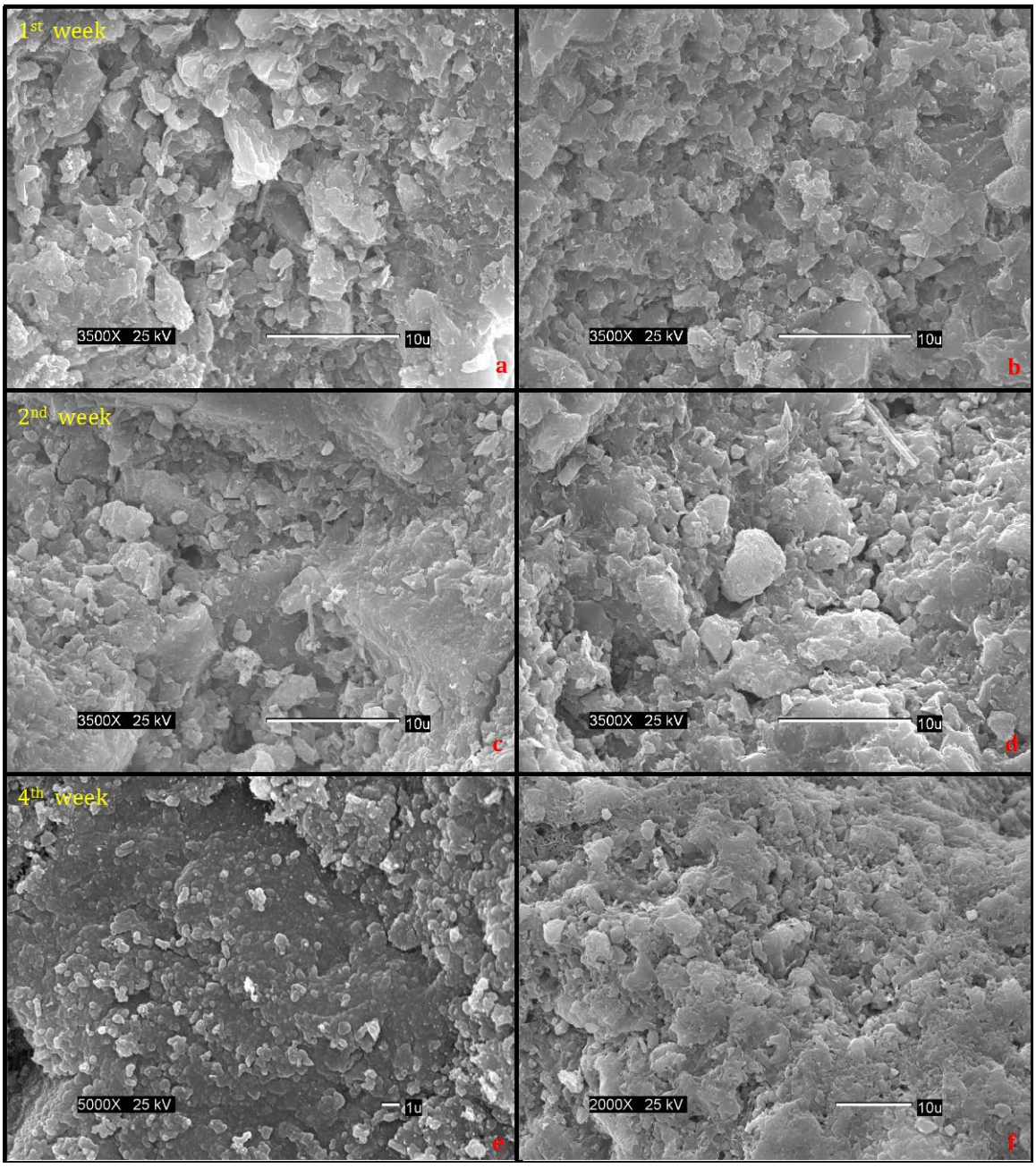


Figure 4.34 Visualization of the biofilm development over the sand grains during one month.

In the second week, a clear increase in the particles over the grain surface was observed (Figure 4.34c and d), which could indicate the colonization of the sand surface. In

the image (c) and (d), there is the presence of what could be filamentous bacteria. In the images obtained from the 4th week (e, f), it is evident that the surface of the sand grain was covered with a developed biofilm, with what it seems are cocci bacteria. As shown in the image (f) the diversity of the microorganisms established over the sand surface increased over the time. Those results could indicate the heterogeneity of the biofilm developed in the IBSF. Making a comparison between the image (e) and the right image in Figure 4.34, the sand surface is no longer visible at the last week of sampling.

4.5.2 SAND GRAINS SURFACE VISUALIZATION THROUGH THE IBSF DEPTH

Sand samples were obtained with the purpose to visualize the sand grains surface changes through the sand bed layer, after 96 days of ripening period in filter FA. The samples were observed under the SEM and the results are presented in Figure 4.35. All images from the first column are at the same magnification while the other two columns have higher magnification. The top sample was taken from the top surface of the sand bed. Some of the shapes that could be microorganism were identified with yellow circles.

In the samples from the top of the sand bed, some particles that seem to be rod, cocci and bacillus bacteria were identified. In the image (c), some material that has the form of flakes is present. This could be part of the biofilm or it could be soil particles that were suspended in the feeding water and get trapped in the surface of the sand bed.

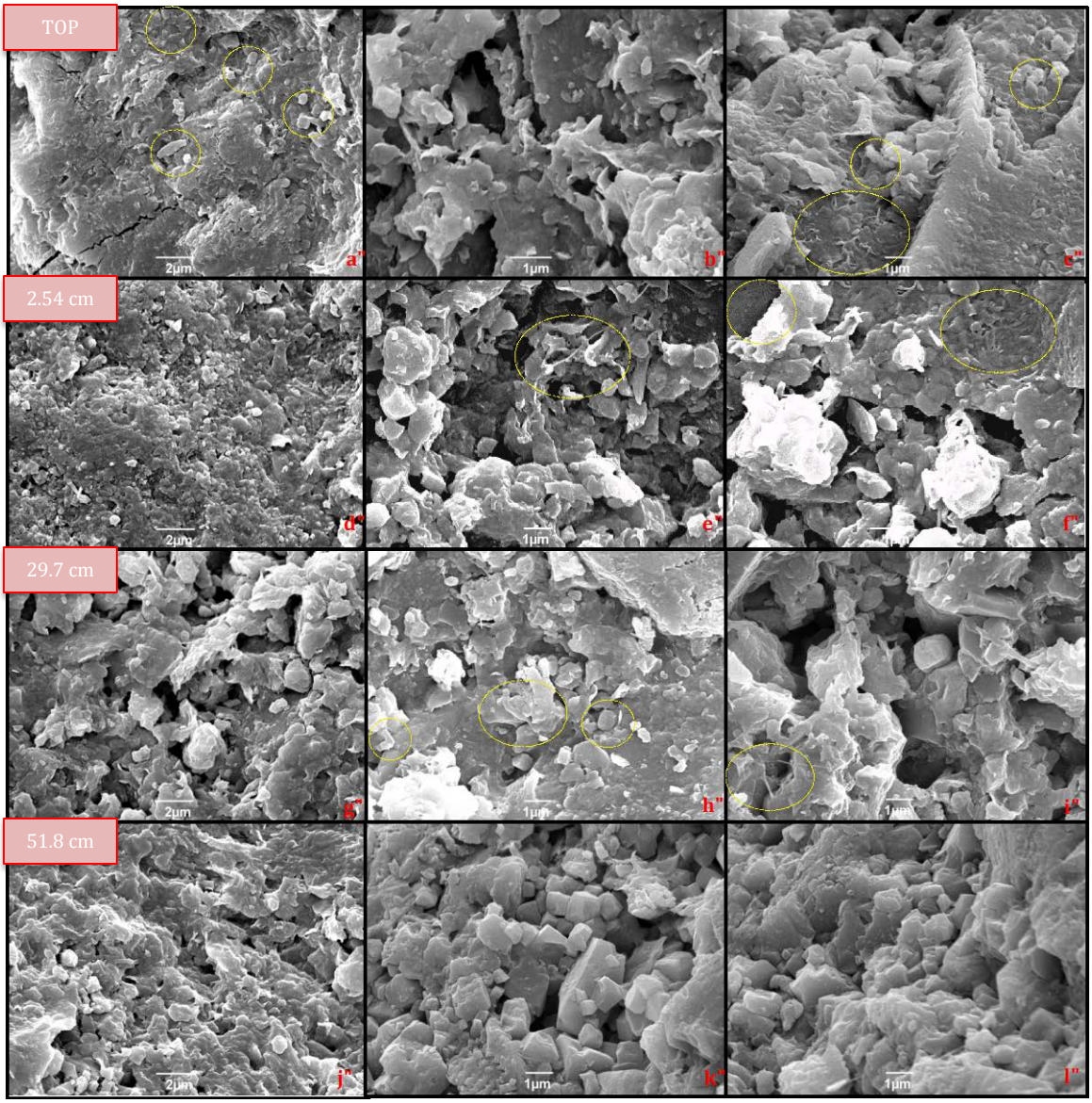


Figure 4.35 Visualization of the sand grains surface through the sand bed depth under the SEM.

The images obtained from the 2.54 cm sand sample, mainly the (d) looks very similar to the image obtained in Figure 4.34 in the 4th week of the filter ripening period. The sand grain surface is completely covered with some round particles that could be bacteria. In the images (e) and (f), which shows a closer look, it can be observed what seem to be extra polymeric substances.

In sand samples obtained at 29.7 cm depth from the top surface of the sand bed, some particles that appear to be bacteria were found. In the image (h), some particles of 1 – 2 μm that exhibit the form of bacillus, vibrio, rod and filamentous bacteria were identified. Additionally, image (i) shows particles with a similar shape of palisade bacteria and a substance that could be extra polymeric substance. In the sand sample obtained from the deeper sand layer (at 51.8 cm), most of the particles are larger than 2 μm and have symmetrical and sharp edges, which could be a sign that they are soil particles instead of microorganism.

Unfortunately, with the obtained results there is no microorganism that could be precisely identified. Further investigation is needed in order to relate the visual development of the biofilm over the sand grains and the impact of this development in the water quality. In addition other microbiological identification techniques should be used to characterize the biofilm (i.e. fluorescence microcopy).

Although an identification of specific type of bacteria was not possible with the obtained SEM images, the visual results matches with the organic matter content present in the sampled sand layers. According to Figure 4.36, the organic matter content percent is

higher at the 2.54 cm sampling point because it is located in the biolayer, where the maximum establishment of the microorganism was observed.

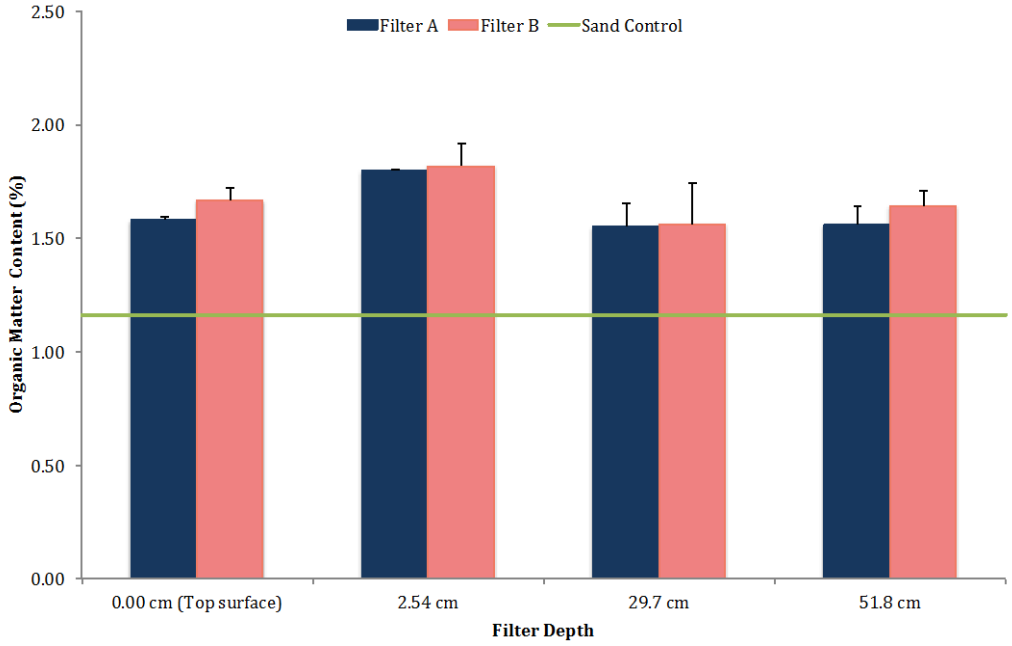


Figure 4.36 Organic matter content percent (After 96 day of IBSF ripening).

Even though the higher organic matter content was found in the 2.54 sampling point, the other sampled sand layers (at 29.7 cm and 51.8 cm) increased in organic matter content compared to the percent present in the sand control sample (green line Figure 4.36). Both filters FA and FB had very similar results in terms of the organic matter content percent through the sand bed depth.

5 CONCLUSIONS AND RECOMMENDATIONS

"A conclusion is simply the place where you got tired of thinking"

-Dan Chaon

The results of this research reveal several evidences about the behavior and effectiveness of the IBSF to treat raw water. The monitoring of the *E. coli* and *Enterococcus* levels as function of the DO concentrations, filter sand bed depth and time made possible the following conclusions:

- The flow of the top of the IBSF filter corresponds to more of a mixing behavior, while the flow near the bottom of the filter corresponds to more of a plug flow-type behavior with some dispersion. (Refer to Section 4.2.2)
- The turbidity removal rate in IBSF is up to 99.77% since the first week of use and low hydraulic flow rates do not seem to affect the turbidity removal. (Refer to Section 4.3.2)
- There is a strong correlation between the raw feed water quality and the immediately resulting IBSF removal efficiency on turbidity, *E. coli* and *Enterococcus*. (Refer to Sections, 4.3.2, 4.3.7, 4.3.8)
- There is a significant decrease in the concentration of DO between the feed water and the first 2.54 cm of the sand bed depth, and anoxic conditions could be reached in the biolayer. (Refer to Section 4.3.6)

- There is no significant difference between the DO concentration and the DO deficit in the middle and the bottom of the sand bed depth, which suggest uniform oxygen consumption rates below the upper region. (Refer to Section 4.3.6)
- The *E. coli* population removal percent by the IBSF is up to 98%. It is not only an effect of the biolayer, but could be also caused by other mechanisms, such as mechanical trapping. (Refer to Section 4.3.7)
- Comparing the *E. coli* removal rate at the middle and at the end of the sand bed, the removal appears to be very similar until near the day 30 of filter use. After day 30, the removal rate increases with time and depth. (Refer to Section 4.3.7)
- The IBSF is capable to remove up to 98.7% of The *Enterococcus* population, having the highest reduction rate at the biolayer. It seems that there is no difference between the *Enterococcus* reductions at the middle and the end of the IBSF sand bed depth. The Enterococcus removal seems not to be influenced by the lack of DO concentration, but rather, it may be strongly influenced by adsorption mechanisms. (Refer to Section 4.3.8)
- Iron particles seem to have a high impact on the *Enterococcus* removal (no detection levels), but not in the *E. coli* removal (no removal).
- The IBSF removes up to 86.4% of the phosphate present in the feed water. The phosphate removal could not be significantly correlated with the biological activity mechanism or with the anoxic conditions inside the IBSF. One of the possible capture mechanisms for the phosphate removal in the IBSF could be adsorption.

