Greywater treatment for reuse by slow sand filtration : study of pathogenic microorganisms and phage survival

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Greywater treatment for reuse by slow sand filtration: study of pathogenic microorganisms and phage survival

Traitement des eaux grises par filtration lente pour leur réutilisation : étude de la survie des micro-organismes pathogènes et des bactériophages

JURY

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DEDICATION

To my father, Shipat elhamd Bahig KHALAPHALLAH. Rest in peace

To my mother, Rawhia kamel Ahmed. Allah Save for us

To my brothers and sisters

To my friends

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Publications

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Résumé et mots-clés

Dans les dernières décennies, la plupart des pays du monde ont connu une pénurie d'eau et l'augmentation du taux de consommation. Aujourd'hui, tous les pays dans le monde essayent de trouver des alternatives pour remédier à cette pénurie. Une solution consiste en la réutilisation des eaux grises (GW) pour l'irrigation après traitement. Les GW correspondent aux eaux usées générée dans une maison à l'exception de l'eau des toilettes. Les risques associés à la réutilisation de ces eaux est la présence de microorganismes pathogènes qui peuvent infecter les humains, les animaux et les plantes. Dans cette thèse centrée sur l'étude de la survie des représentants d'agents pathogènes, comme *E. coli, P. aeruginosa*, et le bactériophage MS2 qui sont trouvés dans les eaux grises. Il a été étudié l'effet de quelques facteurs physico-chimiques tels que; température (6 ± 2,23 ± 2 et 42 ± 2 ° C), la salinité (1,75 and 3.5% de NaCl), de l'oxygène (aérobie et anaérobie), des éléments nutritifs (milieu riche et de milieux pauvres), la lumière avec la photocatalyse (lampes UV et visible) et filtre à sable lent (sable du désert égyptien et le sable piscine). Une combinaison de la température, la lumière du soleil et de haute photocatlysis sont principalement responsables de la baisse rapide des bactéries et du coliphage MS2. Le filtre à sable lent a une influence nettement moindre sur la survie des bactéries dans les eaux grises, mais il est efficace pour diminuer la turbidité et de la DCO.

Mots-clés : Filtration lente, filtre à sable, eaux grises, réutilisation, survie microbienne (*E. coli, P. aeruginosa*, coliphage MS2), facteurs abiotiques, photocatalyse, UV.

Résumé et mots-clés en anglais

In recent decades, most countries of the world have experienced a shortage of water and increase its rate of consumption. Today, every country in the world are interested in this problem by trying to find alternatives to address this shortage. One solution is reuse greywater (GW) for irrigation after treatment. GW is all water generated from Houshold except toilet water. The risks associated with the reuse of these waters are the presence of pathogens that can infect humans, animals and plants. In this thesis focused on studying treatment by slow sand filtration and the survival of representatives of pathogens, such as *E. Coli, P. aeruginosa , E. Faecalis* and Bacteriophage MS2 which could be found in the greywater. The study factors was a physico-chemicals factors such as; temperature (6±2,23±2,42±2°c), salinity (1.75 and 3.5% Nacl), oxygen (aerobic and anaerobic condition), nutrient (rich media , 50%: 50% salt and poor media), light with photocatalysis (UV and Visible lights) and slow sand filter (egyptian desert sand and swimming pool sand). A combination of high temperature, sunlight and photocatlysis are mainly responsible for the rapid decline of bacteria and MS2 coliphage. Slow sand filter have clearly less influence on the survival of bacteria in the greywater, but it effective to decline turbidity and COD for short times.

Key words: Slow sand filtration, Greywater, Reuse, Abiotic factors, Microbial survival (*E;coli, P. aeruginosa*, coliphage MS2), Photocatalyse, UV.

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ABBREVIATIONS AND NOTATION

Abbreviations

AOPs	Advanced Oxidation Processes
BOD	Biological Oxygen Demand
COD	Chemical Oxygen Demand
CFU	Colony Forming Unit
DW	Dionized Water
DAL	Double Agar layer
EC	*Esechrechia Coli*
EDS	Egyptian Desert Sand
FNU	Formazin Nephelometric Units
FAO	Food and Agriculture Organization
GW	Greywater
HLR	Hydraulic Loading Rate
HRT	Hydraulic Retention Time
NB	Nutrient Broth Media
MMG	Mimum Mineral with Glucose Media
MS2 Phage	Phage attack *E.coli* Hfr
NA	Nutrient Agar
OD	Optical Density
PSD	Particle Size Ditibution
PA	*Pseudomonase Aeruginosa*
PFU	Plaque Forming Unit
RTD	Residence Time Distribution
SEM	Scanning Electron Microscope
SPS	Swiming Pool Sand
SSF	Slow Sand Filter
TTC7	Triphenyl Tetrazolium Chloride Media
TYG	Trypron Yest-extract Glucose Media
TOC	Total Organic Carbon
TSS	Total Suspended Solids
Tot.P	Total Phosphorous
Tot.N	Total Nitrogen
WW	Wastewater
WHO	World Health Organization
UV	Ultra Violet
Vis	Visble Light

Notation

$\mathbf{d_{90}}$	Particle diameter below which lies a percentage (90 %)
dNt/dt	the rate of change in the number of coliforms with time
D	Diameter
E	Energy
$\mathbf{F_N}$	The frequency number
h	Planck's constant
k	inactivation rate constant
Nt	the number of coliforms at time
rpm	revolutions per minute
t	time.
τ	tortousity
$\mathbf{T_{90}}$	the time requirment to decay 90%
v	frequency
U	Velocity

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CHAPTER ONE

General introduction and outline

1.1 Background

Water scarcity is one of the most significant challenges to human health and environmental integrity in most parts of the world. As the world's population grows and prosperity spreads, water demands increase and multiply without the possibility for an increase in supply. The mounting demand on this finite and invaluable resource has inspired creative strategies for freshwater management, including innovative techniques for wastewater recycling. Greywater reuse is one such strategy, and its usefulness to fulfil non-potable water needs should be thoroughly investigated.

In General, water mismanaged by overconsumption and pollution is factors from water crisis, over 70% of freshwater consumption is devoted to agricultural activities (FAO 2008). Recently, declining productivity of commercial farms has led international policy networks to recommend the promotion of urban and peri-urban agriculture as an escape from food crisis situations (FAO 1999). Greywater treatment and reuse at the individual level can provide a combined solution to these problems by supplying the water and nutrients needed for household food production. Greywater treatment and reuse for irrigation may well hold the key to easing demand on limited freshwater reserves while improving the food production capacity of households and farms.

However, there are significant concerns about the safety of greywater reuse for irrigation purposes. The key issue involved is the potential for damaging effects of poor quality water on soil, plants and humans. Water quality requirements for agricultural irrigation are a subject of much interest to researchers in theory; agricultural water need not be of potable quality, opening the door to greywater and surface water irrigation (WHO 2006).

However, the microbial population of untreated water is very diverse, and dangerous organisms can be present. Microorganisms that can cause illness or disease, collectively known as pathogens, are usually associated with human or animal faecal which reach to greywater by washing hand, clothes or vegetables and surface water sources. Greywater contains a large number of pathogenic bacteria (Rose *et al.*, 1991; Birks *et al.*, 2004; Jefferson *et al.*, 2004) that reach them through the leak from the bathroom or washing vegetables or washing hands and contaminated clothing. Irrigation water contaminated with pathogens has often been blamed for outbreaks of foodborne illness. It is important to carefully manage this

risk when promoting the reuse of non-potable water sources to fulfill the water demand of agricultural irrigation activities.

In most country of the world, greywater reuse is not yet a common practice because the water use regulation are severe and the treatment processes did not reached to them. The greywater production rates for each country are different according to household activities and the personal habits. Today there are signs that some of countries' historically privileged access to fresh water may be changing. As fresh water levels in this countries drop and pollution increases, prices are rising and efficient use of water is becoming more important to businesses, farmers and homeowners. As a result, interest in greywater reuse technologies is growing rapidly.

1.2. Need for research

"Greywater", which refers to used water flowing from sources such as showers, washing machines, and bathroom sinks, often represents over 2/3 (60-70%) of household wastewater but is considered to be only weakly contaminated by pathogenic organisms and other potentially dangerous substances (WHO 2006 ; Friedler 2004 ; Gulyas et al., 2004). In the context of a densely populated like as a most of cities, full greywater treatment and reuse is rarely feasible at the household level due to the high space requirement and technological of greywater treatment. Source separation of household wastewater into streams of grey and black waters is a strategy that has the potential to reduce the space and investment required to achieve water reuse at the domestic level.

Domestic greywater reuse schemes allow the two factors mentioned above (water wastage and high irrigation demand) to be twinned, putting excess wastewater to new use in the irrigation. There is also an economic benefit to this activity, as it provides significant savings in water use and sewage disposal. The health and environmental hazards of this form of water reuse are not enough clear and limited studies. The use of greywater for watering vegetables and other edible plants is the subject of some concern. While greywater may appear benign, it can contain hazardous elements such as heavy metals, pathogenic microorganisms, and toxic chemicals that could pollute garden soils and contaminate edible crops (Eriksson et al. 2002).

This study is focus on greywater treatment by slow sand filter technology. Moreover various factors from the environment, such as abiotic factors, or the use of solar light irradiation with catalyst have been studied. On this basis, the work has to overcome the problem of pathogenic microbes in the water. In order to promote water recycling practices as part of the effort to ease water demand in our growing cities (Egypt), research is needed to fully understand the benefits and risks of greywater irrigation.

1.2.1. Objectives and scope

This thesis aims to propose a way of treatment by a rustic and robust treatment process bathroom greywater and to understand the survival and the disinfection of pathogen microorganisms (including phage). The global objective is to provide safe, pathogen-free water for greywater reuse. Accordingly, the following objectives were identified (Figure 1.1):

1. To assess current knowledge of the pathogens present in greywater and the suitability of disinfection technologies for their removal.
2. To determine the microbial quality and presence of specific pathogens in bathroom greywater
3. To evaluate the microorganism removal performance of leading contender greywater treatment technologies i.e sand filtration and photocatalyse
4. To assess the efficacy of established and novel disinfectants for the disinfection of grey water under environmental factors.

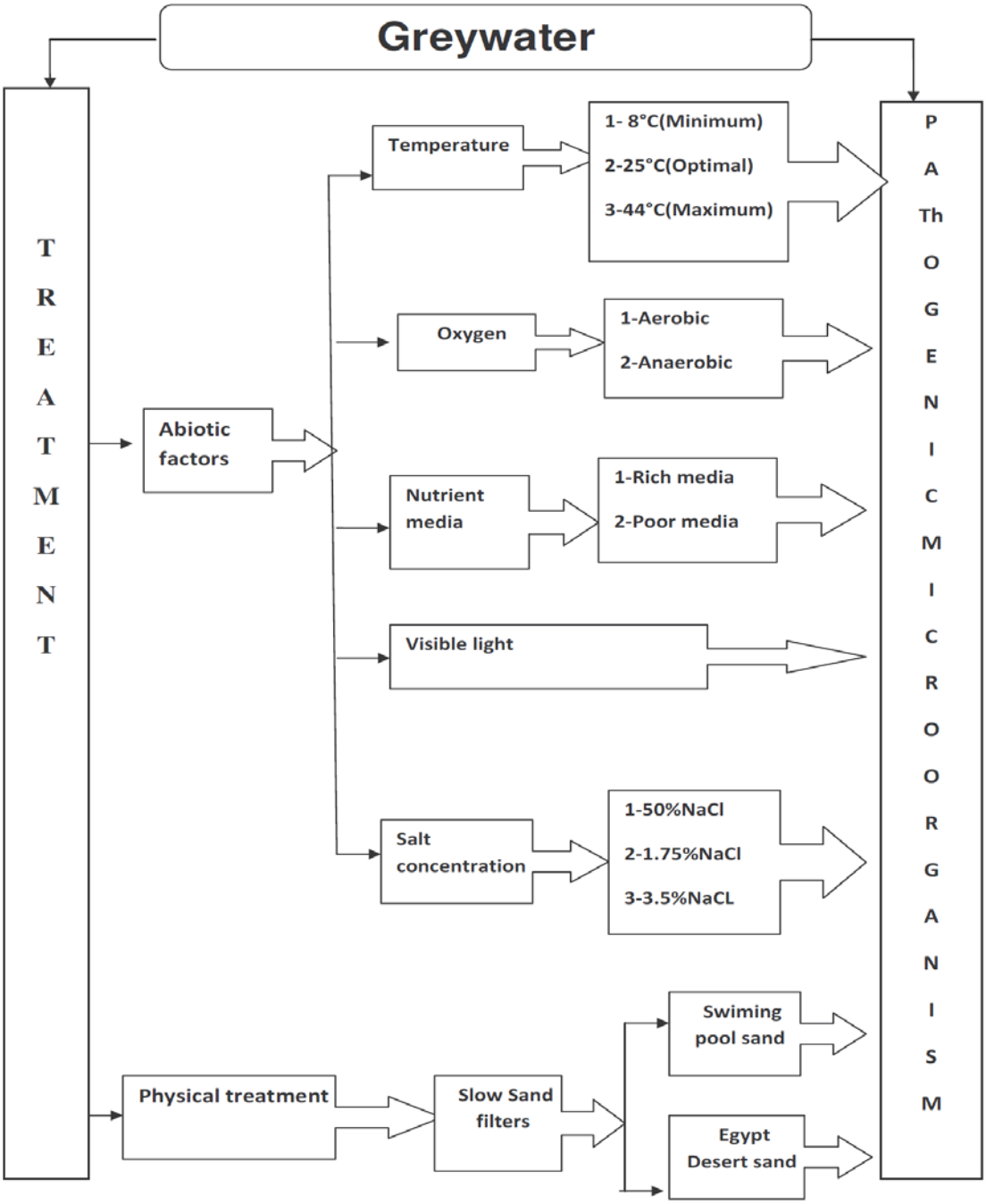


Figure 1.1 General scheme of the proposed studied of greywater treatment for safe reuse.

The study fits into a growing body of research in the field of water reuse strategies and provides data on the quality of greywater . It is one of the first focused investigations into the reuse of greywater by used a new technologies like as light with catalyst and slow sand filter,

which may become a vital survival strategy for residents of water-scarce regions in years to come.

1.2.2. Outline of the thesis

This thesis is organized in five chapters as depicted in Figure 1.2 in addition to Chapter 1.General introduction and outline.

Chapter 2. Reviews the literature of pathogens present in greywaters, characterizes greywater, greywater treatment technologies regarding operation, affordability and efficiency.

Chapter 3 Treatment of greywater by slow sand filtre ,this chapter divided to two part , the first is clean filter media and sand filter, second part is slow sand filtration.

Chapter 4. Effect of abiotic factors on survival of pathogenic bacteria in greywater.

Chapter 5 Inactivation of pathogenic bacteria and coliphages by NF- TiO2 with solar light.

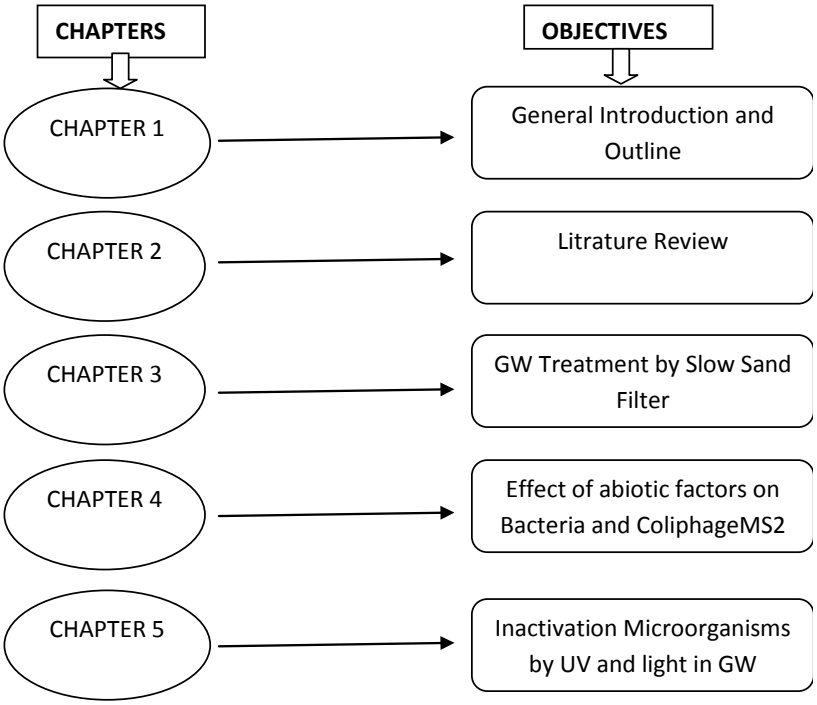


Figure 1.2 Chapters and Objectives

CHAPTER TWO

Literature review

2.1 Introduction

Greywater treatment and recycling of useful products, i.e. water, nutrients and organic matter, limit both water shortages and environmental pollution. Related to an increasing global population, scarcity of water resources, mismanagement and climate change, water shortage and water pollution have become a global issues (Falkenmark, 1990; Arnell, 1999; Bouwer, 2000). Industrial and domestic wastewaters' constituents contribute to water resource and soil pollution (Metcalf and Eddy, 2003). Greywater is any household wastewater with the exception of wastewater from toilets, which is known as blackwater. 50-80% of household wastewater is greywater from kitchen sinks, dishwashers, bathroom sinks, tubs and showers.

Greywater contribution to domestic wastewater is 60-75% of the water volume (Gulyas et al., 2004), and includes release of 9-14%, 20-32%, 18-22% and 29-62 % of N, P, K and organic matter respectively (Kujawa-Roeleveld and Zeeman, 2006). Several issues emerge with greywater, namely, reusing with or without simple treatment (Christova-Boal et al., 1995; Al-Jayyousi, 2002). The first issue is recycling for indoor use such as flushing toilets, washing clothes and/or bathing (Christova-Boal et al., 1995; Bingley, 1996; Nolde, 1999; Jefferson et al., 1999; 2001; Shrestha et al., 2001a and b; Li et al., 2003; Cui and Ren, 2005). The second issue is for outdoor use such as irrigating domestic gardens, lawns on college campuses, athletic fields, cemeteries, parks and golf courses, washing vehicles and windows, extinguishing fires, feeding boilers, developing and preserving wetlands and recharging ground water (Christova-Boal et al., 1995; Bingley, 1996; Fittschen and Niemczynowicz, 1997; Nolde, 1999; Otterpohl, 1999; Okun, 2000; Jefferson et al., 2001; Shrestha et al., 2001a and b; Eriksson et al., 2002; Al-Jayyousi, 2002; 2003). The third issue is (standards) mainly related to health and social aspects in order to improve the control of the recycling process (Nolde, 1999; Jefferson et al., 1999; 2000; 2001; Li et al., 2003; Cui and Ren, 2005). The fourth issue is obtaining affordable treatment technologies to cope with the quantity and quality variation of greywater sources (Imura et al., 1995; Eriksson et al., 2002), and the recycling requirements (Nolde, 1999; Jefferson et al., 1999; 2000; 2001; Li et al., 2003; Cui and Ren, 2005).

Knowledge of the pathogen content of greywater is limited. However, specific pathogens and significant numbers of indicator bacteria have been reported (Rose et al., 1991; Birks et al., 2004; Jefferson et al., 2004), indicating that the disinfection of greywater prior to reuse is

essential to reduce the risk to public health. This review, therefore, examines various greywater treatment methods with the aim of coming up with an efficient, simple and affordable treatment method with safe effluent for use. A treatment method is considered efficient if it produces the required effluent quality, is simple in operation with minimum maintenance, and affordable due to its low energy.

2.2 Greywater composition

Greywater composition varies widely from household to household, depending on the personal habits of residents and the products used in the home, reflecting the lifestyle of the residents and the choice of house-hold chemicals for laundry, bathing, etc. The percentages of greywater and blackwater (corresponding to water contaminated with feces and urine) from a household wastewater production are presented in Figure2.1.

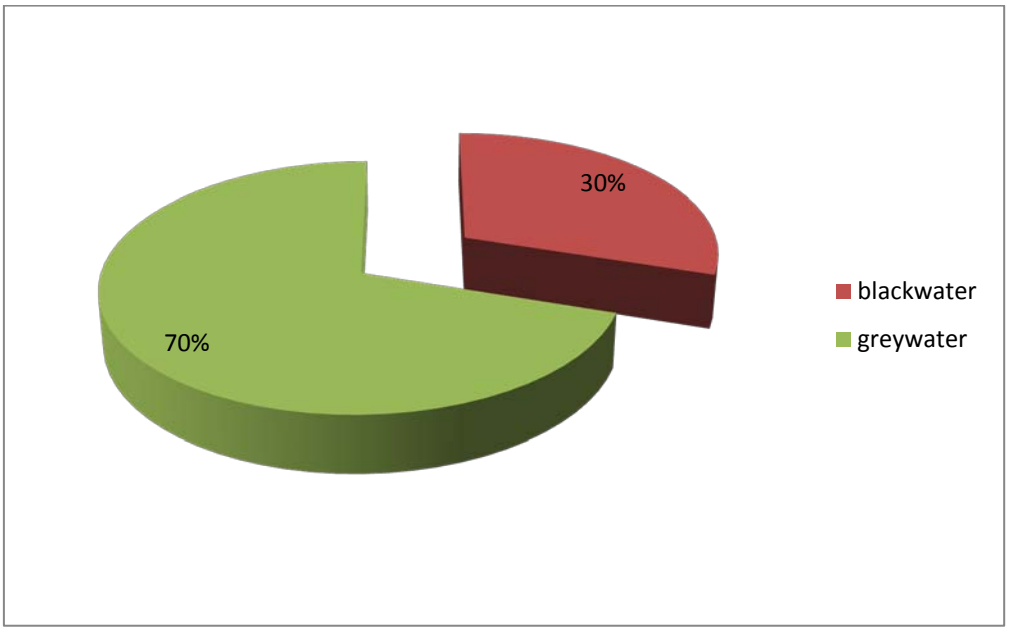


Figure2.1. Percentages of greywater and blackwater from wastewater produce in a household (according to Friedler2004)

Greywater represents the largest potential source of water savings in domestic residences, accounting for as much as 50–80% of the total water uses (Al-Jayyousi et al., 2003; Boghos et al., 2004; Christova-Boal et al. 1996; Flowers et al., 2004). In general, it contains often high concentrations of easily degradable organic material, i.e. fat, oil and other organic substances,

residues from soap, detergents, cleaning agents, etc. and generally low concentrations of pathogens (Ridderstolpe 2004). Greywater in general has low content of any metals or organic pollutants, but depending on the building it can increase with the addition of environmentally harmful substances (Ridderstolpe 2004). The content of metals or organic compounds greatly depends on usage of substances like paints, solvents, etc. (Ridderstolpe 2004).

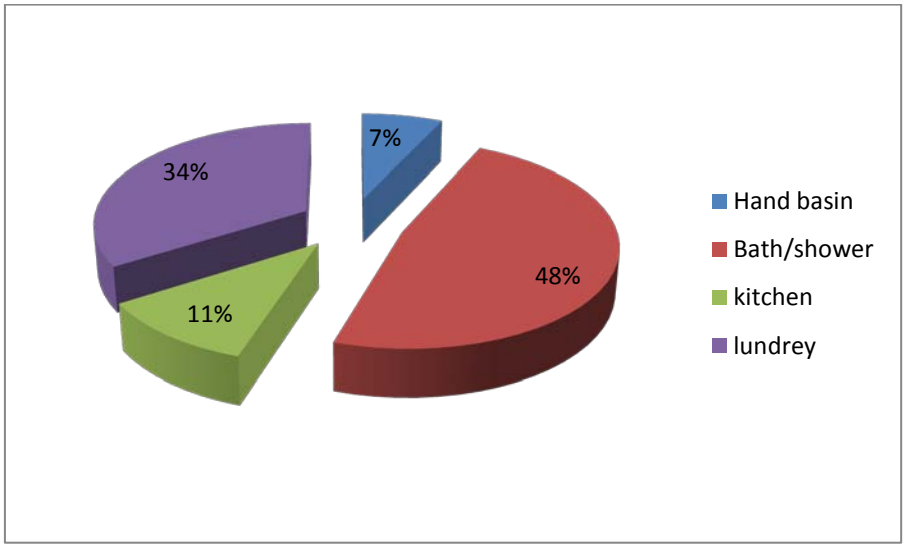


Figure 2.2. Percentages of greywater resources from household wastewater

Greywater composition also varies according to source, (Figure 2.2). The first source is: greywater from bathroom, water used in hand washing and bathing generates around 50-60% of total greywater and is considered to be the least contaminated type of greywater. Common chemical contaminants include soap, shampoo, hair dye, toothpaste and cleaning products. It also has some faecal contamination (and the associated bacteria and viruses) through body washing. The second source is: greywater from kitchen contributes about 10% of the total greywater volume. It is contaminated with food particles, oils, fats and other wastes. It readily promotes and supports the growth of micro-organisms. Kitchen greywater also contains chemical pollutants such as detergents and cleaning agents which are alkaline in nature and contain various chemicals. Therefore kitchen wastewater may not be well suited for reuse in all types of greywater systems. The third source is: greywater from cloth washing, water used in cloth washing generates around 25-35% of total greywater. Wastewater from the cloth washing varies in quality from wash water to rinse water to second rinse water. Greywater generated due to cloth washing can contain, in addition to soap and detergent, a large amount

of dyes and can also have faecal contamination with the associated pathogens and parasites such as bacteria.

The household's activities varies according to socio-economic status, cultural practices, cooking habits, cleaning agents used, as well as demography. For example, a household that uses phosphate-free laundry detergent will produce a greywater that is much lower in phosphate than one that does not. Greywater usually contains surfactants (anionic, cationic and amphoteric) which come from shampoos, detergent formulations, etc.(Eriksson et al.,2002). Personal care products are usually found in bathroom greywaters while detergents are the main constituents of laundry greywaters. Several technologies have been developed for greywater treatment and reuse in the literature (Winword et al., 2008) including natural treatment systems (Gross et al.,2007), basic coarse filtration (Kim et al.,2007, chemical (Winword et al.,2008) and biological processes (Merz et al., 2007).

2.3 Greywater characteristics

Greywater treatment is a prerequisite for reuse; treatment requirements vary based on biological and chemical characteristics and intended use of treated greywater. The aim of treatment is to overcome on all problems, which are caused by pathogenic microorganisms such as *P.aeruginosa, E.coli* and coliphage MS2, chemical compounds such as organic matter (soluble and solid), nitrogen, phosphorus, to meet reuse standards. The variation in chemical and microbial quality of greywater depends on source types. The chemical characteristics of greywater and bathroom greywater are presented in Table 2.1. Greywater pollutants, measured as COD, have, depending of the sources, an anaerobic and aerobic biodegradability of respectively 72-74% (Elmitwalli and Otterpohl, 2007; Zeeman et al., 2008) or 84±5% (Zeeman et al., 2008) or 33% (Chaillou et al.,2011). Furthermore, 27-54% is dissolved, 16-23% colloidal, and 28-50% suspended (Elmitwalli and Otterpohl, 2007; Zeeman et al., 2008). Greywater can contain recalcitrant organic matter (Friedler et al., 2006; Hernandez et al., 2007). For example anionic and cationic surfactants are slowly or non-biodegradable under anaerobic conditions (Garcia et al., 1999; Matthew et al., 2000). This compares with 2.2±0.6 for typical domestic sewage and 3–10 for final effluent and indicates that greywater is likely to contain a higher non biodegradable content than sewage (Metcalf and Eddy, 1991). Storing greywater for 48 hours at 19 to 26 ºC deteriorates its quality, (Dixon et al., 1999); biological

degradation produces malodorous compounds, causing an aesthetic problem (Kourik, 1991; van der Ryn, 1995; Christova-Boal et al., 1995; Dixon et al., 1999), pathogen growth (Christova-Boal et al., 1995; Rose et al., 1991; Dixon et al., 1999) and mosquito breeding (Christova-Boal et al., 1995), which are a health threat.

Paremeter	Unit	[a]Typical Greywater		[b]Bathroom Greywater	
		Range	Mean	Range	Mean
Suspended solids	Mg/L	45 - 330	115	-	-
Turbiditiy	NTU	22 - >200	100	9-68	28
BOD5	Mg/L	9 - 290	160	NA	NA
COD	Mg/L	100-633	456.7	116-233	170
Toal Nitrogen	Mg/L	2.1-31.5	12	2.5-33	14
Total Phosphorous	Mg/L	0.6-87	8	-	0.27
pH	-	6.6-8.7	7.5	7.6-7.9	7.8
Conductivity	mS/cm	325-1140	600	360-465	399

[a] *Based on Jeppersen and Solley (1994)*

[b]*Based on Khalaphallah,et al.(2011)* ; NA – Not Applicable

Table 2.1. Typical composition of greywater compared with bathroom greywater

However, the most important facet of greywater with regard to biodegradation is its nutrient imbalance. COD:N:P ratio for the bath, shower and hand basins sources were averaged at 100:2.25:0.06, 100:2.91:0.05 and 100:1.77:0.06 respectively. Greywater has a significantly lower concentration of inorganic nutrients (N and P) which can limit biological treatment of this material (Jefferson, et al., 2001). Comparison with the literature suggest ratios between 100:20:1 (Metcalf and Eddy, 1991), 250:7:1 (Franta et al., 1994) and 100:10:1 with trace sulphur (Beardsley and Coffey, 1985) indicating that greywater is deficient in both nitrogen and phosphorus in roughly equal proportions. The nitrogen and phosphorus deficiencies are to be expected as the majority of nitrogen compounds are excreted into the toilet bowl during urination and so not normally expected in greywater. Similarly most phosphorus originates in detergents used in washing powders and so will only be present if the laundry is included in the source waters. Turbidity profiles were similar to the solids concentrations with suspended solids to turbidity ratios of 1.04, 0.96 and 0.93 which is much lower than normally observed for sewage or potable water systems but is only slightly lower tertiary effluent (Metcalf and

Eddy, 1991). Zinc was also included as levels approaching or above the guideline values for irrigation have been found in greywater (Christova-Boal, 1996; Hypes, 1974). The very high concentration sources are normally related to either clothes washing operations such as washing machines or hand washing or kitchen sinks (Eriksson et al., 2002). Greywater characteristics also vary according to source: each fixture contributing to the greywater collection system will carry its own particular contaminant load. Friedler (2004) recommends excluding fixtures like the kitchen sink and dishwasher from a greywater system because they constitute only 25-30% of greywater volume but contribute nearly half of its COD content. For this reason, the least contaminated streams of household greywater are usually prioritized for reuse.

Treatment is therefore required (Eriksson et al., 2002) and the treatment level depends on the reuse options (Pidou et al., 2007). A larger fraction of the organic load in greywater is poorly biodegradable (soaps, greases, etc.) giving greywater a higher COD:BOD ratio than domestic sewage (COD:BOD ratio as high as 4:1) (Sayers, 1998; Brandes, 1978; Christova-Boal , 1996). Greywater has a significantly lower concentration of inorganic nutrients (N and P) which can limit biological treatment of this material (Jefferson, et al., 2001). A biological treatment system is appropriate for stabilizing the organic matter (Nolde, 1999; Jefferson et al., 1999; 2004). Biological oxygen demand varied widely (BOD) 410-6250 mg L^{-1} (Kiplagat Kotut et al., 2011). Greywater treatment does not aim at providing water of drinking water quality but at water for toilet flushing, laundry, lawn irrigation, windows and car washing, ground water recharge, or fire extinguishing (Jefferson et al., 1999; Eriksson et al., 2002). Finally, both the quantity of greywater generated per day and the composition of the greywater fluctuates greatly depending on the geographical location, demographics and level of occupancy of the household (Jefferson, Judd, and Diaper, 2001; Olsson, Karlgren, and Tullander, 1968). Greywater characteristics also vary according to source: each fixture contributing to the greywater collection system will carry its own particular contaminant load. The WHO (2006) guidelines for reuse of grey water for irrigation are combined with guidelines for safe practice; e.g. applying drip irrigation techniques, covering the soil with mulch, avoiding contact with wet soil.

2.3.1. Microbiology characteristics

The focus of most microbiological characteristics of household greywater have received much attention in recent research (Burrows et al. 1991, Birks et al. 2007, Cassanova et al. 2001, Dixon et al. 1999, Ottosson 2003, Rose et al. 1991). The pollution loads in greywater are known to be highly variable (Jefferson et al. 2004) but dark greywater is typically more polluted (Friedler, 2004). To illustrate, COD values range from 100 to 645 $mg.L^{-1}$ in light greywater and from 361 to 1815 $mg.L^{-1}$ in dark greywater. Because of their capacity to cause human illness, microbial pathogens are often considered the most significant health concern associated with greywater reuse. At their lowest, the water quality parameters for greywater compare to a poor quality treated wastewater, and at their highest, they are comparable to untreated wastewater entering a sewage treatment facility. Rose, et al. (1991) found that the total coliform count of used shower water was higher than that of laundry wash and rinse water, averaging respectively 105, 199, and 56 CFU/100mL. Ottosson and Stenstrom, (2003) outlines the full spectrum of hazardous microbial agents potentially present in household greywater and provides an outline for assessing the health risks they represent (Table 2.2).

Wastewater origin	Total coliforms	Thermotolerant coliforms	*E. coli*	Faecal enterococci
Bath, hand basin			4.4	1.0–5.4
Laundry	3.4–5.5	2.0–3.0		1.4–3.4
Shower, hand basin	2.7–7.4	2.2–3.5		1.9–3.4
Greywater	7.9	5.8		2.4
Shower, bath	1.8–3.9	0–3.7		0–4.8
Laundry, wash	1.9–5.9	1.0–4.2		1.5–3.9
Laundry, rinse	2.3–5.2	0–5.4		0–6.1
Greywater	7.2–8.8			
Hand basin, kitchen sink		5.0		4.6
Greywater		5.2–7.0	3.2–5.1	
Greywater, 79% shower	7.4	4.3–6.9		
Kitchen sink		7.6	7.4	7.7
Greywater		5.8	5.4	4.6

Table 2.2 Reported numbers of indicator bacteria in greywater (log_{10}. mL^{-1}) (Ottosson and Stenstrom, 2003)

Data for indicator bacteria in greywater varies greatly, with total coliform concentrations ranging from as low as 1.7 $log_{10}.100mL^{-1}$ (Rose et al., 1991; Dixon et al., 1999) up to 8.8 $log_{10}.100mL^{-1}$ (Gerba et al., 1995). Total coliform ranges reported for individual bathroom and laundry streams are similar at 1.7 to 5.8 log_{10}. $100mL^{-1}$, whereas combined bathroom streams are generally higher at 2.7 to 7.4 $log_{10}.100mL^{-1}$ (Christova- Boal et al., 1996; Dixon et al., 1999; Birks et al., 2004). The pathogen population of greywater from the shower, bath, wash basin, and washing machine will include those pathogens colonizing the body surface

and orifices such as the nose and mouth. Faecal contamination of greywater is common, meaning that enteric pathogens may be present in greywater. (Ottoson and Stenström 2003) estimated the faecal load of greywater from all streams combined to be 0.04g.person^{-1}.day^{-1}. In general, the number of faecal coliforms in greywater is low unless greywater is generated from washing nappies or clothes contaminated with faeces or vomit (Jeppesen and Solley, 1994). This suggests that the numbers of harmful pathogens are also low.

2.3.1.1 Pathogenic microorganisms and indicator bacteria in Greywater

Pathogens bacteria essentially exist in the greywater fraction if contaminated by faeces. Still, the greywater environment is favorable for bacterial growth which means that it needs to be treated before reuse. Furthermore, untreated greywater easily turns anaerobic (its oxygen is depleted) and thus creates foul odors. The focus of most microbiological analysis has been on fecal pollution and enteric pathogens. Rose et al., (1991) reported total coliform concentrations in greywater ranged from 10^4–10^6 CFU.100 mL^{-1}. Cassanova et al., (2001) found higher concentrations and reported a mean of 8.03 10^7 CFU 100 ml^{-1} in their study of greywater. On the other hand, Siegrist et al., (1976) found total coliforms in the range of 10^1 - 10^4 CFU 100 ml^{-1} and Burrows et al. (1991) reported concentrations as low as 85 CFU 100 ml^{-1} (range 85- 890,000) in a study of laundry greywater.

In addition to enteric pathogens, there are several human-associated opportunistic pathogens, such as *Staphylococcus aureus* and *Pseudomonas aeruginosa* (Figure 2.4), which was reported by Casanova et al., (2001) who analyzed twenty greywater samples from a single household over a seven-month period for *P. aeruginosa* and *S. aureus*.

The average concentrations of indicator bacteria in greywater are similar to those of secondary treated wastewater effluent, with median total coliform values of 4.7 $\log_{10}$.100 mL^{-1} for greywater. *E. coli* is reported in almost all of the literature studies and in all types of greywater stream, demonstrating that faecal contamination of greywater is not an occasional occurrence but is to be expected. Concentrations of *E. coli* (Figure 2.3) in greywater are also comparable with those of secondary treated wastewater effluent, although slightly higher, with median values of 3.3 $\log_{10}$.100mL^{-1}. There is great variability in the concentrations of indicator bacteria reported in the literature for different greywater streams and between different studies examining similar greywater streams.

Figure 2.3. *Escherishia coli* colonies on TTC7 petri dishes media

Pseudomonas aeruginosa is ubiquitous being widely distributed in aquatic and terrestrial habitats. It can be found in the gut of many warm-blooded mammals, although it is not a dominant member of the intestinal microbiota. It is even able to proliferate in distilled water. Concentrations of *P. aeruginosa* in sewage may exceed 10^5 CFU.100 mL^{-1} (Howard et al., 2004). While usually not a significant risk to healthy individuals, *P. aeruginosa* (Figure 2.4) has been associated with cases of folliculitis, dermatitis, and ear and urinary infections. In addition, it causes 10–11% of all nosocomial infections in hospitals and has the highest fatality rate of all hospital-acquired bacteremias (Ayi and Dworzack, 2004).

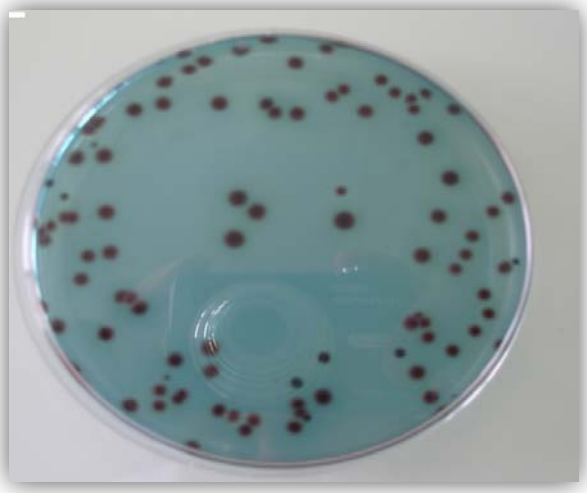


Figure 2.4. *Pseudomonas aeruginosa* colonies on TTC7 petri dishes media

Several attempts have been made to identify specific pathogenic microorganisms within grey water but no real trends have been observed. *Candida albicans*, *Pseudomonas aeruginosa* and *Straphylococcus aureus* have been identified but are commonly found in the mouth, nose and throat of humans (Eriksson et al., 2002). The detection of these pathogens in greywater requires an affected individual contributing to the greywater tested, which is more probable with a larger contributing population (Birks et al. 2004). However, the greywater produced by a larger contributing population will dilute enteric pathogens, resulting in lower concentrations and requiring sampling of large quantities of greywater for pathogen detection. Higher concentrations of specific pathogens in greywater are more likely to occur in smaller reuse schemes due to a lower dilution effect. The demographic of the contributing population has also been shown to affect the microbiological quality of greywater. Households with young children have been shown to produce greywater with higher levels of total and faecal coliforms than households with no children (Rose et al., 1991; Casanova et al., 2001). For example, Rose et al. (1991) reported average total coliform concentrations of 5.5 $\log_{10}$.100 mL^{-1} in greywater produced by families with young children, compared to around 2 $\log_{10}$.100 mL^{-1} for households with no children. A further important issue is whether pathogens in greywater regrow or die-off during storage time.