- There is no significant change in the *E. coli* population over the idle time in the biolayer, suggesting that the *E. coli* is removed in the biolayer is due to mechanical trapping or by their attachment to the extra polymeric substances.
- The *Enterococcus* removal in the biolayer, appears to be more related with biological processes from other biofilm microorganism due to a significant decrease in their population over the idle time.
- Removal rates of *E. coli* and *Enterococcus* were nearly uniforms in the middle and lower layers of the IBSF sand bed, suggesting that an IBSF with a total sand bed depth of 29.7 cm could be equally effective that one IBSF with 51.8 cm if the minimum idle time is 16 hours to have significant *Enterococcus* removal. In the case of *E. coli*, the sand bed reduction could be effective with a minimum idle time of 24 hours.
- Since the first week of the IBSF use, the development of the biofilm over the sand grains was observed until 29.7 cm of the sand bed depth, which appears to provide evidence that the biolayer extends more deeply than expected. The biofilm in the IBSF have a high diversity of microorganisms that increase over time. (Refer to Section 4.5)

Slight variations in the pH water inside the IBSF were observed. Further investigation is need to confirm the nitrification and denitrification process in the IBSF by measuring the NH_4^+, NO_2^- and NO_3^- concentrations through the sand bed depth and conducting experiments in order to identify the presence of ammonia-oxidizing bacteria inside the

IBSF. Additional studies are needed to monitor the impact of the IBSF maintenance process in water quality parameters like the turbidity, pH, dissolved oxygen and phosphate removal, to have results that support the explanations to the changes in bacteria removals after the filter maintenance.

Further research is needed in order to identify the microorganisms that compose the biofilm to relate microorganism presence with specific impact of this in the water quality. In addition, other microbiological identification techniques should be used to characterize the biofilm, fluorescence microscopy, isolation and molecular identification could be some of the options.

Experimental design should be also conducted to verify if a reduction in the sand bed depth (from 51.8 to 29.7 cm) could impact the bacterial and turbidity removal and the water quality of the IBSF treated water. Because of the strong relation found between the feeding water quality and the IBSF efficiency, the impact on IBSF efficiency by different feeding water sources should be studied as well.

6 OUTREACH IMPACT

"Being good is commendable,
but only when it is combined with
doing good is it useful"

-Anonymous

For some time, I have been interested in water treatment systems, especially those for rural areas in "developing" countries. These situations require creative solutions to water quality problems because the available resources are very limited. Also, I find that working on solutions to provide basic needs like water are very inspiring and meaningful. In the summer of 2012, I started working with the project Graduate Research and Education for Appropriate Technology: Inspiring Direct Engagement and Agency (GREAT IDEA), which is an interdisciplinary project at the University of Puerto Rico at Mayagüez funded by the National Science Foundation (#1033028), in a research were I had the opportunity to combine basic science with broader impacts to the community of Duchity, Haiti, and to rural communities in Puerto Rico.

6.1 OUTREACH IN DUCHITY, HAITI

I visited Duchity on three occasions to understand the context of water quality engineering and science as applied to a community that lacks access to clean water. Duchity was selected in part because there is an active group called YoutHaiti (youthaiti.org), directed by Gigi Pomerantz, which is addressing sanitation by building dry composting toilets in the region. Duchity is a small community of approximately 10,000 people, whose

access to safe and clean water is very limited. This community has several schools, churches, a large public market, and health and dental clinics. However, the people who live in Duchity have problems accessing clean water. Figure 6.1 shows the geographic location of Duchity.

Figure 6.1 Duchity geographic location.

Most of the people derive their daily water supply from the river La Sous, either by collecting the water directly from the river or from the village pipeline system that delivers water from the river, by gravity, to several faucets in the community. In desperate situations, water is collected from puddles in the road. Figure 6.2 illustrates people from Duchity collecting water.

During our first visit in November 2012, we (GREAT IDEA team), established a laboratory in the community health clinic to test the water for basic water quality

parameters like turbidity, pH, temperature, total and fecal coliforms and cholera. The purpose of this was to learn about the conditions of water quality in Duchity, as well as to develop a capacity among local people to understand water quality testing the results.

Figure 6.2 People collecting water in Duchity.

Part of the logistics to perform the water quality testing was the selection of the most appropriate equipment and methods to obtain reliable results under the non continuous electricity service and non sterile conditions in the facilities in were the laboratory was established. After a rigorous search of equipment appropriate for this purposes, we selected an incubator for the bacteriological analysis that is capable to work with battery and incubate samples for up to 24 hours, a turbidity tube to measure the turbidity, a membrane filtration system for the bacteriological analysis, including reusable

aluminum petri dishes, and a colorimetric method to measure the water pH. For the sterilization of the materials for the bacteriological analysis, we used boiling water, alcohol, Clorox and a lighter. The water quality testing was necessary to understand baseline conditions for the research and became an opportunity to teaching.

In addition to the establishment of the laboratory, training about water quality testing and collection of water samples was offered to two young men from the community, Julien Linot and Rony François, with the goal of enabling independent water testing when we are not present in the community. The training included water samples collection in the field and water quality testing in the laboratory. Figures 6.3 and 6.4 shows the training of the technicians and the laboratory facilities.

Figure 6.3 Training of the laboratory technicians**.**

Figure 6.4 Laboratory facilities in Duchity and a water quality testing.

At the end of the training, the technicians obtained an official certificate from the GREAT IDEA project. Figure 6.5 presents the moment where the certificates were given to the technicians.

Figure 6.5 Moment where the certificates were given to the technicians in April 2013.

I was really impressed at how two young men with limited knowledge about water quality and water testing performed the procedures correctly. This indicates that with training, dedication and effort, we as engineers can provide the tools and knowledge for people to continue the projects and activities independently.

There were also some difficulties, challenges and lessons learned. The water quality results obtained from samples taken at several points where people typically collect water, were alarming. Although the results for the cholera testing where negative, the water samples showed high contamination of fecal coliform bacteria. Equally concerning was that there was no one to inform officially about the results. We, as people outside from the community, were unclear as to whom these results should be given and what should be the correct and appropriate action from us. No one was surprised that the results showed contamination, but there was no plan or resources to renovate the pipeline system. This further underscores the importance of bringing filters to the community.

In addition to training two individuals, I believe it is also important to create consciousness about water quality in the community as a whole, and why consuming unsafe water can impact the wellbeing of the community. For this reason, as I reflect back, it would have been better to train more people from the community in the testing, and to inform many more people about our activities and findings. Of course, it is important to develop this process together with the community leaders so that information is shared in an unthreatening manner that enhances peoples' capabilities.

In 2013, nearly 50 IBSF's were already installed in households. During our second visit to Duchity, as part of our work, with the aid of a local technician, Patrick Louis, we installed two IBSF's in the community. This opportunity allows us to identify the challenges and requirements of users and technicians when using this technology. This experience provided strong reasons to conduct the study presented in here, particularly because of the transportation challenges I witnessed caused by carrying the very heavy IBSF's. In Figure 6.6 some of the photos obtained during the IBSF installation is presented.

Figure 6.6 Installation of IBSF in Duchity.

6.2 IBSF SURVEY

A survey was designed in collaboration with Felix Despange, who manages a technical arts school in Duchity (in partnership with the Vermont Haiti project) with the intention to be used in Duchity or has a tool and reference for future research of the IBSF in similar communities, in Haiti or in other countries. It was redacted in English and in Haitian Creole. Both versions are included. This survey can help to provide information to researchers and community officials while at the same time raising consciousness within the community regarding the importance of safe water.

6.2.1 ORAL INFORMED FORM

Oral Informed form ***Date:***

Before continuing with the study, we want to make sure the person interviewed clearly understands what is this study about. After the explanation of the study objectives, you can decide whether you want to participate or not.

This study has the following objectives:

1) Identify potential improvements for the biosand filter design.
2) Identify possible water contamination sources while using the biosand filter.
3) Verify the user's biosand filter operating procedure.
4) Develop a schedule adapted to the biosand filter user for the batch input time.

Study benefits:

1) To obtain recommendations for future improvements to the biosand filter design in base of the user needs, resources, and water sources available.
2) To identify errors in user's operating procedure of the biosand filter that could contribute to the selection of topics for future educational workshops.

Study Risks:

During the survey, personal information of the participant could be exposed. The investigators will make sure to manage this information in a private and confidential manner.

If you do not want to continue with this study you can choose not to proceed with the survey at any moment. If you do not wish to answer a question, the interviewer will move on to the next one.

Do you want to continue with this study?

Yes. (Fingerprint) Signature of the investigator

Fòm Enfòmasyon oral ***Dat:***

Anvan nou koumanse ak etid la, nou vle asire nou ke moun nap entèwoje yo byen konprann sou kisa etid sa a baze. Konsa, apre nou fin eksplike tout objektif etid lan, chak moun lib pou'l deside sil vle patisipe ou non.

Etid la gen objektif sa yo:

5) Idantifye divès mwayen ki ka amelyore aparèy filtè a
6) Idantifye tout bagay ki ka kontamine dlo filtre nou pral benifisye a.
7) Verifye kouman itilizatè yo sèvi avèk sistem filtè sa.
8) Elabore yon orè pou filte sad la pou konnen kilè pou met dlo nan sistèm nan.

Avantaj etid la:

3) Pou jwenn rekòmandason nan fason pou amelyore yon sistèm ki filtre dlo avèk sab, tou depan de itilizatè, resous ak sous dlo ki genyen diponib nan yon kominote.
4) Idantifye tout erè yon moun ka fè pandan la'p itilize yon aparèy filtè ki vin bay nesans a lòt deba.

Risk etid la:

Pandan etid la, enfòmasyon pèsonèl patisipan yo ka toutfwa devwale. Men, investigatè yo pral fè tout sa yo kapab pou yo kenbe enfòmasyon sa yo pèsonèl e konfidansyèl. Si yon moun pa vle kontinye, ou ka chwazi pa patisipe nenpòt lè. Si'w pa anvi reponn yon kesyon wap jis mande moun kap poze kesyon yo pou pase a kesyon ki swivan an.

Ou vle kontinye ak etid la?

Wi. (Anprent) Siyati envèstigatè a

6.2.2 PHOTO CONSENT FORM

Photo Consent form ***Date:***

By signing this waiver, you give us the right to photograph your biosand filter and water storage container. However, you can participate in the survey even though you do not authorize the pictures.The photos taken will be used for future presentations, educational workshops and research reports. Your personal information will be kept private and confidential.

Do you want to approve the taking and use of the photos?

Yes. (Fingerprint) Signature of the investigator

Foto pa konsantman ***Dat:***

Lèw siyen fòm sa a, ou ba nou dwa pou'n pran foto sistèm ki filtè dlo ou an ak tout rezevwa dlo ou genyen an. Sepandan, ou ka toujou patisipe nan etid la menm si ou pa otorize nou pran foto sa yo.Nou pral itilize foto nou pran yo nan prezantasyon ke nou pral gen pou nou fè, nan rapò, ak nan atelye pwofesyonèl. Enfòmasyon pèsonèl ou ap toujou rete prive e konfidansyèl.

Ou aksepte nou pran foto sa yo?

Wi. (Anprent) Siyati envèstigatè yo

6.2.3 SURVEY

Survey

A. Respondent Information

Date	
Respondent name	
Role in family	
Family members	
Children aged 0-5 years	

Etid

A. *Patisipan*

Dat	
Non patisipan an	
Rol ou nan fanmiw lan	
Kantite moun nan kay la	
Timoun mwens ke senk an	

B. Water source

1) What water sources do you have available?

Rain	
Well	
River	
Other	

2) Which one is your preferred water source?

Rain	
Well	
River	
Other	

3) Why do you like it better?

Better Quality	
Closer	
Other	

4) How far away is the water source?

Time	

5) How much water you use daily?

Quantity	

6) Who goes to collect the water?

Mother	
Father	
Child	
Other	

7) What container is used to collect the water?

Bucket with lid	
Bucket without lid	
Plastic bottle	
Glass bottle	
Other	

8) Do you think this water is clean?

Yes	
No	

9) Do you treat your water to make it safe?

Yes	
No	

10) If so, which water treatment do you use?

Boiled	
Solar Exposure	
Ceramic Filter	
Biosand filter	
Chlorination	
Other	

11) How much do you pay for this treatment?

Quantity	

12) Do you prefer a water treatment at household level or community level?

Household level	
Community level	

13) How much you are willing to pay for a water treatment at community level?

Quantity	

C. Biosand Filter Use

14) How long have you owned the biosand filter?

Time	

15) How many people use this filter?

Quantity	

16) Is your biosand filter working?

Yes		*Go question 21*
No		*Go question 18 -22*

17) For how long has it been out of order?

Quantity	

18) What caused it to malfunction?

_____

_____

19) Why hasn't it been repaired?

_____

_____

20) Are you using another technology to treat the water?

Yes	
No	

21) How much did you pay for the biosand filter?

Quantity	

22) You use the filtered water for:

Cooking	
Drinking	
Bathing	
Washing hands	
All of the above	
Other	

23) Do you pre-treat the water before adding it to the biosand filter?

Yes	
No	

24) What treatment do you use?

Settling	
Other	

25) How many times a day do you add water to the biosand filter?

One	
Two	
Three	
Four	
More than four	

26) How much time you wait to add a new bucket of water to the biosand filter?

Just after the last one	
One hour after last one	
Six hours after last one	
More than six hours after the last one	

27) Does the water produced by the biosand filter satisfy your needs? In quantity

Yes	
No	

28) Describe the process you follow to use the filter.

_____

_____

_____

_____

_____

_____

_____

29) Have you had problems with the biosand filter?

Yes		*Go next question.*
No		*Go question 33.*

30) Describe the problem.

_____

_____

31) Did you require a technician assistance to repair the filter?

Yes	
No	

32) Is the overall filter operating procedure easy to follow?

Yes	
No	

33) Do you treat the water after the biosand filter?

Yes		*Go next question.*
No		*Go question 36.*

34) Which post-treatment do you use?

Boiling	
Solar Exposure	
Chlorination	
Other	

35) What changes to the biosand filter do you recommend in order to satisfy your needs?

_____

_____

_____

_____

D. Biosand Filter Maintenance

36) Which indicator do you use to do maintenance on the biosand filter?

Flow rate decrease	
Water filtered seems dirty	
Sand seems dirty	
Water taste bad	
Water smells bad	
Get sick using filtered water	
Other	

37) How often do you need to do maintenance on the filter?

Weekly	
Each two weeks	
Each tree weeks	
Monthly	
More than a month	

38) Do you do the filter maintenance on your own or do you require the help of a technician?

By my own	
Technician	

39) Describe the maintenance procedure.

_____

_____

40) Is it easy to contact the technician for help?

Yes	
No	

E. Water storage

41) You store the non-treated water in a:

Bucket with lid	
Bucket without lid	
Plastic bottle	
Glass bottle	
Other	

42) You store the treated water in a:

Bucket with lid	
Bucket without lid	
Plastic bottle	
Glass bottle	
Other	

43) How often do you clean your water storage container?

Twice each day	
Each day	
Each week	

When it seems dirty	
Other	

44) Which water you use to clean the storage container?

Treated water	
Untreated water	

45) What do you use to clean the storage container?

Just water	
Chlorine and water	
Other	

E. User schedule

46) Do you have a job or stay at home?

Job	
Stay at home	

47) At what time do you usually wake up?

5:00 am	
6:00 am	
7:00 am	
8:00 am	
After 8:00 am	
Other	

48) At what time do you usually go to sleep?

8:00 pm	
9:00 pm	
10:00 pm	
11:00 pm	
After 12:00 pm	
Other	

49) At what time do you come home from work?

3:00 pm	
4:00 pm	
5:00 pm	
6:00 pm	
After 7:00 pm	
Other	

50) How many times a day do you add water to the filter?

One	
Two	
Three	
Four	
More than four	

51) Any additional comments or recommendations about the biosand filter?

_____

_____

_____

B. Kote ou jwenn dlo

1) Ki kote ou abitye jwenn dlo pou sèvi?

Dlo lapli	
Dlo tiyo	
Dlo rivyè	
Lòt sous	

2) Kijan de dlo ou pi renmen?

Dlo lapli	
Dlo tiyo	
Dlo rivyè	
Lòt sous	

3) Poukisa se tip de dlo sa ou prefere?

Bon kalite	
Pi pre lakay	
Lòt	

4) Ki distans dlo sa ye de lakay ou?

Distans:	

5) Ki kantite dlo ou itilize pa jou?

Kantite:	

6) Kiyès ki al chèche dlo pou met nan kay la?

Manman	
Papa	
Timoun yo	
Lòt	

7) Ki rezèvwa ou gen pou konsève dlo a?

Bokit ak kouvèti	
Bokit san kouvèti	
Boutèy plastik	
Boutèy an glas	
Lòt	

8) Ou panse dlo sa pwòp?

Wi	
Non	

9) Eskew trete dlo sa?

Wi	
Non	

10) Siw reponn wi, kisa ou itilize pout trete dlo sa a?

Bouyil	
Metel nan solèy	
Filtrel ak seramik	
Filtrel ak sitem sab	
Met kloroks	

Lòt	

11) Konbyen ou peye pou trètman sa a?

Kantite:	

12) Ou prefere yon filtè ki trete dlo a lakay ou, oubyen youn ki trete dlo pou tout kominote a?

Lakay mwen	
Tout kominote a	

13) Konbyen ou pare pou ta peye pou yon filtè ki trete dlo pou tout kominote a?

Kantite:	

C. Jan ou itilize filtè a

14) Depi konbyen tan ou posede sistèm filtè a sab la?

Tan:	

15) Konbye moun ki itilize sistèm sa a?

Tan:	

16) Eske sistèm filtè sab sa a mache?

Wi		*Pase nan kesyon 21*
Non		*Pase nan kesyon 18 a 22*

17) Konbyen sa genyen depi nou pa jwenn sistèm sa ankò?

Tan:	

18) Kisak fèl pa mache byen?

_____

_____

_____

19) Poukisal pa repare?

_____

_____

20) Eske ou gen yon lòt sistèm pou trete dlo?

Wi	
Non	

21) Konbyen ou peye pou filtè sab sa a?

Kantite:	

22) Ou itilize dlo filtre pou:

Fè manje	
Bwè	
Benyen	
Lave men	
Tout opsyon ki sot site yo	
Lòt	

23) Eske ou pre-trete dlo a avan ou metel nan filtè a?

Wi	
Non	

24) Ki tretman li ye?

Kite dlo a poze	
Lòt	

25) Konbyen fwa pa jou ou itilize filtè a?

1 fwa pa jou	
2 fwa pa jou	
3 fwa pa jou	
4 fwa pa jou	
Plis ke 4 fwa pa jou	

26) Chak kilè ou chanje rezèvwa dlo a?

Toutswit	
Inèdtan apre dlo a fini	
Sizèdtan apre dlo a fini	
Plis ke sizèdtan apre dlo a fini	

27) Kameite dlo ki trete pa siltè sad la?

Wi	
Non	

28) Kijan ou itilize filtè a.

_____

_____

_____

_____

_____

_____

29) Eskew te gen ak system filtè a?

Wi		*Pase nan kesyon suivan an.*
Non		*Pase nan kesyon 33*

30) Dekri pwoblem nan.

_____

_____

31) Eskew te potel bay yon teknisyen pou reparel?

Wi	
Non	

32) Eske li fasil pou ititlize filtè a?

Wi	
Non	

33) Eskew trete dlo ankò aprew fin pasel nan filtèa?

Wi		*Pase nan kesyon suivan*
Non		*Pase nan kesyon 36*

34) Kilot tretman ou ititlize?

Bouyil	
Sèl pran solèy	
Mett klorox	
Lòt	

35) Ki chanjman ou ta renmen yo sè nan sistem filtè a pou'l vin pi bon?

_____

_____

_____

_____

D. Antretyen filtè sad la

36) Kijan ou sè konnen kilè pouw repare aparey filtè a?

Dlo a koule piti	
Filtr lan sanble sal	
Sab lan sanble sal	
Dlo a gen move gou	
Dlo a bay move odè	
Dlo filtre a fè moun malad	
Lòt	

37) Chak kilè ou fè sèvis nan filtè a?

Chak semèn	
Chak de semèn	
Chak twa semèn	
Chak mwa	
Plis ke yon mwa	

38) Ou repare sistèm filtè a pou kont ou, ou byen ou rele yon teknisyen?

Mwen fel pou kont mwen	
Teknisyen	

39) Di kijan ou reparel.

_____

_____

_____

_____

_____

_____

_____

40) Eske li fasil pou jwenn yon teknisyen pou repare filtè a?

Wi	
Non	

E. Konsèvasyon dlo a

41) Ki kote ou mete dlo ki pa trete a?

Bokit avek kouveti	
Bokit san kouveti	
Boutey plastic	
Boutey an glas	
Lòt	

42) Ki kote ou mete dlo trete a:

Bokit avek kouveti	
Bokit san kouveti	
Boutey plastik	
Boutey an glas	
Lòt	

43) Chak kilè ou netwaye rezèvwa dlo a?

2 fwa pa jou	
Chak jou	
Chak semen	
Lè li sal	
Lòt	

44) Ki dlo ou itilize pou netwaye rezèvwa a?

Dlo trete	
Dlo ki pa trete	

45) Kisa ou itilize pou netwaye rezèvwa a?

Dlo selman	
Dlo ak klorox	
Lòt	

F. Orè

46) Ou gen yon travay ou byen ou rete lakay?

Travay	
Rete lakay	

47) A kilè ou leve nan kabann?

5:00 am	
6:00 am	
7:00 am	
8:00 am	
Apre 8:00 am	
Lòt	

48) A kilè ou abitye al dòmi?

8:00 pm	
9:00 pm	
10:00 pm	
11:00 pm	
Apre 12:00 pm	
Lòt	

49) A kilè ou sot travay?

3:00 pm	
4:00 pm	
5:00 pm	
6:00 pm	
Apre 7:00 pm	
Lòt	

50) Konbyen fwa ou met dlo nan filtè a pa jou?

1 fwa pa jou	
2 fwa pa jou	
3 fwa pa jou	
4 fwa pa jou	
Plis ke 4 fwa pa jou	

51) Kisaw ta rekòmande nou fè pou filtè a pi bon?

_____

_____

_____

6.3 OUTREACH IN PUERTO RICO

In addition to the work and efforts in Duchity, we expanded our work to the communities here in Puerto Rico. With the collaboration of the Coastal Training Program of the National Estuarine Research Reserves, trainings about water quality, the IBSF installation and operation were offered to the general community in Jobos Bay Reserve and in the University of Puerto Rico at Mayagüez. Those trainings provided information to the general community about this type of filter as a simple alternative to treat the water here in Puerto Rico. Even though most communities in Puerto Rico have access to municipal water systems, some remote communities in the mountains do not and depend on wells and rainwater. Filters can potentially serve to these communities as a reliable means to purify water. Moreover, even in places with access to municipal water supplies, filters offer a cost effective emergency means to purify water in the aftermath of earthquakes and hurricanes, both of which occur in Puerto Rico with some regularity. For these reasons, the IBSF can be considered an appropriate technology for Puerto Rico.

Figure 6.7 Flyer announcing the trainings about water quality and the IBSF to the general community. (Prepared by the Costal training program).

6.4 PERSONAL REMARK

After completing this study and having the experiences that I obtained during this research, I believe the appropriate technology concept can open the engineer's minds to wide list of factors that can influence the success of a project. It not only the ability of a technology to perform a task or function, but it is also how the community or the people that are affected by the technology will be impacted by it's implementation. As part of this

research I contributed a brief reflection on my work in a multi-author article written by the GREAT IDEA team, which I close with here:

> "*My work with the GREAT IDEA project is the best professional and personal opportunity of my life. We, as engineering students, need courses and research experiences focused in the development of skills to work with and for the community. Engineers should work to solve problems in the simplest way and always keep in mind what the user really needs, rather than the creation of new products to make the user depend on it. For example with the IBSF, the basic need of safe water can be provided without sophisticated technology and power, even though the scientific basis of its operation is highly technical. As part of my work with GREAT IDEA, I had been exposed to meetings in where engineers discuss the ideas with the community. From this experience I learned that for the project to succeed, engineers have to understand what the community needs and how they want it, but also the community need to trust the engineers because they have the required knowledge for design. In a community project engineers need to develop effective communication skills with the community people and include as part of the work, the evaluation of the social impact of the project in the community. Because of my experience in GREAT IDEA, now I know that I have more career alternatives than just the industry"* (Papadopoulos et al., 2014).

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8 APPENDIX

In this section additional graphs with the obtained results from the R Statistical Analysis are presented. In addition the R program code is included for the analysis of both bacteria, *E. coli* and *Enterococcus*.

APPENDIX A: R Statistical Analysis Additional Graphs

A.1 *E. coli* removal

A.2 *Enterococcus* removal

APPENDIX B: R Statistical Analysis Codes

B.1 For *E. coli* population

B.2 For *Enterococcus population*

APPENDIX A: R Statistical Analysis Additional Graphs

A.1 *E. coli* removal:

Figure 8.1 Normality and Homocedasticy for log [y/(1-y)] of *E. coli*.

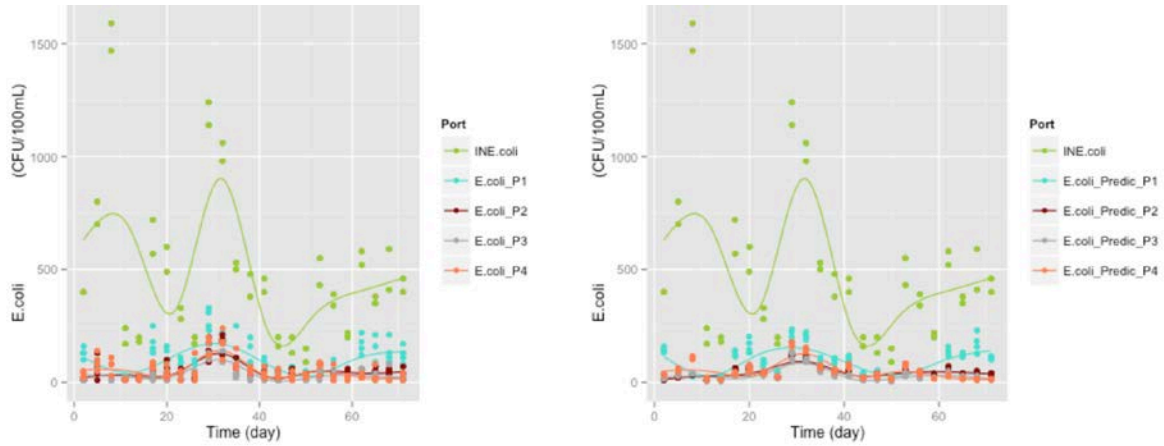

Figure 8.2 *E. coli* model approximations.

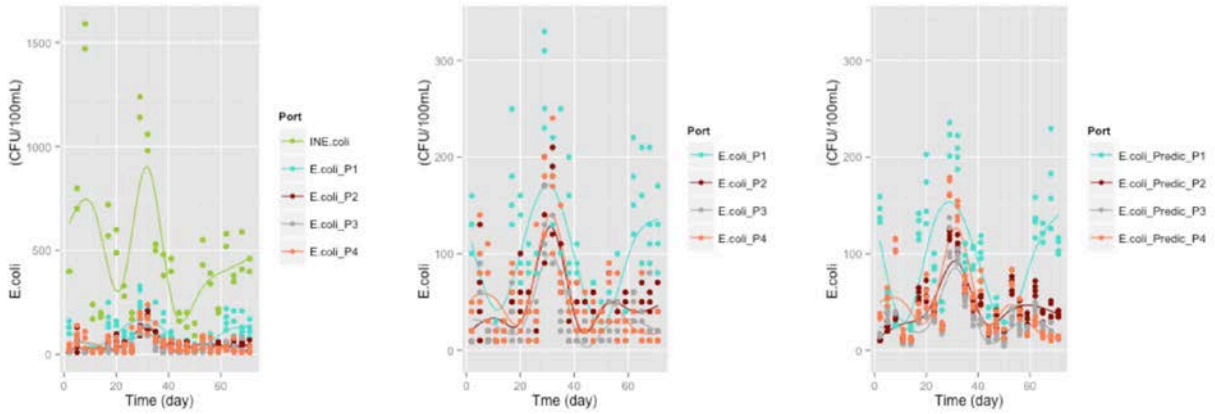

Figure 8.3 *E. coli* population through IBSF in time at each sampling point.