2.3.1.2 Bacteriophages

Viruses are small infectious particles, typically 20– 200 nm consisting of a nucleic acid core (single or double stranded RNA or DNA) enclosed by a protein coat (capsid) and in some cases a lipid envelope (Singleton and Sainsbury, 2002). Bacteriophages are viruses that infect bacterial host cells and are harmless to humans. Many enteric viruses are more resistant in the environment, as well as to different treatments, than bacteria. They are also smaller, which assigns them different transport features. Therefore bacteriophages have been suggested as indicator organisms to predict the presence and behavior of enteric viruses in the environment (Havelaar et al., 1991). Contact with the host cell occurs by passive diffusion. Phage adsorption and entry are mediated by specific receptors such as carbohydrates, proteins and lipopolysaccharides on the surface of the host cell (Marks and Sharp, 2000). Bacteriophages used as indicators for faecal pollution have been divided into three groups: the first group, Somatic coliphages, infecting various *E. coli* and related strains by attachment to the cell wall as the first step in the infection process, are used as an indicator of faecal contamination as

well as an index organism for virus reduction over treatment and in sediments. Somatic coliphages were analysed by the double agar method (Adams, 1959) according to (ISO 10705-2, 2000). For potential enteric virus contamination of recreational waters, ground water, drinking water and shell fish (Lees, 2000; Baggi et al., 2001; IAWPRC, 1991). The second group; F specific RNA bacteriophages are capable of infecting bacteria possessing the F-plasmid (sex plasmid) by adsorption to the F-pili as the first step in the infection process. Their presence indicates pollution by wastewater contaminated by human or animal faeces (Havelaar et al., 1986). F-specific RNA bacteriophages were analyzed by the double agar method (Adams, 1959) according to (ISO 10705-1, 1995 Figure 2.5). The third group; Phages infecting anaerobic bacterium *Bacteroides fragilis* attach to molecules in the cell wall of host bacteria as the first step in the infection process. Bacteriophages are highly abundant in the aquatic environment ranging from 10^4 ml^{-1} to in excess of 10^8 ml^{-1} (Bergh et al., 1989). Numbers are typically 3–10 times greater than the bacterial counts, although there is substantial variation between ecosystems (Weinbauer, 2004). A relationship with bacterial numbers and activity implies that the majority of aquatic viruses may be phages. Furthermore, numerous viral abundance studies show seasonal (Bergh et al., 1989; Cochran and Paul, 1998; Hofer and Sommaruga, 2001) and diet variations (Jiang and Paul, 1994; Weinbauer et al., 1995). Early studies (Dias and Bhat, 1965) indicated that *E. coli* phages (coliphage) were not functional in laboratory scale activated sludge systems. Within 2 h of aeration coliphage abundance declined 10-fold from 2460 to 230 PFU ml^{-1}, stabilizing thereafter for a further 23 h. Coliphages infectious for *Enterobacteria* present in wastewater, are removed during activated sludge treatment (Bitton et al., 1999). Over many year *E .coli* and fecal streptococci have used as indicators for viral pollution. But there have been cases when epidemics have occurred even when the microorganism indicators were removed fully from the effluents. In 1974, this idea led some authors to suggest that coliphages might serve as indicators of fecal pollution. (Berrego et al., 1986) developed a highly specific, sensitive and rapid technique for detection of *E.coli.*

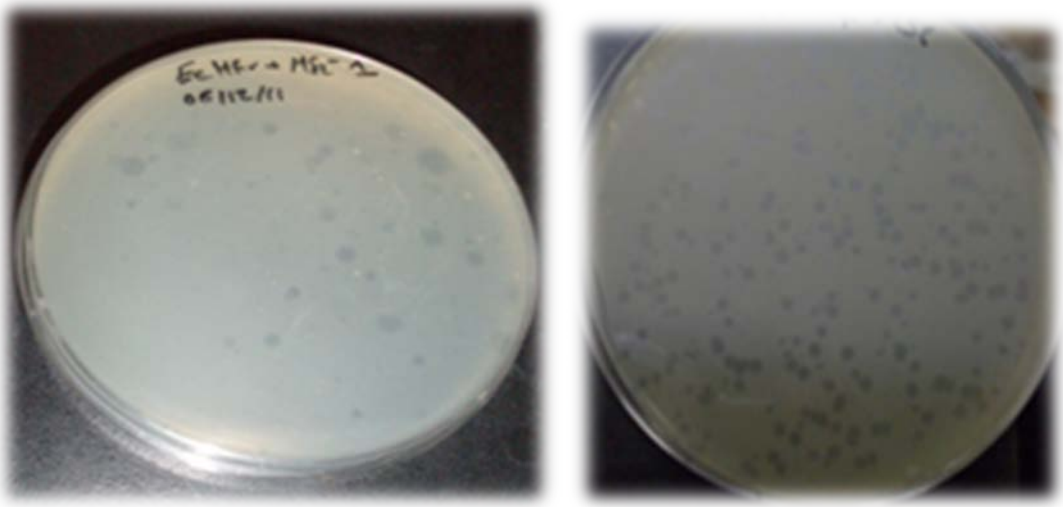


Figure 2.5. Coliphage MS2 plaques by double layer agar on TYG agar media

2.3.1.3 Pathogenic microorganism parameters

2.3.1.3.1 Particle cell size distribution

Nutrient availability is one of the strongest determinants of cell size. When grown in rich media, single cell organisms such as yeast and bacteria can be up to twice the size of their slow growing counterparts. The size distribution of microbial cells provides objective and valuable information on molecular mechanisms of cell division. Moreover, it can be used to test various hypotheses regarding the replication cycle of prokaryotes (Bernander & Nordstrom, 1990; Keasling et al., 1995; Koch, 1993). Cell size variation is also a sensitive parameter reflecting the physiological and molecular-genetic state of a microbial population, e.g. it can change due to the number of plasmid copies in the recombinant strains (Lyncha et al., 2000) or the temperature shock (Scherbaum, 1956), osmotic stress (Elmoazzen et al., 2005) or various pollutants (Biggs et al., 1978; Ting et al., 1991). Therefore, the analysis of size distribution has numerous applications in biotechnological, biomedical and environmental research

2.3.1.3.2 Electrostatic charges on the cell surface (Zeta potential measurement)

The electrokinetic potential (zeta potential, Z) is the potential drop across the mobile part of the double layer that is responsible for electrokinetic phenomena, for example electrophrosis (motion of colloidal particles in an electric field). It is assumed that the liquid adhering to the solid (particle) surface and the mobile liquid are separated by a shear plane (slipping plane). The electrokinetic charge is the charge on the shear plane (Stumm, 1992). At the interface between a charged surface and an electrolytic solution, it exist a solution layer called double layer, it has different properties than an electrolyte. This layer is constituted by the compact layer corresponding to adsorbed ions in the surface of the solid in the way of the mono-layer of Langmuir, and the diffuse layer. Several models have been elaborated to describe the charge evolution ($\sigma 0$) and the surface potential ($\Psi 0$) of the double layer, and it is the triple layer model that allows understanding the zeta potential signification (Stumm and Morgan, 1996). The triple layer model was introduced in 1947, the compact layer in sub divided in two parts (Figure 2.6). The first part is placed between the surface and the Helmholtz Internal Plane (HIP). Just one layer or the ions that present a high interaction with the surface can be received, and they loss partially or totally their hydration sphere (internal sphere complexes). The center of these ions is located at HIP level. The second part is between the HIP and the Helmholtz external plane (HEP) and it does not take into account the hydrated ions retained by the electrostatic forces (extern sphere complexes).

One of the common methods to determine a microorganism's net electric charge is to obtain its electrophoretic mobility, which is a measure of the microorganism's movement in a solution when subjected to an externally applied electric field (Richmond and Fisher, 1973). The isoelectric point or the zeta potential or the electrophoretic mobility at pH 4 usually characterizes the surface charge of bacteria (Krekeler et al., 1989). It is possible to calculate the surface charge density of a microorganism from its zeta potential (Adamson, 1960; Brinton and Laufer, 1959). However, most researchers use only zeta mobility data, because of the uncertainties regarding several factors needed for the calculations. Experiments employing isoelectric equilibrium analysis have shown that the number of net negative charges on unmodified cells of *Escherichia coli* was tens of thousands per cell (Sherbet and Lakshmi, 1973). No fundamental differences between the isoelectric point of Gram-positive and Gram-

negative microorganisms were observed (Krekeler et al., 1989). The surface charge on biological particles can also be characterized by colloid or polyelectrolyte titration.

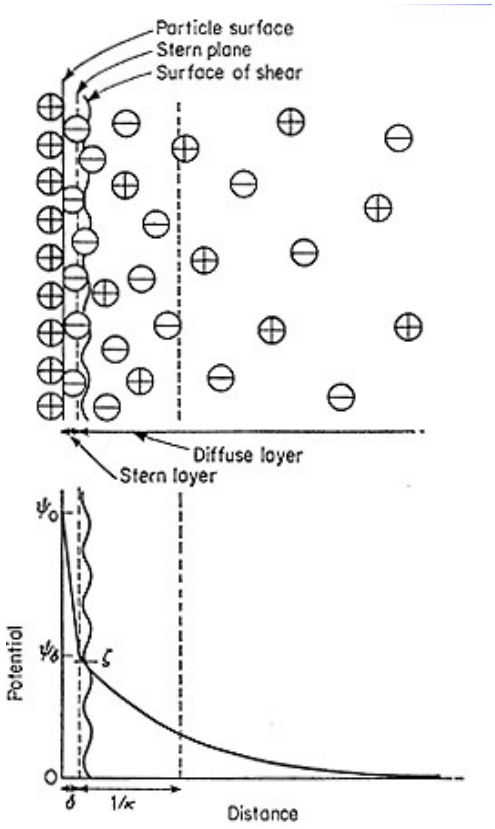


Figure 2.6. Triple layer zeta model.

This method is based on the fact that polyelectrolytes of opposite charge form complexes in a stoichiometric way and the endpoint of the complex formation can be determined colorimetrically by using indicators (Krekeler et al., 1989). Through utilizing this method Noda, et al., (1984) determined the surface charge of *Micrococcus luteus*, and Van der Wal, et al., (1997) found that the cell wall charge density in Gram-positive bacteria can be as high as 0.5-1.0 C/m. However, the accuracy of this method is usually not sufficient and, thus, colloid titration finds limited applications. Another popular method for investigating the bacterial charge is by electrostatic interaction chromatography. This method is simpler than the zeta potential measurement and can be used to examine interactions on parts of the cell surface, i.e., to determine localized charges (Hermanssonvet al, 1982). It has been noted, however, that electrophoretic measurements and interaction chromatography give different data about the surface charges on the bacteria (Hermansson et al., 1982; Jones et al.,, 1996).

2.4. Greywater treatment and microorganisms' survival

In order to minimize potential negative impacts, it is strongly recommended that greywater be treated before reuse. Treatment systems for greywater exist in many forms, varying in their complexity, treatment method, and location within or outside the home, and should be designed in accordance with greywater source, quality, site specifications, and reuse patterns. Greywater treatment systems range in sophistication from simple branched-drain garden irrigation networks to full tertiary treatment systems that can filter water to nearly potable levels of quality. While greywater treatment is a relatively new concept now, it has been practiced for several years in places where water is less abundant or expensive to use. In the southern US, Australia, and many Middle Eastern countries, simple greywater diverting schemes are common as a means of irrigating landscape plants in arid regions. Many different treatment mechanisms have been studied, with varying degrees of success. Nolde (2000) recommends aerated biological treatment, while Pidou et al. (2007) received good results with chemical treatment involving coagulation and magnetic ion exchange. Hernandez-Leal et al. (2007) experimented with anaerobic greywater treatment in their study and found it less effective than comparable aerobic processes, likely because of the interference of surfactants with anaerobic bacteria. Whatever the treatment method, complete system design ideally includes a tertiary disinfection stage, usually either chlorine or UV radiation, to protect against bacterial regrowth in treated water. To date, there is no generally accepted design for greywater treatment systems, but several manufacturers around the globe provide plug-and-go solutions for homeowners, indicating that some private sector researchers may have found effective, safe solutions for greywater recycling.

2.4.1 Effect of abiotic factors on survival growth of bacteria

The influence of factors on the survival of bacteria in water is still not well understood despite the large number of studies which have previously been carried out. Nevertheless, several factors have been proposed as making a significant contribution to the decline of bacteria in the aquatic environment, though their relative importance is not always agreed upon by the various authors. At the risk of duplicating the efforts of many previous investigators, the most frequently documented factors are discussed in this chapter. Existing reviews on this subject

are numerous (Waksman & Hotchkiss, 1937; Greenberg, 1957; Carlucci & Pramer, 1959; Jones, 1971; Mitchell & Chamberlin, 1975, 1978; Elliot & Colwell, 1985). Carlucci and Pramer (1959) summarized the major factors which influence the decline of enteric bacteria in water. These factors are: temperature, available oxygen, salinity and nutrients compositions.

2.4.1.1 Temperature

Every microbe requires optimal temperature for its metabolic rates. Whenever temperature increases metabolic rate of microbe decreases and dies. In low temperature microbes will be in inactive form. It doesn't die but stay dormant. Extremes of temperature seem to be most disruptive to bacterial survival (McFeters et al., 1974; Flint, 1987). Several investigators have noted that at lower temperatures bacteria survive longer than at higher temperatures, in fresh waters (McFeters & Stuart, 1972; Auban et_al., 1983; Barcina et al., 1986a), in estuarine waters (Faust et al., 1975; Vasconcelos & Swartz, 1976) and in marine waters (Pike et al., 1970; Evison & Tosti, 1980). Pike et al. (1970) or Mancini (1978) commented on the direct relationship which appeared between temperature and die-off rates of coliforms in fresh and sea water laboratory experiments under conditions of darkness. Mortality rate increases linearly with increasing temperature between 4° C and 24°C for faecal coliforms in seawater in the dark (Gameson, 1984). That survival is inversely proportional to temperature has been reported over a number of temperature ranges (McFeters & Stuart, 1972; Faust et al., 1975; Evison & Tosti, 1980). Survival of bacteria decreases with increasing temperature (Kristiansen et al 1981, Stenstre et al 1982, Ostrolenk et al 1947, Shah et al 1994). Incubation of *E. coli* in soil at 5°C, 10°C, 20°C and 37°C showed best survival at 5°C (Sjogren et al, 1994). In a survival experiment with *Pseudomonas sp.* in soil, Vandenhove et al, (1991) found no difference between 5°C and 15°C, but a significant reduction of the bacterial number at 25°C. However, conflicting evidence demonstrating prolonged survival of *E.coli* and salmonella in sterile and non-sterile estuarine waters at warm temperatures (>18° C) has been presented (Rhodes & Kator, 1988) suggesting that survival is directly proportional to temperature, an observation which supports those previously made by Anderson et al. (1983). It is believed that temperature exerts its effect on survival through its influence on other factors (EPA, 1985) and also through its control of the metabolic rate of cells (Jones, 1971). At 45° C cells have long since stopped functioning, with major organ failure occurring at much lower temperatures, making this temperature pathogenically irrelevant (Nguyen, 2006). In sterile waters, the inverse relationship between survival of *E.coli* and temperature is not

apparent (Barcina et al., 1986). Anderson et al. (1983) found that survival of *E.coli* is directly related to temperature in the absence of eukaryotes. These workers concluded that the interactions between experimental bacteria and autochthonous organisms and the occurrence of sublethal stress may account for the discrepancies in temperature-related survival data. Evidence in support of this was obtained by (Flint 1987) who concluded that though temperature was a major factor affecting the survival of bacteria introduced into the natural environment, its importance is secondary to that of biotic influences. Undoubtedly, temperature is still considered by many to contribute significantly to bacterial die-off in the environment. The influence of this factor should be considered in connection with the time of year and location of release of bacteria into the environment.

2.4.1.2 Nutrient deficiencies

Nutrition is the provision to cells and organisms, of the materials necessary to support life. Many common health problems can be prevented or alleviated with a healthy diet. The study of nutrition is increasingly concerned with metabolism and metabolic pathways: the sequences of biochemical steps through which substances in living things change from one form to another. Alexander (1986) suggested that in addition to susceptibility to antagonistic and predative agents, nutrient deficiencies may be responsible for the decline of those bacteria which are relatively resistant to the effects of abiotic factors. Increased survival of bacteria has been attributed to the nutrient content of the water (Slanetz & Bartley, 1965; Hendricks, 1972; Yanagita & Takagi, 1980). When seawater was supplemented with inorganic salts and organic matter, the decline of *Escherichia coli* was reduced (Carlucci & Pramer, 1960). This effect was found to be even more pronounced in freshwater (Hendricks, 1972). The addition of amino acids to freshwater could increase survival of *E.coli* up to 3 fold. The addition of ammonium or nitrate has a limited effect but no carbon or phosphorus source is effective to the same degree (Lim & Flint, 1989). The chemical composition of water greatly influences the survival of enteric bacteria. The composition of seawater varies much less than that of freshwater (Mitchell & Chamberlin, 1978). The type and concentration of nutrients available have different effects on different species, for example, the addition of peptone to *E.coli* in seawater increases its survival but the addition of glucose reduces its survival (Carlucci & Pramer, 1960). *E.coli* and other coliforms require only low concentrations of organic matter to grow, whereas faecal streptococci require much higher concentrations (Allen et al., 1952).

The difference in survival rates of different species of bacteria may be due to their varying abilities to find and compete for growth limiting nutrients in a particular environment (Alexander, 1986). The most successfully competitive organism is likely to be the one with the fastest growth rate under the prevailing conditions (Alexander, 1986). For this reason, enteric bacteria are unable to compete adequately with autochthonous bacteria for low concentrations of nutrients. However if bacterial predators preferentially consume nutrients, then the effect of predation on bacterial mortality will be reduced (Sinclair & Alexander, 1984). Evidence exist which shows that some allochthonous bacteria are capable of growth after being discharged into rivers and marine waters depending on the presence of appropriate nutrients(Hendricks & Morrison, 1967). Under conditions of high nutrient concentrations, the adverse effects of salinity and temperature are less pronounced for coliform bacteria (Anderson et al., 1979). Humic substances have been accredited with the stimulation of various processes of cell metabolism. At low concentrations they can stimulate the growth of phytoplankton but at high concentrations may inhibit growth altogether (Prakash & MacGregor, 1983). The utilization of humic materials by some microorganisms as an energy source has been reported (Shapiro, 1957) and its able to increased growth of *Pseudomonas spp.* In some freshwater systems microbial growth has been attributed to the metabolism of fulvic acids (De Haan, 1974). Phosphorus and nitrogen occur as functional groups and structural components of humic acids which may be released as soluble forms (Malcolm, 1985). Seawater also contains humic acids but in very low concentrations (Harvey & Horan, 1985). It is believed that the main reason for the death of allochthonous bacteria in the aquatic environment is their inability to lower their metabolic requirements in a situation of low nutrient availability (Klein & Casida, 1967). The survival of *E.coli* in nutrient-free water or seawater is dependent on the age of the cells at the time of harvesting and exposure (Gauthier et al., 1989). Chai (1983) showed that even natural seawater could support the weak growth of *E.coli* under laboratory conditions and that the sensitivity of these cells to certain chemicals was different to that of cells grown in a rich medium.

2.4.1.3 Dissolved oxygen

The dissolved oxygen (DO) is oxygen that is dissolved in water. The oxygen dissolves by diffusion from the surrounding air; aeration of water that has tumbled over falls and rapids; and as a waste product of photosynthesis.

Biologically speaking, however, the level of oxygen is a much more important measure of water quality than feacal coliform. Dissolved oxygen is absolutely essential for the survival of all aquatic organisms not only fish but also invertebrates such as crabs, clams, zooplankton, etc. Moreover, oxygen affects a vast number of other water indicators, not only biochemical but esthetic ones like the odor, clarity and taste. Consequently, oxygen is perhaps the most well-established indicator of water quality. Dissolved oxygen (DO) levels of water vary with the season of the year, lower DO levels occurring in summer and higher DO levels during winter. The survival of *E.coli* is directly proportional to the DO concentration of the water, the decline in cell numbers being lowest at high DO levels (Faust et al., 1975). However, the shock of leaving an oxygen-poor medium such as sewage and entering an oxygen-rich medium like seawater promotes rapid decay of coliform bacteria.

2.4.1.4 Salinity

The influence of salinity appears to be due to either an osmotic effect, or to toxicity of specific ions (Carlucci & Pramer, 1960). The effect to which salt concentration causes changes in bacterial growth depends on the osmotic balance required for such growth. Some bacteria require an astonishingly high level of salt to begin growth, whereas other bacteria would be immediately killed in high levels of salt. The salinity of surface seawater is approximately 3.5% (ZoBell, 1946), suggesting a high concentration of potentially toxic inorganic salts. The physiological effects of salinity are very diverse, as discussed by Rheinheimer (1985). Pike et al. (1970) found that the T_{90} (corresponding to time for a decrease of 90 % of the microbial population) values for coliforms in a river were approximately double those for seawater. Early laboratory studies reported by Gameson (1984) also showed that T_{90} values for total coliforms in the dark had a direct correlation with salinity. An inverse, linear relationship was also found when faecal coliforms were exposed in water of various salinities, to daylight and to an artificial light source (Gameson & Gould, 1985). Evison and Morgan (1982), however, provided evidence which suggested that the relationship between die-off rate and salinity was not linear for most bacteria. Maximum survival was recorded for those bacteria tested at 0.05% salinity. At 0%, survival time was shorter. It was also found that sublethal stress could develop in *E.coli* exposed to waters of high salinity (Kapuscinski & Mitchell, 1981).

2.4.2 The effect of light irradiation on microorganisms inactivation

2.4.2.1 Inactivation by visible light

With the exception of Reynolds (1964), who quite by chance observed differences in coliform mortality rates between day and night, most workers dismissed the effect of sunlight on bacterial mortality as being insignificant for reasons previously mentioned. It was not until 1967 that higher mortality rates of coliforms were found when exposed to daylight than when kept in the dark (Gameson & Saxon, 1967). Consequently, after numerous shore-based, submerged bottle and in situ experiments had been performed, the conclusion was that sunlight had an important effect on bacterial mortality in seawater. About half the lethal effect of sunlight can be attributed to wavelengths below 370 nm (ultraviolet), a quarter to the near-visible ultraviolet (370-400 nm) region and a quarter to the blue-green region of the visible spectrum (400- 500 nm). However, this observation may be invalid at lower depths of the water or sea because of the greater attenuation of UV light by water (Gameson and Gould, 1985). The probability of inactivation decreases, as wavelength increases, and concluded that 265 nm is perhaps the most lethal wavelength for *E.coli* (Hollaender 1943). However, light of wavelength 293 nm is the lowest found at sea level (Henderson, 1970) and 300 nm is probably the lowest wavelength of light occurring at British latitudes (Gould & Munro, 1980). Between 1965 and 1975, more than 300 laboratory beaker experiments on the mortality of coliform bacteria exposed to sunlight and a further 400 on the mortality of coliforms in the dark, the results of which were published in a series of technical reports by Gameson, (1984) and Gameson & Gould (1985). The conclusions formed as a result of these experiments being carried out were discussed (Gameson and Gould 1975) and are as follows:

1) The mortality curves for coliform bacteria in natural waters were approximately log-linear, following first-order kinetics.
2) In laboratory experiments, it was frequently found that *E.coli* exhibited a lag phase or steadily increasing mortality rate.
3) It was found that the T_{90} value of bacteria in the dark decreased with increasing temperature.
4) T_{90}s as short as 20 minutes were recorded in bright summer sunshine.
5) It was found that the rate of decay (K) is approximately proportional to light intensity but decay rates were not found to be significantly different for bright sunshine and over cast sky.

6) The temperature does not exert a significant effect on the value K between 2 and 29° C in sunlight. (Mitchell and Chamberlin 1975, 1978) used the following mathematical model to summarize the observations made by (Gameson and Gould 1975 and Alkan et al., 1995).

The most common parameters to characterize rate of bacteria growth and decay are:

(i) The decay rate K (h) were calculated according to Chick's law which is depicted as

$$- Kt = (\text{Log } N_t / N_0)$$

Where: N_0 and N_t is respectively the bacterial Numbers initial and at time t measured in hours

(ii) The T_{90}, the time required for 90% of the initial population has disappeared.

$$T_{90} = - t / \log (N / N_0)$$

Where: N_0 and N are respectively the bacterial Numbers initial and at time t measured in hours.

The influence of sunlight on mortality rate of microorganisms is related to depth with an effective attenuation coefficient of approximately $0.22m^{-2}$, directly proportional to light intensity, and a first order reaction with respect to coliform concentration according to Mitchell and Chamberlin (1978). They assumed that light intensity decreased exponentially with depth. In conclusion, the variability of coliform decay rates in sea water can be attributed to the variability of factors influencing the depth profile of light intensity, and bacterial concentration. Mancini (1978) developed a decay model for coliforms which also took the contribution of the dark mortality rate into consideration. Mitchell and Chamberlin (1978) believed that light was the single most influential factor acting upon bacterial decay rate but not to the exclusion of the role of all other factors. These workers also pointed out that only actively metabolizing coliforms are sensitive to light so that the effect of light would be substantially reduced in nutrient-poor waters and it is conceivable that light only injures coliforms rendering them more susceptible to other factors. It has been suggested that light intensity becomes an increasingly important influence on bacterial die-off above a certain threshold intensity, and that this value may be quite low (Anson & Ware, 1975; Sieracki, 1980).

2.4.2.2 Inactivation by ultraviolet light

Inactivation of bacterial cells by UV light is principally due to localized lesions in DNA caused by the formation of pyrimidine dimers particularly thymine (Figure 2.7), by direct absorption of UV light, resulting in mutation or loss of the ability to replicate (Smith & Hanawalt, 1969). Ultimately, damage to the DNA results in death of the cell. Setlow and Pollard (1962) provided indirect evidence for the involvement of DNA in the lethal action of UV light by comparing the UV adsorption spectrum of DNA and the action spectrum for UV inactivation. Both spectra peaked at 260 nm used simulation photoreactors in laboratory under control of factors like temperature, oxygen, and wavelength.

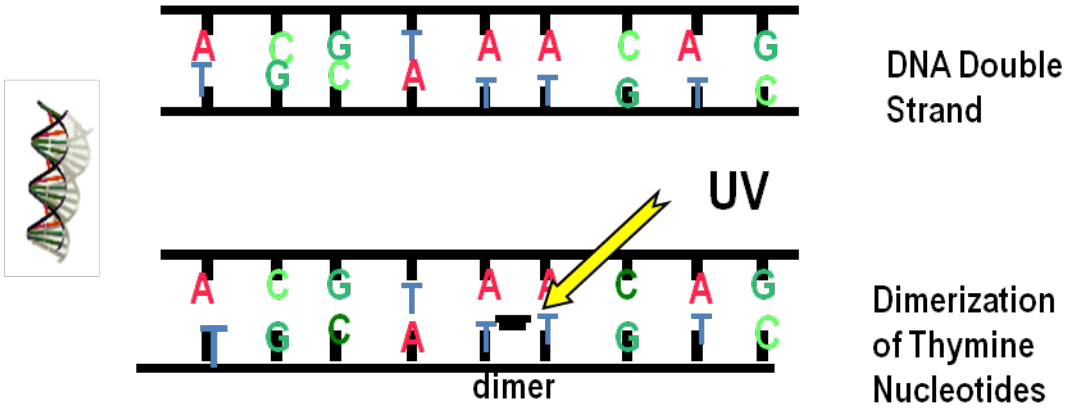


Figure 2.7. Direct Effect of UV on cell's DNA

2.4.2.3 Dark recovery

The studies of Setlow (1967, 1968) provided experimental evidence for repair of UV-damaged cells in the dark. Defective regions in the DNA strand are excised and replaced with normal nucleotides, using the information on the complementary, intact strand for base pairing. This process is known as excision-repair and occurs without the aid of light. The steps involved in excision-repair are discussed in detail (Setlow 1968 & Smith and Hanawalt 1969). Dark repair is not usually as effective as photoreactivation (Setlow, 1968).

2.4.2.4 Mechanisms of inactivation

Mechanisms of inactivation bacteria are important concept to more knowledge about influence of photoinactivation of microorganisms. Numerous paper have reviewed the

literature describing the mechanisms of light inactivation of bacteria by UV and visible light, (Harrison 1967; Eisenstark,1971 ; Krinsky 1977 & Chamberlin and Mitchell 1978; Gameson and Gould 1985).

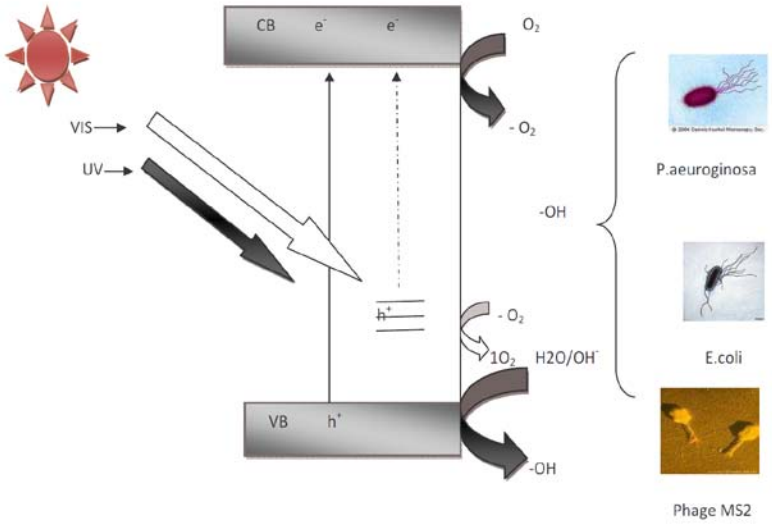


Figure 2.8. Scheme of photocatalytic microorganisms inactivation mechanisms

The lethal effects of sunlight on bacteria, in particular the ultra-violet component of sunlight were established very early (Figure 2.8) and involve the formation reactive species of oxygen (Ward, 1893; Gates, 1930; Gaarder & Sparck, 1931; Kelner, 1949). Many types of cell damage have been induced by the photodynamic action of UV light, including loss of colony-forming ability, damage to DNA, damage to the cell membrane, and inactivation of enzymes (Smith & Hanawalt, 1969). Photodynamic action requires the presence of a photosensitizing pigment and oxygen. The effect of light in the near visible and visible part of the spectrum (370-500 nm) on bacteria in sea water received little attention for many years. Some authors (Greenberg 1956 ; Carlucci and Pramer 1960) believed that the effect of sunlight on bacterial mortality was negligible. This was due in part, to the high attenuation coefficients of the UV and visible components in sea water, and partly to the misconceptions that only far ultraviolet light wavelength (280 nm) was harmful to bacteria and that nucleic acids were the only sites of damage. It was later confirmed that visible light was lethal to -many bacteria and that the addition of photosensitisers such as fluorescent dyes, and the presence of oxygen enhanced mortality (Eisenstark, 1971). Only UV light can react directly with DNA. The effects of visible light are due to the presence of natural endogenous photosensitisers which behave

analogously to exogenous photosensitisers involved in photodynamic action. In an extensive review of the literature (Eisenstark 1971) compared the effects of photodynamic action and the effects of visible light, (Figure 2.8). His observations were:

i) The oxygen demand for killing by UV light was low compared to that for visible light.

ii) Photoreactivation occurred after exposure to UV light but not to visible light.

iii) The action spectrum for visible light was 300-380nm for division delay and 338nm for growth inhibition (c.w. 260nm).

iv) DNA does not undergo rapid degradation when exposed to visible light.

2.4.2.5 Developments in photocatalytic water treatment

Greywater treatment technologies must be robust to handle variations in organic and pathogen concentration in water influent, and to consistently produce an effluent of an appropriate and safe quality to meet the required microbial standards for reuse (Jefferson et al., 2008). Those characteristics are easily achieved by some Advanced Oxidation Processes (AOPs), and more specifically by photocatalytic processes. Photocatalytic oxidation is an emerging technology that could be suitable to remove recalcitrant organic compounds (Gaya et al., 2008) found in greywaters (Zhu et al.,2008). Furthermore, the disinfection of wastewater could be carried out with this advanced oxidation process (Paeologou et al., 2007). The irradiation of titanium dioxide dispersions can lead to the formation of highly reactive hydroxyl radicals which attack the pollutant molecule to degrade it into carbon dioxide, water and mineral acids. This technology has advantages, such as, the integration into small places, low maintenance and easy operation (Figure 2.9). Since the discovery of photocatalytic effect on water splitting (Fujishima and Honda 1972) using TiO_2 electrode, numerous researches have evolved to synthesis TiO_2 catalyst of different scale, characterize its physical properties and determine its photooxidation performances to the surface oriented nature of photocatalysis reaction (Fujishima and Honda, 1972; Kondo et al., 2008; Hosono et al., 2004; Joo et al., 2005; Wang et al., 1999). The TiO_2 catalyst in nano-dimensions allows having a large surface area-to-volume ratio and can further promote the efficient charge separation and trapping at the physical surface (Nagaveni et al., 2004).

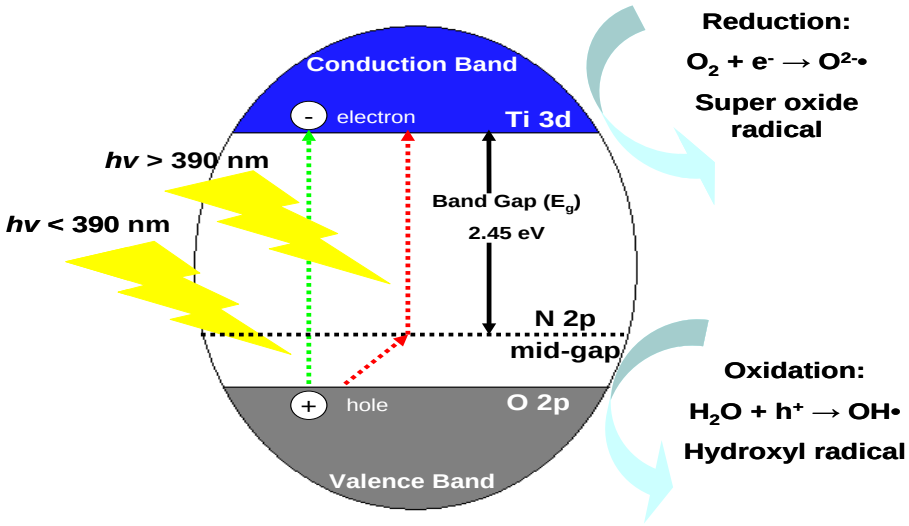


Figure 2.9. Electron hole pair between valence and conduction band

The light opaqueness of this nano-scale TiO_2 catalysts was reported to have an enhanced oxidation capability compared to the bulk TiO_2 catalysts (Siddiquey et al., 2008). Although the nano-scale TiO_2 catalysts show considerable improvement in terms of their physical and chemical properties, their particle size and morphology remains the main problem in a large scale water treatment process (Byrne et al., 1998; Yu et al., 2002).

2.4.2.6 Photocatalyst modification and doping

As TiO_2 photocatalytic reactions take place under ambient operating conditions, photoactivity is usually constrained by the narrow wavelength spectrum for photonic activation of catalysts. The higher-end of UV spectrum required for catalysts activation is usually accompanied by high operating costs. One attractive option is to utilize the vast abundance of outdoor solar irradiation for catalyst activation in a suitably designed photoreactor system.

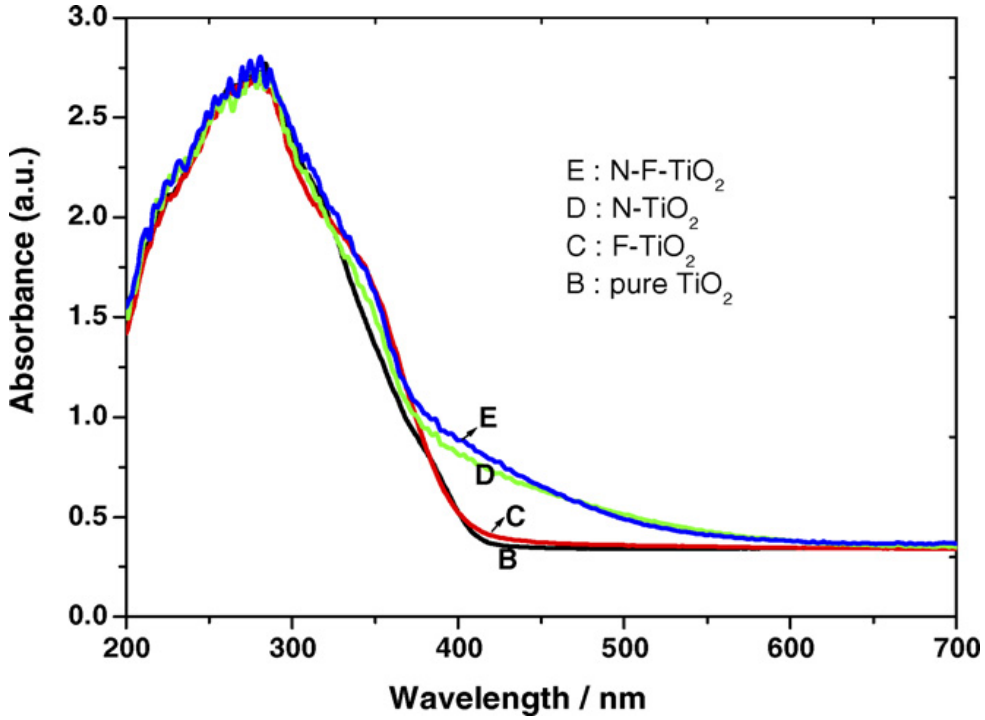


Figure 2.11. UV–vis absorption spectra of: (B) TiO_2; (C) F–TiO_2; (D) N–TiO_2; (E) N–F codoped TiO_2 prepared by sol–gel-solvothermal method (Huang et al.2006)

To broaden the photoresponse of TiO_2 catalyst for solar spectrum, various material engineering solutions have been devised, including composite photocatalysts with carbon nanotubes (Yu et al., 2005), dyed sensitizers (Vinodgopal et al., 1996), noble metals or metal ions incorporation (Ni et al., 2007), transition metals (Litter, 1999) and non-metals doping (Figure 2.11, 2.12) (Fujishima et al., 2008). Similarly, noble metals (e.g. Ag, Ni, Cu, Pt, Rh, and Pd) with Fermi level lower than TiO_2 catalyst have also been deposited on the TiO_2 surface for enhanced charge separation (Ni et al., 2007).

Figure 2.11. N-F codoped TiO2 prepared by sol- gel solvothermal method

These metals were reported to enhance electron transfer, but require good knowledge on the optimal deposited amount needed during the fabrication process. Although noble metals coupling could be efficient in prolonging the surface charge separation, their cost-effectiveness for an industrial application is usually replaced by more economical transition or non-metals doping. The mechanism of such transition and non-metals doping, however, is different from the noble metals coupling as the TiO_2 is incorporated into the TiO_2 crystal lattice (Asahi et al., 2001; Irie et al., 2003; Ihara et al., 2003)

2.4.3 Treatment by Slow Sand Filtration

Slow sand filtration is a simple and reliable process. They are relatively inexpensive to build, but do require highly skilled operators. The process percolates untreated water slowly through a bed of porous sand, with the influent water introduced over the surface of the filter, and then drained from the bottom. Properly constructed, the filter consists of a tank, a bed of fine sand, a layer of gravel to support the sand, a system of under drains to collect the filtered water, and a flow regulator to control the filtration rate. No chemicals are added to aid the filtration process.

Slow sand filtration has been used for water treatment for hundreds of years. The first known water treatment system to use elements of slow sand filtration was constructed in Lancashire, England as part of bleach works circa 1790 (Weber-Shirk & Dick, 1997). This filter's sole purpose was to improve the aesthetic quality of the water. The first slow sand filter used in a public water supply was constructed in 1804 in Paisley, Scotland. Water entering this filter flowed from a settling basin through a gravel filter, through a sand filter and into a holding chamber; thus incorporating both pretreatment and slow sand filtration (Baker, 1982). It was recognized in 1885 that slow sand filtration could remove bacteria. Particle and bacterial removal by straining was thought to be the main removal mechanism of the slow sand filter, with "bacterial action" proposed as a second explanation (Graham in 1850 ; Weber-Shirk and Dick, 1997). The first large scale demonstration of the effectiveness of slow sand filtration occurred during a cholera epidemic in Germany in 1892. Two cities, Altona and Hamburg drew their water from the Elbe River. Even though Altona's water intake was downstream from Hamburg's sewer outfalls, cholera cases occurred at a rate of 230 per 100,000 in Altona and 1,344 per 100,000 in Hamburg. The difference, Altona used slow sand filtration. The

majority of cholera cases occurring in Altona could be traced to source waters in Hamburg (Logsdon, 2002). The importance of the slow sand filter's "schmutzdecke", roughly translated from German as "dirt blanket," began to be investigated around the turn of the century. The currently accepted definition of "schmutzdecke" refers to the thin layer of bacteria and soil particles located at the sand-water interface in a slow sand filter. It was recognized that the undeveloped filter cake (the sand bed of the filter not including the level of silt and biological organisms directly covering it) could not remove impurities as well as a filters containing a "gelatinous film." Early literature regarding the function of this layer is often quite confusing, as researchers developed their own definitions. The advent of other drinking water treatment system unit processes, such as, coagulation, rapid filtration and sedimentation technologies during the early 20th century decreased interest in slow sand filtration. Slow sand filtration research slowed during the middle of the 20th century, with no significant research completed between 1915 and 1970. During the early 1980s a resurgence of interest was stimulated by increasingly stringent EPA surface water treatment guidelines (Weber-Shirk & Dick, 1999), as well as the discovery that slow sand filtration could be a viable means of removing *Giardia lambia*, an intestinal parasite, from water sources (Logsdon et al. 2002). A freshly packed filter (not biologically mature) can remove 99% of *Giardia* cysts. The 1985 Bellamy et al. Study showed that only 26 cysts/L passed all the way through a slow sand filter with an influent *Giardia* concentration of 2,770 cysts/L (hydraulic loading rate = 0.47 m3/m2/hr). Rapid filtration (hydraulic loading rate =14 m3/m2/hr) resulted in less than 50% removal of *Giardia* cysts, showing that hydraulic loading rate is a critical variable influencing water quality in slow sand filters (Bellamy et al. 1985). Current interest in slow sand filtration focuses on pretreatment of water sources as well as applications to water treatment in small communities and in developing countries.

2.4.3.1 Mechanisms of microorganisms Removal by Slow Sand Filters (SSF)

Simple straining is the main removal mechanism in slow sand filtration. Contaminant particles larger than the pores between sand particles become trapped at the water-filter bed interface and are thus removed from the water, forming a filter cake. As finer particles become trapped in the filter cake, pore spaces in the filter cake become smaller and particle removal increases. While particle and contaminant removal increases, filtration rate decreases (Weber-Shirk et al., 1997). A severely decreased filter rate signals the need for filter

maintenance. In addition to mechanical straining, several biological mechanisms are thought to be at work in slow sand filters. The importance of a mature biological community's presence in a slow sand filter bed has been demonstrated, though there is a dearth of conclusive evidence on the subject (Haarhoff and Cleasby, 1991). One study (Bellamy et al. 1987), showed that a sand filter devoid of biological activity induced by high level of chlorination added during the experiment removed bacteria at a rate of 60%, in comparison with the 98% rate of removal observed in the control filter. The biological contaminants that were not removed are assumed to be smaller than the typical 2 μm pore spaces between sand particles (Bellamy et al., 1987). The effectiveness of a slow sand filter depends on several bacterially-mediated processes. It has also been suggested that bacteria entering the filter via source water produce extracellular polymers and attach to media in the filter, though this is considered to make a very insignificant contribution to removal (Logsdon 2002). Many have hypothesized that bacteria and particles are removed from source water via attachment to sticky biofilms, though this removal has not been directly measured (Weber-Shirk et al., 1997). Other biological processes with possible effects on filtered water quality may include :(1)- Death of influent bacteria (2)- Metabolic breakdown of organic carbon substrates by bacteria existing in the filter column. The bacterial population in a filter appears to be able to metabolize incoming bacteria effectively until a threshold concentration is reached.

2.4.3.2 The Biosand Filter

The Biosand filter was developed in the early 1990s by David Manz while working as a civil engineer at the University of Calgary. Biosand filters were first used for water treatment in 1993, when one was installed in each home in Valler de Menier, Nicaragua. The efficacy of the filter was clearly demonstrated in 1996, when Manz working for the NGO "Samaritan's Purse" reported that no one in Valler de Menier contracted cholera while many people in other portions of the country died from the disease. Recognizing the Biosand's potential for success as a simple and sustainable household water treatment technology, Samaritan's Purse has since installed 26,000 Biosand filters worldwide. At the end of 2001, various church groups and NGOs, including Samaritan's Purse, had installed more than 50,000 Biosand filters in more than 40 countries worldwide, including Haiti, the Dominican Republic, Nepal, and Nicaragua (CAWST, 2003).

Tse-Lue Lee's 2001 thesis focused on coliform and turbidity removal efficiencies of the same BioSand filter pilot program (Lee, 2001). Heather Lukacs' 2002 thesis continued evaluation of the BioSand filters in Nepal (Lukacs, 2002). Finally Melanie Pincus' 2003 thesis continued the work of Paynter, Lee and Lukacs, as well as evaluating a BioSand-based filter pitcher she developed (Pincus, 2003).

2.5 Option of greywater reuse

Since the emergence of source separation technology, it has been generally assumed that because greywater excludes toilet wastes, it should be technologically simpler and also more space-efficient to treat and recycle at the household and community level (Eriksson et al. 2002). While recent research has cast doubt on the first assumption, the second remains because of the exclusion of solid human wastes, greywater treatment can be achieved on less land than that required for full wastewater treatment (Toze 2006). This factor makes greywater treatment and reuse feasible at the individual household scale, even in urban settings and multi-unit dwellings. On the demand side, multiple end-uses for recycled greywater have been identified for both indoor and outdoor reuse. These include toilet flushing, laundry, and garden irrigation at the household level, and cooling, firefighting, and industrial washing at the commercial scale. Especially in water-scarce regions of the world, greywater recycling is increasingly common and commercial treatment.

2.5.1 Greywater storage

Storage of greywater prior to reuse is discouraged because it can affect the pathogen load of both raw and treated greywater. Dixon et al. (2000) tested a model for predicting quality changes in stored greywater, based on observed processes of settlement of suspended solids, aerobic microbial growth, anaerobic release of soluble COD from settled organic matter, and atmospheric re-aeration. The study suggests that storage of greywater for 24h could potentially improve water quality, but storage for more than 48h could seriously deplete dissolved oxygen (DO) levels and lead to what they call "aesthetic problems", including anaerobic processes and associated smells. Rose et al. (1991) found a 1-2 log increase in total and fecal coliform counts over the first 48h of greywater storage.

2.5.2 Greywater Recycling

To recycle greywater safely, users must understand the nature of the grey water itself as well as the natural cycles and processes involved in the purification of it. Each set of circumstances requires its own unique recycling system for optimum results. Possible applications identified for the reuse of greywater at the household level include most commonly toilet flushing, cars washing, and lawn and/or garden irrigation. These reuse applications alone have the potential to significantly reduce domestic water consumption. Potential impacts of these most common forms of greywater reuse have been outlined by (Christova-Boal et al. 1996). With regard to reuse for toilet flush water, possible hazards include physical clogging of toilet inlet pipes and anaerobic decomposition of insufficiently treated water in the toilet tank. (Lazarova, et al., 2003) nevertheless emphasize the appropriateness of toilet-flushing as an end-use for recycled greywater in a review that outlines successful examples of water reuse for toilet flushing in large developments around the world.

2.5.3 Greywater reuse for irrigation

Greywater contains contaminants, which vary in their effect from beneficial to detrimental for irrigation of plants. These contaminants include nitrogen, phosphorous and potassium, which in most cases are beneficial to plants, except for a small number of native plants that have a unique low phosphorous or nutrient tolerance from having evolved and developed in marginal nutrient depleted soils. Greywater also generally has a slightly alkaline pH making it preferable not to use greywater to irrigate acid loving plants, unless the pH is managed, by digging soil conditioners such as peat or compost into the soil. Suitable plants and soil conditioners should be discussed with your local gardening centre. When greywater is being reused for garden and landscape irrigation is by far the most common mode of domestic greywater reuse (Madungwe & Sakuringwa 2007). Research into the implications of greywater reuse for irrigation often focuses on physical properties of the greywater that could potentially affect the long-term quality and productivity of soils. Elements of health and environmental significance that become important when greywater is discharged outdoors have been identified by (Toze, 2005 & Roesner et al., 2006). Environmentally important factors include pathogens, pH, salinity, metals and organic chemicals that could accumulate in receiving soils (Roesner et al,. 2006). Of these, enteric pathogens are identified as the most

significant direct risk to human health, especially when there is potential for residents to come into direct contact with the re-circulated water.

2.6 CONCLUSION

A survey of the literature has indicated that the primary focus of research has been on the assessment of survival of bacteria in the water and greywater. Therefore many previous studies have not taken into account the presence and effects of abiotic factors on those bacteria and coliphage MS2. Only a few such studies have been carried out. However, it is already apparent that the rate at which bacteria and coliphages MS2 is influenced by temperature and salinity (Roszak, 1986) and by low intensity visible light (Barcina et al., 1989). The following study was undertaken with the broad objective of investigating the influence of some of the more important environmental factors, i.e., temperature, salinity and sunlight, on the survival of bacteria and coliphage under conditions of low nutrient concentrations and treatment by sand filter. The following observations have been based on this concept: in water, a combination of sunlight and photocatlysis are mainly responsible for the rapid decline of *E.coli*, *Pseudomonas aeruginosa* and MS2 coliphage though temperature and sedimentation also contribute. Survival is greater in estuarine waters (Fujioka et al., 1981), probably because of reduced salinity and the protective effect of turbidity against sunlight. Survival in freshwater is less clearly understood but temperature (Mancini, 1978) and salinity (Fujioka et al., 1981) may be important. Darkness and nutrient have clearly less influence on the survival of bacteria in the greywater for short times. That bacteria and coliphageMS2 in waters and greywater can be detected using traditional techniques for bacterial counts and coliphage culture is evident(Adams, 1959) according to (ISO 10705-2, 2000), In addition to investigating the influence of the more important environmental factors, investigating treatment of greywater by inactivation microorganisms and removal bacteria using slow sand filter and photo-inactivation. Also the final objective of the present study was on greywater storage and applications. It is vital that we establish safe practices for reusing our water in order to remain healthy and productive in a water scarce future.

CHAPTER THREE

Treatment of greywater by slow sand filtration

3.1. INTRODUCTION

The treatment of greywater is a valuable ecnomical source of water in arid and semi arid country. In recognition of the importance of conserving its water resources, greywater consideration a little source is polluted. Currently, more and more countries suffer a depletion of their reserves. There is more to a sharp increase in water demand due to increasing world population and consumption of each individual. These issues important to water led to the development of solutions for recycling of wastewater. They reduce water withdrawals to meet the needs of populations, as drinking water is replaced with recycled water in applications where a lower quality is sufficient.

Greywater is defined as domestic wastewater (ERD) to the exclusion of water containing urine and feces, which they are called black water. This greywater is characterized according to their origin, which allows to adopt a specific treatment can be implemented to purify these waters for reuse for non-potable uses such as irrigation. This work divided to two parts: A first part focus to clean filter and sand filter. In this part was studied characterization of sand and Hydrodynamic parameters like as RTD, HTD and porosity. A second part is dedicated to the study of slow sand filter for the treatment of greywater. In this part was measured the physico-chemical parameters like as; turbidity, COD, pH and conductivity. Survival studied of microorganisms like as *E.coli, P.aeruginosa, E. Faecalis* and total bacterial flora and biofilm development was also investigated.

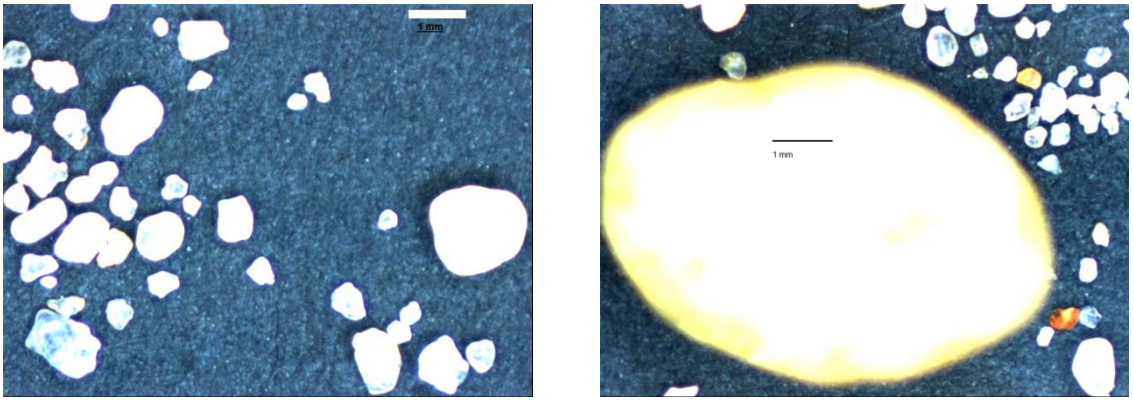
3.1.1. Sand characterization

Two types of sand were used in the manufacture of filter media:

- The first is a commercial sand frequently used in the filtration of swimming pools (SPS);
- The second from the Egyptian desert (EDS) has previously been prepared before implementation.

Because this sand contains very fine particles, a preparation was needed before it could be used. This sand has been washed to remove all the fine clay particles, and it was placed in an oven at 100 ° C for 24 hours before undergoing sieving. Sieving was then performed using a series of sieves with opening size ranging from 2500 µm to 200 µm. The tests sieves are arranged in a stack with the largest mesh openings at the top of the stack. The sample is placed on the top sieve. After sieving, all particles passing through the sieve with 200 µm

opening were rejected. It should be noted that after removal of much finer grains, the sand used is heterogeneous and contains some gravel of size up to several millimeters (Figure 3.1).



A **B**

Figure 3.1. (A) Example of EDS samples treated in image analysis; (B) Example of a EDS sample used in image analysis with a gravel larger than 6mm.

The both sand were analyze by X ray energy dispersive fluorescence (EDXRF) to compare their elemental composition. The obtained results are presented table 3.1. The two sand presents a high content in silicate and alumina. The biggest difference is the contents in iron and titanium present in EDS and in magnesium which is present in SPS.

Analyte	EDS	SPS
SiO_2	87.41 %	85.33 %
Al_2O_3	5.87 %	2.16 %
SO_3	2.44 %	2.29 %
MgO	nd	8.60 %
P_2O_5	1.25 %	1.26 %
K_2O	0.91 %	0.04 %
CaO	0.87 %	0.24 %
Fe_2O_3	0.87 %	0.06 %
TiO_2	0.34 %	nd
ZnO	0.02 %	0.02 %
ZrO_2	0.01 %	nd
SrO	0.01 %	nd

Table 3.1. Comparison of the elemental composition obtain by EDXRF (nd; not detected).

Measurement techniques for characterization of particle shape have been developed with time in many application areas, in basic research and for industrial purposes. Sieving is a classical method for measuring grain-size distributions. However, the method is not suitable for the fine fractions. In this study, image analysis was used to measure various grain dimensions such as the mean diameter. For each type of sand, hundreds of particles were observed and images were recorded by a CCD camera. The image obtained is processed using commercial image analysis software (OPTIMAS 6-Bioscan). The number of particles studied is rather important to represent the whole sample, the criterion chosen to estimate this number is based on the calculation of the standard deviation which must be stabilized.

A thin layer of gold coating (15 nm) was then deposited using an Edwards sputter coater (S15OB) prior to the morphological examination using a scanning electron microscope (SEM) (Carl Zeiss EVO 40) operating at 15 kV. Pictures of SPS grain recorded after SEM observations are shown in Figure 3.2.

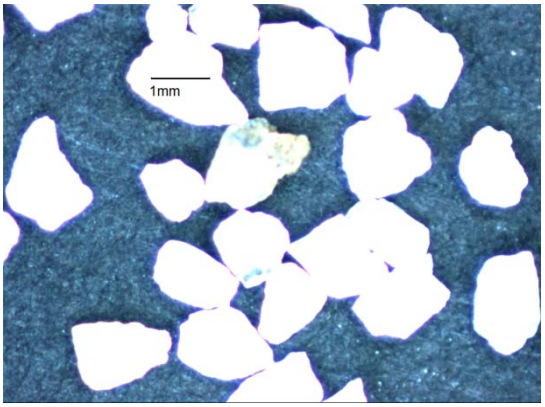


Figure 3.2. SEM images of SPS used

Grading parameters are reported in Table 3.2. The technique used allows to measure the projected area of particle, and the equivalent characteristic defined is the equivalent diameter surface. In our calculation, the equivalent mean diameter of the grain is defined as the geometrical diameter of a sphere that has the same surface as the particle. Representative particle size distribution of different sands is presented in Figures 3.3, 3.4. The average

particle size, i.e. the average equivalent diameter denoted dg and obtained by calculation of arithmetic mean. d_{10}, d_{60} and d_{90} represent the grain diameter in µm, for which, 10%, 60% and 90% of the sample respectively, are finer than. The size distribution is represented by the uniformity coefficient, which enables you to see how well graded your sand sample is (that is, whether there is a whole different range of sizes, or whether most of the sample is only one size). This is calculated by taking the d_{60} and dividing by the d_{10}. For slow sand filtration, some degree of uniformity is desirable in order to ensure that the pore sizes between the grains are reasonably regular and that there is sufficient porosity.

Circularity values were calculated from 2D sand images. The circularity is defined as the ratio of the area perimeter length (P) squared divided by the surface area (A) (i.e., $C=P^2/A$). This is a dimensionless number with a minimum value of four pi (12.57) achieved only for circular boundaries (the value is 16 for square boundaries and 20.78 for equilateral triangular boundaries). Circularity values of sand grains are ranged from 13.8 to 44.8 with a mean value close to 16.9.

Sand	d_{10} (µm)	d50 (µm)	d90 (µm)	d_{min} (µm)	d_{max} (µm)	d_m (µm)	Uniformity coefficient	Circularity
EDS	254	452	856	22.4	6660	515	2.02	16.9
SPS	669	1170	1631	120	2143	1150	1.89	17.7

Table 3.2. The different parameters obtained by image analysis for the two sands

For a given particle size, *x*, the Q_0-value represents the percentage of the particles finer than *x*. The particle size distribution (PSD) is displayed as a diagram. The range of measured particle sizes is discretized into n size intervals and the particles are sorted into the corresponding size class. The frequency number, denoted f_N, is the ratio between the number of particles per class and the width of the class. It is normalized by total number of particles. The normalized frequency number for each size class i is defined by/

$$f_N(i) = \frac{\text{total number of grains in the class i}}{(\text{total number}) \text{ x } (\text{width of class i})} \qquad \text{Eq. 3.1.}$$

Graphical representations of the size distribution of the two sands are presented in Figures 3.3, 3.4 and 3.5.

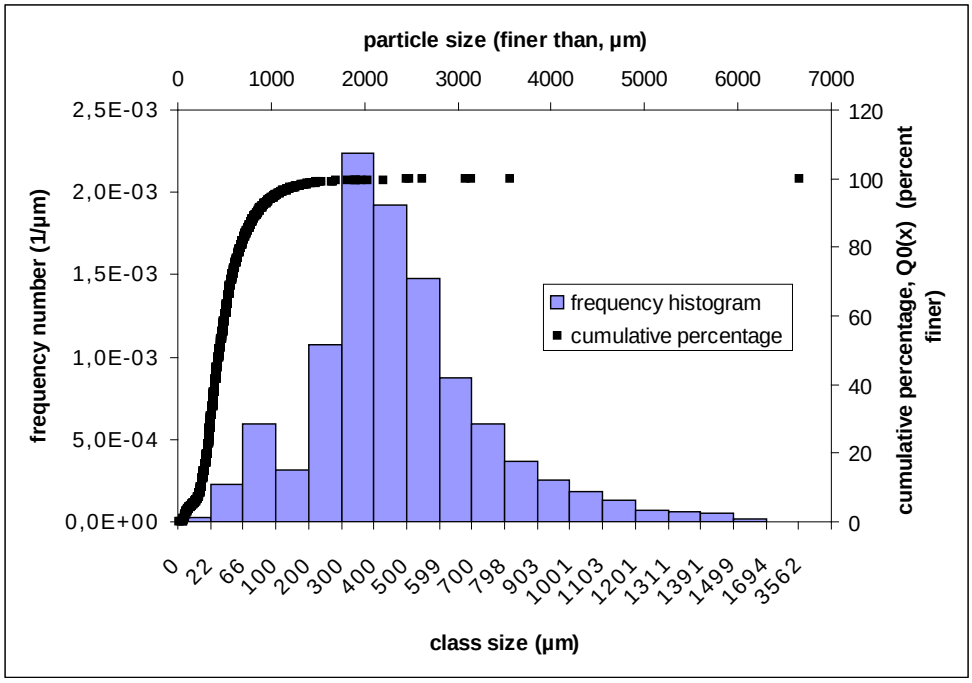


Figure 3.3. Grain size distribution of the EDS

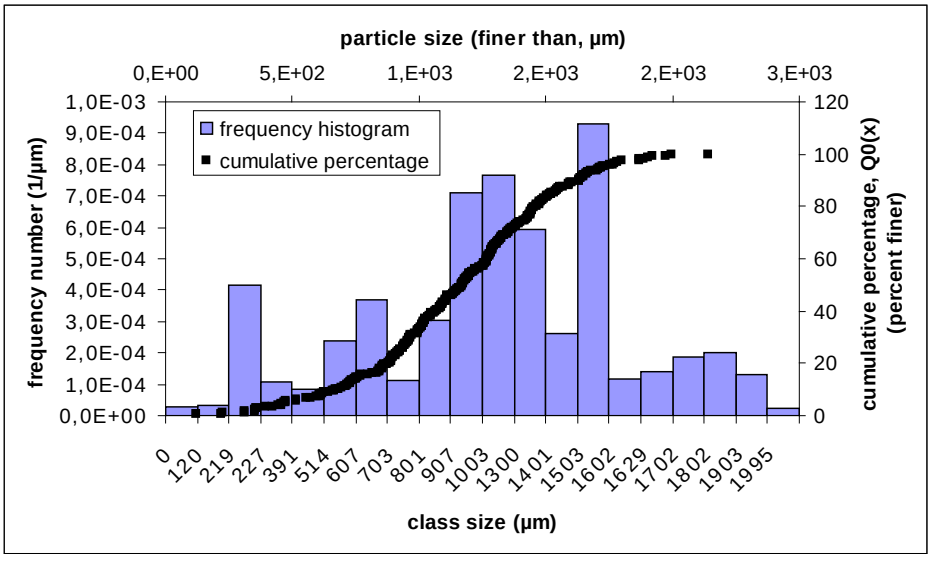


Figure 3.4. Grain size distribution of the SPS

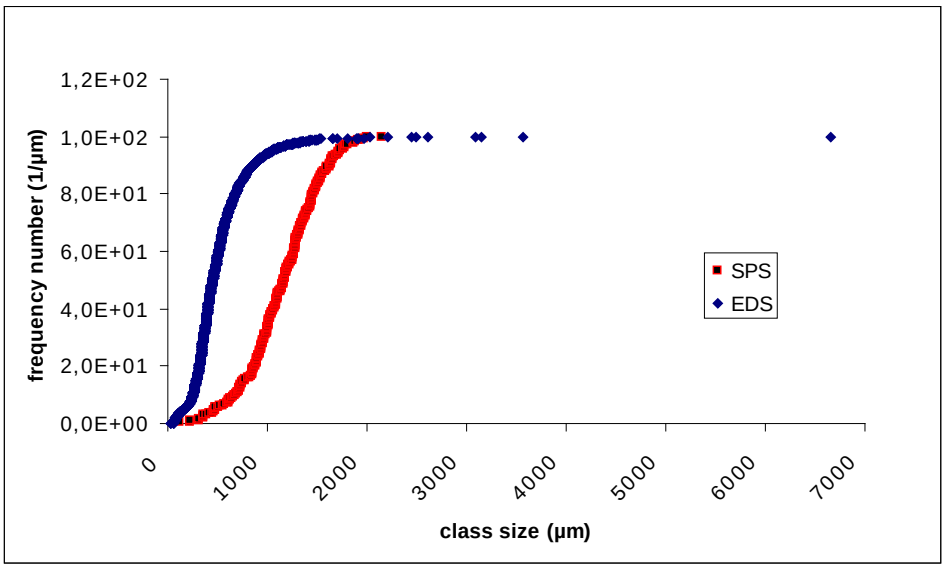


Figure 3.5. Comparison of the particle size distributions of the two sands

3.1.2. Hydrodynamic characterization of clean filter media

The sand filter used for the biofiltration has been previously characterized to better understand the behavior in the process of retention. Measurements of pressure drop were carried out during the flow of water through sand packing. Two types (EDS and SPS) of sand were used to prepare the beds in our fixed pressure drops experiments. The aim of these measurements is to evaluate the structural parameters of the clean filter media. Figure 3.6 shows a schematic diagram of the experimental setup used in our tests. The sand is contained in a plexiglass column (inner diameter of 60 mm) and the pressure measurements were carried out, using differential pressure sensors DeltaBar (ENDRESS and HAUSER), on different sections 10 cm in length. The pressure sensors can perform measurements on nominal measuring range from 0 to respectively 25, 100 and 500 mbar with an accuracy of 0.1% from the top of the scale. The measurements were repeated three times to ensure reproducibility. Porosity was determined after packing the column, prior to experiments. It was calculated by measuring the volume of the solid phase needed to pack a column of known total volume and was found to be 0.42 ± 0.01 and 0.38 ± 0.01 respectively for the SPS and EDS. The water was circulated in the downward direction through the fixed beds using a volumetric displacement pump. The liquid flow rate was measured by a rotameter. The experiments were performed at 18°C, the value of the fluid temperature was controlled at the inlet of the filter.

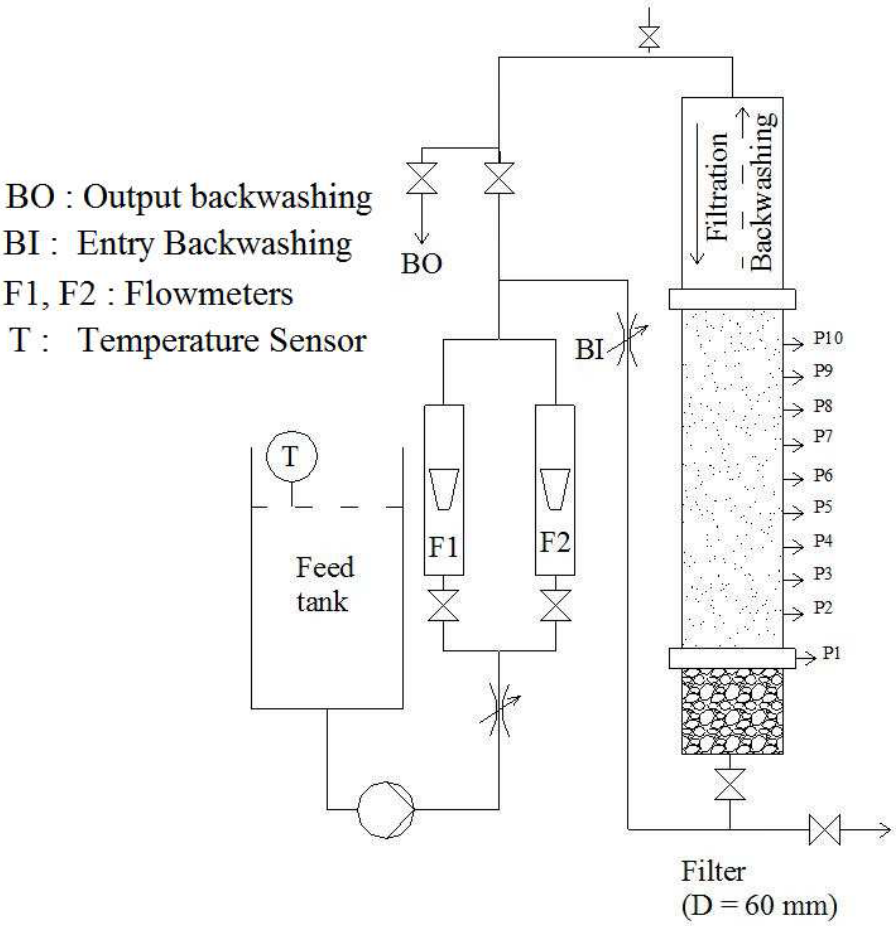


Figure 3.6. Diagram of the experimental setup used for pressure drop measurements.

For both types of sand, we adopted the following representation for the pressure gradient:

$$\frac{\Delta P}{H} = A\, u_0 + B\, u_0^2 \qquad \text{Eq. 3.2}$$

The coefficients A and B of both viscous and inertial terms of pressure gradient were determined experimentally.

We then applied an existing model based on the representation of capillary porous media proposed by Comiti and Renaud, (1989). In this model, the bed is considered as a bundle of identical cylindrical tortuous pores of diameter d and length L (m). If H is the height of the column (m), the tortuosity factor of the porous medium is defined as: $\tau = L / H$. The diameter of the pores is calculated by assuming that their developed surface area is identical to the fixed bed surface area actually reached by the fluid flow:

$$d_{pore} = \frac{4\varepsilon}{a_{vd}(1-\varepsilon)}$$ Eq.3.3

a_{vd} is the dynamic specific surface area of the porous medium (m^{-1}). It is defined as:

$$a_{vd} = \frac{surface\ area\ presented\ by\ the\ particle\ to\ the\ flow\ (m^2)}{volume\ of\ solid\ (m^3)}$$ Eq 3.4

The mean velocity u ($m.s^{-1}$) in the pore is:

$$u = \frac{u_o \tau}{\varepsilon}$$ Eq. 3.5

This representation is associated to a model for pressure drop evaluation for Newtonian fluid flow. The general equation of the model allowing the calculation of pressure gradient, taking into account a correction for wall effects with a column diameter *D*, is:

$$\frac{\Delta P}{H} = N u_o + M u_o^2$$ Eq. 3.6.

with, $$N = 2\mu \frac{(1-\varepsilon)^2}{\varepsilon^3} a_{vd}^2 \ \tau^2 \left[1 + \frac{4}{a_{vd} D\ (1-\varepsilon)}\right]^2$$ Eq. 3.7.

and $$M = \left[0.0413\ \ (1-(1-\frac{d_g}{D})^2) + 0{,}0968\ \ (1-\frac{d_g}{D})^2\ \right] \tau^3 a_{vd} \rho \frac{(1-\varepsilon)}{\varepsilon^3}$$ Eq. 3.8.

Obtaining the experimental values of A and B and using the capillary model described before allowed us to evaluate both structural factors of the porous medium: τ and a_{vd}. Results concerning structural parameters obtained for each sand from pressure drop measurements are summarized in Table 3.3.

The pressure gradient across the different sections of 10 cm thickness is presented in Figure 3.7 and 3.8. For SPS, measurements of pressure drop occurred for superficial velocities comprised between 4.10-3 m/s and 6.10-2 m/s. For this sand we have carried out the experiments in inertial regime, this is confirmed by the correlation obtained.

For the finest EDS we could not exceed the Darcy regime. For this reason, in order to estimate the dynamic specific area, we chose to do a rough calculation by setting reasonable value of tortuosity $\tau = 1.50$ (Comiti & Renaud, 1989).

Permeability (K) in the first section (upper section) of each sand filter was determined using the results in Darcy regime.

Obtaining the experimental values of A and B and using the capillary model described before allowed us to evaluate both structural factors of the porous medium: τ and a_{vd}. Results concerning structural parameters obtained for each sand from pressure drop measurements are summarized in Table 3.3.

Sand	**Coefficient A x 10^{-6} (SI)**	**Coefficient B x 10^{-7} (SI)**	**d_g (µm) and range**	**Tortuosity (τ)**	**a_{vd} (m^{-1})**	**Pore diameter (µm)**	**K (m^2)**
SPS	1.148	2.322	120 – 2143 1150	1.69	6570	440	
EDS	2.230	/	22.4 – 6660 515	1.50	26 599	92	

Table 3.3. Structural parameters of the beds studied.

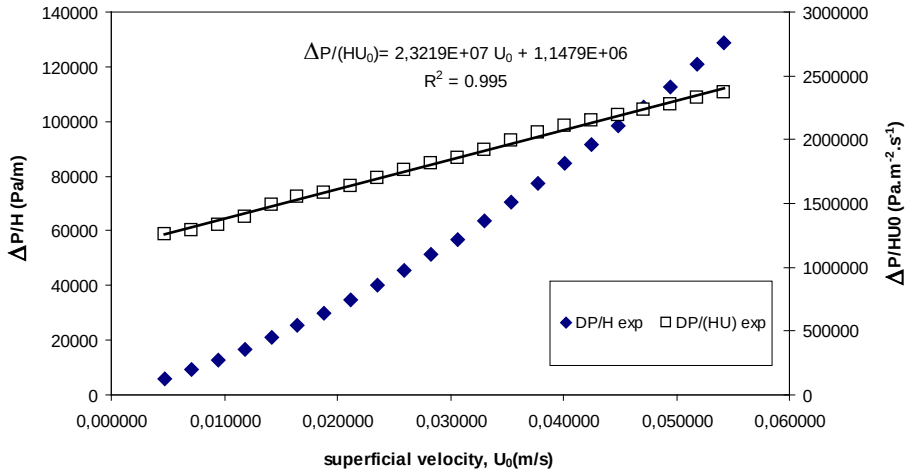


Figure 3.7. Pressure gradient across the fixed bed as a function of superficial velocity (SPS at 23.3 °C).

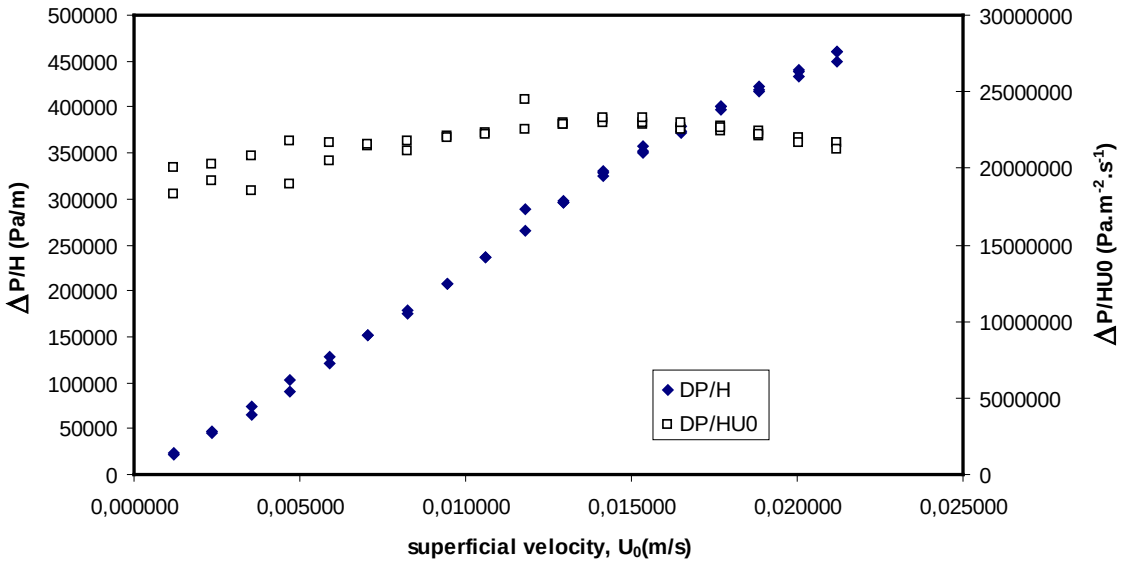


Figure 3.8. Pressure gradient across the fixed bed as a function of superficial velocity (EDS at 23.3 °C)

The value of the dynamic specific surface area obtained for the two sands is greater than the geometric surface area ($a_s = 6/d_g$) calculated from the grain average diameter d_g. The pressure drop measurements were performed in the upper portion of the sand packing and in this region are located finer particles due to the fluidization of particles before starting the measurements.

3.1.3. Experimental determination of residence time distribution (RTD)

Knowledge of the actual behavior of the filter can provide information to better understand the filtration process. To model the flow in the filter, the experimental determination of the residence time distribution (RTD) is performed using a tracer substance. In the next section we recall at first the theoretical elements concerning the distribution of residence time in a reactor.

The response to an injection pulse type gives direct access to the function E (t).

In a pulse input, an amount of tracer No is suddenly injected in one shot into the feed stream entering the reactor in as short time as possible. The outlet concentration C is then measured

as a function of time. The effluent concentration-time curve is referred the C curve in the RTD analysis.

For a selected increment of time Δt sufficiently small than the transit time, the amount of tracer material, ΔN, leaving the reactor between time t and t + Δt is:

$$\Delta N = C(t)Q\Delta t \qquad \text{Eq. 3.9.}$$

where, Q is the effluent volumetric flow rate. In other words, Δ*N* is the amount of material that has spent an amount of time between *t* and *t* +Δ*t* in the reactor. If we now divide by the total amount of material that was injected into the reactor, *N*0, we obtain

$$\frac{\Delta N}{N_0} = \frac{QC(t)}{N_0}\Delta t \qquad \text{Eq. 3.10.}$$

which, represents the fraction of the material that has a residence time in the reactor between time *t* and *t* + Δ*t*.

For a pulse injection we define $E(t) = \frac{QC(t)}{N_0}$ Eq. 3.11

So that $\frac{\Delta N}{N_0} = E(t)\Delta t$ Eq. 3.12.

This quantity *E*(*t*) is called the residence time distribution function. It describes in a quantitative manner how much time different fluid elements have spent in the reactor.
Re-writing the above equations in the differential form,

$$dN = QC(t)dt \qquad \text{Eq. 3.13.}$$

and integrating, we obtain

$$N_0 = \int_0^\infty QC(t)dt \qquad \text{Eq. 3.14.}$$

The volumetric flow rate Q is constant, and so we can define *E*(*t*) as: The response to an injection pulse type gives direct access to the function E (t):

$$E(t) = \frac{C(t)}{\int_0^\infty C(t)dt} \qquad \text{Eq. 3.15.}$$

The integral in the denominator is the area under the C curve.