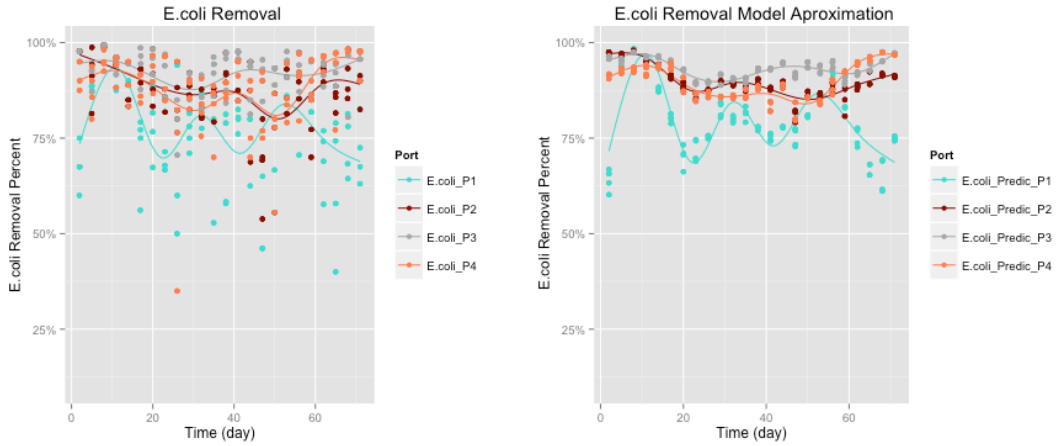

Figure 8.4 *E. coli* removal percent through IBSF in time.

A.2 *Enterococcus* removal:

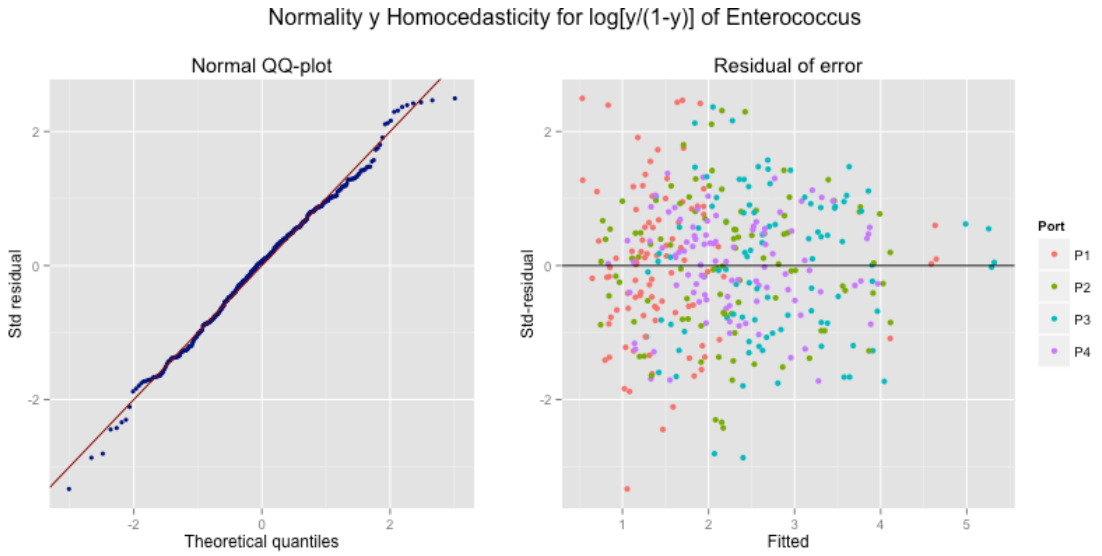

Figure 8.5 Normality and Homocedasticy for log [y/(1-y)] of *Enterococcus*.

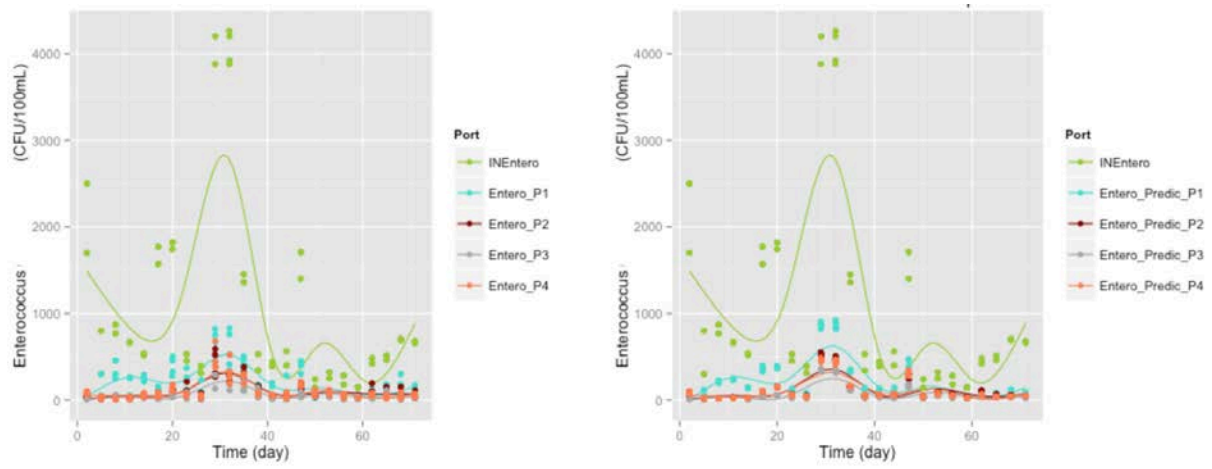

Figure 8.6 *Enterococcus* model approximations.

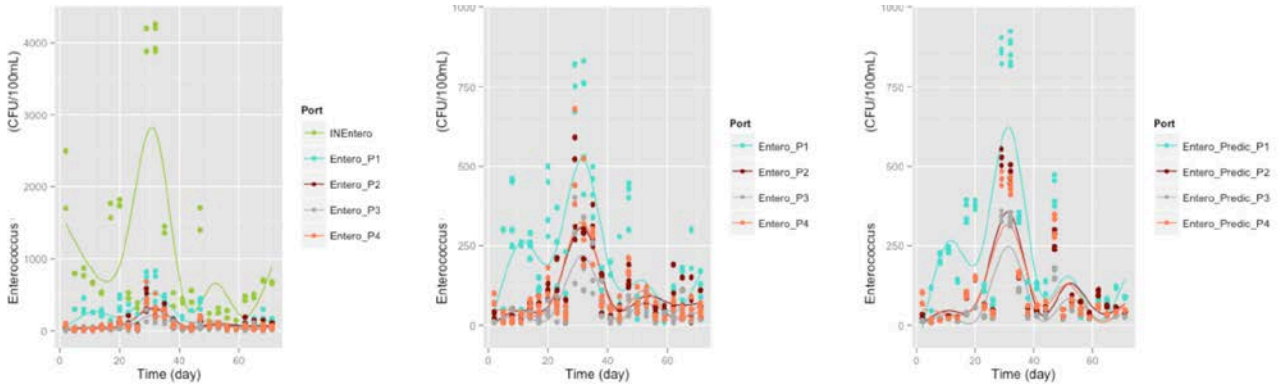

Figure 8.7 *Enterococcus* population through IBSF in time at each sampling point.

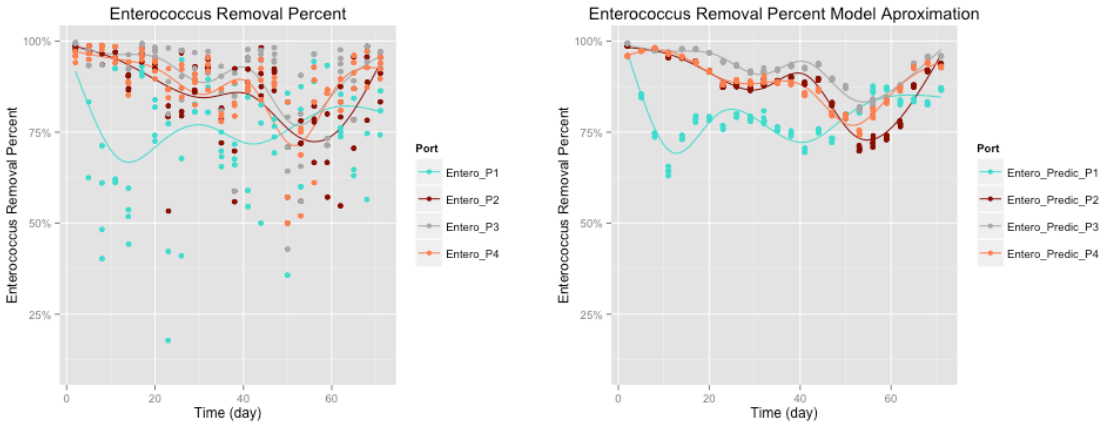

Figure 8.8 *Enterococcus* removal percent through IBSF in time.