Detectors generally do not measure the concentration C (t) but a parameter y (t) proportional to C (t) and E (t) is then:

$$E(t) = \frac{y(t)}{\int_0^\infty y(t)dt} \qquad \text{Eq. 3.16.}$$

Figure 3.9 and 3.10. gives typical example of response.

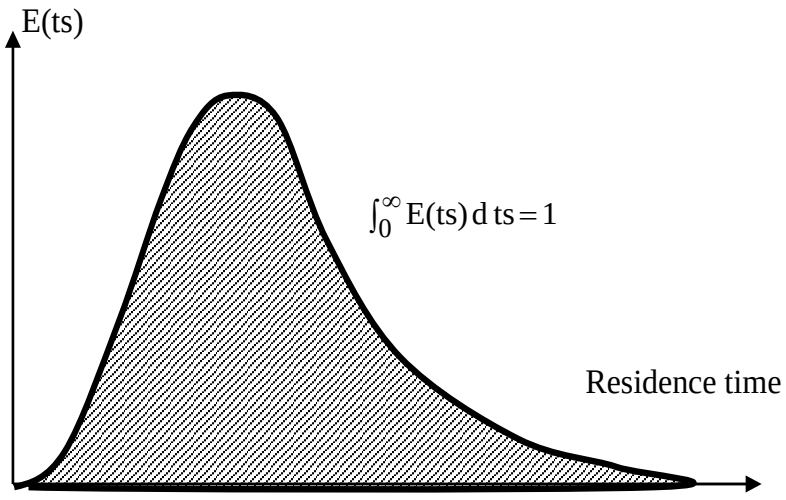


Figure 3.9. Typical example of response

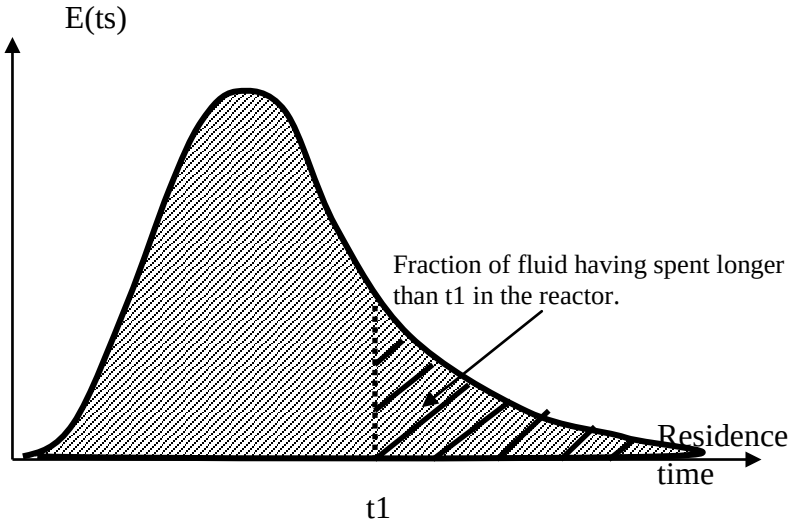


Figure 3.10. Typical example of response

We can also determine the average residence time:

$$\bar{t}_s = \frac{\int_0^\infty tC(t)dt}{\int_0^\infty C(t)dt} = \frac{\sum_{i=1}^{n} t_i C_i(t)\Delta t_i}{\sum_{i=1}^{n} C_i(t)\Delta t_i} \qquad \text{Eq. 3.17}$$

The variance is defined as (Eq. 3.18.):

$$\sigma^2 = \int_0^\infty (t - \bar{t}_s)^2 E(t).dt = \frac{\int_0^\infty (t-\bar{t}_s)^2\, y\, dt}{\int_0^\infty y\, dt} = \frac{\int_0^\infty t^2\, y\, dt}{\int_0^\infty y\, dt} - \bar{ts}^2 = \frac{\sum_{i=1}^{n} ti^2 yi\Delta ti}{\sum_{i=1}^{n} yi\Delta ti} - \bar{t}_s^2$$

In our experiments, two columns were used for measurements:

3.1.4. Residence time distribution (RTD) test in the case of filtration rate between 2.9 10^{-3} and 7 10^{-3} m / s: Experiments and results

The first laboratory-scale packed bed is a column with an inside diameter of 60 mm: the height of the packing is 970 mm and the distance between the injection and sampling point output is 910 mm. This column was used for tests with relatively high flow rates (between 50 and 120 mL / min).

After steady state flow was reached a pulse type injection is performed using fluorecein ($C_{20}H_{12}O_5$) solution of concentration 10^{-3} mol/L as tracer. The tracer is injected into a

narrowed area to ensure a turbulent flow and mixing. The amount of tracer injected was evaluated according to the amount of tracer that can be analyzed out, and taking into account the input stream into the filter. A volume of 5 mL of this solution is manually injected at very short time intervals at the filter input. Samples are taken in the last section of the packing to avoid taking into account the perturbations from the circuit downstream of the filter in the measurement of DTS. The outlet flow absorbance is measured using a spectrophotometer (490 nm) at 10 seconds intervals for a total period exceeding the transit time calculated theoretically. More than 40 samples are carried out, which provides a representative residence time distribution.

We performed for each sand DTS measurements at different flow-rates and with two different volumes of tracer injected (50 mL and 5 mL). In order to have time and concentration scales independent of the flow reactor and of the amount of injected tracer, we adopted a normalized representation. In this representation, we used:

- In abscissa coordinate, the reduced time as the ratio of the real-time and mean hydraulic time T_h:

$$\mathbf{T_h = \frac{interstitial\ \ volume}{flow-rate} = \frac{\varepsilon\ apparent\ volume}{flow-rate} = \frac{\varepsilon\left(L\frac{\pi D_c^{\,2}}{4}\right)}{Q}}$$

- In y coordinate, the reduced concentration: $C(t)/C_0$

Where C_0 is defined by:

$$C_0 = \frac{Q}{V_i}\sum_{i=1}^{n} C_i(t)dt \quad \text{and} \quad \frac{C(t)}{C_0} = \tau\, E(t)$$

The results are presented in Figures (3.11; 3.12). The tracer response curves for the various tests are different. This is due to the difference in the configuration and structure of the packing after backwash. The difference is much less pronounced in the case of commercial sand; this can be attributed to sand size distribution and homogeneity. The size distribution for SPS is less spread out than the EDS.

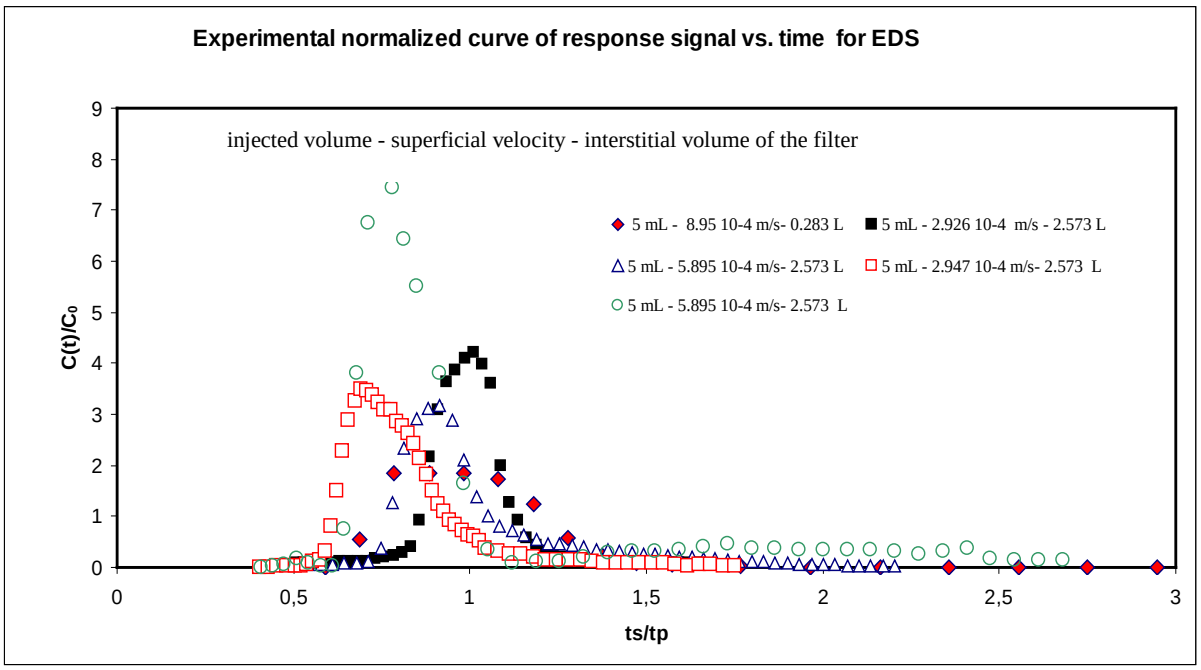


Figure 3.11. Experimental normalized curve of response for EDS

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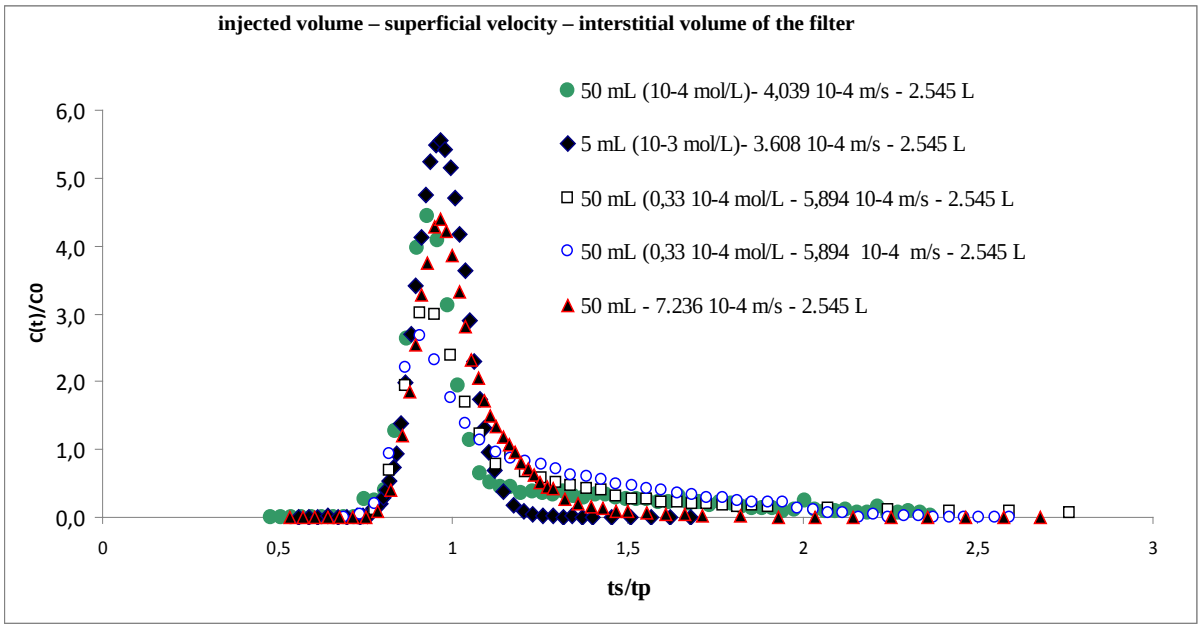

Figure 3.12. Experimental normalized curve of response signal vs. time obtained for SPS

The function E(ts) is calculated from experimental data using Eq. (3.16). The mean residence time ts is calculated using Eq; (3.17) and compared to the predicted hydraulic time based on the flow rate and the volume of the porous medium. The variance of the RTD is calculated from Eq. (3.18). The experimental function E(ts) is compared with the two reactors models:

the Plug Flow with Axial Dispersion (PFAD) and the Continuous Stirred Tank Reactors in series. The different curves for E (ts) obtained for different flow rates are presented in Figures (3.13 - 3.22).

- The Plug flow with Axial Dispersion (PFAD) depends on one parameter and the mathematical solution of E(ts) is given by:

$$E(t) = \frac{1}{2}\sqrt{\frac{Pe}{\pi \tau t}} \exp\left(- \frac{Pe(\tau - t)^2}{4 \tau t} \right)$$

The global Peclet number, Pe, is defined as the ratio of advection to dispersion:

Pe =UL/Dax where L is the total vertical distance between the tracer injection and sample points. The axial dispersion coefficient Dax can be obtained from the Peclet number.

The Peclet number is estimated by minimizing the SSE (sum of the squared errors) between the model and the data by changing Pe or J.
(or)

The experimental RTD is well reproduced by the PAD model

In the case of the Continuous Stirred Tank Reactors CSTR in series, E(ts) is given by:

$$E(t) = \left(\frac{J}{\theta} \right)^J \frac{t^{J-1} \exp(-Jt/\theta)}{(J-1)!}$$

The number J of CSTR in series is estimated by minimizing the SSE (sum of the squared errors) between the model and the data. All experimental parameters obtained are summarized in Table 3.4.

The results show that the plug flow model without axial dispersion appears best suited to represent the flow in the present conditions.

Sand	*Flow rate (mL/min)*	*Residence time seconds*	*Hydraulic time*	*superficial velocity (m/s)*	*Peclet number*	*J*	Variance	Ecart-type
EDS	**50**	**1196**	**1188**	**2.93E-04**	**210**	**95**	***29319***	***171***
EDS	**49.64**	**1249**	**1188**	**2.95E-04**	**157**	**75**	***47266***	***217***
EDS	**50**	**153**	**152.68**	**8.95E-04**	**44**	**21**	***1282***	***36***
EDS	**100**	**610.1**	**590**	**5.89E-04**	**300**	**90**	***31985***	***179***
EDS	**100**	**610.7**	**590**	**5.89E-04**	**145**	**70**	***25699***	***160***
SPS	**68.52**	**1103**	**1003**	**4.04E-04**	**250**	**90**	***104569***	***323***
SPS	**61.2**	**1042**	**1073**	**3.61E-04**	**400**	**100**	***1089136***	***1044***
SPS	**122.75**	**573**	**560**	**7.24E-04**	**260**	**100**	***6137***	***78***
SPS	**100**	**837**	**695**	**5.89E-04**	**180**	**90**	***89947***	***300***
SPS	**100**	**816**	**695**	**5.89E-04**	**130**	**65**	***56960***	***239***

Table 3.4. Results of experimental study of the RTD for high flow rates (50 – 100 mL/min).

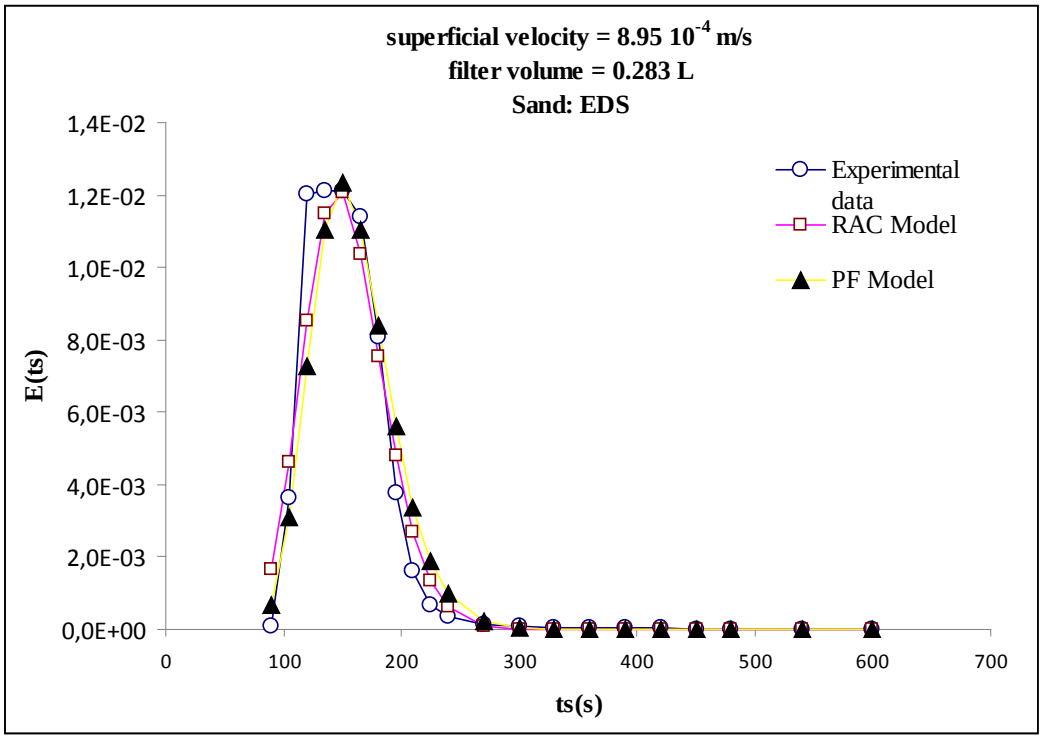

Figure 3.13. E(t) vs time for EDS

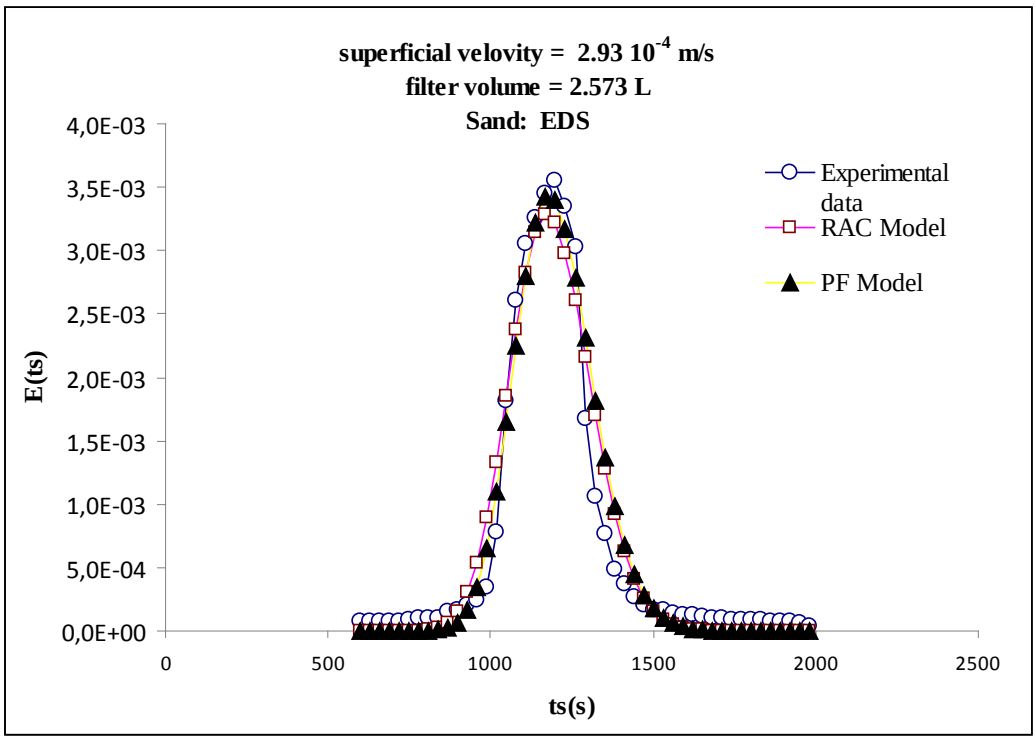

Figure 3.14. E(t) vs time for EDS

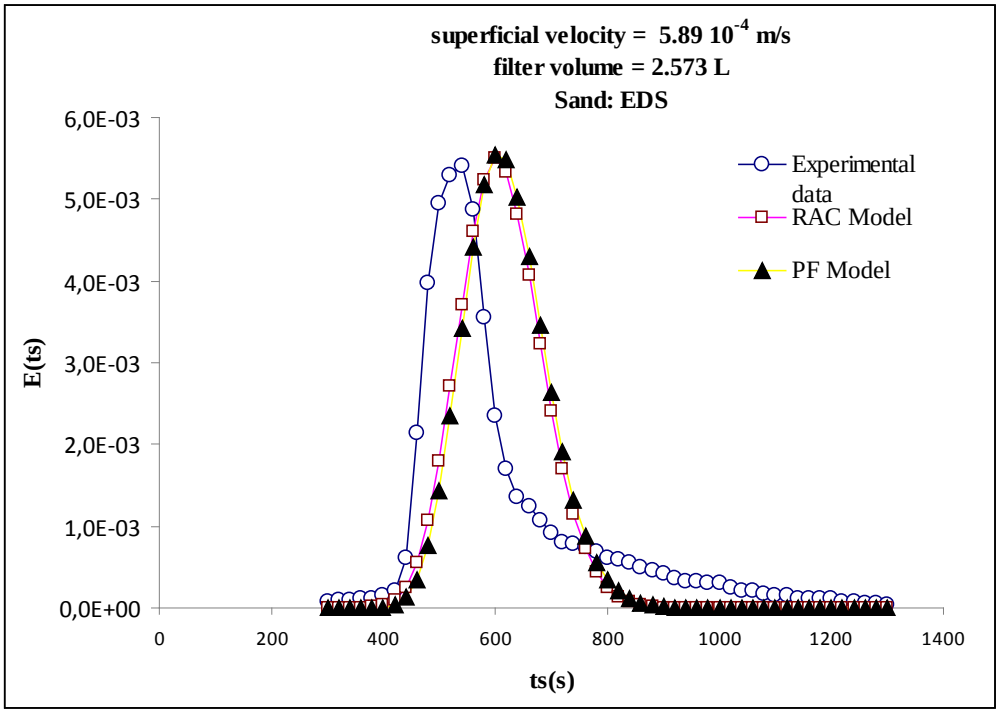

Figure 3.15. E(t) vs time for EDS

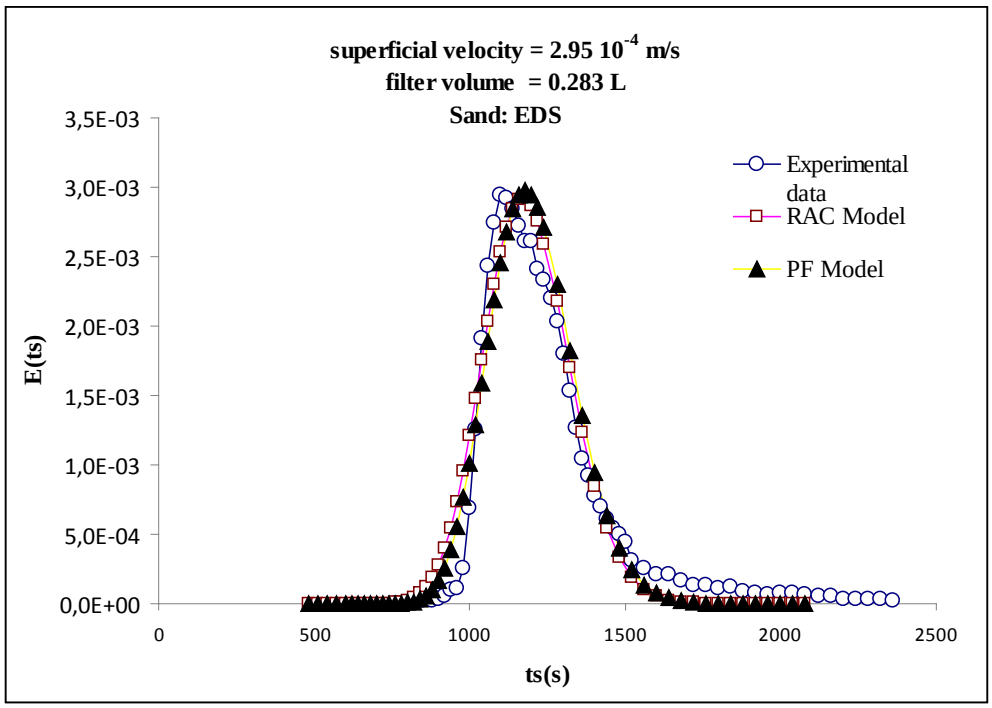

Figure 3.16. E(t) vs time for EDS

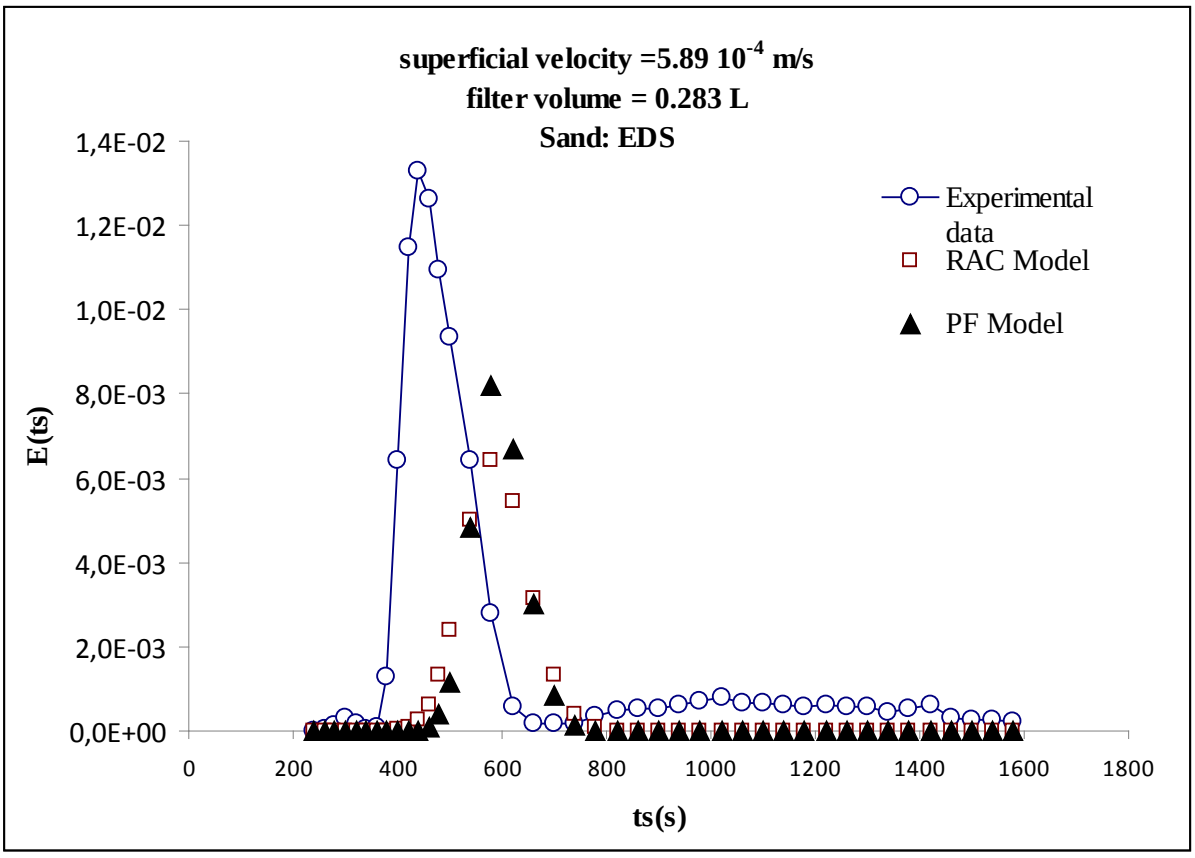

Figure 3.17. E(t) vs time for EDS

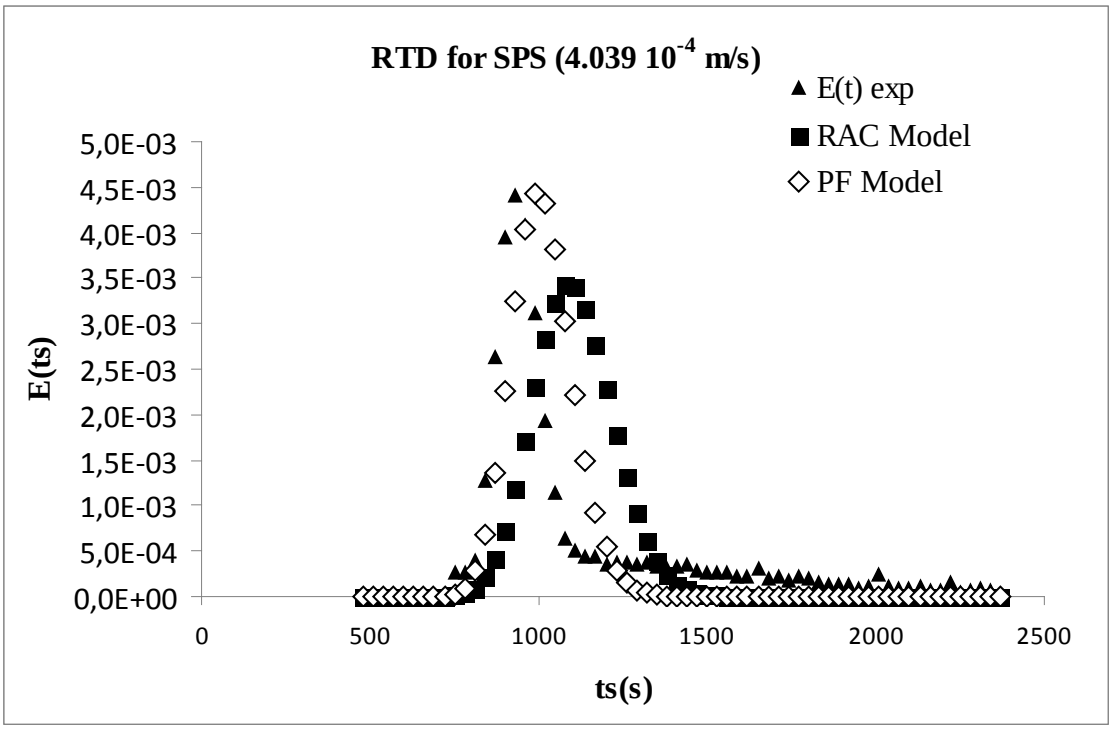

Figure 3.18. E(t) vs time for SPS

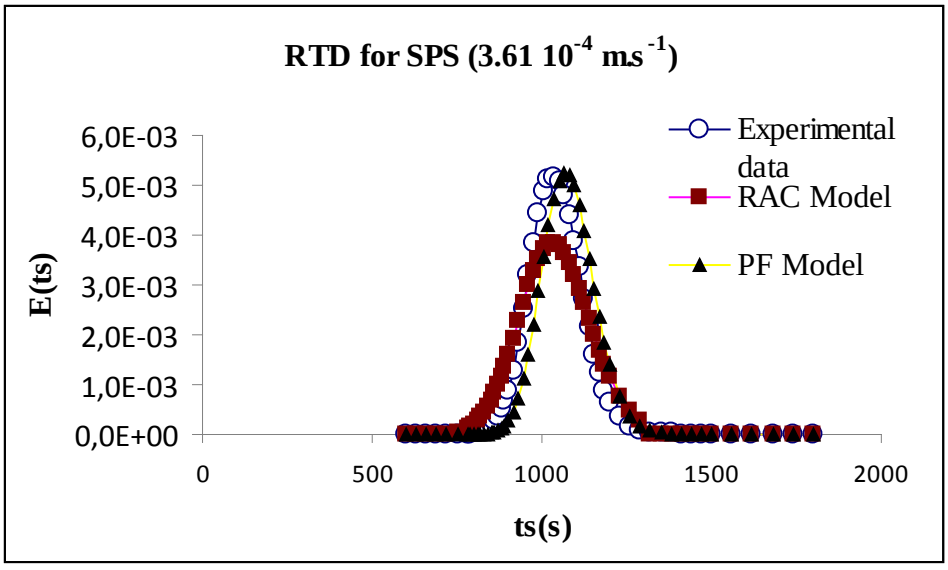

Figure 3.19. E(t) vs time for SPS

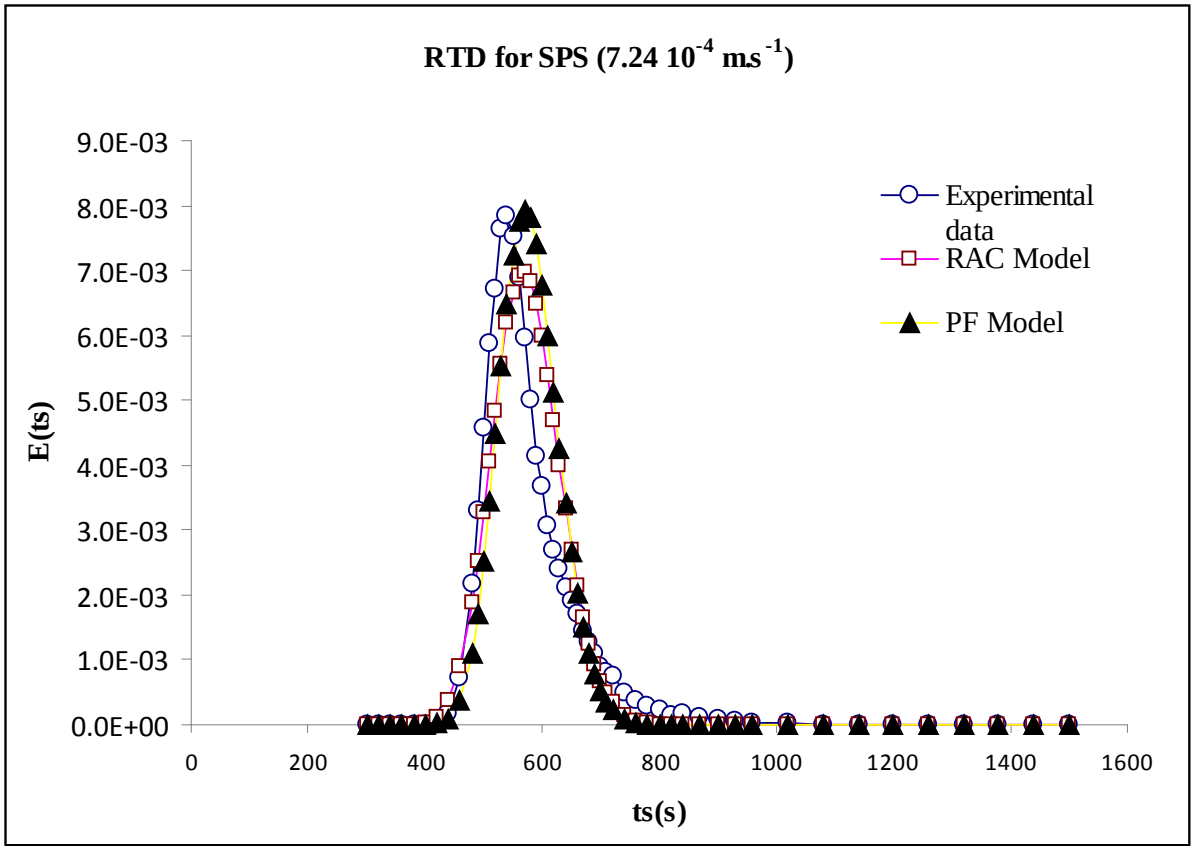

Figure 3.20. E(t) vs time for SPS

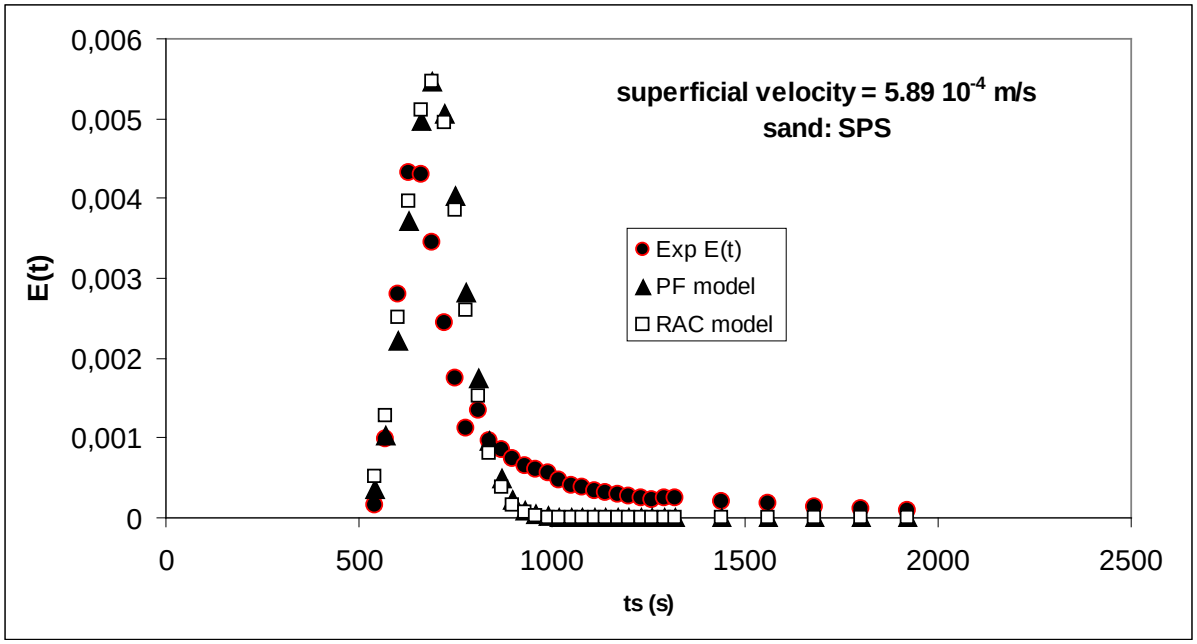

Figure 3.21. E(t) vs time for SPS

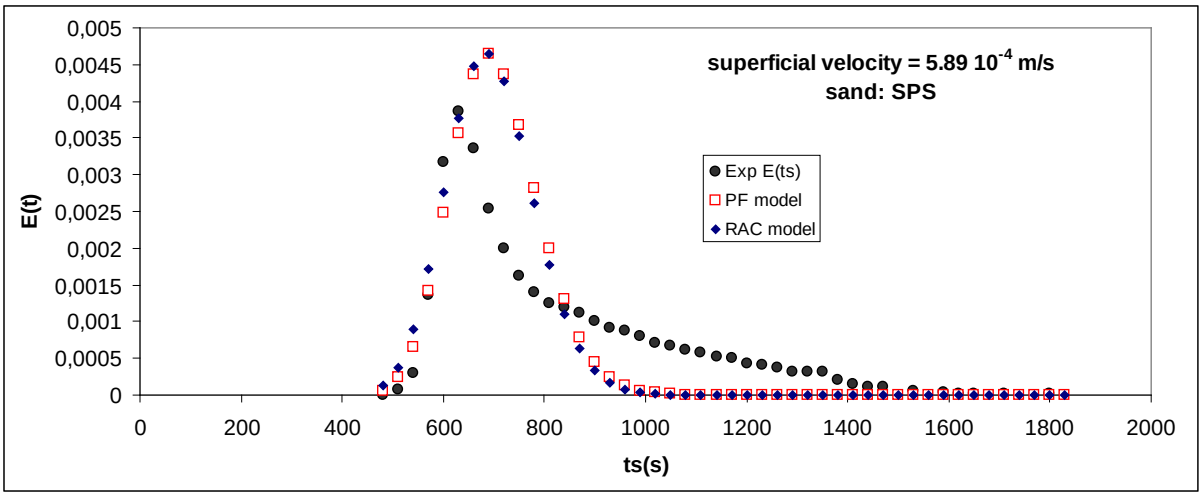

Figure 3.22. E(t) vs time for SPS

3.2. SLOW SAND FILTER (SSF)

3.2.1. Materiel and Methods

3.2.1.1 Slow Sand Filters and experiment methods

The experiment was carried out at laboratory scale process using a slow sand filter (SSF) to treat domestic greywater. The SSF was placed adjacent to the raw greywater tank, and fed with raw greywater with constant flow pump. Flux was set at around 10 ml. min^{-1} and the operation was carried out at ambient temperature and pH. Excess greywater was manually changed 2 times a week, so that the parameters might be changes depending on used hygiene product (soap or shower gel). The preparation of filter beds corresponding with the column packing, with deferential amount of sand. A mass of 4.8 kg of swimming pool sand (SPS) and 5.1 kg of Egypt desert sand (EDS) was used to fill the both column. The connection of the feeding tank is at the base of the filter bed so that the supply takes place from bottom to top. This testing ground allows a sluggish circulation, and therefore a low flow rate in the bed and a supply of the biofilm development. The samples will then at 63 cm from the entrance, just above the filter bed (Figure 3.24).