APPENDIX B: R Statistical Analysis Codes

B.1 For *E. coli* population

```
############################################################
# library
############################################################
library(multcomp)
library(nlme)
library(mvtnorm)
library(survival)
library(TH.data)
library(reshape2)
library(ggplot2)
library(RColorBrewer)
library(scales)
library(gridExtra)
library(splines)
library(mgcv)
multiplot <- function(..., plotlist=NULL, file, cols=1, layout=NULL) {
  require(grid)
  # Make a list from the ... arguments and plotlist
  plots <- c(list(...), plotlist)
  numPlots = length(plots)
  # If layout is NULL, then use 'cols' to determine layout
  if (is.null(layout)) {
    # Make the panel
    # ncol: Number of columns of plots
    # nrow: Number of rows needed, calculated from # of cols
    layout <- matrix(seq(1, cols * ceiling(numPlots/cols)),
                     ncol = cols, nrow = ceiling(numPlots/cols))
  }
  if (numPlots==1) {
    print(plots[[1]])

  } else {
    # Set up the page
    grid.newpage()
    pushViewport(viewport(layout = grid.layout(nrow(layout),
ncol(layout))))

    # Make each plot, in the correct location
    for (i in 1:numPlots) {
      # Get the i,j matrix positions of the regions that contain this
subplot
      matchidx <- as.data.frame(which(layout == i, arr.ind = TRUE))
```

```
      print(plots[[i]], vp = viewport(layout.pos.row = matchidx$row,
                                      layout.pos.col = matchidx$col))
    }
  }
}

BaseDatosJRS_FINAL_COL<-
 read.csv("~/Desktop/datos/BaseDatosJRS_FINAL_COL.csv")

data_t=BaseDatosJRS_FINAL_COL[,c(2,6,10,33,14,37,18,41,22,45)]
data=BaseDatosJRS_FINAL_COL[,c(6,10,33,14,37,18,41,22,45)]

cols2 <-
 c("INE.coli"="yellowgreen","E.coli_P11"="turquoise","E.coli_P12"="turq
 uoise", "E.coli_P21"="darkred",
 "E.coli_P22"="darkred","E.coli_P31"="darkgray","E.coli_P32"="darkgray"
 , "E.coli_P41"= "navy", "E.coli_P42"= "coral")
minimo=min(data)
maximo=max(data)
k=30
met <- melt(data_t, id = "Tiempo", measure =
 c("INE.coli","E.coli_P11","E.coli_P12", "E.coli_P21",
 "E.coli_P22","E.coli_P31","E.coli_P32", "E.coli_P41", "E.coli_P42"))
p1<-qplot(Tiempo, value, data= met, colour = variable, size=I(0.6))+
  stat_smooth(method = "gam", formula = y ~ s(x, bs = "cs", k = 8)
 ,fill=NA, size =0.5)+
  geom_point(position=position_jitter(width=0.1))+
  ylim(minimo-k,maximo+k)+
  xlab("Time (day)") +
  ylab("E.coli population [CFU/100mL]") +
  scale_colour_manual(values =
 cols2,labels=c("INE.coli","E.coli_P11","E.coli_P12", "E.coli_P21",
 "E.coli_P22","E.coli_P31","E.coli_P32", "E.coli_P41", "E.coli_P42")) +
  labs(colour="Port-Filter")+
  scale_linetype_manual(values =
 c("solid","solid","dashed","solid","dashed","solid","dashed","solid","
 dashed"))+
  ggtitle("E. coli population ")

data1=BaseDatosJRS_FINAL_COL[,c(10,33,14,37,18,41,22,45)]
cols3 <- c("E.coli_P11"="turquoise","E.coli_P12"="turquoise",
 "E.coli_P21"="darkred",
 "E.coli_P22"="darkred","E.coli_P31"="darkgray","E.coli_P32"="darkgray"
 , "E.coli_P41"= "navy", "E.coli_P42"= "coral")
minimo=min(data1)
maximo=max(data1)
k=30
met <- melt(data_t, id = "Tiempo", measure =
 c("E.coli_P11","E.coli_P12", "E.coli_P21",
```

```
 "E.coli_P22","E.coli_P31","E.coli_P32", "E.coli_P41", "E.coli_P42"))
p2<-qplot(Tiempo, value, data= met, colour = variable, size=I(0.6))+
  stat_smooth(method = "gam", formula = y ~ s(x, bs = "cs", k = 8)
 ,fill=NA,size =0.5)+
  geom_point(position=position_jitter(width=0.1))+
  ylim(minimo-k,maximo+k)+
  xlab("Time (day)") +
  ylab("E.coli population [CFU/100mL]") +
  #   theme(legend.key.size = unit(0, "cm"))+
  scale_colour_manual(values =
 cols3,labels=c("E.coli_P11","E.coli_P12", "E.coli_P21",
 "E.coli_P22","E.coli_P31","E.coli_P32", "E.coli_P41", "E.coli_P42")) +
  labs(colour="Port-Filter")+
  scale_linetype_manual(values =
 c("solid","dashed","solid","dashed","solid","dashed","solid","dashed")
 )+
  ggtitle("E. coli population Sampling Ports")

multiplot(arrangeGrob(p1,p2, ncol=2,
 widths=c(5/10,5/10),main=textGrob(" E. coli population throught the
 IBSF depth in time",gp=gpar(fontsize=16))))

BaseDatosJRS_mean_filtros<-
 read.csv("~/Desktop/datos/BaseDatosJRS_mean_filtros.csv")

minimo=min(BaseDatosJRS_mean_filtros[,c(2,4,6,8,10)])
maximo=max(BaseDatosJRS_mean_filtros[,c(2,4,6,8,10)])
cols1 <- c("INE.coli"="yellowgreen","E.coli_P1"="turquoise",
 "E.coli_P2"="darkred","E.coli_P3"="darkgray", "E.coli_P4"= "coral")
k=10
met <- melt(BaseDatosJRS_mean_filtros, id = "Tiempo", measure =
 c("INE.coli","E.coli_P1", "E.coli_P2","E.coli_P3", "E.coli_P4"))
p1<-qplot(Tiempo, value, data= met, colour = variable, size=I(0.6))+
  stat_smooth(method = "gam", formula = y ~ s(x, bs = "cs", k = 8)
 ,fill=NA, size =0.5)+
  geom_point(position=position_jitter(width=0.1))+
  ylim(minimo-k,maximo+k)+
  xlab("Time (day)") +
  ylab("E. coli population (CFU/100mL)") +
  scale_colour_manual(values = cols1,labels=c("INE.coli","E.coli_P1",
 "E.coli_P2","E.coli_P3", "E.coli_P4")) +
  labs(colour="Sampling Point")+
  scale_linetype_manual(values =
 c("solid","solid","solid","solid","solid"))+
  ggtitle("E. coli population ")

cols2 <- c("E.coli_P1"="turquoise",
 "E.coli_P2"="darkred","E.coli_P3"="darkgray", "E.coli_P4"= "coral")
minimo1=min(BaseDatosJRS_mean_filtros[,c(4,6,8,10)])
```

```
maximo1=max(BaseDatosJRS_mean_filtros[,c(4,6,8,10)])
k=10
met <- melt(BaseDatosJRS_mean_filtros, id = "Tiempo", measure =
 c("E.coli_P1", "E.coli_P2","E.coli_P3", "E.coli_P4"))
p2<-qplot(Tiempo, value, data= met, colour = variable, size=I(0.6))+
  stat_smooth(method = "gam", formula = y ~ s(x, bs = "cs", k = 8)
 ,fill=NA,size =0.5)+
  #   geom_smooth(fill=NA,size =0.5) +
  geom_point(position=position_jitter(width=0.1))+
  ylim(minimo1-k,maximo1+k)+
  xlab("Time (day)") +
  ylab("E. coli population (CFU/100mL)") +
  scale_colour_manual(values = cols2,labels=c("E.coli_P1",
 "E.coli_P2","E.coli_P3", "E.coli_P4")) +
  labs(colour="Sampling Point")+
  scale_linetype_manual(values = c("solid","solid","solid","solid"))+
  ggtitle("E. coli population Sampling Ports")

multiplot(arrangeGrob(p1,p2, ncol=2,
 widths=c(5/10,5/10),main=textGrob("E.coli population throught IBSF
 depth vs Time (FA and FB Averaged)",gp=gpar(fontsize=16))))

BaseDatosJRS_FINAL_COL<-
 read.csv("~/Desktop/datos/BaseDatosJRS_FINAL_COL.csv")
data1=BaseDatosJRS_FINAL_COL$INE.coli-
 BaseDatosJRS_FINAL_COL[,c(6,10,33,14,37,18,41,22,45)]
data2=data1[,c(-1)]
data3=100*data2/BaseDatosJRS_FINAL_COL$INE.coli
Tiempo=BaseDatosJRS_FINAL_COL$Tiempo
data4  = data.frame (cbind(Tiempo,data3))

cols1 <- c("E.coli_P11"="turquoise","E.coli_P12"="turquoise",
 "E.coli_P21"="darkred",
 "E.coli_P22"="darkred","E.coli_P31"="darkgray","E.coli_P32"="darkgray"
 , "E.coli_P41"= "navy", "E.coli_P42"= "coral")
minimo1=min(data4)
maximo1=max(data4)
k=0.1
met <- melt(data4, id = "Tiempo", measure =
 c("E.coli_P11","E.coli_P12", "E.coli_P21",
 "E.coli_P22","E.coli_P31","E.coli_P32", "E.coli_P41", "E.coli_P42"))
p1<-qplot(Tiempo, value, data= met, colour = variable, size=I(0.6))+
  #   geom_smooth(fill=NA,size =0.5) +
  stat_smooth(method = "gam", formula = y ~ s(x, bs = "cs", k = 8)
 ,fill=NA,size =0.5)+
  geom_point(position=position_jitter(width=0.1))+
  xlab("Time (day)") +
  ylab("E. coli Removal Percent") +
  scale_y_continuous(breaks=c(25,50,75,100), labels=c("25%",
```

```
"50%","75%","100%"),limits=c(25, 100))+
  scale_colour_manual(values =
cols1,labels=c("E.coli_P11","E.coli_P12", "E.coli_P21",
"E.coli_P22","E.coli_P31","E.coli_P32", "E.coli_P41", "E.coli_P42")) +
  labs(colour="Port-Filter")+
  scale_linetype_manual(values =
c("solid","dashed","solid","dashed","solid","dashed","solid","dashed")
)+
  ggtitle("FA and FB E.coli Removal Percent")

BaseDatosJRS_mean_filtros<-
 read.csv("~/Desktop/Filtros/datos/BaseDatosJRS_mean_filtros.csv")
data1=BaseDatosJRS_mean_filtros$INE.coli-
 BaseDatosJRS_mean_filtros[,c(1,4,6,8,10)]
data2=data1[,c(-1)]
data3=100*data2/BaseDatosJRS_mean_filtros$INE.coli
Tiempo=BaseDatosJRS_mean_filtros$Tiempo
data5= data.frame (cbind(Tiempo,data3))

cols2 <- c("E.coli_P1"="turquoise", "E.coli_P2"="darkred",
 "E.coli_P3"="darkgray", "E.coli_P4"= "coral")
minimo=min(data5[,c(2,3,4,5)])
maximo=max(data5[,c(2,3,4,5)])
k=0.1
met <- melt(data5, id = "Tiempo", measure = c("E.coli_P1",
 "E.coli_P2", "E.coli_P3", "E.coli_P4"))
p2<-qplot(Tiempo, value, data= met, colour = variable, size=I(0.6)) +
  stat_smooth(method = "gam", formula = y ~ s(x, bs = "cs", k = 8)
 ,fill=NA,size =0.5)+
  geom_point(position=position_jitter(width=0.1)) +
  xlab("Time (day)") +
  ylab("E. coli Removal Percent") +
  scale_y_continuous(breaks=c(25,50,75,100), labels=c("25%",
"50%","75%","100%"),limits=c(25, 100))+
  scale_colour_manual(values = cols2,labels=c("E.coli_P1",
"E.coli_P2", "E.coli_P3", "E.coli_P4")) +
  labs(colour="Sampling Port") +
  scale_linetype_manual(values = c("solid","solid","solid","solid")) +
  ggtitle("FA and FB Averaged")

multiplot(arrangeGrob(p1,p2, ncol=2,
 widths=c(5/10,5/10),main=textGrob("E.coli Removal Percent throught
 IBSF depth vs Time",gp=gpar(fontsize=16))))

BaseDatosJRS_ANC <- read.csv("~/Desktop/datos/BaseDatosJRS_ANC.csv")
y =(BaseDatosJRS_ANC$INE.coli -
 BaseDatosJRS_ANC$E.coli)/BaseDatosJRS_ANC$INE.coli
y1=log(y/(1-y))
```

```
Filtro.lme <- lme(y1 ~ factor(Filtro) +  factor(Puerto)*bs(Tiempo,
 df=7, degree=3) + log(INOxigeno) + log(Oxigeno), random = ~
 1|Filtro_Puerto, data=BaseDatosJRS_ANC)
summary(Filtro.lme)

Filtro.anova.lme <- aov(y1 ~ factor(Filtro) +
 factor(Puerto)*bs(Tiempo,  df=7, degree=3) + log(INOxigeno) +
 log(Oxigeno), random = ~ 1|Filtro_Puerto, data=BaseDatosJRS_ANC)
summary(Filtro.anova.lme)

ks.test(residuals(Filtro.lme), "pnorm", mean = 0, sd = 1)
shapiro.test(residuals(Filtro.lme))

-2*logLik(Filtro.lme)
AIC(Filtro.lme)
BIC(Filtro.lme)

Filtro.lme1 <- lm(y1 ~ factor(Filtro) +  factor(Puerto)*bs(Tiempo,
 df=7, degree=3) + log(INOxigeno) + log(Oxigeno),
 data=BaseDatosJRS_ANC)

p1 <-ggplot(Filtro.lme1, aes(sample = .stdresid)) + stat_qq(colour =
 "navy",size=1.5) +
  geom_abline(colour = "darkred") +
  labs(title="Normal QQ-plot", x="Theoretical quantiles", y="Std
 Residual")

p2<- qplot(.fitted, .stdresid, data = fortify(Filtro.lme1,
 BaseDatosJRS_ANC),size=I(0.1),
           xlab="Fitted",ylab="Std-residual", colour = factor(Puerto))
 +
  geom_point(position=position_jitter(width=0.3)) +
  scale_colour_hue("Port",drop=FALSE)+
  labs(title="Residual of error") +
  geom_hline(yintercept =0)

multiplot(arrangeGrob(p1,p2, ncol=2,
 widths=c(4/10,5/10),main=textGrob("Normality and Homocedasticity for
 log[y/(1-y)] of E. coli",gp=gpar(fontsize=16))))

qqnorm(Filtro.lme, ~ resid(., type = "p") | Filtro_Puerto, abline =
 c(0, 1), xlab="Theoretical quantiles", ylab="Std
 residual",main="Residual of error")
qqnorm(Filtro.lme, ~ resid(., type = "p") | Filtro, abline = c(0, 1),
 xlab="Theoretical quantiles", ylab="Std residual",main="Residual of
 error")
qqnorm(Filtro.lme, ~ resid(., type = "p") | Puerto, abline = c(0, 1),
 xlab="Theoretical quantiles", ylab="Std residual",main="Residual of
 error")
```

```
plot(Filtro.lme)

plot(Filtro.lme, resid(., type = "p") ~fitted(.) |Filtro_Puerto,
 abline = 0)
plot(Filtro.lme, resid(., type = "p") ~fitted(.) |Filtro, abline = 0)
plot(Filtro.lme, resid(., type = "p") ~fitted(.) |Puerto, abline = 0)
plot(Filtro.lme, Filtro_Puerto ~resid(., type="p"))
BaseDatosJRS_ANC <- read.csv("~/Desktop/datos/BaseDatosJRS_ANC.csv")
BaseDatosJRS_ANC$Puerto <- factor(BaseDatosJRS_ANC$Puerto)
BaseDatosJRS_ANC$Filtro <- factor(BaseDatosJRS_ANC$Filtro)
BaseDatosJRS_ANC$Filtro_Puerto <-
 factor(BaseDatosJRS_ANC$Filtro_Puerto)
y = (BaseDatosJRS_ANC$INE.coli -
 BaseDatosJRS_ANC$E.coli)/BaseDatosJRS_ANC$INE.coli
y1=log(y/(1-y))

E.coli_P1=as.vector(y1[c(1:48,193:240)])
E.coli_P2=as.vector(y1[c(49:96,241:288)])
E.coli_P3=as.vector(y1[c(97:144,289:336)])
E.coli_P4=as.vector(y1[c(145:192,337:384)])
data=
 data.frame(cbind(Tiempo=as.vector(BaseDatosJRS_ANC[1:96,2]),Replica=as
 .vector(BaseDatosJRS_ANC[1:96,3]),E.coli_P1,E.coli_P2,E.coli_P3,E.coli
 _P4))

Filtro.lme <- lme(y1 ~ factor(Filtro) +  factor(Puerto)*bs(Tiempo,
 df=7, degree=3) + log(INOxigeno) + log(Oxigeno), random = ~
 1|Filtro_Puerto, data=BaseDatosJRS_ANC)
Fit.lme.predic=predict(Filtro.lme)

E.coli_Predic_P1=as.vector(Fit.lme.predic[c(1:48,193:240)])
E.coli_Predic_P2=as.vector(Fit.lme.predic[c(49:96,241:288)])
E.coli_Predic_P3=as.vector(Fit.lme.predic[c(97:144,289:336)])
E.coli_Predic_P4=as.vector(Fit.lme.predic[c(145:192,337:384)])
data.fit=
 data.frame(cbind(data,E.coli_Predic_P1,E.coli_Predic_P2,E.coli_Predic_
 P3,E.coli_Predic_P4))

cols2 <- c("E.coli_P1"="turquoise", "E.coli_P2"="darkred",
 "E.coli_P3"="darkgray", "E.coli_P4"= "coral")
minimo=min(data.fit[,c(1,3,4,5,6)])
maximo=max(data.fit[,c(1,3,4,5,6)])
met <- melt(data.fit, id = "Tiempo", measure = c("E.coli_P1",
 "E.coli_P2", "E.coli_P3", "E.coli_P4"))
p1<-qplot(Tiempo, value, data= met, colour = variable, size=I(0.6)) +
  stat_smooth(method = "gam", formula = y ~ s(x, bs = "cs", k = 8)
 ,fill=NA,size =0.5)+
  geom_point(position=position_jitter(width=0.1)) +
  ylim(-1,6)+
```

```
  xlab("Time (day)") +
  ylab("log[y/(1-y)], where y=(INE.coli-E.coli)/INE.coli") +
  scale_colour_manual(values = cols2,labels=c("E.coli_P1",
 "E.coli_P2", "E.coli_P3", "E.coli_P4")) +
  labs(colour="Sampling Port") +
  scale_linetype_manual(values = c("solid","solid","solid","solid")) +
  ggtitle(" E. coli Removal")

cols3 <-
 c("E.coli_Predic_P1"="turquoise","E.coli_Predic_P2"="darkred","E.coli_
 Predic_P3"="darkgray","E.coli_Predic_P4"="coral")
minimo1=min(data.fit[,c(1,7,8,9,10)])
maximo1=max(data.fit[,c(1,7,8,9,10)])
met <- melt(data.fit, id = "Tiempo", measure =
 c("E.coli_Predic_P1","E.coli_Predic_P2","E.coli_Predic_P3","E.coli_Pre
 dic_P4"))
p2<-qplot(Tiempo, value, data= met, colour = variable, size=I(0.6)) +
  stat_smooth(method = "gam", formula = y ~ s(x, bs = "cs", k = 8)
 ,fill=NA,size =0.5)+
  geom_point(position=position_jitter(width=0.1)) +
  ylim(-1,6)+
  xlab("Time (day)") +
  ylab("Model Aproximations") +
  scale_colour_manual(values =
 cols3,labels=c("E.coli_Predic_P1","E.coli_Predic_P2","E.coli_Predic_P3
 ","E.coli_Predic_P4")) +
  labs(colour="Sampling Port") +
  scale_linetype_manual(values = c("solid","solid","solid","solid")) +
  ggtitle("E. coli Removal Model Aproximation ")

multiplot(arrangeGrob(p1,p2, ncol=2,
 widths=c(4.6/10,5/10),main=textGrob(" E. coli Removal throught the
 IBSF in Time, where y=(INE.coli-
 E.coli)/INE.coli",gp=gpar(fontsize=16))))

BaseDatosJRS_ANC <- read.csv("~/Desktop/datos/BaseDatosJRS_ANC.csv")

E.coli_P1=as.vector(BaseDatosJRS_ANC$E.coli[c(1:48,193:240)])
E.coli_P2=as.vector(BaseDatosJRS_ANC$E.coli[c(49:96,241:288)])
E.coli_P3=as.vector(BaseDatosJRS_ANC$E.coli[c(97:144,289:336)])
E.coli_P4=as.vector(BaseDatosJRS_ANC$E.coli[c(145:192,337:384)])

INE.coli=as.vector(BaseDatosJRS_ANC$INE.coli[c(1:96)])

data=
 data.frame(cbind(Tiempo=as.vector(BaseDatosJRS_ANC[1:96,2]),E.coli_P1,
 E.coli_P2,E.coli_P3,E.coli_P4))
Filtro.lme <- lme(y1 ~ factor(Filtro) +  factor(Puerto)*bs(Tiempo,
 df=7, degree=3) + log(INOxigeno) + log(Oxigeno), random = ~
```

```
 1|Filtro_Puerto, data=BaseDatosJRS_ANC)

Fit.lme.predic=predict(Filtro.lme)

yP1=exp(as.vector(Fit.lme.predic[c(1:48,193:240)]))/(1+exp(as.vector(F
 it.lme.predic[c(1:48,193:240)])))
yP2=exp(as.vector(Fit.lme.predic[c(49:96,241:288)]))/(1+exp(as.vector(
 Fit.lme.predic[c(49:96,241:288)])))
yP3=exp(as.vector(Fit.lme.predic[c(97:144,289:336)]))/(1+exp(as.vector
 (Fit.lme.predic[c(97:144,289:336)])))
yP4=exp(as.vector(Fit.lme.predic[c(145:192,337:384)]))/(1+exp(as.vecto
 r(Fit.lme.predic[c(145:192,337:384)])))

E.coli_Predic_P1=(BaseDatosJRS_ANC$INE.coli-
 BaseDatosJRS_ANC$INE.coli*yP1)
E.coli_Predic_P2=(BaseDatosJRS_ANC$INE.coli-
 BaseDatosJRS_ANC$INE.coli*yP2)
E.coli_Predic_P3=(BaseDatosJRS_ANC$INE.coli-
 BaseDatosJRS_ANC$INE.coli*yP3)
E.coli_Predic_P4=(BaseDatosJRS_ANC$INE.coli-
 BaseDatosJRS_ANC$INE.coli*yP4)

data.fit.lme=
 data.frame(cbind(data,INE.coli,E.coli_Predic_P1,E.coli_Predic_P2,E.col
 i_Predic_P3,E.coli_Predic_P4))

cols2 <- c("INE.coli"="yellowgreen","E.coli_P1"="turquoise",
 "E.coli_P2"="darkred", "E.coli_P3"="darkgrey", "E.coli_P4"= "coral")
minimo=min(data.fit.lme[,c(2,3,4,5,6,7,8,9,10)])
maximo=max(data.fit.lme[,c(2,3,4,5,6,7,8,9,10)])

k=10
met <- melt(data.fit.lme, id = "Tiempo", measure =
 c("INE.coli","E.coli_P1", "E.coli_P2", "E.coli_P3", "E.coli_P4"))
p1<-qplot(Tiempo, value, data= met, colour = variable, size=I(0.6)) +
  stat_smooth(method = "gam", formula = y ~ s(x, bs = "cs", k = 8)
 ,fill=NA,size =0.5)+
  geom_point(position=position_jitter(width=0.1)) +
  ylim(minimo-k,maximo+k)+
  xlab("Time (day)") +
  ylab("E.coli population (CFU/100mL)") +
  scale_colour_manual(values = cols2,labels=c("INE.coli","E.coli_P1",
 "E.coli_P2", "E.coli_P3", "E.coli_P4")) +
  labs(colour="Port") +
  scale_linetype_manual(values =
 c("solid","solid","solid","solid","solid")) +
  ggtitle("E.coli population ")

cols3 <-
```

```
c("INE.coli"="yellowgreen","E.coli_Predic_P1"="turquoise","E.coli_Pred
ic_P2"="darkred","E.coli_Predic_P3"="darkgray","E.coli_Predic_P4"="cor
al")

met <- melt(data.fit.lme, id = "Tiempo", measure =
c("INE.coli","E.coli_Predic_P1","E.coli_Predic_P2","E.coli_Predic_P3",
"E.coli_Predic_P4"))
p2<-qplot(Tiempo, value, data= met, colour = variable, size=I(0.6)) +
  stat_smooth(method = "gam", formula = y ~ s(x, bs = "cs", k = 8)
,fill=NA,size =0.5)+
  geom_point(position=position_jitter(width=0.1)) +
  ylim(minimo-k,maximo+k)+
  xlab("Time (day)") +
  ylab("E.coli population (CFU/100mL)") +
  scale_colour_manual(values =
cols3,labels=c("INE.coli","E.coli_Predic_P1","E.coli_Predic_P2","E.col
i_Predic_P3","E.coli_Predic_P4")) +
  labs(colour="Port") +
  scale_linetype_manual(values =
c("solid","solid","solid","solid","solid")) +
  ggtitle("E.coli population Model Aproximation")

multiplot(arrangeGrob(p1,p2, ncol=2,
widths=c(4.6/10,5/10),main=textGrob(" E. coli population throught the
IBSF in Time",gp=gpar(fontsize=16))))

cols2 <- c("E.coli_P1"="turquoise", "E.coli_P2"="darkred",
"E.coli_P3"="darkgray", "E.coli_P4"= "coral")
minimo=min(data.fit.lme[,c(2,3,4,5,7,8,9,10)])
maximo=max(data.fit.lme[,c(2,3,4,5,7,8,9,10)])

k=10
met <- melt(data.fit.lme, id = "Tiempo", measure = c("E.coli_P1",
"E.coli_P2", "E.coli_P3", "E.coli_P4"))
p3<-qplot(Tiempo, value, data= met, colour = variable, size=I(0.6)) +
  stat_smooth(method = "gam", formula = y ~ s(x, bs = "cs", k = 8)
,fill=NA,size =0.5)+
  geom_point(position=position_jitter(width=0.1)) +
  ylim(minimo-k,maximo+k)+
  xlab("Tme (day)") +
  ylab("E.coli population (CFU/100mL)") +
  scale_colour_manual(values = cols2,labels=c("E.coli_P1",
"E.coli_P2", "E.coli_P3", "E.coli_P4")) +
  labs(colour="Port") +
  scale_linetype_manual(values = c("solid","solid","solid","solid")) +
  ggtitle("E.coli population ")

cols3 <-
c("E.coli_Predic_P1"="turquoise","E.coli_Predic_P2"="darkred","E.coli_
```

```
 Predic_P3"="darkgray","E.coli_Predic_P4"="coral")
met <- melt(data.fit.lme, id = "Tiempo", measure =
 c("E.coli_Predic_P1","E.coli_Predic_P2","E.coli_Predic_P3","E.coli_Pre
 dic_P4"))
p4<-qplot(Tiempo, value, data= met, colour = variable, size=I(0.6)) +
  stat_smooth(method = "gam", formula = y ~ s(x, bs = "cs", k = 8)
 ,fill=NA,size =0.5)+
  geom_point(position=position_jitter(width=0.1)) +
  ylim(minimo-k,maximo+k)+
  xlab("Time (day)") +
  ylab("E.coli population (CFU/100mL)") +
  scale_colour_manual(values =
 cols3,labels=c("E.coli_Predic_P1","E.coli_Predic_P2","E.coli_Predic_P3
 ","E.coli_Predic_P4")) +
  labs(colour="Port") +
  scale_linetype_manual(values = c("solid","solid","solid","solid")) +
  ggtitle("E.coli population ")

multiplot(arrangeGrob(p3,p4, ncol=2,
 widths=c(4.6/10,5/10),main=textGrob("E.coli population throught IBSF
 in Time (Sampling Ports)",gp=gpar(fontsize=16))))


multiplot(arrangeGrob(p1,p3,p4, ncol=3,
 widths=c(4/12,4/12,4.3/12),main=textGrob("E.coli population throught
 IBSF in Time (Sampling Ports)",gp=gpar(fontsize=16))))

data1 =100*((data.fit.lme$INE.coli -
 data.fit.lme[c(2,3,4,5,7,8,9,10)])/data.fit.lme$INE.coli)
data2 =cbind(Tiempo=data.fit.lme$Tiempo,data1)

cols2 <- c("E.coli_P1"="turquoise", "E.coli_P2"="darkred",
 "E.coli_P3"="darkgray", "E.coli_P4"= "coral")
minimo=min(data2[,c(2,3,4,5,7,8,9)])
maximo=max(data2[,c(2,3,4,5,7,8,9)])

met <- melt(data2, id = "Tiempo", measure = c("E.coli_P1",
 "E.coli_P2", "E.coli_P3", "E.coli_P4"))
p1<-qplot(Tiempo, value, data= met, colour = variable, size=I(0.6)) +
  stat_smooth(method = "gam", formula = y ~ s(x, bs = "cs", k = 8)
 ,fill=NA,size =0.5)+
  geom_point(position=position_jitter(width=0.1)) +
  scale_y_continuous(breaks=c(25,50,75,100), labels=c("25%",
 "50%","75%","100%"),limits=c(10, 100))+
  #   ylim(minimo-k,maximo+k)+
  xlab("Time (day)") +
  ylab("E.coli Removal Percent") +
  scale_colour_manual(values = cols2,labels=c("E.coli_P1",
 "E.coli_P2", "E.coli_P3", "E.coli_P4")) +
```

```
  labs(colour="Port") +
  scale_linetype_manual(values = c("solid","solid","solid","solid")) +
  ggtitle(" E.coli Removal")
cols3 <-
 c("E.coli_Predic_P1"="turquoise","E.coli_Predic_P2"="darkred","E.coli_
 Predic_P3"="darkgray","E.coli_Predic_P4"="coral")
met <- melt(data2, id = "Tiempo", measure =
 c("E.coli_Predic_P1","E.coli_Predic_P2","E.coli_Predic_P3","E.coli_Pre
 dic_P4"))
p2<-qplot(Tiempo, value, data= met, colour = variable, size=I(0.6)) +
  stat_smooth(method = "gam", formula = y ~ s(x, bs = "cs", k = 8)
 ,fill=NA,size =0.5)+
  geom_point(position=position_jitter(width=0.1)) +
  #   ylim(minimo-k,maximo+k)+
  scale_y_continuous(breaks=c(25,50,75,100), labels=c("25%",
 "50%","75%","100%"),limits=c(10, 100))+
  xlab("Time (day)") +
  ylab(" E.coli Removal Percent") +
  scale_colour_manual(values =
 cols3,labels=c("E.coli_Predic_P1","E.coli_Predic_P2","E.coli_Predic_P3
 ","E.coli_Predic_P4")) +
  labs(colour="Port") +
  scale_linetype_manual(values = c("solid","solid","solid","solid")) +
  ggtitle(" E.coli Removal Model Aproximation")
multiplot(arrangeGrob(p1,p2, ncol=2,
 widths=c(4.6/10,5/10),main=textGrob(" E.coli Removal Percent throught
 IBSF in Time",gp=gpar(fontsize=16))))
```