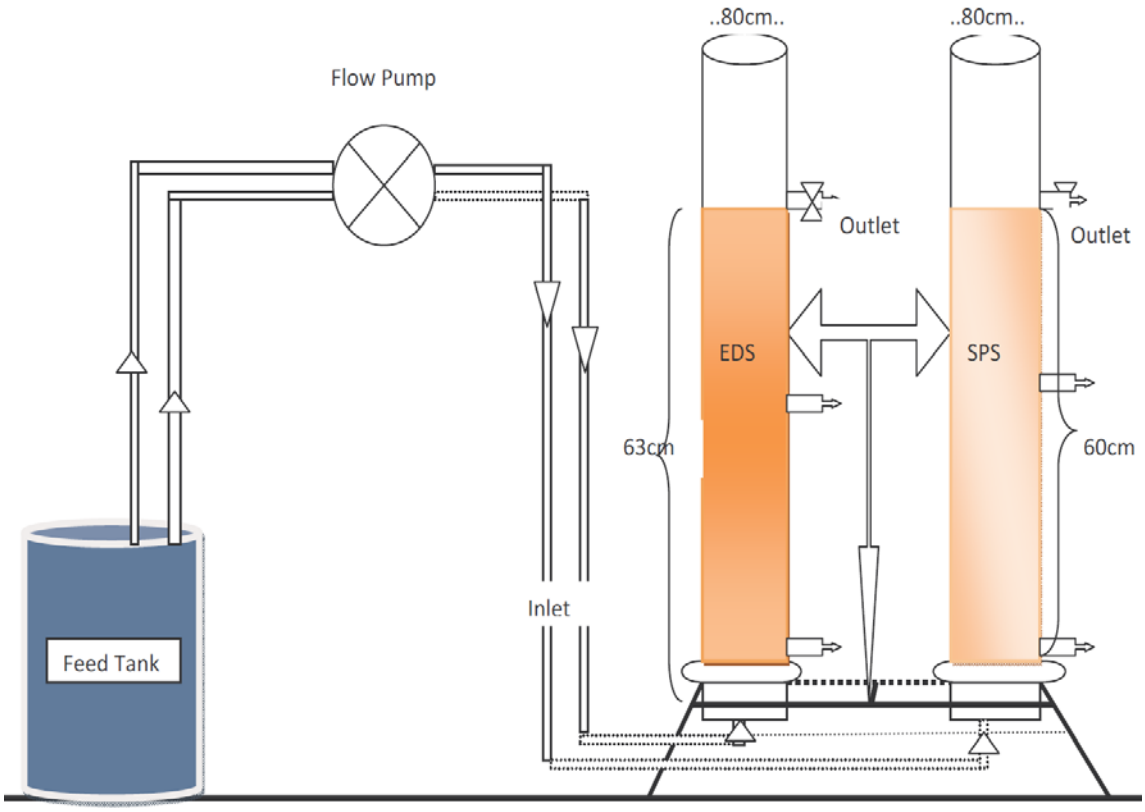

Figure 3.24. Schematic diagram of the slow sand filtration

There are several important elements that should be observed when carry out slow sand filters:

- The greywater supply feeding the filter should be able to maintain a constant head of water above the filter bed, thus there will be a constant pressure pushing water through the filter.
- The raw greywater source must therefore be able to supply a flow rate greater than the flow rate through the filter
- The sand filter bed should be at least 0.6 m deep and should contain sand of an appropriate size and size distribution.
- The under drainage system must support the filter bed while providing the minimum resistance to flow.
- The resistance of the filter bed will increase during use as the pores between sand grains become blocked by the material being removed from the raw greywater.
- The flow rate through the bed should be controlled by a regulating value placed in a pipe after the filter. The amount of head above the filter should not be used to regulate flow.

3.2.1.2. RTD and flowrate

3.2.1.2.1. Fluorescein Tracing

Fluorescein ($C_{20}H_{12}O_5$) is a hydrologic tracer used to plot the underground streams, resurgences and even leaks. The effect is a function of the fluorescent dye concentration. However beyond a certain amount that fluorescence tends to disappear. The experimental protocol is as follows: Desired concentration: 10^{-3} mol L^{-1} Molecular weight: 376.27g/mol which gives us a mass concentration of 0.376 g L^{-1} We prepared in 250 ml, the massed weighing is then: m = (0,376 * 250) / 1000 m = 0.094g or 94 mg. We then concentrated the solution four times, which we have to weigh 376 mg fluorescein. As part of our experiment, we used to determine the transit time of effluent in the columns that correspond to the time that the solution is in the column from its injection until discharged. The transit time will in turn determine the residence time of water in the gray columns. We then follow the evolution of the solution through the columns at a flow rate of 10 ml/min. the sample were taken every

30 minutes after that record the concentration by a spectrophotometer to determine absorbance.

3.2.1.2.2. Flow rate

The flow rate was set at around 10 ml. min^{-1} to keep on biofilm in the sand bed, we also follow its variations, as it allows us to check if a blockage in the pores. The experimental protocol is as follows: The sampling of the effluent is done through two pre-weighed beakers. After a filling time of the beakers, we weigh again beakers filled with filtered greywater through the filter bed containing swimming pool sand (SPS) and containing the Egyptian desert sand (EDS).

3.2.2. Physicochemical parameters

Chemical components analyzed for all of two filters tested are shown in Table 3.6. The parameters were selected based on a literature review of components of greywater and investigation of greywater components likely to have detrimental impacts on soils, plant life and other water bodies (Jeppesen 1996). The sand type to which the greywater is applied will have an effect on the impacts of different chemical constituents. In general, EDS is more affected by high salinity, basic character and metals content (Table 3.1.). On the other hand, in SPS all these parameters are more likely to affect the treated greywater. Turbidity was also measured, as this is an easy way to measure indicators of colloidal and suspended residual material, and provides a quick check of filter technology performance. The electrical conductivity was measured as a surrogate measure for total dissolved solids, which provides a measure of the dissolved salt content or salinity. Salinity will affect both plant growth and soil structure. pH can also impact plant growth and soil structure and also provides an easy check that a technology is operating correctly. Chemical oxygen demand (COD) was included as it provides a good surrogate measurement of biologically degradable, organic and none biologically degradable components. The inclusion of these parameters for analysis when the tested technology utilizes chemical disinfection is recommended.

3.2.3. Microbiological analysis

Microbial populations were enumerated by viable counts using Colony forming Unit (CFU/ml). Four strains of bacteria were studied for get of biofilm construction.

3.2.3.1. Total Bacterial Flora

Bacterial Flora is a population of bacteria that exists on or in the body, and possesses a unique ecological relationship with the host. Bacterial flora encompasses a wide variety of microorganisms coming from the surface tissues, i.e., skin and mucous membranes, which are constantly in contact with environmental organisms and become readily colonized by various microbial species. The human skin contains microbes that reside either in or on the skin and can be residential or transient. Resident microorganism types vary in relation to skin type on the human body. A majority of bacteria reside on superficial cells on the skin or prefer to associate with glands. These glands such as oil or sweat glands provide the bacteria with water, amino acids, and fatty acids that provide nutrients for the microbes. In addition, resident bacteria can be pathogenic and are characteristically gram positive bacteria such as certain gram positive bacteria can be associated with oil glands that play a role in acne and skin disease (Willey et al 2011). Moreover, human sweat is by nature odorless, but bacteria associated with the skin play a role in producing body odor. The bacteria count on nutrient agar after incubation at 37 ° C for 24 h. Some bacteria could also come from the tap water.

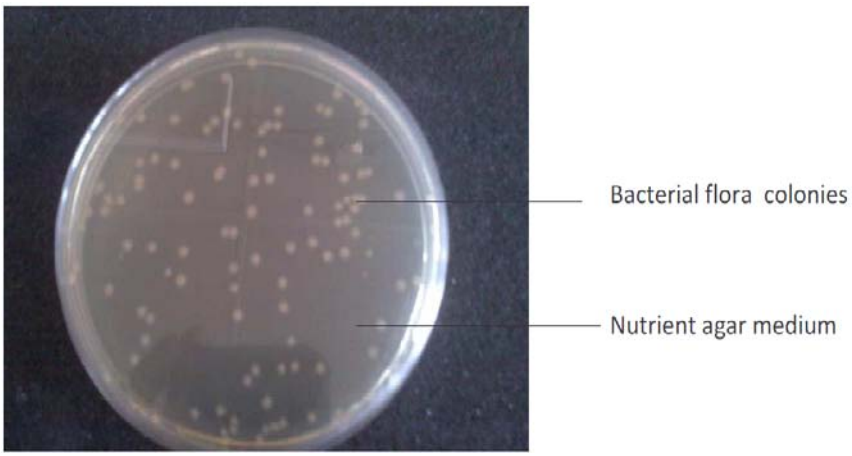

Figure 3.25. Colonies of bacterial flora on nutrient agar media

3.2.3.2. *Escherichia coli* and *Pseudomonas aeruginosa*

In this study, were chosen two bacterial species *Escherichia coli* and *Pseudomonas aeruginosa*. *E. coli* is as pathogenic indicators, commonly found in the lower intestine of warm-blooded organisms; it is gram negative, facultative anaerobic and non-sporulating bacteria. Most *E. coli* strains are harmless, but some serotypes can cause serious food poisoning in humans, and are occasionally responsible for product recalls due to food contamination. *P. aeruginosa* is widely distributed in aquatic and terrestrial habitats. Concentrations of *P. aeruginosa* in sewage may exceed 105 CFU/100 mL (Howard et al. 2004). While usually not a significant risk to healthy individuals, *P. aeruginosa* has been associated with cases of folliculitis, dermatitis, and ear and urinary infections. Both of bacteria grow on TTC Tergitol 7 Agar media (incubation at 44 ° C, for 24 h).

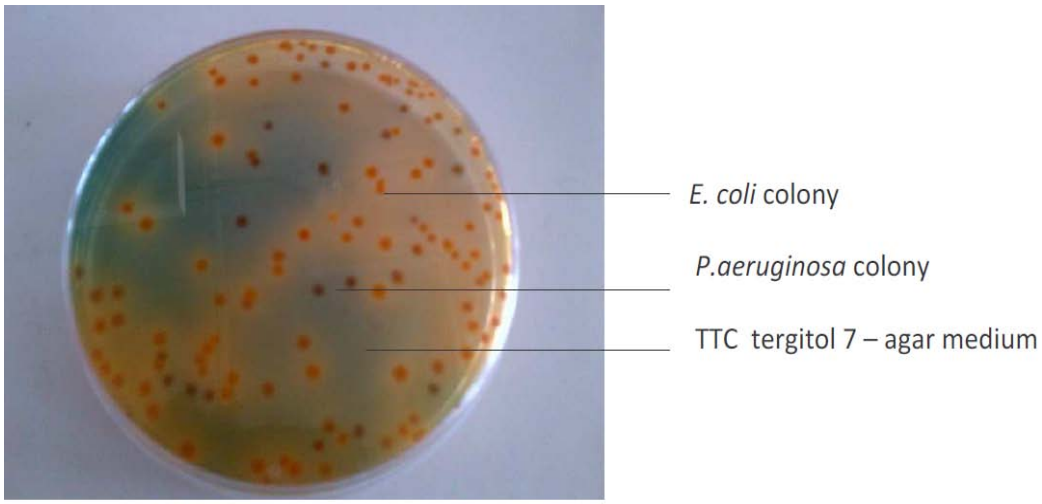

Figure 3.26. Colonies of *E.coli* and *P.aeruginoa* on TTC7 agar media

3.2.3.3. *Enterococcus faecalis :*

Enterococcus faecalis is a gram positive, facultative anaerobic microbe; it ferments glucose without gas production, and does not produce a catalase reaction with hydrogen peroxide. commenseal bacterium inhabiting the gastrointestinal tracts of humans and other mammals. *Ent. faecalis* can cause life-threatening infections in humans, especially in the nosocomial (hospital) environment, where the naturally high levels of antibiotic resistance found in *E. faecalis* contribute to its pathogenicity. *E. faecalis* can cause endocarditis and bacteremia, urinary tract infections (UTI), meningitis, and other infections in humans. The bacterial colonies are red color (Figure 3.27) and grow on Slanetz and Barley at 37°C for 48 h.

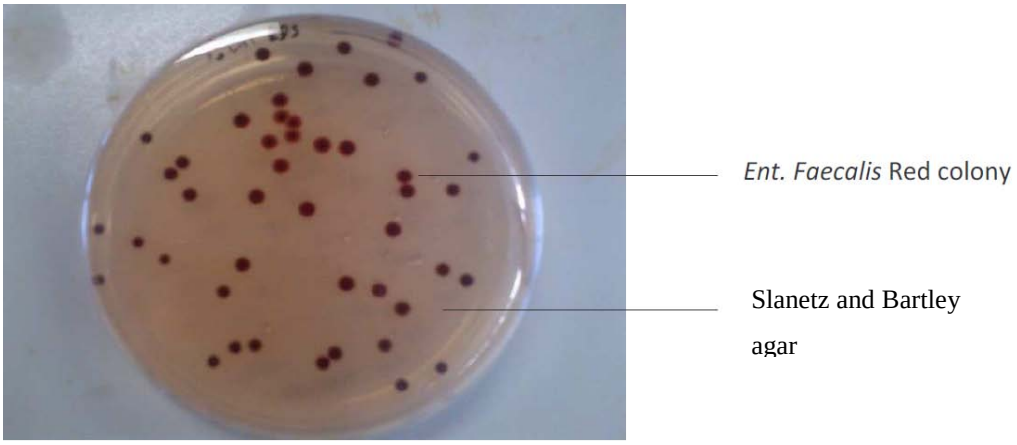

Figure 3.27. Colonies of *E.faecalis* on Slanetz and Bartley agar media

3.2.4. Biological Action and biofilm construction

Biofilm construction inside of the filter bed is due to microorgarnisms provided by the raw greywater and takes mostly place at the inlet of the column. Slow sand filters have small flow rates hence most solid particles are removed in the top 0.5 cm of sand. This top layer of sand develops into a biologically active area known as the *schmutzdecke* (which translates roughly from German as 'dirty layer') and in the bottom and middle of the filter bed. While most of the biological activity occurs in this region some activity continues down to a bottom, although faster flow rates will carry organic food, that sustains bacteria, even deeper into the sand bed. The Schmutzdecke is perhaps the single most important feature of the slow sand filter and is a sticky reddish brown layer consisting of decomposing organic matter, iron, manganese and silica. It acts as a fine filter to remove fine colloidal particles from the raw water and is also the initial layer of bioactivity. The schmutzdecke takes a while to form and ripen, this may take 2- 3 weeks depending on the temperature and the biological content (bacteria and organic material) of the raw greywater. Once functioning the schmutzdecke should remain undisturbed until the experiment finish, in our case probably it take 8 weeks. The effectiveness of the schmutzdecke relies on there being adequate food (organic material in the raw greywater), a high enough oxygen content and a sufficient water temperature. The following points should be observed when operating a slow sand filter:

- The flowrate must be at 10ml/min (minimum flow rate) to keep the schmutzdecke
- The sand must be kept wet to keep the essential micro-organisms alive in the biological zone.

- Ensure there is a continual flow of water through the filter, and Provide an aeration treatment before, or as, the raw water enters the filter.
- *E.coli, P.aeruginosa , E. faecalis* and bacterial flora needs food , therefore row greywater should be continally fed in and the filter be run continuously to 12 h after that recycle until anther determination.
- The biological zone (biofilm) needs adequate oxygen for the metabolism of biodegradable components and the consumption of photogenes. If the oxygen content of the filter drops too far anaerobic decomposition occurs producing hydrogen sulphide, ammonia and other products that affect the taste and odors of the water

The biological layer becomes less effective at lower temperatures. When the air temperature drops to below 2°C for any prolonged period the filter should be covered to prevent heat loss or chlorination should be used on the filtered water as a safeguard.

3.2.5. Results and discussions

3.2.5.1. RTD test in the case of slow sand filtration (SSF): rate filtration ~ 3.31 10^{-5} m / s

The measurements of the residence time distribution were also performed with the same column which was used as a biofilter. The experimental setup consisted essentially of a rigid Plexiglas column of 8 cm of diameter, fed at the base at ambient temperature. The *biofilter* columns were operated at room temperature (20–21 °C) by imposing an upward flow to avoid any malfunction which may be related to clogging over time. 2.5 ml of fluorescein concentration of 39 10^{-4} mol / L were injected at 3 cm from the support grid of the packing at the bottom of the column. Given the relatively low flow rates, the samples were performed every 10 minutes for a period of 8 hours.

Experimental results for EDS are presented in Figures 3.23. The values of the Peclet number (Table 3.5) are much too low (~5 – 6) compared to those obtained with higher flows. The residence time appears to be greater and approximately twice the theoretical hydraulic time. The results indicate that the residence time distribution has a long tail which could mean that there are stagnant volumes.

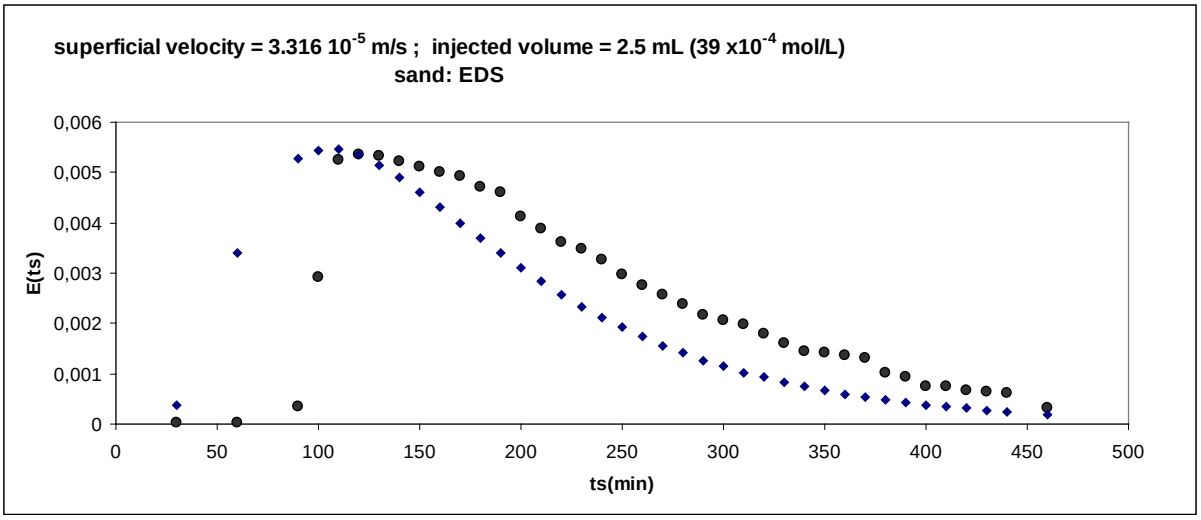

Figure 3.23. Residence time distribution with flow rate used for bio-filtration: comparison between experiment and plug model.

Sand	*Flow rate (mL/min)*	*Residence time minutes*	*Hydraulic Time minutes*	*superficial velocity (m/s)*	*Peclet number*
EDS	**10**	**220**	**127**	**3.32E-05**	**5,5**
SPS	**10**	**225**	**127**	**3.32E-05**	**5,5**

Table 3.5. Results of experimental study of the RTD for low flow rate 10 mL/min.

3.2.5.1. Physico-chemical and microbiological parameters

All samples were analyzed as soon as possible after harvesting. The raw results of the physico-chemical and microbiological greywater bathrooms are reported (Annex 1). Reasons for the large number of results it was essential to determine the mean values of various parameters before filtration and after filtration for Egyptian sand (EDS) and (SPS). These values are reported in Table 3.6. Indeed, given the results obtained out of range for some samples, their case is specifically addressed at the end of this part of chapter.

Parameters	EDS		SPS	
	Before filtration	After filtration	Before filtration	After filtration
Turbidity (FNU)	75.7 (4.7-281)	7.2 (1.37-17)	75.7 (4.7-281)	26.7 (3.3-75)
pH	7.5	7.7	7.5	7.6
Conductivity (µs/cm)	390.9 (358-426)	419.8 (387-485)	394.8 (358-426)	388.9 (376-412)
COD (mg/L)	221 (69-285)	77.9 (36-129)	224.9 (69-285)	101 (34-158)
E.coli (CFU/ml)	7.92E+04	4.44E+03	8.97E+04	4.73E+03
P.aeruginosa (CFU/ml)	1.03E+05	8.29E+02	1.06E+05	2.90E+03
E.faecalis (CFU/ml)	2.14E+02	>10	1.60E+02	> 10
Bacterial Flora (CFU/ml)	2.04E+05	1.41E+04	2.32E+05	8.38E+04

FNU: Formazin Nephelometric Units; COD: Chemical Oxygen Demand; CFU: Colony Forming Unit

Table 3.6. Comparison between the mean values of physico-chemical and microbiological samples with References values

An overview on the composition of greywater has been realized in the literature and review part. Data on greywater bathrooms are included in Table 2.1, and reference values and regulatory quality greywater are grouped into minimum and maximum in Table 2.1. These values are still reflected in the results table (Table 3.6).

First, the examination of the greywater turbidity is very heterogeneous; we have no samples with values of turbidity are greater than the values reported in the literature. The values obtained for both columns are still slightly different. Indeed, the average results obtained during analysis of samples from the Egyptian sand, are lower than those from swimming pool sand, 7.16 and 26.67 respectively (Table 3.6). This is certainly due to the characteristic of the different sands; the desert sand is very fine Egyptian less porous and therefore retains large quantities of the suspended solids in the water even though they are very small. Turbidity is easily removed by filtration and then it is due to the MES in the sample, given the negligible amount of suspended solids in the samples, results in turbidity are very satisfactory.

In terms of pH and conductivity, all samples analyzed have values of pH and conductivity which scarcely vary. In general they are around pH 7.9 and 400 μs/cm conductivity. These values are target values between minimum and maximum target values. COD compared to other parameters is subject to special monitoring because it is an essential parameter for following the biodegradation of organic matter. For analyzes of chemical oxygen demand, the mean values are within the ranges of target values are 77.86 mg L^{-1} for the Egyptian desert sand and 101 mg L^{-1} for swimming pool sand. The reduction in COD was from 271 mg L^{-1} (raw greywater) to 83 mg L^{-1} (treated greywater) with a mean removal efficiency of 69.4% for EDS, on ther other hand; the reduction in COD was from 140 mg L^{-1} (raw greywater) to 71 mg L^{-1} (treated greywater) with a mean removal efficiency of 42.1% for SPS. The study results agree with those of Heally et al. (2006) who studied the performance of a stratified sand filter in removal of chemical oxygen demand, total suspended solids and ammonia nitrogen from high-strength wastewater.

Work days(d)	Time (h)	**EDS**		% reduction	**SPS**		% reduction	Flowrate (ml/min)	
		T_0COD (mg/l)	T_5COD (mg/l)		T_0COD (mg/l)	T_5COD (mg/l)		EDS	SPS
d1	0	298	146	51	298	200	32.8	9.9	9.8
d2	16	271	83	69.4	271	157	42.1	10.7	11.3
d3	20	140	73	47.9	140	71	49.3	10.5	11
d4	27	285	98	65.6	285	146	48.8	9.1	9.5
d5	30	220	71	67.7	220	115	47.7	4.9	9.4
d6	36	214	98	54.2	214	186	13.08	9.9	9
d7	39	190	78	58.9	190	138	27.4	14.9	14

Table 3.7. The values of COD and flowrate (T_0: initial; T_5 after 10 hours of treatment)

The results demonstrate the presence of biofilm on the filter bed and therefore a significant degradation of organic matter by bacteria. Comparing these values of chemical oxygen demand with flow variation, it has been shown that when the flow increases, the values of COD increase and decrease when the flow decreases (Figure 3.28). This could be explained by the modification of the RDT which allowed not enough time to the pollutant transfer to the biofilm. Indeed, when the flow increases, the velocity increases and thus given the fragility of the biofilm, it detaches from the filter bed and therefore bring with him all bacteria capable of degrading organic matter.

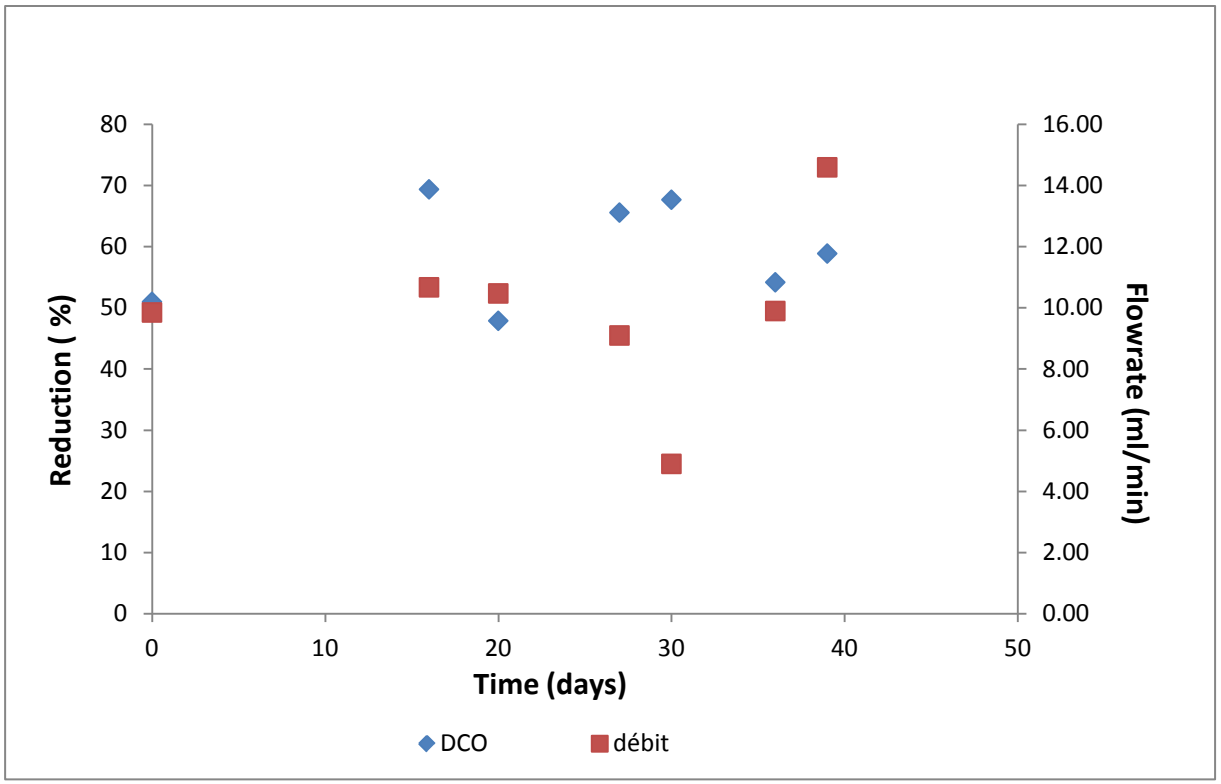

Figure3.28. Curve of COD and flowrate of Egyptian desert sand

The Figure 3.28 described the changes in the chemical oxygen demand and flow versus time. We can then notice that the percentage reduction of COD increases when the flow decreases. Indeed, it evolves over the biofilm to a limited lifetime. What we can say that this method is effective in removing biodegradable organic matter, but at some point the biofilm and thus weakens unclogged.

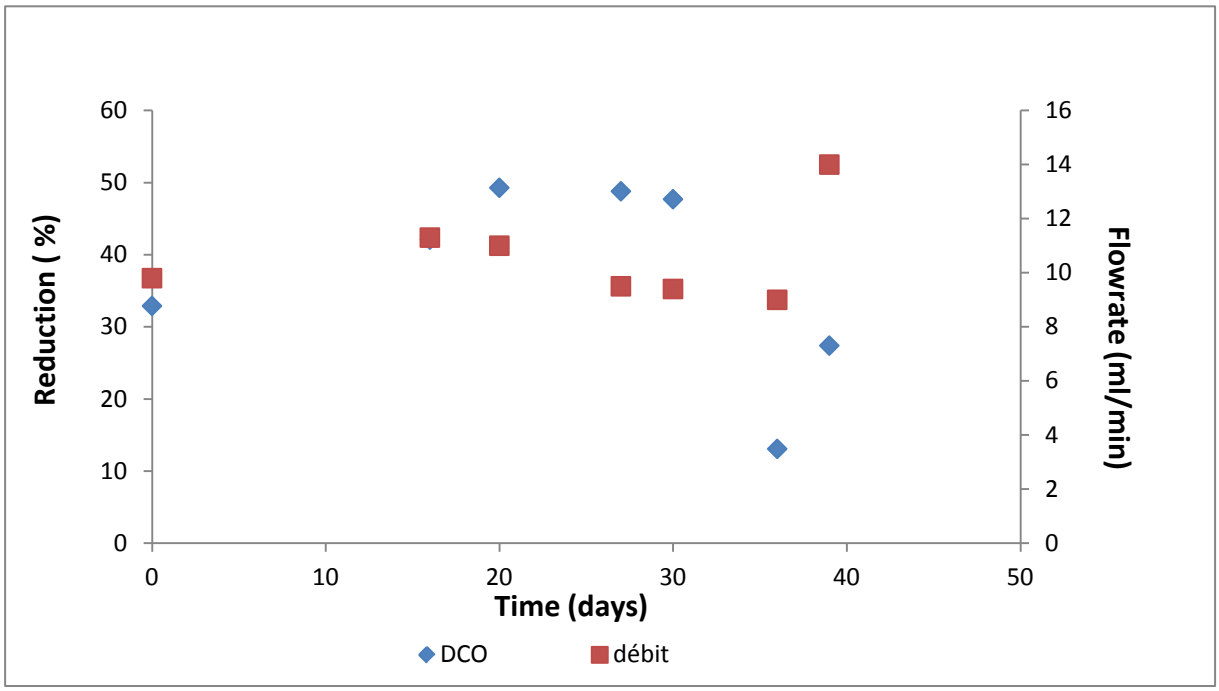

Figure 3.29. Curve of COD and flowrate of Swimming Pool sand

The same goes for swimming pool sand, the percentage reduction of COD increases when the flow decreases, except that the first day of analysis that was not the case. This is certainly due to the absence of biofilm. Indeed, the biofilm may develop slowly over the filter bed.

By comparing the results of the two sands, one can say that the sands of Egypt, is more efficient because it has a maximum value of percentage reduction of 70% and a minimum value of 47% (Figure 3.28), unlike the values of percentage reduction of swimming pool sand (SPS) varies between 22% and 50% (Figure 3.29). As for microbiological analyzes, they demonstrate the significant quantities of microorganisms such as *E.coli*, *Pseudomonas aeruginosa*, *Enterococcus faecalis* and total bacterial flora present in the greywater samples (Table 3.6). The magnitudes of the target values are not observed in our samples. While for the total flora and *P.aeruginosa* target values are not determined, there is a significant amount of these bacteria in the samples. All these results emphasize the need to eliminate bacteria in the treatment chosen for the recycling of greywater.

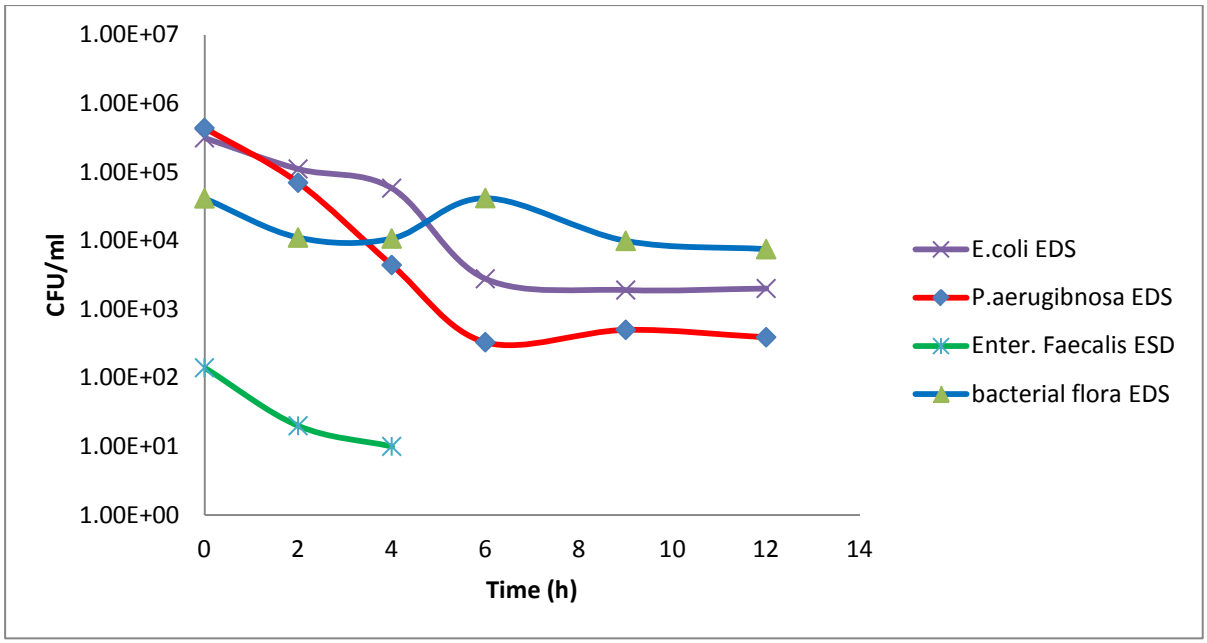

Figure 3.30. Variation of bacterial colonies of Egyptian Desert Sand

E. coli and bacterial flora can sometimes be found in abundance in greywater bathroom because the products used in the shower (soap or shower gel) allows their development from organic materials biodegradation. This process is not effective for microbiological treatment because the amount of bacteria before filtration and after filtration did not decrease significantly. Figures 3.30; 3.31 clearly shows that the microbiological parameters are not fully addressed in this process. The amount of bacteria decreases almost no before and after filtration. Indeed, the porosity of the different sands does not allow retention of bacteria in diameter very low.

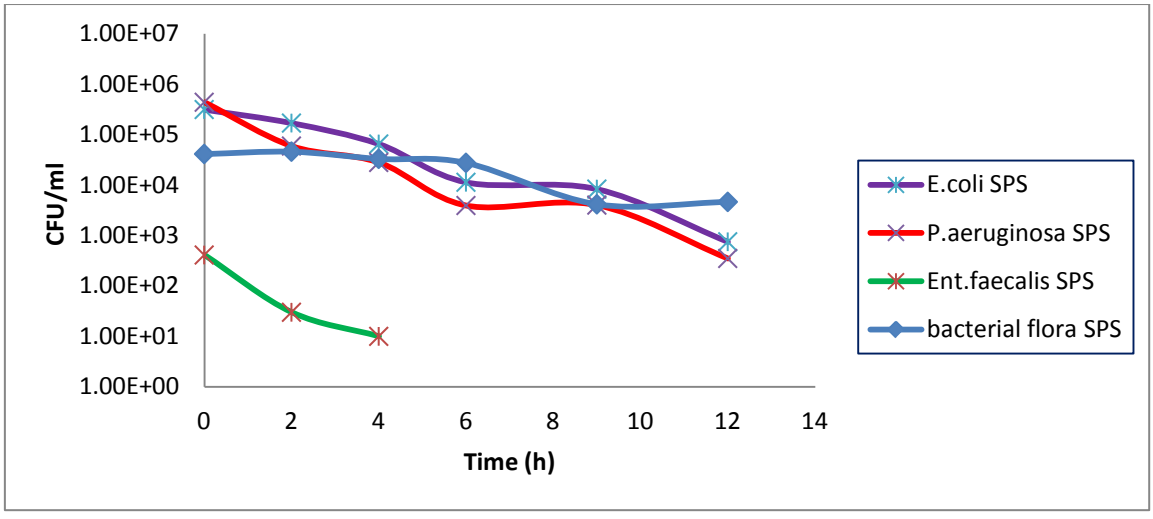

Figure 3.31. Variation of bacterial colonies of Swimming Pool Sand

There are two cases there the biofilm could be removed and found in the effluent of the filter bed: (i) then the filter bed clog; (ii) then is a decrease of carbon sources.

3.3. CONCLUSION

The physico-chemical and microbiological characteristics of greywater depends on the sources of production. Habits of producers, amount and types of product used, water consumption are determining factors. By comparing the data samples from the sand filtration of the Egyptian desert to the samples from the swimming pool, we find that the results differ greatly. It is better to make a greywater treatment by the Egyptian desert sand; respect it more or less the points for which a system would work: Minimize the impact on the environment, affordable and ensure the safety and hygiene.

In general, the cost of treatment technologies for greywater varies with the complexity of the system that is closely related to the final application of recycled water and therefore its quality. For this study, it would consider moving towards a path for individual habitats ensuring water quality is average because the subject of a single treatment (filtration more or less rough with exposure to high temperatures). In this, the local structures that have greywater must be found outside the building has to take advantage of abiotic factors such as temperature at which a great influence on the reproduction of microorganisms and the rules of Hygiene standards are met. A disinfection step can also be set up after filtration. It can be done either by chemical, physical (UV) or electroperoxydation. Time storage of recycled water remains to be defined, to prevent odors and microbial

CHAPTER FOUR

I- The effect of various abiotic factors on the survival growth of *Escherichia coli* and *Pseudomonas aeruginosa* in bathroom greywater.

II- Coliphage MS2 inactivation by abiotic factors in Greywater

4.1 The effect of various abiotic factors on the survival growth of *Escherichia coli* and *Pseudomonas aeruginosa* in bathroom greywater

4.1.1 Introduction

Greywater (GW) is usually defined as all the wastewater produced in a household except toilet wastes (black water). Typically, GW includes water from bathroom sinks, baths and showers, and may also include water from laundry facilities and dishwashers. Some definitions include wastewater from kitchen sinks although there is no consensus on this (Queensland Government 2003). GW represents the largest potential source of water savings in domestic residences, accounting for as much as 50–80% of the total water uses (Christova-Boal et al. 1996; Kargi & Dincer 1996; Eriksson et al. 2002; Jenssen & Vrale 2003; Flowers 2004). This study focuses on bathroom GW, which comes from hand washing and bathing and generates 50–60% of total GW and is considered to be the least contaminated type of GW. Common chemical contaminants include soap, shampoo, hair dye, toothpaste and cleaning products. It also has some faecal contamination (and the associated bacteria and viruses) through body washing. It may further contain microbial agents that present a public health hazard, according to previous studies (Rose et al. 1991; Dixon et al. 1999; Casanova et al. 2001; Friedler et al. 2006; Gilboa & Friedler 2008). In addition to enteric pathogens, there are several human associated opportunistic pathogens present in GW. Because of the large volume produced and low pollutant concentration, many people propose that GW could be used as irrigation water in dry regions. However, there has been little consideration of the survival of potential pathogenic microorganisms and use of GW without treatment. Few studies have investigated the effect of abiotic factors on pathogenic bacteria in real GW. In this study we choose two bacterial species *Escherichia coli* and *Pseudumonas aeruginosa*. *E. coli* is commonly found in the lower intestine of warm-blooded organisms; it is one of the most diversen bacterial species with several pathogenic strains with different symptoms and only 20% of the genome is common to all strains (Lukjancenko et al. 2010). *P. aeruginosa* is widely distributed in aquatic and terrestrial habitats. Concentrations of *P. aeruginosa* in sewage may exceed 10^5 CFU/100 mL (Howard et al. 2004). While usually not a significant risk to healthy individuals, *P. aeruginosa* has been associated with cases of folliculitis, dermatitis, and ear and urinary infections. Many factors could affect the growth rate or

survival of bacteria. This paper focuses on four factors: temperature, aeration, salt concentration and nutrient concentration. Cell size and zeta potential for *E. c*oli and *P. aeruginosa* were also determined in relation to the medium composition.

4.1.2 Material and Methods

4.1.2.1 Bacterial growth conditions and Parameters

The experimental work concerning pathogenic microorganisms in GW was carried out with *E. coli* and *P. aeruginosa*. Both species have been isolated from bathroom GW from Nantes, France. *E. coli* and *P. aeruginosa* were generally grown in different media: nutrient broth (NB: composition g/L, beef extract 3.0 g, peptone 5.0 g); minimum mineral medium with glucose (MMG: composition g/L, Na_2HPO_4 4.8 g, KH_2PO_4 4.4 g, MgSO4 $7H_2O$ 0.5 g, NH_4Cl 1.0 g, glucose 1 g (Mian et al. 1978)); and GW. The survival or growth measurement was done by plate counting using a selective medium (TTC Tergitol 7Biokar diagnostics) at 37 or 44 °C. To characterise the rate of bacteria decay and the time required for 90% reduction in bacterial cell, we used decay rate K (h) and T_{90}: (i) The decay rate K (h) were calculated according to Chick's law (Alkan et al., 1995) which is depicted as

$$- Kt = (\text{Log } N_t / N_0) \qquad \text{Eq. 4.1}$$

Where: N_0 and N_t is respectively the bacterial Numbers initial and at time t measured in hours

(iii) The T_{90}, the time required for 90% of the initial population has disappeared.

$$T_{90} = - t / \log (N / N_0) \qquad \text{Eq. 4.2}$$

Where: N_0 and N are respectively the bacterial Numbers initial and at time t measured in hours.

4.1.2.2 Greywater samples

GW samples were collected from a domestic bathroom shower. The samples were immediately stored at 4 °C in a dark polyethylene bottle. The typical chemical composition of GW which was used in this study is presented in Table 4.1 (Chaillou et al. 2O11). For each experiment, the strain was first cultured in NB, harvested by centrifugation and washed with

deionised water (DW) and added to GW at the appropriate initial concentration; all experimental survival curves was done in triplicate.

4.1.2.3 Temperature

To investigate the effect of temperature, threeErlenmeyer flasks were filledwith 250 mL of GW seeded with *E. coli* or *P. aeruginosa* suspension at pH 7 and incubated in the dark at different temperatures (6 ± 2, 23 ± 2 and 42± 2 °C) under shaking at 300 rpm. The three temperatures were chosen depending on the mean temperatures in France and Egypt, and on the known survival characteristics of *E. coli* and *P. aeruginosa*.

4.1.2.4 Aeration

Two bottles were used for *E. coli* and *P. aeruginosa*. The bottles were each filled with 250 mL of GW, seeded with *E. coli* or *P. aeruginosa* suspension and incubated at 23 ± 2 °C. To produce anaerobic and aerobic conditions, one bottle was closed air-tight and the other closed with a cotton-wool plug to allowed air to enter with shaking at 300 rpm.

4.1.2.5 Salinity

Three salinity concentrations were investigated: 1.75 and 3.5% and GW dilution at 50% GW:50% DW seeded with *E. coli* or *P. aeruginosa* suspensions.

Parameters	Average (range)
COD (mg/l)	170 (116–233)
Turbidity (FNU)	28 (9–68)
pH	7.8 (7.6–7.9)
Conductivity (µS/cm)	399 (360–465)

Table 4.1. Chemical analysis of greywater samples used in this study (Chaillou et al. 2011) **(COD**: Chemical Oxygen Demand. **FNU**: Formazin Nephelometric Units).

Samples	Temperature (°C)	pH	Zeta potential (mV)
EC GW	25	7.47	−14.5
EC NB	25	6.72	−33.3
PA GW	25	7.13	−23.2
PA NB	25	6.82	−6.61

Table 4. 2. Zeta potential and pH for *E. coli* and *P. aeruginosa* in NB and GW media at 25 °C (EC: *Escherichia coli;* PA: *Pseudomonas aeruginosa*; GW: Greywater; NB: Nutrient broth).

The flasks are protected from light and incubated at 23 ± 2 °C with shaking (300 rpm). The two levels of salinity were chosen according to the mean levels of salt in seawater: 3.5% salinity is the mean seawater salinity; half of this concentration (1.75% salinity) represents the effect of dilution by GW.

4.1.2.6 Zeta potential and cell size measurement

The zeta potential and the cell size of the study microorganisms were measured by dynamic light scattering with a Zetasizer Nano ZS (Malvern Instruments Ltd, UK). For this experiment, the microorganisms were grown in NB as a nutrient-rich medium or in MMG and GW as a nutrientpoor medium at 25 °C with shaking at 300 rpm. The bacteria were harvested by centrifugation for 10 min at 8,545 × g rpm and washed twice with NaCl solution (9 g L^{-1}). The measures were recorded after 6 days for GW and after the end of exponential growth phase for other media.

4.1.3 Results and discussions

4.1.3.1 Effect of temperature on survival of *E.coli* and *P.aeruginosa*

Effects of temperature on the survival of *E. coli* and *P. aeruginosa* To study the effects of temperature on abundances of *E. coli* and *P. aeruginosa*, we incubated these species in GW at different temperature (6 ± 2, 23 ±2 and 42±2 °C). As shown in Figures 4.1, 4.2 and 4.3, the survival of bacteria decreased with increasing temperature, in accordance with other studies (Ostrolenk et al. 1947; Kristiansen 1981; Stevik et al. 2004). *E. coli* survived better at 6 ±2

and 23± 2 °C. The survival period was close to 860 h for both temperatures. *P. aeruginosa* also survived better at 6 ±2 and 23± 2 °C, with survival periods of 552 and 720 h, respectively. At low temperature, the bacteria limit energy expenditure by reducing metabolic activities, allowing much longer survival than at high temperatures. Many authors have shown that low temperatures prolong the survival of pathogenic bacteria, in freshwater (Barcina et al. 1986; Wang & Doyle 1998; Mizunoe et al. 1999), in estuarine water (Faust et al. 1975) and in marine water (Pike & Gameson1970). The results also show that 42 ± 2 °C has a greater effect on the survival of *E. coli* and *P. aeruginosa* than other study temperatures.

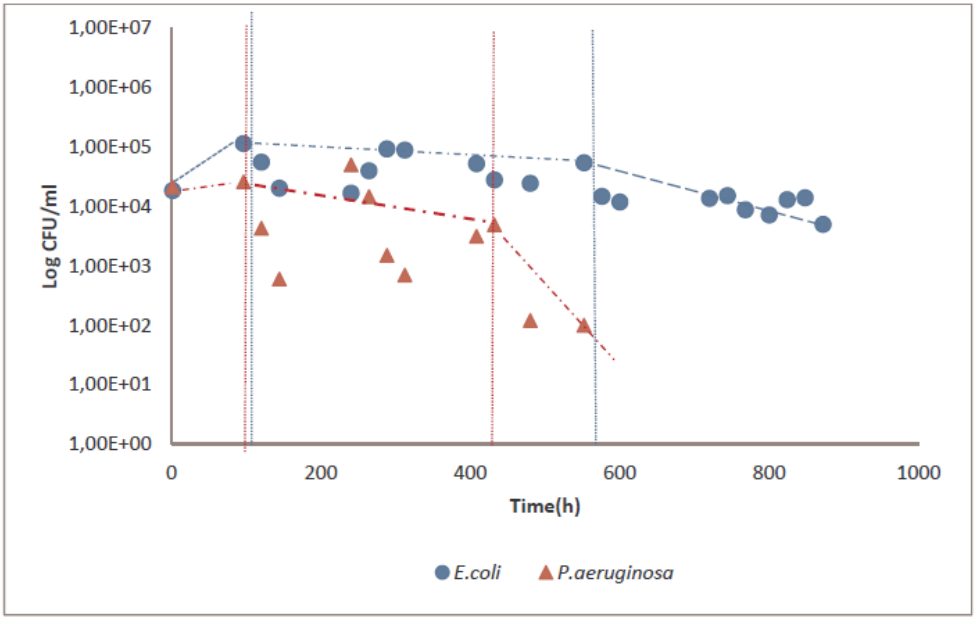

Figure 4.1 Effects of temperature on growth curve of *P. aeruginosa* and *E. coli* at 6±2 °C. (The vertical lines indicate change in survival stage).

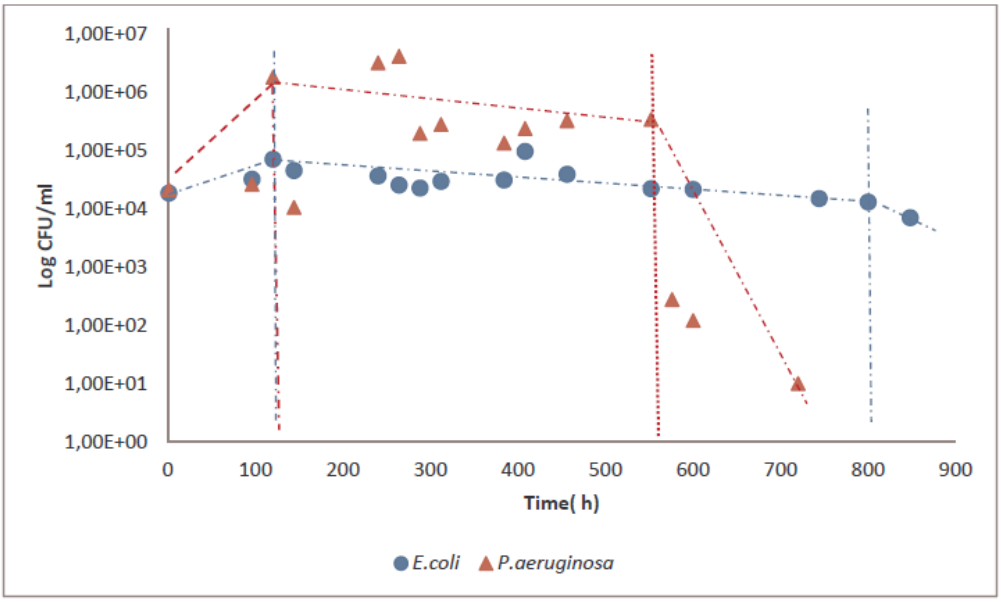

Figure 4.2. Effects of temperature on growth curve of *P. aeruginosa* and *E. coli* at 23 ±2 °C. (The vertical lines indicate change in survival stage).

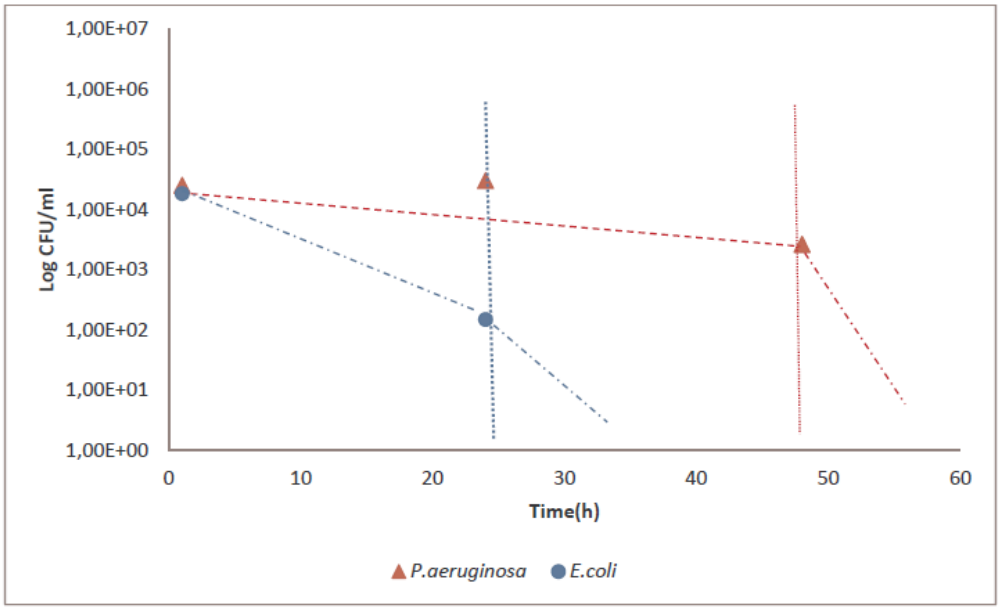

Figure 4.3. Effects of temperature on growth curve of *P. aeruginosa* and *E. coli* at 42 ±2 °C. (The vertical lines indicate change in survival stage).

Extremes of temperature seem to be most disruptive to bacterial survival (McFeters et al. 1974; Flint 1987). Three distinct stages were observed during the incubation; a short survival growth stage, a stationary survival stage and decrease or death stage (Figures 4.1, 4.2, and 4.3). During the initial stage the bacteria grow rapidly, so the cell numbers increase. Then cell

numbers reach the stationary phase, then slowly decrease (K1), followed by a rapid decrease (K2). So the overall decrease in numbers could be determined by two rate factors, the two decay rates constant, a slow one K1 (h^{-1}) and a rapid one K2 (h^{-1}). There are limited data in the literature for these constants. Tables 4.3 and 4.4 present the constant values for *E. coli* and *P. aeruginosa* determined for the three experimental temperatures. The main observations, is that for both species the optimal survival condition is 23 ± 2 °C. In this case the time required for loss of 90% of bacterial cells (T_{90}) is relatively similar at 730 h for *E. coli* and 590 h for *P. aeruginosa*. At 6± 2 °C the two species also show good survival, better one for *E. coli* (T_{90} ≈ 590 h) than *P. aeruginosa* (T_{90} ≈ 460 h). In contrast, *P. aeruginosa* is more resistant than *E. coli* at 42 ± 2 °C. The time required for 90% reduction in cell viability is lower for *E. coli* than *P. aeruginosa*. Incubation at 42 ±2 °C has the strongest effect on the bacterial mortality.
These results are in agreement with Abdulkarim et al. (2009) who investigated the effect of 44 °C on *E. coli.*

		Parameters		
Factors		$K1(h^{-1})$	$K2(h^{-1})$	$T_{90}(h)$
Temperature	6 ± 2 °C	1.6	8.0	590
	23 ± 2 °C	2.0	13.3	730
	42 ± 2 °C	0.01	-	11
Salinity	50% DW:50% GW	0.4	5.6	680
	1.75% NaCl	0.4	5.6	150
	3.5% NaCl	0.4	-	43
Aeration	Aerobic	2.0	12.4	490
	Anaerobic	2.0	13.7	380

Table 4.3. The absolute magnitude values T_{90} and K for *Escherichia coli* incubation in laboratory conditions K: Decay rate (h). T_{90}: Time require for 90% reduction in cell viability.

Factors		Parameters		
		$K1(h^{-1})$	$K2(h^{-1})$	$T_{90}(h)$
Temperature	6 ± 2 °C	7.2	1.6	460
	23 ± 2 °C	2.0	9.2	590
	42 ± 2 °C	0.01	-	48
Salinity	50% DW : 50% GW	0.4	8.5	300
	1.75% NaCl	0.4	6.47	247
	3.5% NaCl	0.4	-	115
Aeration	Aerobic	1.6	9.2	520
	Anaerobic	0.01	4.4	100

Table 4.4. The absolute magnitude Values T_{90} and K of *Pseudomonas aeruginosa* incubation in laboratory condition (K: Decay rate (h). T_{90}: Time require for 90% reduction in cell viability).

4.1.3.2 Effect of oxygen on survival of *E. coli* and *P.aeruginosa*

Figures 4.4 and 4.5 show the effects of oxygen (aerobic and anaerobic conditions) on the survival of *E. coli* and *P. aeruginosa* in GW. Three distinct stages could be noted. The related constants are presented in Tables 4.3 and 4.4. Under anaerobic conditions in GW, *P. aeruginosa* did not show growth, while *E. coli* did grow. The aerobic condition causes a marked decrease in the survival of *E. coli*. In contrast, the anaerobic conditions cause a marked decrease in the survival of *P. aeruginosa*. This is consistent with other studies which had shown that aeration conditions affect the abundance of pathogenic bacteria (Attrassi et al. 1996; Yoon et al. 2002; Mahendran et al. 2005). The numbers of viable cells remains relatively constant in the stationary stage and the decay rate was slow. This was followed by a rapid death stage (greatest K2). In general the rapid die-off could be attributed to the fact that bacterial structures get weaker in the stationary period, so the bacteria were not enough strong to show more resistance to environmental changes. The time required for a 90% decrease in CFU (T_{90}) for *P. aeruginosa* was estimated to be 520 and100 h under aerobic and anaerobic conditions, respectively (Tables 4.3 and 4.4). Thus, *P. aeruginosa* maintained cultivability five times longer when incubated under aerobic compared with anaerobic conditions. For *E. coli*, T_{90} was estimated to be 490 h under anaerobic conditions and 380 h under aerobic

conditions. Thus, *E. coli* survival only decreased 1.2-fold when incubated under anaerobic compared with aerobic conditions.

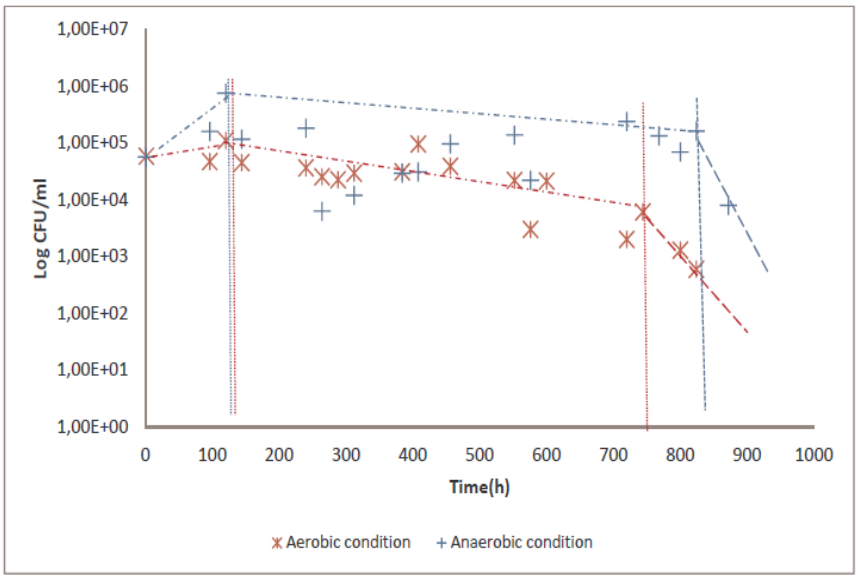

Figure 4.4. Effects of oxygen availability on survival curve of *E. coli.* (The vertical lines indicate change in survival stage).

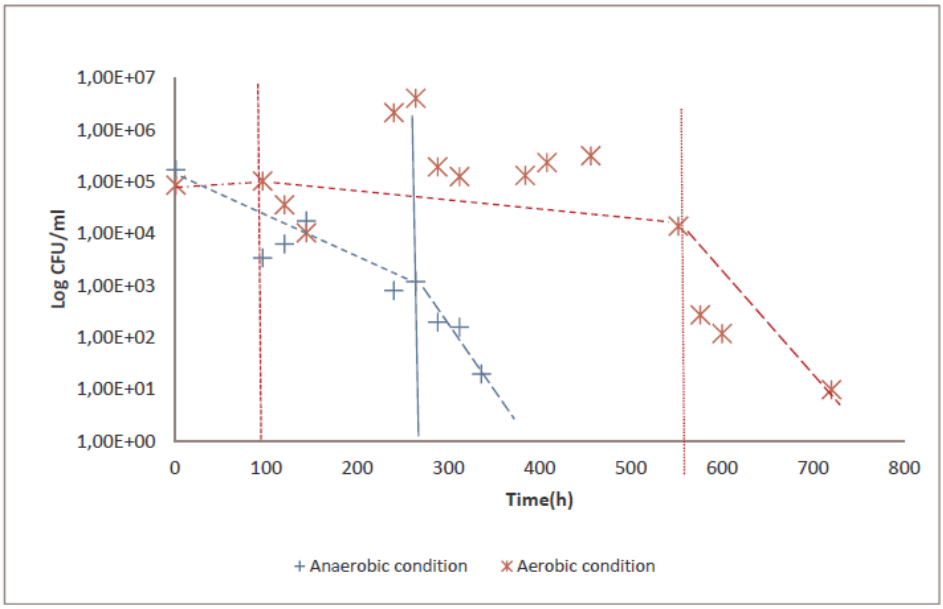

Figure 4.5. Effects of oxygen availability on survival curve of *P. aeruginosa.* (The vertical lines indicate change in survival stage).

4.1.3.3 Effect of Salinity on survival of *E.coli* and *P.aeruginosa*

Salinity is also an important stressor against microorganisms in GW (Gauthier et al. 1987; Rose et al. 1991; Winward et al.2002; Hrenovic & Ivankovic 2009), where the bacteria must restore the osmotic balance between the external environment and the cytoplasm. This restoration involves complex mechanisms, including increasing the concentration of certain solutes (osmo-regulators) in the bacteria. The change in abundance of *E. coli* and *P. aeruginosa* in GW adjusted to different salinities (50% GW: 50% DW, 1.75 and 3.5% salinity) is illustrated in Figures 4.6, 4.7 and 4.8. In laboratory conditions of incubation of the bacteria (in dark at 25 °C), survival curves of *E. coli* and *P. aeruginosa* show the same shape for the different conditions. Bacterial growth goes through three distinct stages: survival growth, stationary survival and death. At the highest salinity level (3.5%) the bacterial abundance decreases very quickly. However at lower salinity (50% GW: 50% DW and 1.75%) the cell numbers decrease very slowly. In Tables 4.3 and 4.4 the results show the adverse effect of increasing salinity on the survival of both species. So for 3.5% salinity, the T_{90} for *E. coli* is only 43 h. This is consistent with other studies which illustrated that at high salinities the survival of faecal coliforms in water is lower (Bordalo et al. 2002). When the salinity decreases by 50% the survival time has shown a two-fold (*P. aeruginosa*) or three-fold (*E. coli*) increase.

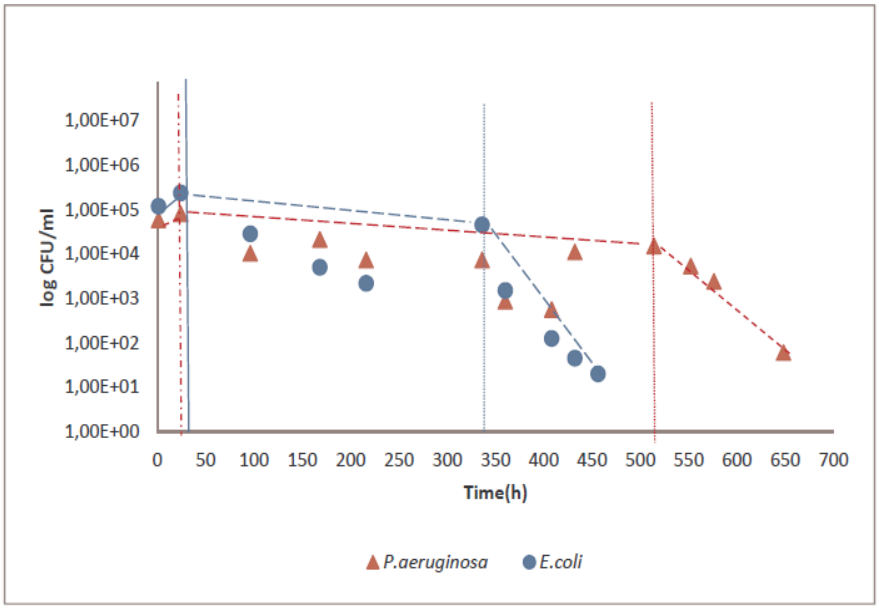

Figure 4.6. Survival curve of *E. coli* and *P. aeruginosa* in 50% GW to 50% DW. (The vertical lines indicate change in survival stage).

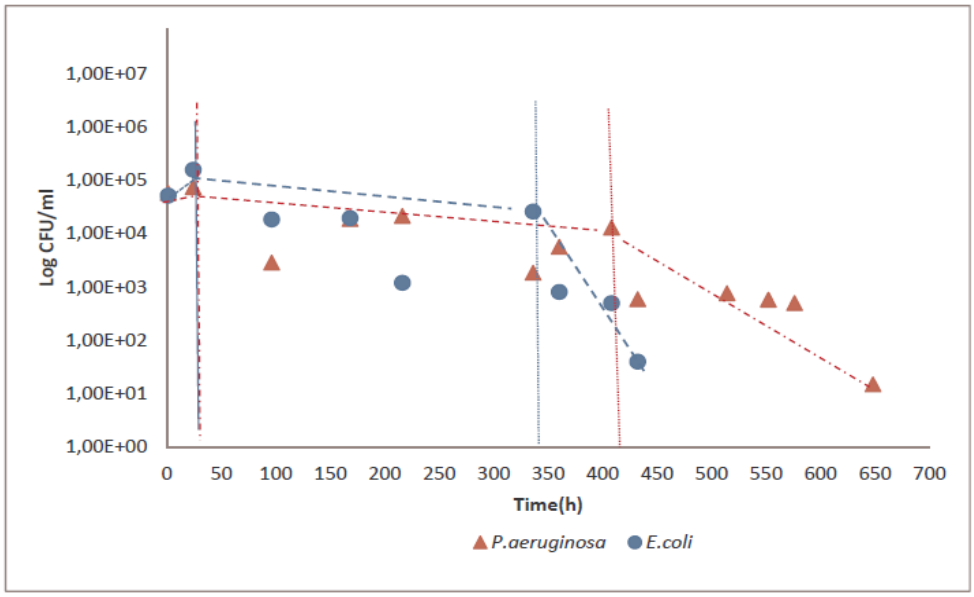

Figure 4.7. Survival curve of *E. coli* and *P. aeruginosa* in 1.7% salinity. (The vertical lines indicate change in survival stage).

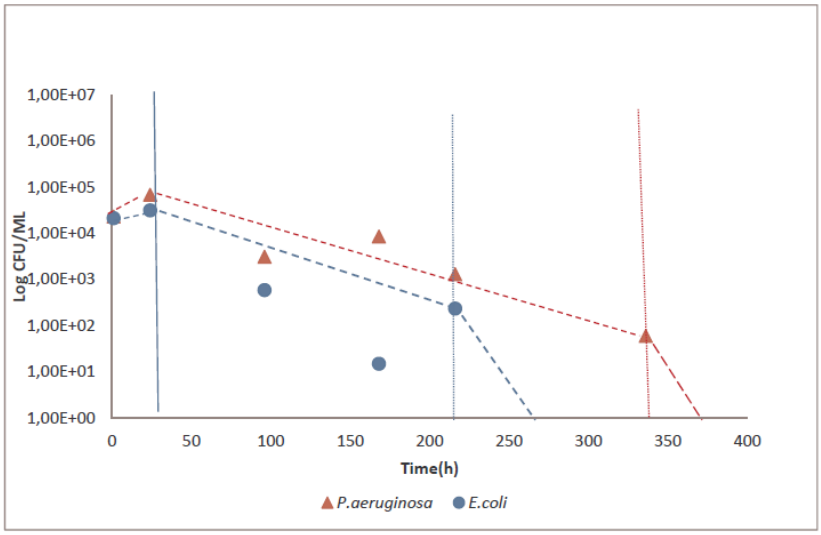

Figure 4.8. Survival curve of *E. coli* and *P. aeruginosa* in 3.5% salinity. (The vertical lines indicate change in survival stage).

Table 4.3 shows the time required for a 90% decrease in CFU (T_{90}) for *E. coli* at low salinity was estimated to 150 and 43 h for 1.75 and 3.5% salinity respectively. For *P. aeruginosa* the T_{90} (Table 4.4) was estimated to 247 and 115 h at low and high salinity respectively; *P. aeruginosa* is more resistant than *E.coli*. Incubation under low nutrient concentrations (50% GW: 50% DW) led to a reduction in both the decay rate (K) and T_{90} (see Tables 4.3 and 4.4).

4.1.3.4 Effect of different media on Zeta Potential and cells Size of *E.coli* and *P.aeruginosa*

The zeta potential of *E. coli* and *P. aeruginosa* exhibit a global negative charge of the cells at pH close to neutrality (Table 4.2). The electric charge on the surface of a bacterial cell is attributable to a large extent to the kind of ionisable groups present on the cell surface and to their spatial distribution. In gram-negative bacteria the major contribution to their surface charge is made by ionisable amino (NH_2) and carboxyl (COOH) groups of proteins exposed at the cell surface (Gittens & James1963; Sherbet & Lakshmi 1973). Such bacteria have been shown to have an overall net negative surface charge. Acid lipopolysaccharides present in the outer membrane of gram-negative bacteria also contribute to the negative charge level (Sutherland 1977). In general, there was no clear influence of the medium on the zeta potential of *E. coli* and *P. aeruginosa*. The cell size distribution of *E. coli* and *P. aeruginosa* cultivated in nutrient-rich (NB, MMG) and poor (GW) media are shown in Figures 4.9 and 4.10. For *E. coli* grown in rich media bacterial cells have mean particle size around 1,050 nm (range 825–1,480 nm). For the same microorganism starved one week in GW the size was smaller and close to 550 nm. For *P. aeruginosa* bacterial cells grown in rich media have mean particle size around 1,050 nm (range 700–1,280 nm). For the *P. aeruginosa* cells maintained one week in GW the size decrease to a value between 450 and 530 nm. These results highlight that if the medium used to develop the bacteria is rich like NB or MMG, the cells size will be bigger than in poor medium like bathroom GW.

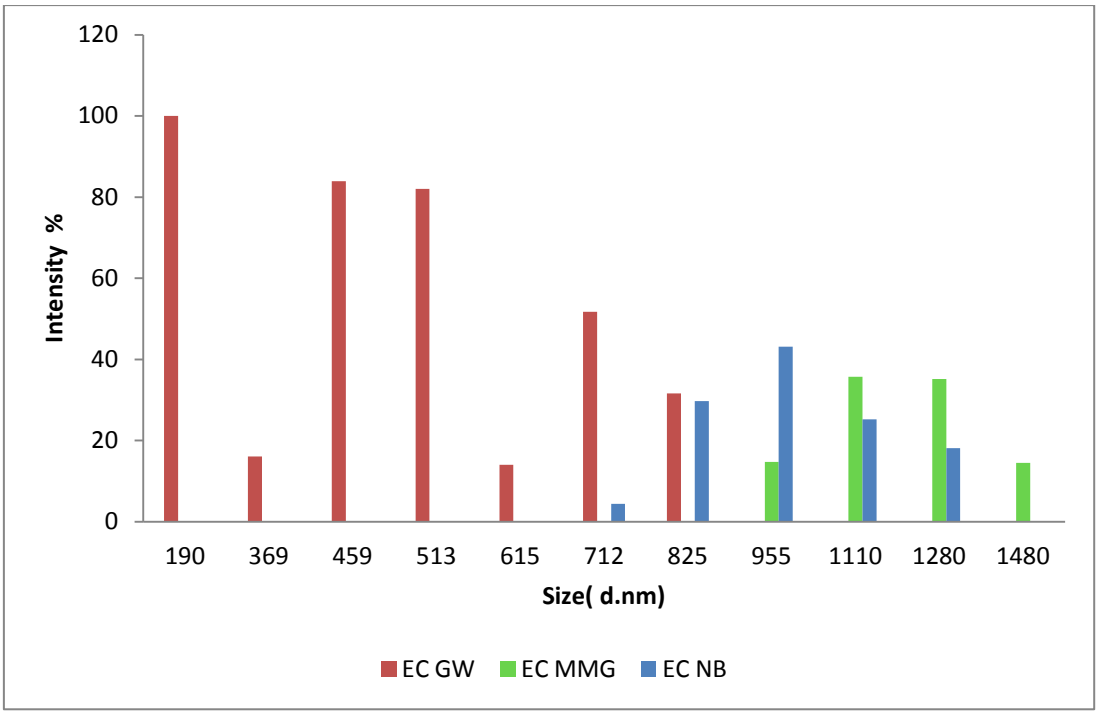

Figure 4.9 Effects of different media on particle size of *E. coli*.

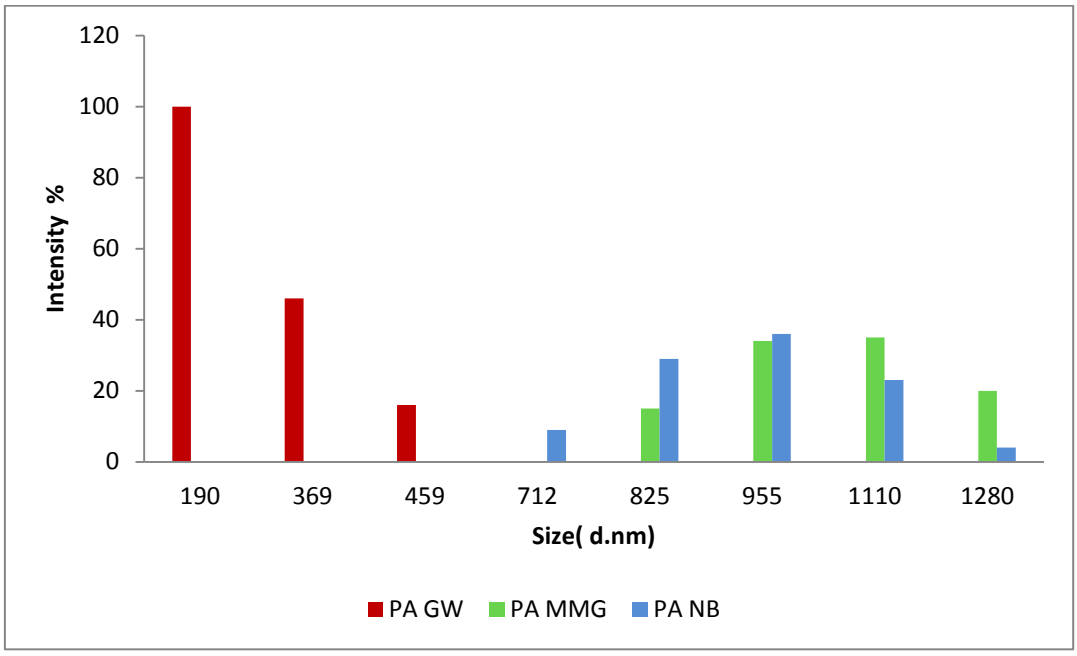

Figure 4.10. Effects different media on particle size of *P. aeruginosa*.

4.1.4 Conclusion

After this study, we can conclude that the treatment of GW with various abiotic factors (temperature, aeration, salt concentrations and nutrient starvation) inhibited the growth of some pathogenic bacteria (*E. coli* and *P. aeruginosa).* We conclude: (1) temperature and salinity have the greatest impact on survival of these bacteria; (2) if the medium has a low concentration of nutrients survival will be affected; (3) substrate and nutrients concentration in the medium also affect cell size and zeta potential. These parameters are of importance in the retention of microorganisms by filtration processes and will condition the interaction between particles and filtration material such as membrane or sand. Our study highlights the fact that for safe reuse of GW it is necessary to remove biological pollution. High temperature is an abiotic factor which could be used as a simple and economic treatment in arid and semi-arid areas in order to make safe use of GW for irrigation. This research could be more widely used and applied in sunny countries such as arid area countries.

4.2 Coliphage MS2 inactivation by abiotic factors in Greywater

4.2.1 Introduction

Abiotic factor has been explored for coliphage MS2 inactivation by used of two factors; temperature and high salts concentration in greywater. Enteric viruses have been responsible for many waterborne disease outbreaks. However, it is often not practical to detect viruses directly because the tests are time consuming, expensive, difficult to perform, and dangerous to operators due to their infectivity (Sowdon, 1989). In view of this, it is desirable to use appropriate virus indicators for assessing the performance of treatment systems in terms of microbial safety. F-Specific bacteriophages (e.g. MS2 coliphage) have been recommended for modeling viral behavior in water because their size and structural properties are similar to many of the human enteric viruses, and they can be quantified more easily and rapidly (Havelaar, 1987 ; Yates, 1985). The coliphage MS2 present a size of 24 nm, and an isoelectric point of pI =3.9 (Dowd et al. 1998). Coliphage MS2 has been used in previous wastewater reuse studies (Mocé-Livina et al. 2003; Arraj et al. 2005) and has well documented detection and enumeration procedures (International Standard Organisation 1995). MS2 is an f-RNA phage. In this study we investigated the relative disinfection efficiencies of different abiotic factors such as temperature (6°C ,25°C,40°C, 65°C) and high salinity (3.5% NaCl), to approach some treatments against coliphage MS2 viruses in laboratory-scale experiments of greywater reuse.

4.2.2 Material and Methods

4.2.2.1 Phage preparation and media

The MS2 coliphage was prepared by adding 1 mL of frozen stock of *E.Coli* HFr host to 100 mL of prewarmed TYGB (per litre : 10g Trypton, 1g Yeat extact and 8g NaCl), $CaCl_2$/glucose solution. The culture was placed in the incubator at 37°C for 2-3 hours until it reached mid log phase. Then 1 mL of MS2 phage (frozen stock) was added and the culture allowed incubating at 37°C overnight. A dilution series was prepared for each of the phage inoculate and a double agar layer assay for MS2 (International Standard Organisation 1995) was carried out to ensure that the concentration range was correct prior to dosing. The

concentrations of MS2 phage had to be at least 10^6 PFU. mL^{-1} so that log 6 removal could be proven by any system tested.

4.2.2.2 ColiphageMS2 and bacterial host

The MS2 bacteriophage used in this study is a male specific, or F+, bacteriophage that infects sex pili or conjugation tubes of specific *Escherichia coli* strains. MS2 bacteriophages have commonly been used as tracer or indicator. *E. coli* Hf (pFamp)R contains an F+ fertility plasmid as well as a plasmid containing resistance genes to ampicillin and streptomycin. Studies have shown nearly 95% host specificity between F+ viruses and the *E. coli* Hf (pFamp) R host (Calci et al., 1998).

4.2.2.3 MS2 Coliphage detection

Coliphages MS2 was quantified by a double –layer –agar technique following the ISO 10705-1 (ISO 1995) for enumeration of F-specific RNA bacteriophages. 200 µl of overnight grown Bacterial host culture *E.coli* Hfr and 200 µl of coliphageMS2 were taken and 0.8% top agar (TYG) semi solid added at 44°C. The mixture was evenly distributed on a Petri plates containing 1.6% bottom agar (TYG) after the agar solidification, the plates were inverted and incubated at 37°C. ColiphagesMS2 was enumerated by counting the clear areas (plaques) on the bacterial lawn, after 8 hours of incubation coliphageMS2 were enumerated by counting the plaques on the bacterial lawn (Figure 4.11).