B.2 For *Enterococcus population:*

```
############################################################
# library
############################################################
library(multcomp)
library(nlme)
library(mvtnorm)
library(survival)
```

```
library(TH.data)
library(reshape2)
library(ggplot2)
library(RColorBrewer)
library(scales)
library(gridExtra)
library(splines)
library(mgcv)

multiplot <- function(..., plotlist=NULL, file, cols=1, layout=NULL) {
  require(grid)

  # Make a list from the ... arguments and plotlist
  plots <- c(list(...), plotlist)

  numPlots = length(plots)

  # If layout is NULL, then use 'cols' to determine layout
  if (is.null(layout)) {
    # Make the panel
    # ncol: Number of columns of plots
    # nrow: Number of rows needed, calculated from # of cols
    layout <- matrix(seq(1, cols * ceiling(numPlots/cols)),
                     ncol = cols, nrow = ceiling(numPlots/cols))
  }

  if (numPlots==1) {
    print(plots[[1]])

  } else {
    # Set up the page
    grid.newpage()
    pushViewport(viewport(layout = grid.layout(nrow(layout),
 ncol(layout))))

    # Make each plot, in the correct location
    for (i in 1:numPlots) {
      # Get the i,j matrix positions of the regions that contain this
 subplot
      matchidx <- as.data.frame(which(layout == i, arr.ind = TRUE))

      print(plots[[i]], vp = viewport(layout.pos.row = matchidx$row,
                                      layout.pos.col = matchidx$col))
    }
  }
}

BaseDatosJRS_FINAL_COL<-
 read.csv("~/Desktop/datos/BaseDatosJRS_FINAL_COL.csv")
```

```
data_t=BaseDatosJRS_FINAL_COL[,c(2,7,11,34,15,38,19,42,23,46)]
data=BaseDatosJRS_FINAL_COL[,c(7,11,34,15,38,19,42,23,46)]

cols2 <-
 c("INEntero"="yellowgreen","Entero_P11"="turquoise","Entero_P12"="turq
 uoise", "Entero_P21"="darkred",
 "Entero_P22"="darkred","Entero_P31"="darkgray","Entero_P32"="darkgray"
 , "Entero_P41"= "navy", "Entero_P42"= "coral")
minimo=min(min(data))
maximo=max(max(data))
k=30
met <- melt(data_t, id = "Tiempo", measure =
 c("INEntero","Entero_P11","Entero_P12", "Entero_P21",
 "Entero_P22","Entero_P31","Entero_P32", "Entero_P41", "Entero_P42"))
p1<-qplot(Tiempo, value, data= met, colour = variable, size=I(0.6))+
  stat_smooth(method = "gam", formula = y ~ s(x, bs = "cs", k = 8)
 ,fill=NA, size =0.5)+
  geom_point(position=position_jitter(width=0.1))+
  ylim(minimo-k,maximo+k)+
  xlab("Time (day)") +
  ylab("Enterococcus population (CFU/100mL)") +
  scale_colour_manual(values =
 cols2,labels=c("INEntero","Entero_P11","Entero_P12", "Entero_P21",
 "Entero_P22","Entero_P31","Entero_P32", "Entero_P41", "Entero_P42")) +
  labs(colour="Port-Filter")+
  scale_linetype_manual(values =
 c("solid","solid","dashed","solid","dashed","solid","dashed","solid","
 dashed"))+
  ggtitle("Enterococcus population ")

data1=BaseDatosJRS_FINAL_COL[,c(11,34,15,38,19,42,23,46)]
cols3 <- c("Entero_P11"="turquoise","Entero_P12"="turquoise",
 "Entero_P21"="darkred",
 "Entero_P22"="darkred","Entero_P31"="darkgray","Entero_P32"="darkgray"
 , "Entero_P41"= "navy", "Entero_P42"= "coral")
minimo=min(data1)
maximo=max(data1)
k=30
met2 <- melt(data_t, id = "Tiempo", measure =
 c("Entero_P11","Entero_P12", "Entero_P21",
 "Entero_P22","Entero_P31","Entero_P32", "Entero_P41", "Entero_P42"))
p2<-qplot(Tiempo, value, data= met2, colour = variable, size=I(0.6))+
  stat_smooth(method = "gam", formula = y ~ s(x, bs = "cs", k = 8)
 ,fill=NA,size =0.5)+
  geom_point(position=position_jitter(width=0.1))+
  ylim(minimo-k,maximo+k)+
  xlab("Tme (day)") +
  ylab("Enterococcus population (CFU/100mL)") +
```

```
  #   theme(legend.key.size = unit(0, "cm"))+
  scale_colour_manual(values = cols3,labels=c("Entero
P11","Entero_P12", "Entero_P21",
"Entero_P22","Entero_P31","Entero_P32", "Entero_P41", "Entero_P42")) +
  labs(colour="Port-Filter")+
  scale_linetype_manual(values =
c("solid","dashed","solid","dashed","solid","dashed","solid","dashed")
)+
  ggtitle("Enterococcus population Sampling Ports")

multiplot(arrangeGrob(p1,p2, ncol=2,
 widths=c(5/10,5/10),main=textGrob("Enterococcus population through
 IBSF in Time",gp=gpar(fontsize=16))))

BaseDatosJRS_mean_filtros<-
 read.csv("~/Desktop/datos/BaseDatosJRS_mean_filtros.csv")

minimo=min(BaseDatosJRS_mean_filtros[,c(3,5,7,9,11)])
maximo=max(BaseDatosJRS_mean_filtros[,c(3,5,7,9,11)])
cols1 <- c("INEntero"="yellowgreen","Entero_P1"="turquoise",
 "Entero_P2"="darkred","Entero_P3"="darkgray", "Entero_P4"= "coral")
k=10
met <- melt(BaseDatosJRS_mean_filtros, id = "Tiempo", measure =
 c("INEntero","Entero_P1", "Entero_P2","Entero_P3", "Entero_P4"))
p1<-qplot(Tiempo, value, data= met, colour = variable, size=I(0.6))+
  stat_smooth(method = "gam", formula = y ~ s(x, bs = "cs", k = 8)
 ,fill=NA, size =0.5)+
  geom_point(position=position_jitter(width=0.1))+
  ylim(minimo-k,maximo+k)+
  xlab("Time (day)") +
  ylab("Enterococcus population (CFU/100mL)") +
  scale_colour_manual(values = cols1,labels=c("INEntero","Entero_P1",
 "Entero_P2","Entero_P3", "Entero_P4")) +
  labs(colour="Sampling Port")+
  scale_linetype_manual(values =
 c("solid","solid","solid","solid","solid"))+
  ggtitle("Enterococcus population ")

cols2 <- c("Entero_P1"="turquoise",
 "Entero_P2"="darkred","Entero_P3"="darkgray", "Entero_P4"= "coral")
minimo1=min(BaseDatosJRS_mean_filtros[,c(5,7,9,11)])
maximo1=max(BaseDatosJRS_mean_filtros[,c(5,7,9,11)])
k=10
met <- melt(BaseDatosJRS_mean_filtros, id = "Tiempo", measure =
 c("Entero_P1", "Entero_P2","Entero_P3", "Entero_P4"))
p2<-qplot(Tiempo, value, data= met, colour = variable, size=I(0.6))+
  stat_smooth(method = "gam", formula = y ~ s(x, bs = "cs", k = 8)
 ,fill=NA,size =0.5)+
  #   geom_smooth(fill=NA,size =0.5) +
```

```
  geom_point(position=position_jitter(width=0.1))+
  ylim(minimo1-k,maximo1+k)+
  xlab("Time (day)") +
  ylab("Enterococcus population (CFU/100mL)") +
  scale_colour_manual(values = cols2,labels=c("Entero_P1",
 "Entero_P2","Entero_P3", "Entero_P4")) +
  labs(colour="Port")+
  scale_linetype_manual(values = c("solid","solid","solid","solid"))+
  ggtitle("Enterococcus population Sampling Ports")

multiplot(arrangeGrob(p1,p2, ncol=2,
 widths=c(5/10,5/10),main=textGrob("Enterococcus population through
 IBSF in Time (FA and FB Averaged)",gp=gpar(fontsize=16))))

BaseDatosJRS_FINAL_COL<-
 read.csv("~/Desktop/datos/BaseDatosJRS_FINAL_COL.csv")
data1=BaseDatosJRS_FINAL_COL$INEntero-
 BaseDatosJRS_FINAL_COL[,c(7,11,34,15,38,19,42,23,46)]
data2=data1[,c(-1)]
data3=100*data2/BaseDatosJRS_FINAL_COL$INEntero
Tiempo=BaseDatosJRS_FINAL_COL$Tiempo
data4  = data.frame (cbind(Tiempo,data3))

cols1 <- c("Entero_P11"="turquoise","Entero_P12"="turquoise",
 "Entero_P21"="darkred",
 "Entero_P22"="darkred","Entero_P31"="darkgray","Entero_P32"="darkgray"
 , "Entero_P41"= "navy", "Entero_P42"= "coral")
minimo1=min(data4[,c(2,3,4,5,6,7,8,9)])
maximo1=max(data4[,c(2,3,4,5,6,7,8,9)])
# k=0.1
met1 <- melt(data4, id = "Tiempo", measure =
 c("Entero_P11","Entero_P12", "Entero_P21",
 "Entero_P22","Entero_P31","Entero_P32", "Entero_P41", "Entero_P42"))
p1<-qplot(Tiempo, value, data= met1, colour = variable, size=I(0.6))+
  #   geom_smooth(fill=NA,size =0.5) +
  stat_smooth(method = "gam", formula = y ~ s(x, bs = "cs", k = 8)
 ,fill=NA,size =0.5)+
  geom_point(position=position_jitter(width=0.1))+
  xlab("Time (day)") +
  ylab("Enterococcus Removal Percents") +
  scale_y_continuous(breaks=c(25,50,75,100), labels=c("25%",
 "50%","75%","100%"),limits=c(10, 100))+
  scale_colour_manual(values =
 cols1,labels=c("Entero_P11","Entero_P12", "Entero_P21",
 "Entero_P22","Entero_P31","Entero_P32", "Entero_P41", "Entero_P42")) +
  labs(colour="Port-Filter")+
  scale_linetype_manual(values =
 c("solid","dashed","solid","dashed","solid","dashed","solid","dashed")
 )+
```

```
  ggtitle("Enterococcus Removal Percents")

BaseDatosJRS_mean_filtros<-
 read.csv("~/Desktop/datos/BaseDatosJRS_mean_filtros.csv")
data1=BaseDatosJRS_mean_filtros$INEntero-
 BaseDatosJRS_mean_filtros[,c(1,5,7,9,11)]
data2=data1[,c(-1)]
data3=100*data2/BaseDatosJRS_mean_filtros$INEntero
Tiempo=BaseDatosJRS_mean_filtros$Tiempo
data5= data.frame (cbind(Tiempo,data3))

cols2 <- c("Entero_P1"="turquoise", "Entero_P2"="darkred",
 "Entero_P3"="darkgrey", "Entero_P4"= "coral")
minimo=min(data5[,c(2,3,4,5)])
maximo=max(data5[,c(2,3,4,5)])
# k=0.1
met <- melt(data5, id = "Tiempo", measure = c("Entero_P1",
 "Entero_P2", "Entero_P3", "Entero_P4"))
p2<-qplot(Tiempo, value, data= met, colour = variable, size=I(0.6)) +
  stat_smooth(method = "gam", formula = y ~ s(x, bs = "cs", k = 8)
 ,fill=NA,size =0.5)+
  geom_point(position=position_jitter(width=0.1)) +
  xlab("Time (day)") +
  ylab("Enterococcus Removal Percents") +
  scale_y_continuous(breaks=c(25,50,75,100), labels=c("25%",
 "50%","75%","100%"),limits=c(10, 100))+
  scale_colour_manual(values = cols2,labels=c("Entero_P1",
 "Entero_P2", "Entero_P3", "Entero_P4")) +
  labs(colour="Port") +
  scale_linetype_manual(values = c("solid","solid","solid","solid")) +
  ggtitle("Enterococcus Removal Percents Sampling Ports")

multiplot(arrangeGrob(p1,p2, ncol=2,
 widths=c(5/10,5/10),main=textGrob("",gp=gpar(fontsize=16))))

BaseDatosJRS_ANC <- read.csv("~/Desktop/datos/BaseDatosJRS_ANC.csv")

y =(BaseDatosJRS_ANC$INEntero -
 BaseDatosJRS_ANC$Entero)/BaseDatosJRS_ANC$INEntero
y1=log(y/(1-y))

Filtro.lme <- lme(y1 ~ factor(Filtro) +  factor(Puerto)*bs(Tiempo,
 df=7, degree=3) + log(INOxigeno) , random = ~ 1|Filtro_Puerto,
 data=BaseDatosJRS_ANC)
summary(Filtro.lme)

Filtro.anova.lme <- aov(y1 ~ factor(Filtro) +
 factor(Puerto)*bs(Tiempo,  df=7, degree=3) + log(INOxigeno) , random =
 ~ 1|Filtro_Puerto, data=BaseDatosJRS_ANC)
```

```
summary(Filtro.anova.lme)

ks.test(residuals(Filtro.lme), "pnorm", mean = 0, sd = 1)
shapiro.test(residuals(Filtro.lme))

-2*logLik(Filtro.lme)
AIC(Filtro.lme)
BIC(Filtro.lme)

Filtro.lme1 <- lm(y1 ~ factor(Filtro) +  factor(Puerto)*bs(Tiempo,
 df=7, degree=3) + log(INOxigeno) , data=BaseDatosJRS_ANC)

p1 <-ggplot(Filtro.lme1, aes(sample = .stdresid)) + stat_qq(colour =
 "navy",size=1.5) +
  geom_abline(colour = "darkred") +
  labs(title="Normal QQ-plot", x="Theoretical quantiles", y="Std
 residual")

p2<- qplot(.fitted, .stdresid, data = fortify(Filtro.lme1,
 BaseDatosJRS_ANC),size=I(0.1),
           xlab="Fitted",ylab="Std-residual", colour = factor(Puerto))
 +
  geom_point(position=position_jitter(width=0.3)) +
  scale_colour_hue("Port",drop=FALSE)+
  labs(title="Residual of error") +
  geom_hline(yintercept =0)

multiplot(arrangeGrob(p1,p2, ncol=2,
 widths=c(4/10,5/10),main=textGrob("Normality y Homocedasticity for
 log[y/(1-y)] of Enterococcus",gp=gpar(fontsize=16))))

qqnorm(Filtro.lme, ~ resid(., type = "p") | Filtro_Puerto, abline =
 c(0, 1), xlab="Theoretical quantiles", ylab="Std
 residual",main="Residual of error")
qqnorm(Filtro.lme, ~ resid(., type = "p") | Filtro, abline = c(0, 1),
 xlab="Theoretical quantiles", ylab="Std residual",main="Residual of
 error")
qqnorm(Filtro.lme, ~ resid(., type = "p") | Puerto, abline = c(0, 1),
 xlab="Theoretical quantiles", ylab="Std residual",main="Residual of
 error")
plot(Filtro.lme)

plot(Filtro.lme, resid(., type = "p") ~fitted(.) |Filtro_Puerto,