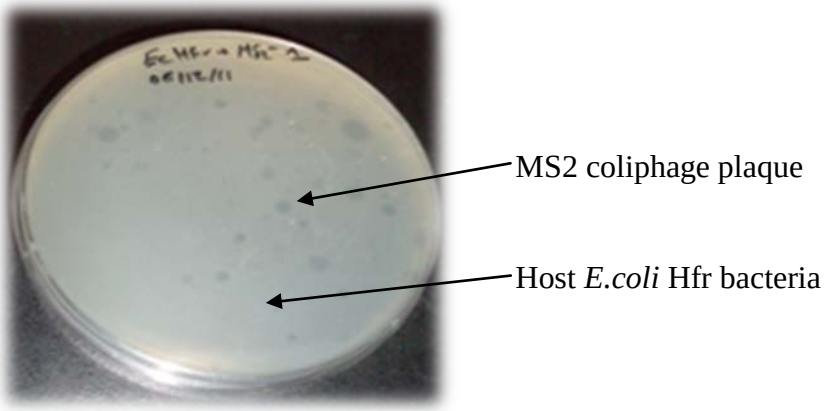

Figure 4.11. MS2 coliphages plaque on TYG double-layer-agar

4.2.2.4 Abiotic factors experiment design:

For the studies on the effect of temperature factors, four greywater samples were prepared at 6°C, 25°C, 40°C,and 65°C. A 10 ml aliquot of MS2 stock solution was added to the various samples at time zero. At time intervals, 1ml samples were assayed to determine concentrations of MS2. The experiments were conducted in triplicate. For salinity factor was used 3.5%NaCl incubation at 25°C.

4.2.2 .5 Data analysis

The efficiency of inactivation was assessed by calculate decay rates K and T90 for each experimental study. The inactivation rate (k) was expressed by the equation: k= (log10 Nt) /N0)/t, where Nt and N_0 are the final and initial numbers of viruses per milliliter volume of water respectively, and t represents time in hours. For non-linear relationships (tailing-off curve), the maximum inactivation rate was calculated by the same equation based on the initial linear phase.

The most common parameters to characterize rate of bacteria growth and decay are:

The decay rate k (h) were calculated according to Chick's law (Alkan et al., 1995) which is depicted as:

$$- kt = (\text{Log } N_t / N_0) \qquad \text{Eq. 4.1}$$

Where; N_0 and N_t is respectively the bacterial Numbers initial and at time t measured in hours

The T_{90}, the time required for 90% of the initial population has disappeared.

$$T_{90} = - t / \log (N / N_0) \qquad \text{Eq. 4.2}$$

Where: N_0 and N are respectively the bacterial Numbers initial and at time t measured in hours.

4.2.3 Results and discussion

4.2.3.1 Effect of abiotic factors on MS2 coliphages:

The results shown in Table 4.5 indicate that k and T_{90} had a significant effect, regardless of the treatment method used for inactivation MS2 Coliphages. In all cases, counts obtained after treatment MS2 by the abiotic factors in the presence of high temperature and salinity were significantly than those obtained with treatment by lower temperature.

MS2 Coliphage	**Parameters**	**Abiotic factors**				
		6°C	**25°C**	**40°C**	**65°C**	**3.5% Nacl**
Greywater	**k(h^{-1})**	**4.9 10^{-4}**	**1.5 10^{-4}**	**5.9 10^{-5}**	**1.0 10^{-3}**	**6.0 10^{-4}**
	T_{90}(h)	**0.5**	**1.3**	**3.0**	**0.5**	**0.5**

Table 4.5. T_{90} and k values for MS2coliphage depending of abiotic factors conditions

Figure 4.12. shows the effect of biotic factors (temperature and salt) on MS2 coliphage the time required for the total inactivation of MS2coliphage was about 180 min. in the presence of both temperatures and salt. The inactivation preceded slowly at temperature 25°C and 40°C and was completed in more than 160 and 180 min, respectively. Table 4.5 the rate decay K or T_{90} for minimum and maximum temperatures of approx. 4.9 10^{-4}, 0.5h and 1.0 10^{-4}, 0.5(h) GW respectively. With 3.5% NaCL the detected reduction in the number of MS2 was close to the same shape than with a temperature of 65 °C. The mechanism of virus inactivation by salts has been reported to affect the stability of the protein coat, and specifically the amino acids tyrosine and histidine (Hsu, 1964 & Cramer et al., 1976).

4.2.4 Conclusion

MS2 coliphage removals by control of environmental factors like temperature and salt concentration have been shown in this work.

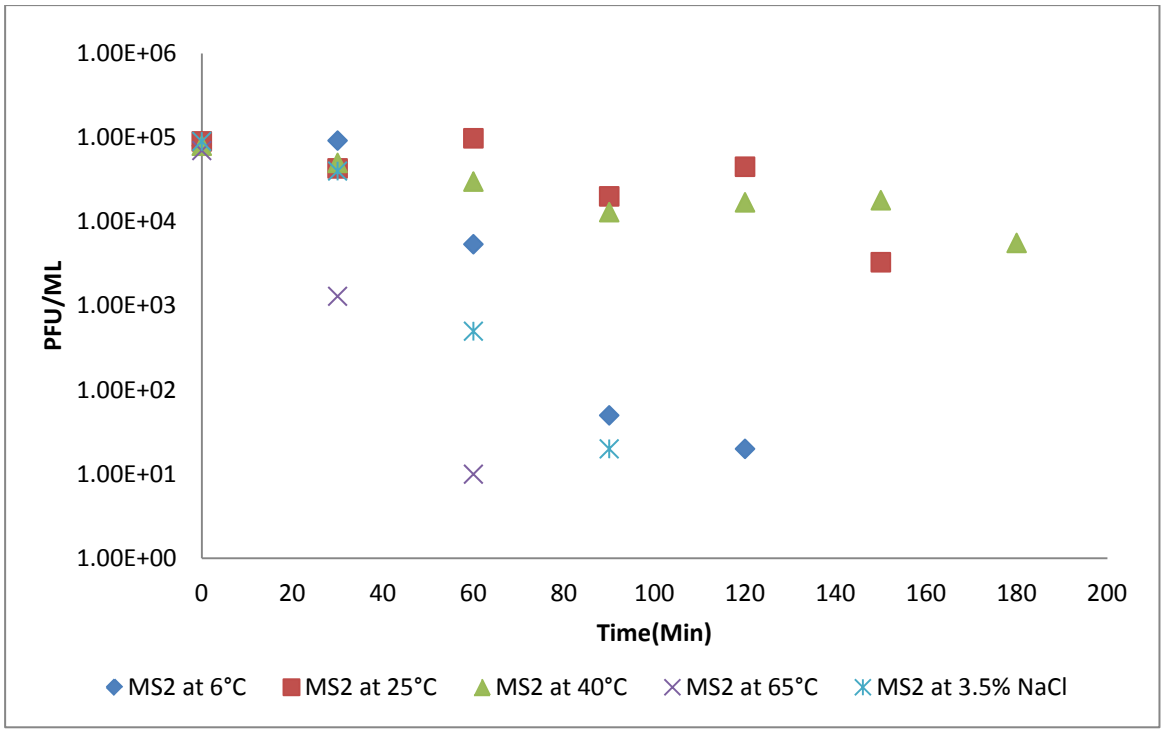

Figure 4.12. Inactivation MS2 coliphage by abiotic factors

CHAPTER FIVE

Inactivation of *E. coli* and *P. aeruginosa* and Bacteriophage MS2 in greywater by NF-TiO_2 photocatalyst under various light irradiation conditions

5.1. INTRODUCTION

World population growth, global warming, long-term droughts, and the rapid development of industrialization lead to a worldwide increase of water demand. Various practical strategies and alternative solutions have been adopted to yield more viable water resources. Arid and semi-arid areas in many countries with abundant sunlight, less rainfall, and long-term droughts experience challenges to preserve viable water resources. This problem affects around 4 billion people worldwide with consequences to have no or little access to clean and sanitized water supply, and millions of people die of severe waterborne diseases annually (Malato et al., 2009). Many research programs are expected to grow in the near future, due to the increasing water contamination by the overwhelming discharge of micropollutants and contaminants into the natural water cycle (Wintgens et al., 2008; Richardson, 2000). The conventional water treatment methods such as coagulation/flocculation/sedimentation, filtration, chemical processes, and membrane technologies involve high operating costs and could generate toxic secondary pollutants into the ecosystem (Gaya and Abdullah, 2008) and in many cases do not don't treat pathogenic microorganisms. Greywater GW) is any type of domestic water that comes from kitchen sinks, baths, washing machines and hand basins excluding black waters (toilets and urinals). GW usually contains surfactants (anionic, cationic and amphoteric) which come from shampoos, detergent formulations, etc. (Eriksson et al., 2002). Several technologies have been developed for GW treatment and reuse in the literature, including natural treatment systems (Gross et al., 2007), basic coarse filtration (Kim et al., 2007), chemical (Winward et al., 2008) and biological processes (Merz et al., 2007). Nevertheless, all these treatment processes need to add a sanitation step. Greywater can be reused in several applications such as toilet flushing, garden irrigation or washing vehicles. In recent years, interest has been focused on the use of semiconductor materials as photocatalysts for the removal of organic and inorganic species from aqueous or gas phase. Recently, modifications towards visible light response of titanium dioxide (TiO_2) has extended the capacity of TiO_2 to utilize a larger portion of the solar spectrum, a renewable source of energy, for a wide variety of applications, including environmental remediation and energy conversion. (Pelaez et al.,2010a). This method has been suggested in environmental protection due to its ability to oxidize the organic and inorganic substrates but also for the inactivation of microorganisms. In heterogeneous photocatalysis, two or more phases are used

in the photocatalytic reaction. A light source emitting appropriate wavelength is used to illuminate the photocatalyst and initiate the photoreaction. The heterogeneous photocatalytic technologies have several advantages over other traditional method such as working under ambient conditions of temperature and pressure, the use of air as oxidant reactant, and the possibility of using solar light to drive the process. All these characteristics present very interesting aspects from the energy consumption and environmental impact viewpoints (Barcina et al., 1989). This study focuses on the disinfection of microorganisms by as the use of novel photocatalysts such as sol-gel based NF-TiO_2 film in the presence of solar light. a new methods of semiconductors materials as phtocatalysts such as sol-gel based NF-TiO_2 film with presences of solar lights. Recently, modifications towards visible light response of titanium dioxide (TiO_2) has extended the capacity of TiO_2 to utilize a larger portion of the solar spectrum, a renewable source of energy, for a wide variety of applications including environmental remediation and energy conversion (Pelaez et al. 2010).this study also investigated affect of UV irradiation on the efficiency inactivation rate (K) and T90 the time require to remove of 90% of coliphage MS2.

This work focuses on the evaluation of novel photocatalyst with enhanced photocatalytic activity for the inactivation of pathogenic microorganisms and efficient disinfection of greywater. The purpose of the study was the comparison of microorganism inactivation under various light source conditions: UV light, visible light, visible light with catalyst, and control in the dark. *Escherichia coli* (EC) and *Pseudomonas aeruginosa* (PA) have been selected as model microorganisms well as bacteriophage MS2.

5.2 METHODOLOGY

5.2.1 Microorganism and growth

Both (EC) and (PA) were isolated from bathroom greywater samples. The species were grown in nutrient broth medium containing (Peptone 10 g/l and yeast extract 5 g/l at 37° C). The cells were grown in a 100 ml Erlenmeyer flask in a shaking incubator at 300 rpm at 37°C. A suitable cell concentration (10^6 colony forming units (CFU) per ml) was inoculated in the one liter of deionized water (DW) or greywater (GW) put in the photoreactor. During the 4 hours of illumination, samples were taken (1.0 mL) at different time intervals. Both irradiated and non-irradiated cell suspensions were diluted sequentially and plated on lactose TTC agar with

tergitol 7. Plates were then incubated at 37°C and colony counts were taken after 24 h of incubation. The GW was harvested from a shower facility. The typical composition is presented Table 5.1.

For Coliphage MS2 preparation and media please referred in the chapter 4

Parameters	Average	Min-max
COD (mg/l)	170	116-233
Turbidity (FUN)	28	9-68
pH	7.8	7.6-7.9
Conductivity(µS/cm)	399	360-465

Table 5.1. Typical greywater composition (COD: Chemical Oxygen Demand ; Chaillou et al., 2011).

5.2.2 Reactor, light source and catalyst

Photocatalytic experiments of bacteria inactivation were carried out using a batch Photoreactor. The photoreactor consisted of a Pyrex beaker of a total volume of 1000 ml with a diameter and the depth of 10 cm and 20 cm, respectively. The reactor was placed on a magnetic stirrer to provide a good mixing. The irradiation was provided by a Xenon lamp (450 W) and by using dichroic mirrors; a proper range of wavelength was selected: 280–400 nm and 350-630 nm. An AM1.5 filter fixed on the output of the beam allowed obtaining the solar spectrum. The light intensity was monitored using a spectroline long wave (320-550 nm) UV and visible light. The light source was placed 26 cm above the surface of the water. An irradiance of 2.35 mW/cm^2 was measured from the light source incident to the top of the beakers by an UV radiometer (IL 390C, International Light Co., USA). The temperature during the experiments was maintained at 20±2°C and the microbial suspension was aerated (Figure 5.1). The photcatalyst NF-TiO_2 was used in a thin film deposited on glass slide (Pelaez et al., 2010b). The used of a doped TiO_2 catalyst with non-metallic elements such as nitrogen and fluorine was chosen because of its ability to absorb light in the visible region (Pelaez et al., 2009).

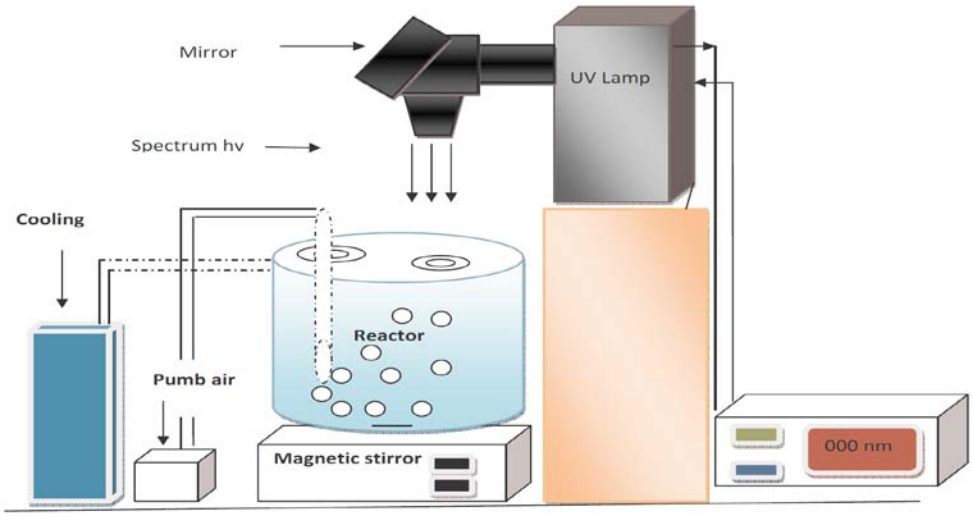

Figure5.1. Schematic diagram of photocatalytic reactor

5.2.3 Heterogeneous photocatalysis reactions

Heterogeneous photocatalysis is a complex sequence of reactions. The oxidation pathway is not yet fully understood. However, (Pirkanniemi 2002) suggested that the heterogeneous photocatalysis reaction follows five steps. These are: (i) diffusion of reactants to the surface, (ii) adsorption of reactants onto the surface, (iii) reaction on the surface, (iv) desorption of products from the surface, and (v) diffusion of products from the surface (Figure 5.2).

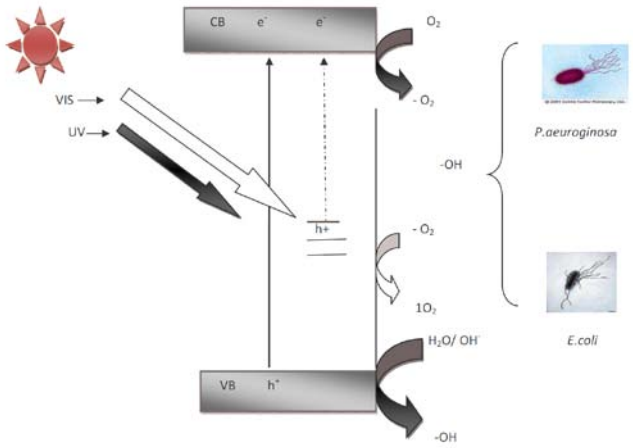

Figure 5.2. Photocatalytic *P.aeruginosa* and *E. coli* inactivation under simulated solar exposition on sol-gel NF doped TiO_2 .

5.2.4 Disinfection Model

The most common parameters to characterize the rate of bacteria growth and decay are:
The decay rate constant k (h) was calculated according to the Chick's law (Alkan et al., 1995), which is depicted as:

$$- kt = \log (N_t / N_0) \qquad \text{Eq. 5.1}$$

Where: N_0 and Nt are respectively the bacterial numbers at the initial condition (time zero) and at time t measured in hours

In this study, T_{90}, which is the time required to cause death to the 90% of the initial population is calculated using the expression below

$$T_{90} = - t / \log (N / N_0) \qquad \text{Eq. 5.2}$$

Where: N_0 and N are respectively the bacterial Numbers at the initial conditions (time zero) and at time t measured in hours.

5.3 RESULTS AND DISCUSIONS

5.3.1 Bacterial inactivation under UV and visible light irradiation in dionezied water

The comparison between inactivation curves of EC and PA in DW under various radiation conditions are presented in Figures 5.3 and 5.4. With time, the cultivable bacteria population slowly decreased in the control experiments (with visible light and in darkness). In these conditions the bacterial mortality could be explained by the mechanical aggression (stirring) of the experimental device. Moreover, the shape of curves obtained under UV or in presence of NF-doped TiO_2 catalyst for visible light irradiation clearly indicates that the cultivable bacterial populations decrease in comparison to visible light irradiation alone or without any light.

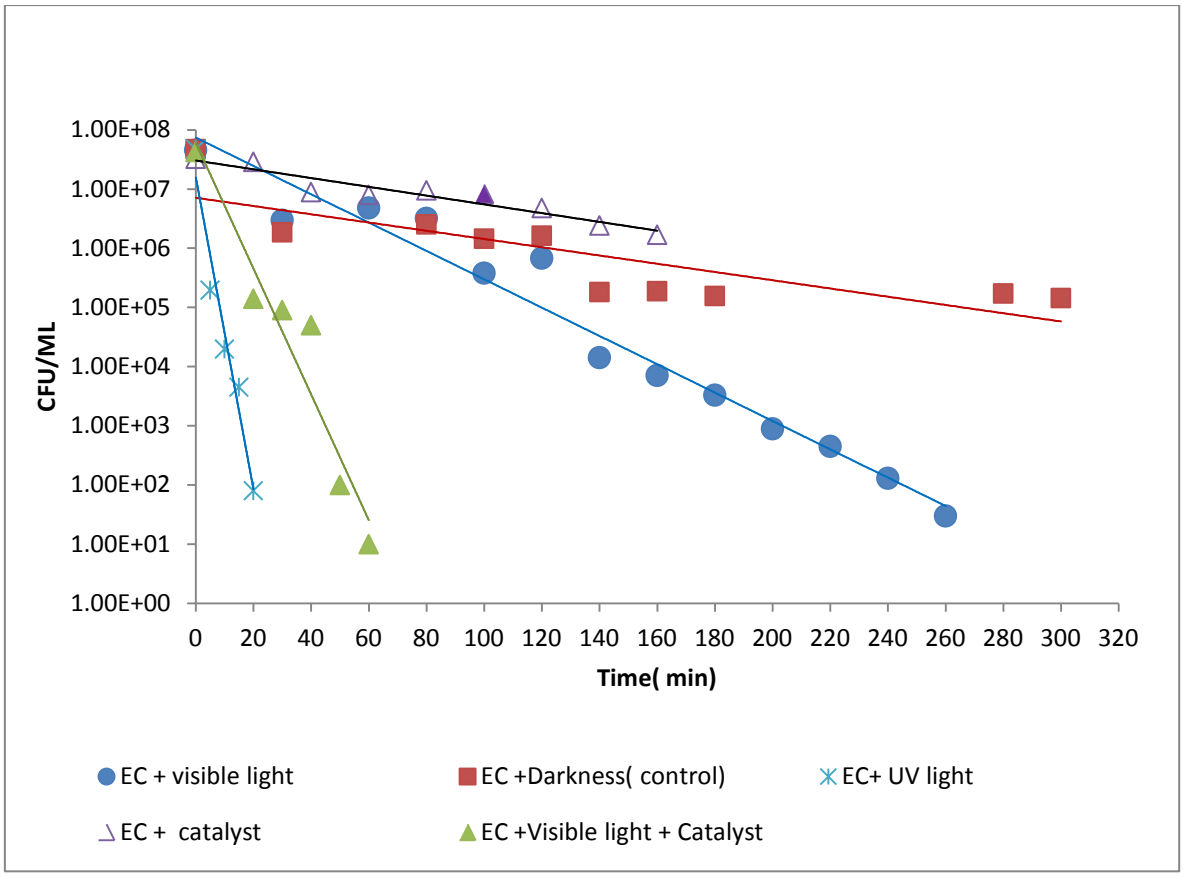

Figure 5.3. *E.coli* mortality in DW under various light irradiation conditions

The beneficial role of UV irradiation or the use of a photocatalysis in visible spectrum for microbial disinfection is evident. In a recent study, a similar effect was observed concerning *E. coli* loss of viability during its contact with NS- TiO_2 photocatalyst (Rengifo-Herrera et al., 2009). For the case of UV irradiation, the main proposed mechanism is that most of the light absorption occurs directly on the nucleic acid and on protein. This energy transfer leads to destruction of the macromolecules, which are indispensable to microbial life.

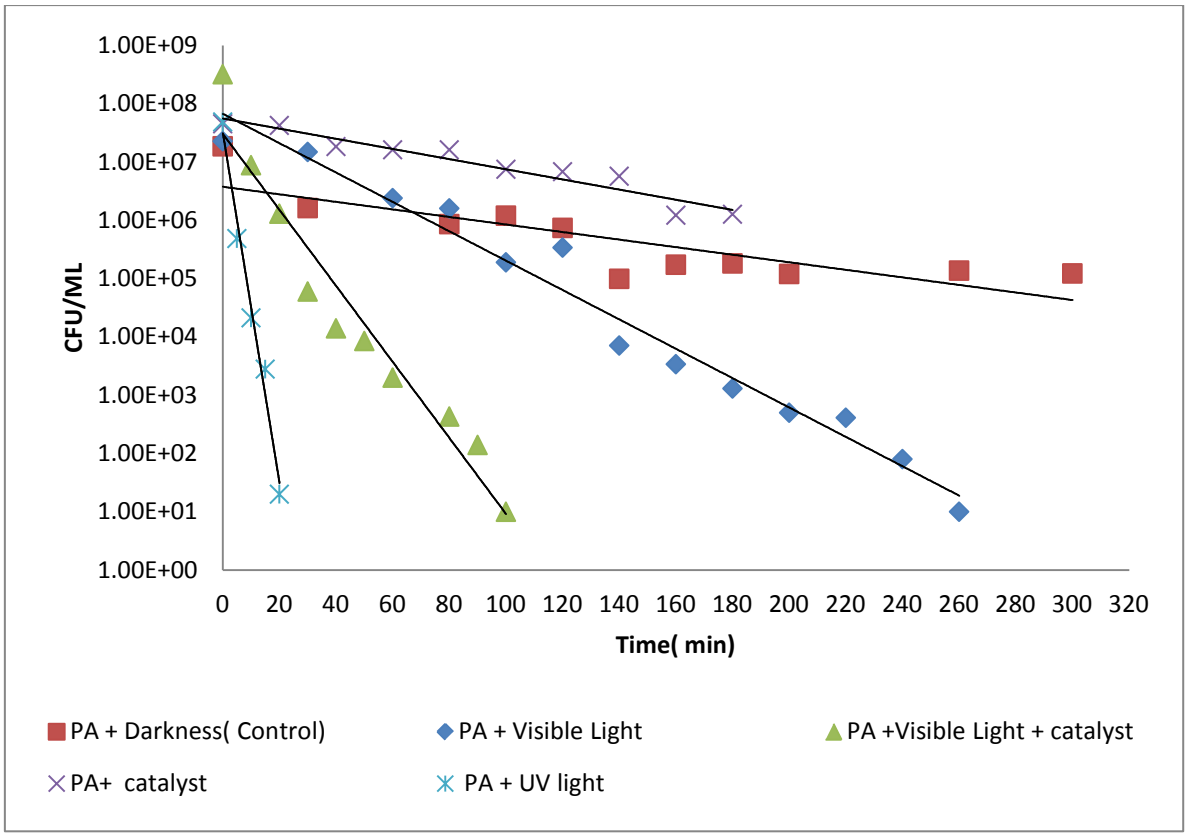

Figure5.4 *P aeruginosa* mortality in DW under various light irradiation conditions

It is generally accepted that hydroxyl radicals HO• are the main oxidative species responsible for the bactericidal action of TiO_2 photocatalysis. However, there is also some evidence that other reactive oxygen species generated photocatalytically, such as superoxide radicals $O_2^{\bullet-}$, perhydroxyl radicals $HO_2^{\bullet}$, and hydrogen peroxide H_2O_2 also contribute to the photocatalytic inactivation (Cho et al., 2004). These results imply that bacteria interacted with the catalyst surface and this interaction resulted in partial degradation of cell membrane integrity and consequently loss of cultivability. A few microorganisms that are resistant to UV-A photolysis have been inactivated successfully by TiO_2 photocatalysis, namely; *Enterobacter cloacae, E. coli, P. aeruginosa* and *Salmonella typhimurium* (Ibanez et al., 2003). Cell membrane is the crucial site of attack for effective inactivation, regardless the oxidative species involved in the process. It has been proposed that the cell wall is initially damaged, followed by a progressive damage of the cytoplasmic membrane and intracellular components, thus eventually leading to loss of essential cell functions (Sunada et al., 2003). For the visible domain,(Rengifo-Herrera et al., 2009) propose the formation of singlet oxygen which can react with the bacterial cell wall compounds.

			Conditions			
			Dark	**Visible light (420-630nm)**	**Visible light and NF TIO_2**	**UV (280-400nm)**
Escherichia. coli	***Deionized Water***	**k (h^{-1})**	**$1.40\ 10^{-4}$**	**$3.96\ 10^{-4}$**	**$1.84\ 10^{-3}$**	**4.8110^{-3}**
		T_{90} (h)	**2.00**	**0.70**	**0.15**	**0.05**
	Greywater	**k (h^{-1})**	**$2.29\ 10^{-4}$**	**$1.89\ 10^{-4}$**	**$8.22\ 10^{-4}$**	**$2.26\ 10^{-3}$**
		T_{90} (h)	**2.15**	**1.47**	**0.34**	**0.13**
Pseudomonas aeruginosa	***Deionized water***	**k (h^{-1})**	**$1.21\ 10^{-4}$**	**$4.08\ 10^{-4}$**	**$1.25\ 10^{-3}$**	**$5.32\ 10^{-3}$**
		T_{90} (h)	**2.30**	**0.68**	**0.22**	**0.05**
	Greywater	**k (h^{-1})**	**$1.85\ 10^{-4}$**	**$1.96\ 10^{-4}$**	**$9.28\ 10^{-4}$**	**$2.26\ 10^{-3}$**
		T_{90} (h)	**1.5**	**1.42**	**0.30**	**0.12**

Table 5.2. T_{90} and k values for EC and PA depending of the illumination conditions

5.3.2 Bacterial inactivation under UV and visible light irradiation in greywater

When the same methodology is applied for GW, the obtained curves present a similar shape (Figure 5.5; 5.6). To be able to compare the efficiency of the various conditions examined, decay constants and T_{90} were determined using eqs. 5.1 and 5.2. The calculated values are summarized in Table 5.2. The light sensitivity of the two studied bacterial species was similar.

For the control essays, the decay is a little bit higher in DW that in GW which could be explained by the presence of nutrients and organic molecules in GW. Under UV or when the catalyst was used in the presence of visible light irradiation, the constants obtained indicate a higher sensitivity of EC compare to PA. Studies in DW show a better efficiency of both processes than in the case of GW. This observation could be explained by the fact that in GW, other compounds present could react with the reactive species produced or by absorbing the incident UV and thus inhibit the rate of bacteria inactivation Figures 5.5 and 5.6. From the T_{90} obtained, it is possible to propose the following order of efficiency in relation to the irradiation conditions: UV > visible light with catalyst > Visible light > dark.

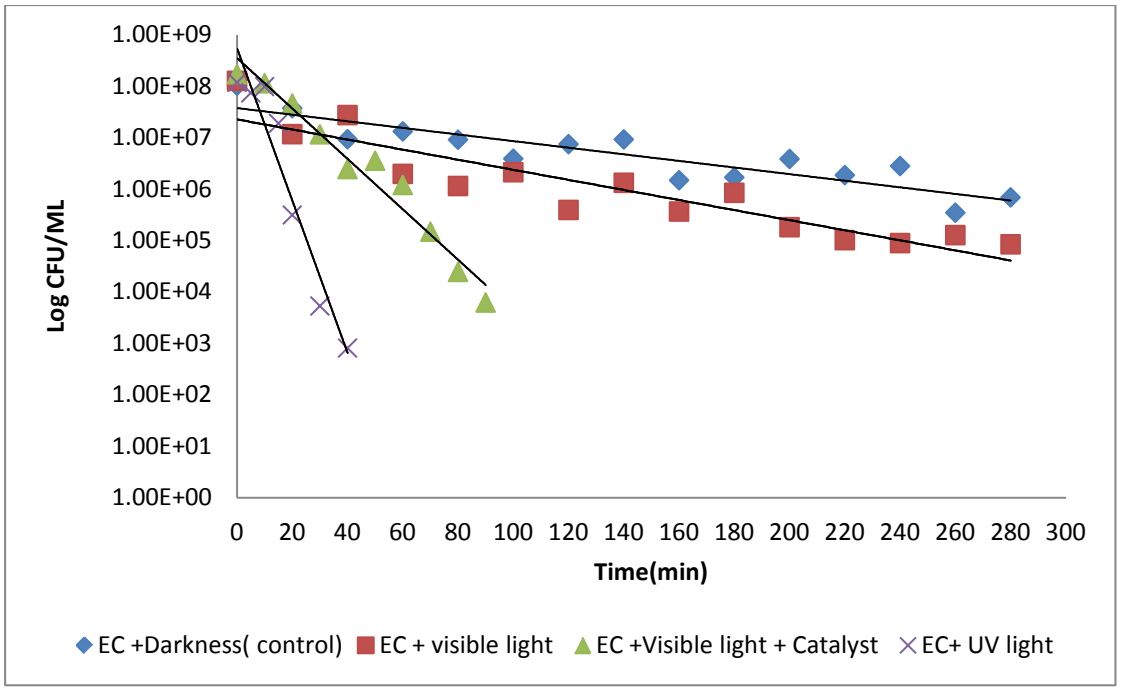

Figure 5.5. *E.coli* mortality under various light irradiation conditions in greywater

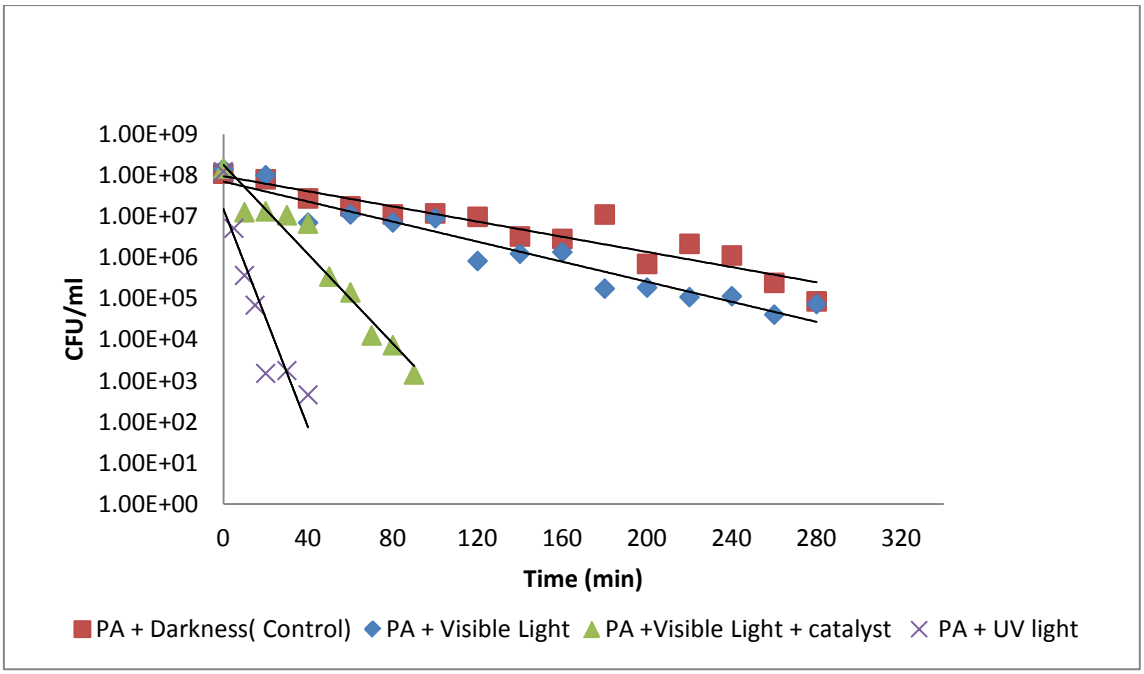

Figure 5.6. *P.aeruginosa* mortality under various light irradiation conditions in greywater

5.3.3 Effect of irradiation on MS2 coliphages in Greywater

Inactivation of coliphage MS2 may be due to changes or damage to their surface structures, such as the protein coat or the attachment sites needed for infection of host cells (*E.coli* Hfr specific host for coliphage MS2). Numbers of coliphage MS2 (log of the relative Plaque population) as a function of time during photocatalytic treatments are shown in Figure 5.7 and 5.8. The time required for the inactivation of coliphage MS2 was approximately 60 min under UV light, while over 180 min were required for the inactivation under visible light with catalysis.

MS2 Coliphge		**Light conditions**			
		darkness	**visible**	**Visile+catalyst**	**UV**
Deionized water	**$k(h^{-1})$**	**$1.4\ 10^{-4}$**	**$3.2\ 10^{-4}$**	**5.410^{-4}**	**1.210^{-4}**
	T_{90}(h)	**1.5**	**0.8**	**0.5**	**0.2**
Greywater	**$k(h^{-1})$**	**1.110^{-4}**	**$2.2\ 10^{-4}$**	**$3.4\ 10^{-4}$**	**$7.8\ 10^{-4}$**
	T_{90}(h)	**1.9**	**1.2**	**0.8**	**0.3**

Table 5.3. T_{90} and k values for MS2colipheg depending of the illumination conditions

Disinfection rate constants were calculated based on Chick's law in which - In (N. No 1) was plotted as a function of time; the inactivation rate constant k. The data show that photocatalysis rates of coliphage at pH 6-8 followed Chick's law (i.e., inactivation rate) with k under UV light 7.4 10^{-4} in greywater values greater than 1.2 10^{-4} in deionized water an exponential regression for all tested (Figure 5.7; 5.8 and Table 5.3).

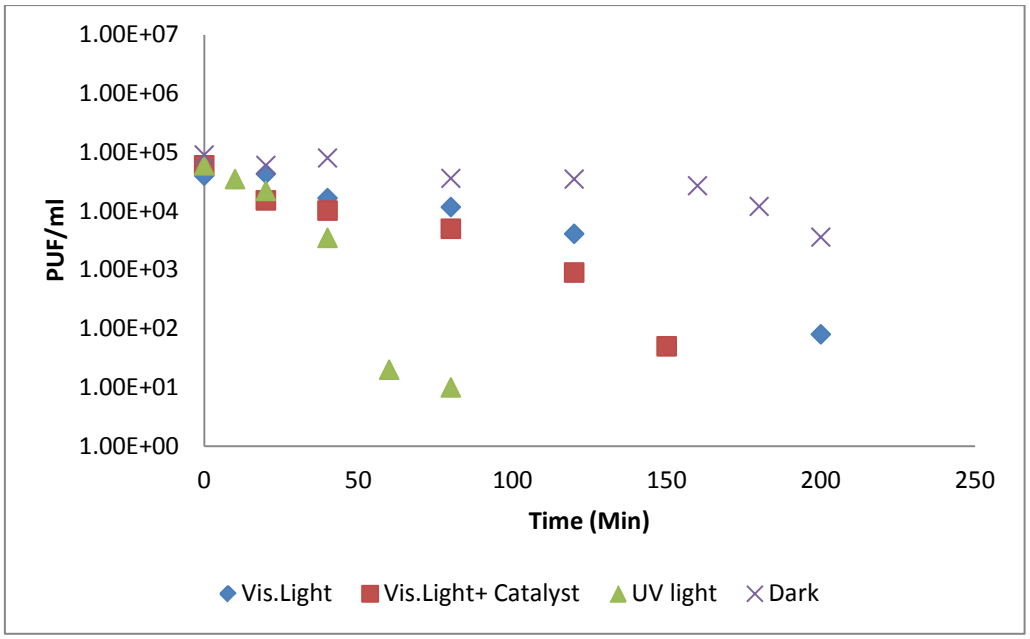

Figure 5.7 inactivation MS2coliphage by Visible and UV light in Greywater

Montogomery (1985) described two primary disinfectant properties that control their efficacy: (1) oxidation and disruption of the cell wall and membrane with resulting disintegration of the cell, and (2) diffusion of the disinfectant into the cell or particle where it may inactivate enzymes, damage intracellular components, interfere with protein synthesis, etc.

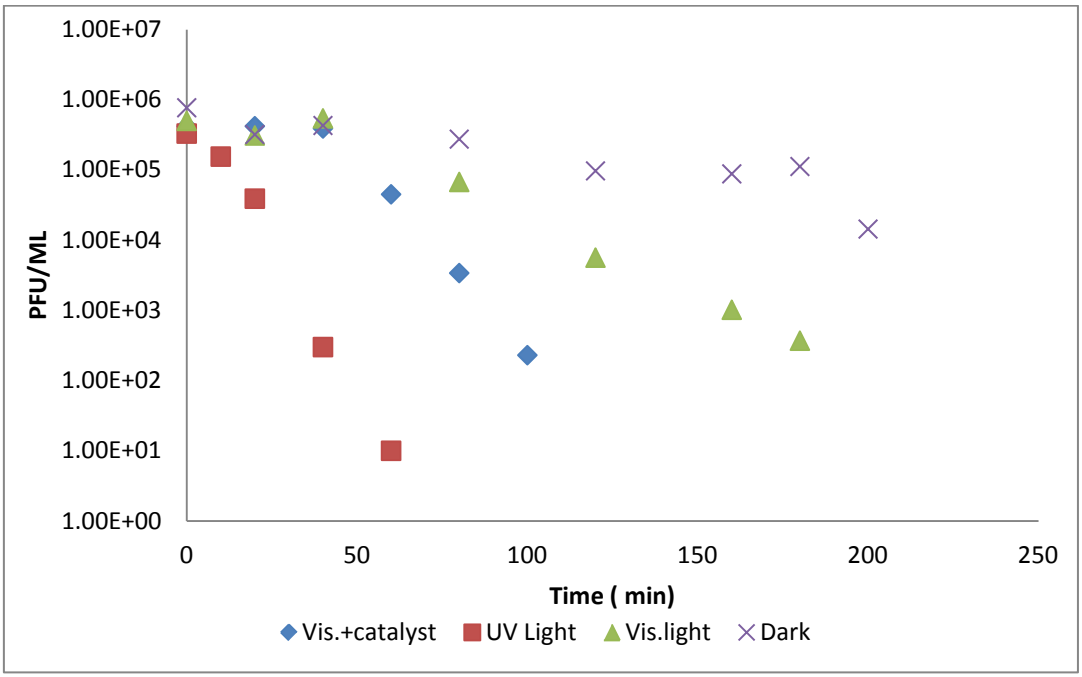

Figure 5.8. inactivation MS2coliphage by Visible and UV light in Deionized water

Titanium dioxide photocatalytic oxidations occur by electron transfer reactions and hydroxyl radical generation at the mineral surface (Ollis, 1985). Hydroxyl radical reacts with most biological molecules at diffusion-controlled rates (Dorfman and Adams, 1973). Therefore, disinfection by photocatalytically generated hydroxyl radicals may be limited by mass transfer through the cell wall or cell membrane. Photocatalytically induced surface oxidations of the cell wall, which may results in altered permeability and leakage of the cytoplasm, are documented as disinfection mechanisms, but potential limited diffusivity into the cell or particle may affect heterogeneous photocatalytic disinfection.