 abline = 0)
plot(Filtro.lme, resid(., type = "p") ~fitted(.) |Filtro, abline = 0)
plot(Filtro.lme, resid(., type = "p") ~fitted(.) |Puerto, abline = 0)
plot(Filtro.lme, Filtro_Puerto ~resid(., type="p"))

BaseDatosJRS_ANC <- read.csv("~/Desktop/datos/BaseDatosJRS_ANC.csv")
```

```
BaseDatosJRS_ANC$Puerto <- factor(BaseDatosJRS_ANC$Puerto)
BaseDatosJRS_ANC$Filtro <- factor(BaseDatosJRS_ANC$Filtro)
BaseDatosJRS_ANC$Filtro_Puerto <-
 factor(BaseDatosJRS_ANC$Filtro_Puerto)
y = (BaseDatosJRS_ANC$INEntero -
 BaseDatosJRS_ANC$Entero)/BaseDatosJRS_ANC$INEntero
y1=log(y/(1-y))

Entero_P1=as.vector(y1[c(1:48,193:240)])
Entero_P2=as.vector(y1[c(49:96,241:288)])
Entero_P3=as.vector(y1[c(97:144,289:336)])
Entero_P4=as.vector(y1[c(145:192,337:384)])
data=
 data.frame(cbind(Tiempo=as.vector(BaseDatosJRS_ANC[1:96,2]),Replica=as
 .vector(BaseDatosJRS_ANC[1:96,3]),Entero_P1,Entero_P2,Entero_P3,Entero
 _P4))

Filtro.lme <- lme(y1 ~ factor(Filtro) +  factor(Puerto)*bs(Tiempo,
 df=7, degree=3) + log(INOxigeno) , random = ~ 1|Filtro_Puerto,
 data=BaseDatosJRS_ANC)
Fit.lme.predic=predict(Filtro.lme)
Entero_Predic_P1=as.vector(Fit.lme.predic[c(1:48,193:240)])
Entero_Predic_P2=as.vector(Fit.lme.predic[c(49:96,241:288)])
Entero_Predic_P3=as.vector(Fit.lme.predic[c(97:144,289:336)])
Entero_Predic_P4=as.vector(Fit.lme.predic[c(145:192,337:384)])
data.fit=
 data.frame(cbind(data,Entero_Predic_P1,Entero_Predic_P2,Entero_Predic_
 P3,Entero_Predic_P4))

cols2 <- c("Entero_P1"="turquoise", "Entero_P2"="darkred",
 "Entero_P3"="darkgray", "Entero_P4"= "coral")
minimo=min(data.fit[,c(1,3,4,5,6)])
maximo=max(data.fit[,c(1,3,4,5,6)])
met <- melt(data.fit, id = "Tiempo", measure = c("Entero_P1",
 "Entero_P2", "Entero_P3", "Entero_P4"))
p1<-qplot(Tiempo, value, data= met, colour = variable, size=I(0.6)) +
  stat_smooth(method = "gam", formula = y ~ s(x, bs = "cs", k = 8)
 ,fill=NA,size =0.5)+
  geom_point(position=position_jitter(width=0.1)) +
  ylim(-2,6)+
  xlab("Time (day)") +
  ylab("log[y/(1-y)], where y=(INEntero-Entero)/INEntero") +
  scale_colour_manual(values = cols2,labels=c("Entero_P1",
 "Entero_P2", "Entero_P3", "Entero_P4")) +
  labs(colour="Port") +
  scale_linetype_manual(values = c("solid","solid","solid","solid")) +
  ggtitle("Enterococcus population ")

cols3 <-
```

```
 c("Entero_Predic_P1"="turquoise","Entero_Predic_P2"="darkred","Entero_
 Predic_P3"="darkgray","Entero_Predic_P4"="coral")
minimo1=min(data.fit[,c(1,7,8,9,10)])
maximo1=max(data.fit[,c(1,7,8,9,10)])
met <- melt(data.fit, id = "Tiempo", measure =
 c("Entero_Predic_P1","Entero_Predic_P2","Entero_Predic_P3","Entero_Pre
 dic_P4"))
p2<-qplot(Tiempo, value, data= met, colour = variable, size=I(0.6)) +
  stat_smooth(method = "gam", formula = y ~ s(x, bs = "cs", k = 8)
 ,fill=NA,size =0.5)+
  geom_point(position=position_jitter(width=0.1)) +
  ylim(-2,6)+
  xlab("Time (day)") +
  ylab("Enterococcus population ") +
  scale_colour_manual(values =
 cols3,labels=c("Entero_Predic_P1","Entero_Predic_P2","Entero_Predic_P3
 ","Entero_Predic_P4")) +
  labs(colour="Port") +
  scale_linetype_manual(values = c("solid","solid","solid","solid")) +
  ggtitle("Enterococcus population Model Aproximations")

multiplot(arrangeGrob(p1,p2, ncol=2,
 widths=c(4.6/10,5/10),main=textGrob("Enterococcus population through
 IBSF in Time where y=(INEntero-
 Entero)/INEntero",gp=gpar(fontsize=16))))

BaseDatosJRS_ANC <- read.csv("~/Desktop/datos/BaseDatosJRS_ANC.csv")

Entero_P1=as.vector(BaseDatosJRS_ANC$Entero[c(1:48,193:240)])
Entero_P2=as.vector(BaseDatosJRS_ANC$Entero[c(49:96,241:288)])
Entero_P3=as.vector(BaseDatosJRS_ANC$Entero[c(97:144,289:336)])
Entero_P4=as.vector(BaseDatosJRS_ANC$Entero[c(145:192,337:384)])

INEntero=as.vector(BaseDatosJRS_ANC$INEntero[c(1:96)])

data=
 data.frame(cbind(Tiempo=as.vector(BaseDatosJRS_ANC[1:96,2]),Entero_P1,
 Entero_P2,Entero_P3,Entero_P4))
Filtro.lme <- lme(y1 ~ factor(Filtro) +  factor(Puerto)*bs(Tiempo,
 df=7, degree=3) + log(INOxigeno), random = ~ 1|Filtro_Puerto,
 data=BaseDatosJRS_ANC)

Fit.lme.predic=predict(Filtro.lme)

yP1=exp(as.vector(Fit.lme.predic[c(1:48,193:240)]))/(1+exp(as.vector(F
 it.lme.predic[c(1:48,193:240)])))
yP2=exp(as.vector(Fit.lme.predic[c(49:96,241:288)]))/(1+exp(as.vector(
 Fit.lme.predic[c(49:96,241:288)])))
yP3=exp(as.vector(Fit.lme.predic[c(97:144,289:336)]))/(1+exp(as.vector
```

```
 (Fit.lme.predic[c(97:144,289:336)])))
yP4=exp(as.vector(Fit.lme.predic[c(145:192,337:384)]))/(1+exp(as.vecto
 r(Fit.lme.predic[c(145:192,337:384)])))

Entero_Predic_P1=(BaseDatosJRS_ANC$INEntero-
 BaseDatosJRS_ANC$INEntero*yP1)
Entero_Predic_P2=(BaseDatosJRS_ANC$INEntero-
 BaseDatosJRS_ANC$INEntero*yP2)
Entero_Predic_P3=(BaseDatosJRS_ANC$INEntero-
 BaseDatosJRS_ANC$INEntero*yP3)
Entero_Predic_P4=(BaseDatosJRS_ANC$INEntero-
 BaseDatosJRS_ANC$INEntero*yP4)

data.fit.lme=
 data.frame(cbind(data,INEntero,Entero_Predic_P1,Entero_Predic_P2,Enter
 o_Predic_P3,Entero_Predic_P4))

cols1 <- c("INEntero"="yellowgreen","Entero_P1"="turquoise",
 "Entero_P2"="darkred", "Entero_P3"="darkgray", "Entero_P4"= "coral")
minimo=min(data.fit.lme[,c(2,3,4,5,6,7,8,9,10)])
maximo=max(data.fit.lme[,c(2,3,4,5,6,7,8,9,10)])

k=30
met1 <- melt(data.fit.lme, id = "Tiempo", measure =
 c("INEntero","Entero_P1", "Entero_P2", "Entero_P3", "Entero_P4"))
p1<-qplot(Tiempo, value, data= met1, colour = variable, size=I(0.6)) +
  stat_smooth(method = "gam", formula = y ~ s(x, bs = "cs", k = 8)
 ,fill=NA,size =0.5)+
  geom_point(position=position_jitter(width=0.1)) +
  ylim(minimo-k,maximo+k)+
  xlab("Time (day)") +
  ylab("Enterococcus population (CFU/100mL)") +
  scale_colour_manual(values = cols1,labels=c("INEntero","Entero_P1",
 "Entero_P2", "Entero_P3", "Entero_P4")) +
  labs(colour="Port") +
  scale_linetype_manual(values =
 c("solid","solid","solid","solid","solid")) +
  ggtitle("Enterococcus population ")

cols2 <-
 c("INEntero"="yellowgreen","Entero_Predic_P1"="turquoise","Entero_Pred
 ic_P2"="darkred","Entero_Predic_P3"="darkgray","Entero_Predic_P4"="cor
 al")
met2 <- melt(data.fit.lme, id = "Tiempo", measure =
 c("INEntero","Entero_Predic_P1","Entero_Predic_P2","Entero_Predic_P3",
 "Entero_Predic_P4"))
p2<-qplot(Tiempo, value, data= met2, colour = variable, size=I(0.6)) +
  stat_smooth(method = "gam", formula = y ~ s(x, bs = "cs", k = 8)
 ,fill=NA,size =0.5)+
```

```
  geom_point(position=position_jitter(width=0.1)) +
  ylim(minimo-k,maximo+k)+
  xlab("Time (day)") +
  ylab("Enterococcus population (CFU/100mL)") +
  scale_colour_manual(values =
 cols2,labels=c("INEntero","Entero_Predic_P1","Entero_Predic_P2","Enter
 o_Predic_P3","Entero_Predic_P4")) +
  labs(colour="Port") +
  scale_linetype_manual(values =
 c("solid","solid","solid","solid","solid")) +
  ggtitle("Enterococcus population Model Aproximation")

multiplot(arrangeGrob(p1,p2, ncol=2,
 widths=c(4.6/10,5/10),main=textGrob("Enterococcus population through
 IBSF in Time",gp=gpar(fontsize=16))))

cols3 <- c("Entero_P1"="turquoise", "Entero_P2"="darkred",
 "Entero_P3"="darkgray", "Entero_P4"= "coral")
minimo=min(data.fit.lme[,c(2,3,4,5,7,8,9,10)])
maximo=max(data.fit.lme[,c(2,3,4,5,7,8,9,10)])
met3 <- melt(data.fit.lme, id = "Tiempo", measure = c("Entero_P1",
 "Entero_P2", "Entero_P3", "Entero_P4"))
p3<-qplot(Tiempo, value, data= met3, colour = variable, size=I(0.6)) +
  stat_smooth(method = "gam", formula = y ~ s(x, bs = "cs", k = 8)
 ,fill=NA,size =0.5)+
  geom_point(position=position_jitter(width=0.1)) +
  ylim(minimo-k,maximo+k)+
  xlab("Time (day)") +
  ylab("Enterococcus population (CFU/100mL)") +
  scale_colour_manual(values = cols3,labels=c("Entero_P1",
 "Entero_P2", "Entero_P3", "Entero_P4")) +
  labs(colour="Port") +
  scale_linetype_manual(values = c("solid","solid","solid","solid")) +
  ggtitle("Enterococcus population Sampling Ports")

cols4 <-
 c("Entero_Predic_P1"="turquoise","Entero_Predic_P2"="darkred","Entero_
 Predic_P3"="darkgray","Entero_Predic_P4"="coral")
met4 <- melt(data.fit.lme, id = "Tiempo", measure =
 c("Entero_Predic_P1","Entero_Predic_P2","Entero_Predic_P3","Entero_Pre
 dic_P4"))
p4<-qplot(Tiempo, value, data= met4, colour = variable, size=I(0.6)) +
  stat_smooth(method = "gam", formula = y ~ s(x, bs = "cs", k = 8)
 ,fill=NA,size =0.5)+
  geom_point(position=position_jitter(width=0.1)) +
  ylim(minimo-k,maximo+k)+
  xlab("Time (day)") +
  ylab("Enterococcus population (CFU/100mL)") +
  scale_colour_manual(values =
```

```
 cols4,labels=c("Entero_Predic_P1","Entero_Predic_P2","Entero_Predic_P3
 ","Entero_Predic_P4")) +
   labs(colour="Port") +
   scale_linetype_manual(values = c("solid","solid","solid","solid")) +
   ggtitle("Enterococcus population Model Aproximation")

multiplot(arrangeGrob(p3,p4, ncol=2,
 widths=c(4.6/10,5/10),main=textGrob("Enterococcus population through
 IBSF in Time Sampling Ports",gp=gpar(fontsize=16))))

multiplot(arrangeGrob(p1,p3,p4, ncol=3,
 widths=c(4/12,4/12,4.3/12),main=textGrob("Enterococcus population
 through IBSF in Time Sampling Ports",gp=gpar(fontsize=16))))

data1 =100*((data.fit.lme$INEntero -
 data.fit.lme[c(2,3,4,5,7,8,9,10)])/data.fit.lme$INEntero)
data2 =cbind(Tiempo=data.fit.lme$Tiempo,data1)

cols2 <- c("Entero_P1"="turquoise", "Entero_P2"="darkred",
 "Entero_P3"="darkgrey", "Entero_P4"= "coral")
minimo=min(data2[,c(2,3,4,5,7,8,9)])
maximo=max(data2[,c(2,3,4,5,7,8,9)])

met <- melt(data2, id = "Tiempo", measure = c("Entero_P1",
 "Entero_P2", "Entero_P3", "Entero_P4"))
p1<-qplot(Tiempo, value, data= met, colour = variable, size=I(0.6)) +
   stat_smooth(method = "gam", formula = y ~ s(x, bs = "cs", k = 8)
 ,fill=NA,size =0.5)+
   geom_point(position=position_jitter(width=0.1)) +
   scale_y_continuous(breaks=c(25,50,75,100), labels=c("25%",
 "50%","75%","100%"),limits=c(10, 100))+
   #   ylim(minimo-k,maximo+k)+
   xlab("Time (day)") +
   ylab("Enterococcus Removal Percent") +
   scale_colour_manual(values = cols2,labels=c("Entero_P1",
 "Entero_P2", "Entero_P3", "Entero_P4")) +
   labs(colour="Port") +
   scale_linetype_manual(values = c("solid","solid","solid","solid")) +
   ggtitle("Enterococcus Removal Percent")

cols3 <-
 c("Entero_Predic_P1"="turquoise","Entero_Predic_P2"="darkred","Entero_
 Predic_P3"="darkgray","Entero_Predic_P4"="coral")
met <- melt(data2, id = "Tiempo", measure =
 c("Entero_Predic_P1","Entero_Predic_P2","Entero_Predic_P3","Entero_Pre
 dic_P4"))
p2<-qplot(Tiempo, value, data= met, colour = variable, size=I(0.6)) +
   stat_smooth(method = "gam", formula = y ~ s(x, bs = "cs", k = 8)
 ,fill=NA,size =0.5)+
```

```
  geom_point(position=position_jitter(width=0.1)) +
  #   ylim(minimo-k,maximo+k)+
  scale_y_continuous(breaks=c(25,50,75,100), labels=c("25%",
 "50%","75%","100%"),limits=c(10, 100))+
  xlab("Time (day)") +
  ylab("Enterococcus Removal Percent") +
  scale_colour_manual(values =
 cols3,labels=c("Entero_Predic_P1","Entero_Predic_P2","Entero_Predic_P3
 ","Entero_Predic_P4")) +
  labs(colour="Port") +
  scale_linetype_manual(values = c("solid","solid","solid","solid")) +
  ggtitle("Enterococcus Removal Percent Model Aproximation")

multiplot(arrangeGrob(p1,p2, ncol=2,
 widths=c(4.6/10,5/10),main=textGrob("Enterococcus Removal Percent
 through IBSF in Time",gp=gpar(fontsize=16))))
```