5.4 CONCLUSIONS

Bactericidal properties of UV and visible light/ NF-doped TiO2 were demonstrated by their ability to inactivate Gram negative bacteria *E. coli* , *P. aeruginosa* and *ColiphageMS2* . The photocatalytic inactivation kinetics was greater for *E. coli* cells. NF-doped TiO_2 in deionized water was found to be a more effective photocatalyst than NF-doped TiO_2 in greywater under similar experimental conditions. Disinfection by TiO_2 photocatalyst may be a promising technology. With further research into its practicality for treatment of water, it may become a possible substitute process for chlorination.

Conclusion

General conclusion and Perspective

This work highlights an important issue which occupies a lot of researchers around the world, namely the issue of lack of usable water. Therefore many studies have been developed for the search of solutions to that problem, such as use of treatment technologies for providing good water quality for water reuse. It was noted that the most difficult challenges of treatment is the presence of microbial pathogens in greywater, which lead to the occurrence of disease and sometimes could lead to death.

First used treatment is by slow sand filter filled by two types of sand (Egyptian desert sand and swimming pools filter sand). A sand characterization was realized by investigated the grains size and sand composition which was examined by SEM photography and X ray respectively. The bed porosity and residence time distribution was also determined. The results show differences between the two used sands. Greywater characteristics and composition were examined such as Chemical Oxygen Demand (COD), Conductivity and Turbidity. In a second section, treatment capacities of the slow sand biofilter were study. COD decrease with filtration time and biofilm formation. Egyptian desert sand seems to present better treatment efficiency. The value of turbidity were different according to hygiene product used (shower gel or soap). The impact of slow sand biofilter process on survival of microorganisms was also investigated and very low effect was observed. The remaining microbial concentration after was too high for a direct water reuse.

The second part of this work was dedicated to the study of the effect of abiotic factors which could affect microorganisms and coliphageMS2 survival. For example, pathogenic indicator bacteria survival at 6±2, 23±2 and 42±2 °C, coliphage MS2 survival at 6±2, 23±2, 39±2 and 63±2°C was explored. The salt concentration effect of bacteria survival at 1.75% and 3.5% NaCl, and coliphage MS2 survival in one salt concentration of 3.5% NaCl was determined. Moreover, available and unavailable oxygen (aerobic and anaerobic) effect onto the bacterial survival was also measured. A nutrient deficiency (rich or poor media) was investigated to better understand the influence of the growth medium on the size and electrical charge of the microorganisms. The results clearly highlight that poor medium and greywater allow the growth and survival of *E. coli* and *P. aeruginosa* but with a cell size reduction until a 2 factor. High temperature shows that it provides the greater inactivation of pathogenic indicator bacteria and coliphageMS2 (42±2°C and 65°C respectively). The salinity increase in the

medium until 3.5 % NaCl presents an inactivation of pathogenic indicator bacteria and bacteriophages.

Finally, light irradiation (Visible and UV) with or without photocatalyst were used for inactivation of pathogenic indicator bacteria and coliphage MS2 in greywater. Visible and ultraviolet light in presence of a catalyst lead to the inactivation of bacteria and coliphages. In General, microorganisms' inactivation light irradiation takes a longer time in greywater than deionized water.

Now the challenge is to improve the slow sand biofilter to stabilize the treatment performance and to choose the better way to reduce the microbial content of the produced greywater. Moreover, economic feasibility to achieve the concept of sustainable development through the reuse of greywater in various applications such as irrigation in agriculture and toilet flushing has also to be investigated.

Future and Perspective

The research presented in this thesis seems to have raised more questions that it has answered. There are several points in many fields study like as photocatalysis, treatments and reuse in agriculture and challenges which faces us when application in arid and semi-arid area. For example, Egypt faces a high population growth and therefore an increasing demand for water. Considering the limited amounts of renewable freshwater resources in Egypt, these resources have to be protected and saved for drinking water. Therefore, the agricultural sector has to be prepared to depend more and more on marginal water resources such as treated greywater.

1- Perspectives

- **Perspectives for photocatalysis and slow sand filter**

  Used widely in disinfection Ag like catalysis could be added in the photocatalyst because it have a more affect on bacteria and more studies on free radicals and it effect on cell wall and cytoplasm. In general, could be used of sun spectrums direct with presence of sheets of aluminum profile or Nano titany to improve the treatment. For greywater treatment by slow sand filter carry out for a long time, therefore influence on biofilm microorganisms.

- **Perspective for reuse greywater in agriculture:**

  Reuse of treated greywater in irrigation has to be study taking into account the health impact and the economic feasibility. Greywater have a composition which make the soil a little bit more basic. The plants which growth and prefer basic soil are olives, acacia, fig and wood trees so it's possible to imagine irrigation of these culture to increase the trees production.

2 - Projects: A pilot project in South Valley University

The project is to recycle greywater by collection in treatment unit and by conveying the greywater treated to irrigate areas via pipes. The project also involves the use of a low capital and operating costs by used sand filter and sun spectrum system, reuse greywater treatment for irrigation (Fig 6).

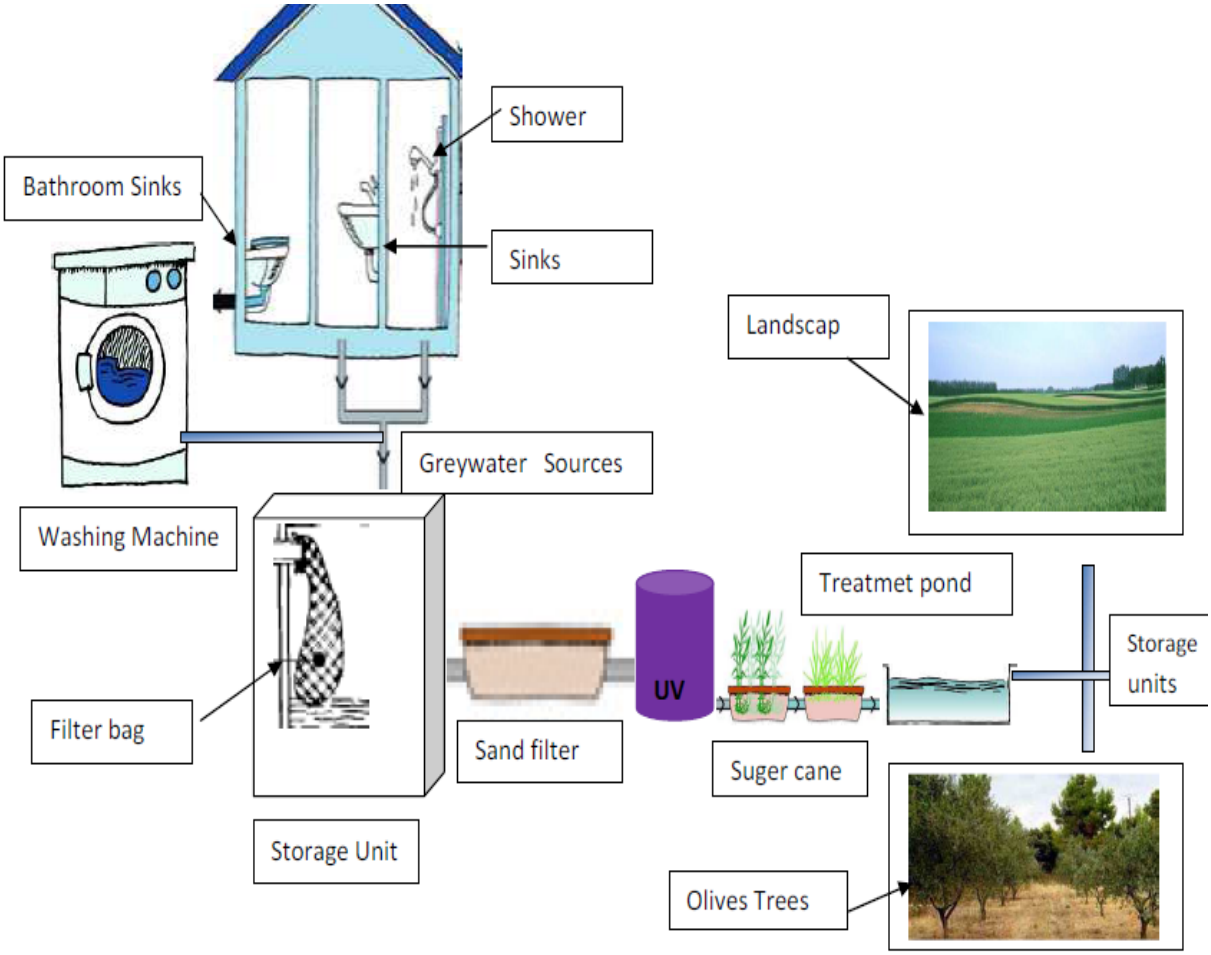

Figure 6 Greywater collection, treatment and reuse for irrigation landscap and economic plant

Traitement des eaux grises par filtration lente pour leur réutilisation : étude de la survie des micro-organismes pathogènes et des bactériophages

Résumé et mots-clés

Dans les dernières décennies, la plupart des pays du monde ont connu une pénurie d'eau et l'augmentation du taux de consommation. Aujourd'hui, tous les pays dans le monde essayent de trouver des alternatives pour remédier à cette pénurie. Une solution consiste en la réutilisation des eaux grises (GW) pour l'irrigation après traitement. Les GW correspondent aux eaux usées générée dans une maison à l'exception de l'eau des toilettes. Les risques associés à la réutilisation de ces eaux est la présence de microorganismes pathogènes qui peuvent infecter les humains, les animaux et les plantes. Dans cette thèse centrée sur l'étude de la survie des représentants d'agents pathogènes, comme *E. coli*, *P. aeruginosa*, et le bactériophage MS2 qui sont trouvés dans les eaux grises. Il a été étudié l'effet de quelques facteurs physico-chimiques tels que; température (6 ± 2, 23 ± 2 et 42 ± 2 ° C), la salinité (1,75 and 3.5% de NaCl), de l'oxygène (aérobie et anaérobie), des éléments nutritifs (milieu riche et de milieux pauvres), la lumière avec la photocatalyse (lampes UV et visible) et filtre à sable lent (sable du désert égyptien et le sable piscine). Une combinaison de la température, la lumière du soleil et de haute photocatlysis sont principalement responsables de la baisse rapide des bactéries et du coliphage MS2. Le filtre à sable lent a une influence nettement moindre sur la survie des bactéries dans les eaux grises, mais il est efficace pour diminuer la turbidité et de la DCO.

Mots-clés : Filtration lente, filtre à sable, eaux grises, réutilisation, survie microbienne (*E. coli*, *P. aeruginosa*, coliphage MS2), facteurs abiotiques, photocatalyse, UV.

1. Introduction

1.1 Contexte

La pénurie d'eau est l'un des défis les plus importants pour la santé humaine et l'intégrité de l'environnement dans la plupart des régions du monde. Alors que la population mondiale croît ainsi que la prospérité, la demande en eau augmentent se multiplie sans la possibilité d'une augmentation de l'offre. La demande croissante sur cette ressource limitée et précieuse a

inspiré des stratégies créatives pour la gestion de l'eau douce, y compris des techniques innovantes pour le recyclage des eaux usées. La réutilisation des eaux usées est une des stratégies, et son utilité pour répondre aux besoins en eau non potable doit être examinée minutieusement.

En général, l'eau mal géré du fait de la surconsommation de même que la pollution sont des facteurs aggravant la crise de l'eau. En effet, plus de 70% de la consommation d'eau douce est consacré aux activités agricoles (FAO, 2008). Récemment, la baisse de la productivité des exploitations agricoles commerciales a entraîné la proposition de politiques internationales de recommandation de la promotion de l'agriculture urbaine et péri-urbaine comme une solution à des situations de crise alimentaire (FAO, 1999). Le traitement des eaux grises et leurs réutilisations au niveau individuel peuvent fournir une solution combinée de ces problèmes en fournissant l'eau et les nutriments nécessaires à la production alimentaire des ménages. Le traitement des eaux grises et leurs réutilisations pour l'irrigation pourraient bien être la clé pour limiter la demande dans les réserves en eau douce tout en améliorant la capacité de production alimentaire des ménages et des exploitations agricoles.

Toutefois, il existe d'importantes préoccupations quant à la sécurité de la réutilisation des eaux grises pour l'irrigation. La question clé est le potentiel d'effets néfastes de la mauvaise qualité de l'eau sur le sol, les plantes et les humains. La qualité de l'eau pour l'irrigation agricole est un sujet de grand intérêt pour les chercheurs. En théorie, l'eau agricole ne doit pas être de qualité potable, ce qui ouvre la porte à l’utilisation des eaux grises pour l'irrigation de surface (OMS, 2006).

Toutefois, la population microbienne de l'eau non traitée est très diversifiée, et des organismes dangereux peuvent être présents. Les micro-organismes qui peuvent causer des maladies, collectivement connus comme pathogènes, sont généralement associées à des matières fécales humaines ou animales qui contaminent les eaux grises par le lavage des mains, des vêtements, des légumes et peuvent provenir directement de contamination de l’eau de surface. En conséquence de quoi il est généralement retrouvé dans les eaux grises un grand nombre de bactéries pathogènes (Rose et al, 1991;. Birks et al, 2004 ;. Jefferson et al, 2004). L'eau d'irrigation contaminée par des pathogènes a souvent été identifiée dans le cas de flambées de maladies d'origine alimentaire. Il est important de bien gérer ce risque lors de la promotion de la réutilisation des sources d'eau non potable pour satisfaire la demande en eau d'irrigation pour des activités agricoles.

Dans la plupart des pays du monde, la réutilisation des eaux usées n'est pas encore une pratique courante parce que la réglementation et la fixation de valeurs guides ou limites tarde à se mettre en place. Les quantités d'eaux grises produites pour chaque pays sont différentes et sont fonction des activités domestiques et des habitudes personnelles. De plus, il existe aujourd'hui des signes, pour certains pays où l'accès aux ressources en eau douce est historiquement privilégié, qui tendent à montrer des signes de pénurie. Comme les niveaux d'eau douce dans ces pays diminue et la pollution augmente, les prix sont à la hausse et l'utilisation efficace de l'eau est de plus en plus important pour les entreprises, les agriculteurs et les usages domestiques. En conséquence, l'intérêt pour les technologies de réutilisation des eaux grises est en croissance rapide.

1,2. Besoin de recherche

«Les eaux grises», qui désignent l'eau utilisée découlant de sources telles que douches, machines à laver, lavabos, représentent souvent plus de 2/3 (60-70%) des eaux usées domestiques, mais est considéré comme faiblement contaminée par des organismes pathogènes et d'autres substances potentiellement dangereuses (WHO 2006; Friedler 2004; Gulyas et al, 2004.). Dans le contexte d'une forte densité de population comme la plupart des villes un, traitement des eaux grises et leurs réutilisations complète est rarement possible au niveau individuel en raison des technologiques de traitement des eaux grises. La séparation à la source des eaux usées ménagères en eaux noires et grises est une stratégie qui présente le potentiel de réduire l'encombrement et les investissements nécessaires pour permettre la réutilisation de l'eau au niveau national.

Les systèmes groupés de réutilisation des eaux grises doivent permettre la réduction de deux facteurs mentionnés ci-dessus : gaspillage de l'eau d'irrigation et usage domestique ne nécessitant pas de l'eau potable. Il ya aussi un avantage économique à la réutilisation à savoir d'importantes économies de consommation d'eau et de traitement des eaux usées. Les risques pour la santé et l'environnement de cette forme de réutilisation de l'eau ne sont pas clairement identifié du fait du nombre limité d'études. L'utilisation des eaux grises pour l'arrosage des légumes et d'autres plantes comestibles est un sujet de préoccupation. Alors que les eaux grises peuvent apparaître bénignes, elles peuvent contenir des éléments dangereux tels que les

métaux lourds, les micro-organismes pathogènes ainsi que de produits chimiques toxiques qui pourraient polluer les sols de jardin et contaminer les cultures comestibles (Eriksson et al. 2002).

Cette étude met l'accent sur le traitement des eaux grises par la technologie de filtration lent avec utilisation de sable. Par ailleurs divers facteurs de l'environnement, tels que les facteurs abiotiques, ou l'utilisation de l'irradiation de lumière solaire avec catalyseur ont été étudiés. Sur cette base, le travail doit permettre d'apporter une réponse quand au problème des microbes pathogènes présent dans l'eau. Afin de promouvoir les pratiques de recyclage de l'eau dans le cadre des efforts visant à réduire la demande en eau dans les villes et les pays à forte croissance de population (Egypte), la recherche est nécessaire pour bien comprendre les avantages et les risques de l'irrigation des eaux grises.
L'Egypte, qui présente une superficie totale 1.000.000 km^2, est située dans la zone aride de la planète avec 94,5% de la surface correspondant à des territoires désertique ce qui laisse seulement 5,5 % à la population pour vivre. En Egypte le taux de la population augmente chaque année de 2% et actuellement la population est de 83 million d'habitants (EAS 2012). La consommation d'eau dans une maison égyptienne et les ablutions pour une personne est estimée à 200-300 L / jour. (National Water Research Center 2007). La conséquence de ces deux facteurs est qu'il existe un déficit de 1,4 milliard de mètre cube par an entre l'eau disponible soit 73,8 milliard de mètre cube et la consommation qui est d'environ de 75,2 milliard de mètre cube.

1.2.1. Objectifs et portée

Cette thèse vise à proposer un mode de traitement des eaux grises par un procédé de traitement rustique et robuste, mais aussi de comprendre paramètres de la survie et la désinfection des micro-organismes pathogènes (y compris les phages). L'objectif global est de fournir un procédé de traitement permettant de produire une eau exempte d'agents pathogènes pour la réutilisation des eaux grises. En conséquence, les objectifs suivants ont été identifiés (figure 1.1):

1. Evaluer les connaissances actuelles sur les agents pathogènes présents dans les eaux grises et l'adéquation des technologies de désinfection pour leurs éliminations.

2. Déterminer la qualité microbienne et la présence d'agents pathogènes spécifiques dans les eaux grises salle de bain.
3. Evaluer la performance d'élimination des micro-organismes par un traitement de filtration des eaux grises et la photolyse / photocatalyse
4. Evaluer l'impacte de facteurs environnementaux abiotiques sur la survie de microorganismes. .

L'étude s'inscrit dans un nombre croissant de recherches dans le domaine des stratégies de réutilisation de l'eau et fournit des données sur la qualité des eaux grises. Elle est l'une des premières enquêtes ont porté dans la réutilisation des eaux grises par les nouvelles technologies utilisées un comme comme la lumière avec un catalyseur et filtre à sable lent, qui peut devenir une stratégie de survie essentiel pour les résidents des régions qui manquent d'eau dans les années à venir.

1.2.2. Grandes lignes de la thèse

Cette thèse est organisée en cinq chapitres comme le montre la figure 1.2, en plus de l'introduction du chapitre 1 :
Chapitre 2. Revue de la littérature des caractéristiques des eaux grises et de l'efficacité des technologies de traitement des eaux grises.
Chapitre 3 Traitement des eaux grises par filtration lente sur sable. Ce chapitre est divisée en deux parties, (1) caractérisation du sable et propriétés hydrodynamique du lit filtrant,(2) traitement par filtration lente d'eaux grises de douche.
Chapitre 4. Effet des facteurs abiotiques sur la survie de bactéries pathogènes et phage MS2 dans les eaux grises
Chapitre 5. Inactivation de bactéries pathogènes et coliphage par NF-TiO2 avec la lumière solaire.

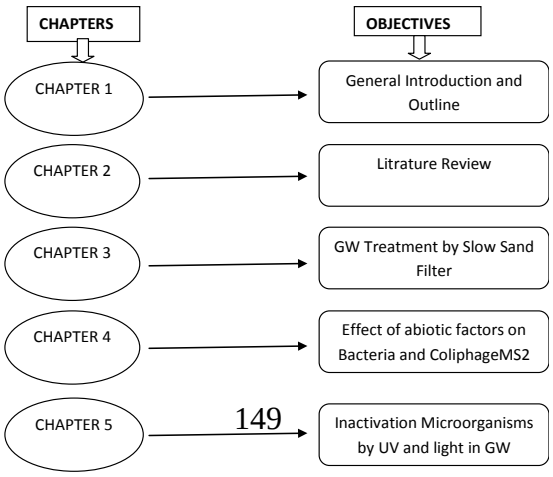

Figure 1.2 chapitres et objectifs

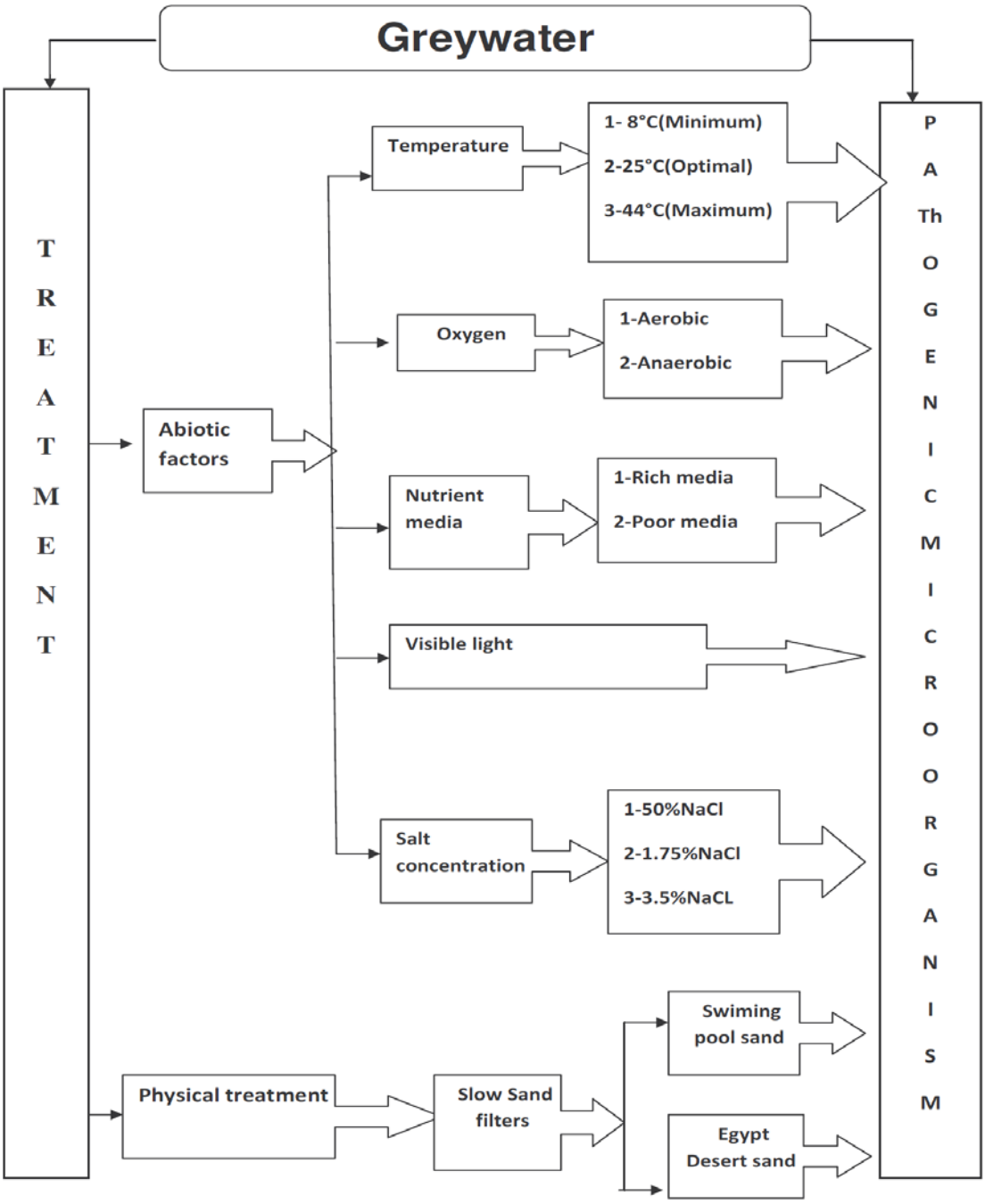

Figure 1.1 Schéma général de l'étude des eaux grises proposé un traitement pour réutilisation en toute sécurité.

2. Résumé de l’état de l’art

Le traitement des eaux grises et le recyclage des produits utiles, à savoir l'eau, les éléments nutritifs doit permettre de limiter les pénuries d'eau et la pollution de l'environnement. Liée à une augmentation de la population mondiale, la raréfaction des ressources en eau, la mauvaise gestion et le changement climatique, la pénurie d'eau et la pollution de l'eau sont devenus un des enjeux mondiaux (Falkenmark, 1990; Arnell, 1999; Bouwer, 2000). Les polluants des eaux usées industrielles et domestiques contribuent à la pollution des ressources en eau et des sols (Metcalf et Eddy, 2003). Les eaux grises sont les eaux usées domestiques à l'exception des eaux usées provenant des toilettes, ce qui est connu sous le nom d'eaux noires. 50-80% des eaux usées domestiques sont des eaux grises provenant des éviers de cuisine, des lave-vaisselles, lavabos, baignoires et douches (Gulyas et al., 2004),. Les eaux grises contiennent de 9-14% de la fraction N rejetée, 20-32% du P, 18-22% de la matière organique et 29-62% du K (Kujawa-Roeleveld et Zeeman, 2006). Plusieurs questions émergent pour la réutilisation des eaux grises, à savoir, réutilisation avec ou sans traitement simple (Christova-Boal et al, 1995;. Al-Jayyousi, 2002). La première possibilité est le recyclage pour une utilisation en intérieur comme les chasses d'eau, lave-linge et / ou de baignade (Christova-Boal et al, 1995;. Bingley, 1996; Nolde, 1999; Jefferson et al, 1999;. 2001; Shrestha et al. , 2001a et b; Li et al, 2003;. Cui et Ren, 2005). La seconde, est pour un usage extérieur tels que l'irrigation des jardins, des pelouses, des terrains de sport, des cimetières, des parcs et des terrains de golf, le lavage des véhicules et des fenêtres, l'extinction des incendies, les chaudières, l'alimentation, des zones humides en voie de développement et la préservation de l'eau souterraine et la recharge des nappes (Christova- Boal et al, 1995;. Bingley, 1996; Fittschen et Niemczynowicz, 1997; Nolde, 1999; Otterpohl, 1999; Okun, 2000; Jefferson et al, 2001;.. Shrestha et al, 2001a et b; Eriksson et al,. 2002; Al-Jayyousi, 2002, 2003). Enfin, se pose la question de quelle qualité pour quel usage. Cette dernière est principalement lié à la réglementions avec pour conséquence la santé et les aspects sociaux. Le but étant de garantir l'efficacité des procédes de recyclage (Nolde, 1999; Jefferson et al, 1999;. 2000; 2001; Li et al, 2003;. Cui et Ren , 2005). Pour ce faire il est important de développer des technologies de traitement efficaces, robustes et économiquement abordables pour faire face à la variation de la quantité et de la qualité des sources d'eaux grises (Imura et al, 1995;.. Eriksson et al, 2002), et les exigences de recyclage (Nolde, 1999;. Jefferson et al, 1999 , 2000, 2001; Li et al, 2003;. Cui et Ren, 2005). La connaissance de la teneur en pathogènes des eaux grises est limitée. Toutefois, les agents pathogènes spécifiques et un nombre important de bactéries indicatrices

ont été rapportés (Rose et al, 1991;. Birks et al, 2004;.. Jefferson et al, 2004), ce qui indique que la désinfection des eaux grises avant de les réutiliser est essentielle pour réduire le risque pour la santé publique. Cette partie présente donc différentes méthodes de traitement des eaux grises dans le but d'arriver à une méthode efficace, un traitement simple et abordable produisant un effluent sans danger. Une méthode de traitement est considéré comme efficace si elle produit la qualité requise des effluents, est d'un fonctionnement simple avec un minimum d'entretien et abordables en raison de sa faible consommation d'énergie.

La revue de la littérature a indiqué que l'objectif principal de la recherche a été mis sur l'évaluation de la survie des bactéries dans l'eau et des eaux grises. Les nombreuses études antérieures n'ont pas pris en compte les effets des facteurs abiotiques sur la survie des bactéries et les coliphages MS2. Seules quelques études ont été ainsi réalisées. Cependant, il est déjà évident que la survie des bactéries et les coliphages MS2 peut-être influencée par la température et de la salinité (Roszak, 1986) et par la lumière visible de faible intensité (Ginger et al., 1989). En conséquence de quoi, cette étude a été entreprise avec l'objectif général de suivre l'influence de quelques-uns des facteurs abiotiques les plus importants de l'environnement, c'est à dire, la température, la salinité et la lumière du soleil, sur la survie des bactéries et des coliphages dans des conditions de faibles concentrations en nutriments et leur traitement par filtre à sable. Les observations suivantes ont été basées sur ce concept: dans l'eau, une combinaison de la lumière du soleil et photocatlysis sont principalement responsables de la baisse rapide de la bactérie *E. coli*, *Pseudomonas aeruginosa* et coliphage MS2 si la température et de la sédimentation également y contribuer. La survie est supérieure dans les eaux estuariennes (Fujioka et al., 1981), probablement à cause de réduction de la salinité et l'effet protecteur de la turbidité des rayons du soleil. La survie en eau douce ainsi qu'en fonction de la température est moins bien comprise (Mancini, 1978) et de salinité (Fujioka et al., 1981) peut être importante. Il est clair qu'une faible quantité de nutriments a moins d'influence sur la survie des bactéries dans les eaux grises pour de courtes périodes. Les bactéries et les coliphageMS2 dans les eaux et les eaux grises peuvent être détectés en utilisant des techniques traditionnelles pour le dénombrement de bactéries et de la culture coliphage (Adams, 1959 ; ISO 10705-2, 2000).En plus de l'étude de l'influence des facteurs les plus importants de l'environnement sur la survie, une étude d'inactivation de micro-organismes des eaux grises et de leurs élimination à l'aide d'un filtre à sable lent et la photo-inactivation a également été réalisée. Aussi l'objectif final de la présente étude était le

traitement des eaux grises en vue de son utilisation en irrigation agricole. De ce fait il est essentiel d'établir des recommandations pour des pratiques sûres dans un avenir rareté de l'eau.

3. Traitement de l'eau grise par filtration lente (sable)

3.1. Introduction

Le traitement des eaux grises est une précieuse source d'eau dans l'économie des pays arides et semi arides. En reconnaissance de l'importance de conserver ses ressources en eau, la prise en compte des eaux grises comme une source peu polluée est primordiale. Actuellement, les pays souffrent de plus en plus d'un épuisement de leurs réserves en eau. Il y a une forte augmentation de la demande en eau en raison de la croissance démographique mondiale et de la consommation de chaque individu. Ces importantes questions vis-à-vis de la ressource en eau conduit à l'élaboration de solutions pour le recyclage des eaux usées. Cette dernière approche réduit les prélèvements d'eau pour répondre aux besoins des populations, ainsi l'eau potable peut être remplacée par de l'eau recyclée dans les applications où une baisse de la qualité est suffisante.

Cette partie du travail est divisée en deux parties: Une première partie de caractérisation avec de l'eau propre du média filtrant et du sable. Dans cette partie il a été étudiée la caractérisation des paramètres hydrodynamiques du sable tel que, la RTD, HTD et la porosité. Une seconde partie est consacrée à l'étude de la filtration lente avec du sable pour traitement d'eaux grises de douches. Dans cette partie ont été mesuré les paramètres physico-chimiques suivant, la turbidité, la DCO, le pH et la conductivité. La survie des micro-organismes étudiés *E. coli, P. aeruginosa, E. faecalis* et flore bactérienne totale et le développement du biofilm a également été mesuré.

3.2. Caractérisation du sable

Deux types de sable ont été utilisés dans la fabrication de médias filtrants:

- Le premier est un sable commercial fréquemment utilisés dans la filtration des piscines (SPS);
- Le deuxième un sable provenant du désert égyptien (EDS) a été mis en œuvre.

Parce que ce sable contient des particules très fines, une préparation est nécessaire avant qu'il puisse être utilisé. Ce sable a été lavé pour éliminer toutes les fines particules d'argile, et il a été placé dans un four à 100 ° C pendant 24 heures avant de subir un tamisage. Tamisage a ensuite été effectuée en utilisant une série de tamis à ouverture de taille allant de 2500 um à 200 um. Les tamis des tests sont disposés en une pile avec les ouvertures plus grandes mailles en haut de la pile. L'échantillon est placé sur le tamis supérieur. Après tamisage, toutes les particules passant à travers le tamis de 200 um d'ouverture ont été rejetées. Il convient de noter que, après l'enlèvement des grains beaucoup plus fins, le sable utilisé est hétérogène et contient un peu de gravier de la taille de plusieurs millimètres

3.3. Caractérisation hydrodynamique des filtres à sable en eau propre.

Pour ce faire il a été réalisé des mesures de perte de charges en fonction du débit ainsi qu'une approche par modélisation à l'aide d'un modèle capillaire afin d'évaluer les paramètres de structure et la caractérisation du media poreux.

Dans ce modèle, le lit est considéré comme un ensemble de pores cylindriques identiques tortueux de diamètre d et de longueur L (m).

Si l'on considère H comme étant la hauteur de colonne (m) le facteur de tortuosité est définie par : $\tau = L/H$

- l'aire de la surface dynamique avd par : $d_{pore} = \dfrac{4\varepsilon}{a_{vd}(1-\varepsilon)}$
- le diamètre de pore dpore par : $a_{vd} = \dfrac{surface\ area\ \ presented\ by\ the\ \ particle\ to\ the\ \ flow\ \ (m^2)}{volume\ of\ \ solid\ \ (m^3)}$

Les principaux résultats obtenue sont regroupé dans le tableau 3.1.

	Tortuosity	***Dynamic surface area*** a_{vd} **(m^{-1})**	**Pore diameter (µm)**
SPS	1.69	6570	440
EDS	1.50	26 599	92

Tableau 3.1: Caractéristiques hydrodynamiques des lits filtrant étudiés.

3.4. Traitement des eaux grises.

3.4.1 Traçage

Le traçage nous a permis de déterminer le temps de passage de l'eau dans les colonnes. Ainsi en comparaison avec le temps de passage théorique, nous pouvons déterminer le temps de séjour de l'effluent dans le lit filtrant avant la sortie. Les résultats sont présentés Figure 3.1 et 3.2.

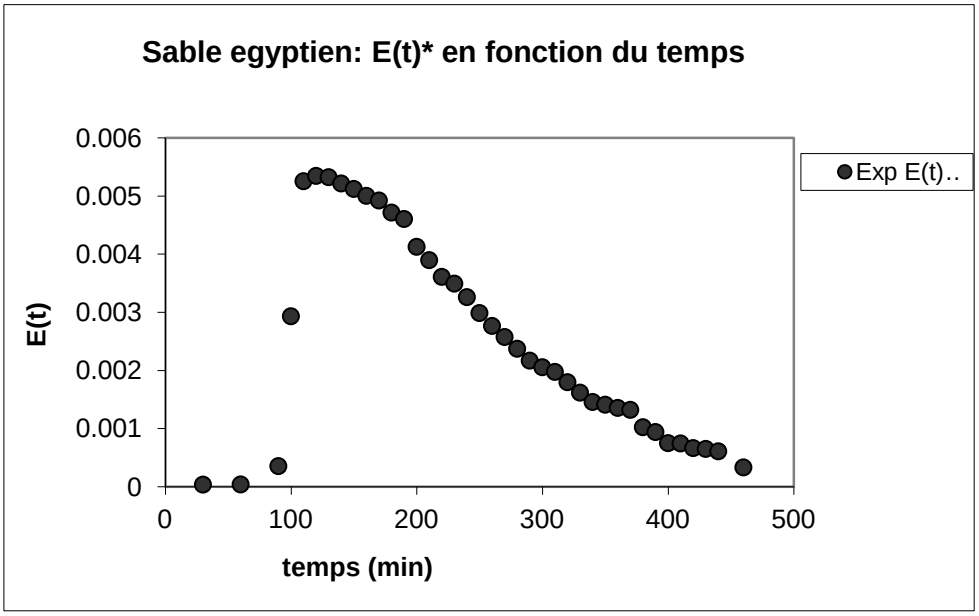

Figure 3.1: courbe de traçage de la fluorescéine du sable égyptien

E(t)= Absorbance/ absorbance(t) $\triangle$ t

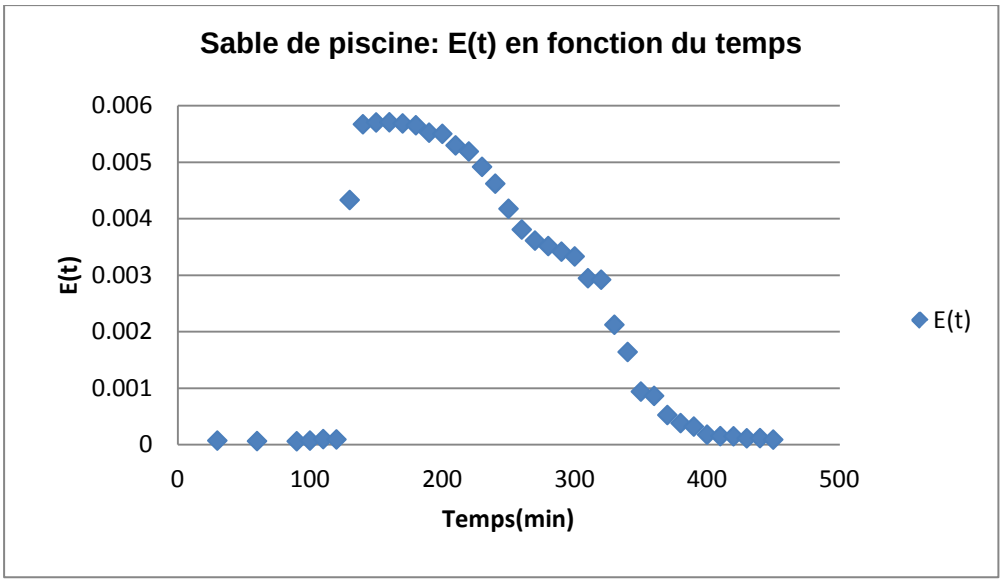

Figure 3.2 : courbe de traçage de la fluorescéine du sable de piscine

Les résultats des ces traçages nous permettent ainsi de déterminer le temps de passage dans la colonne contenant du sable égyptien et dans celle contenant du sable de piscine, qui est respectivement de 220 et 225 min.

3.4.2. Performances épuratoires.

Tout d'abord, l'examen de la turbidité des eaux grises est très hétérogène, ce pendant nous n'avons aucun échantillon dont les valeurs de la turbidité sont supérieur aux valeurs mentionnée dans la littérature. Les valeurs obtenues pour les deux colonnes sont tout de même différentes. En effet, les résultats moyens obtenus lors des analyses des échantillons provenant du sable égyptien, sont plus faibles que ceux provenant du sable de piscine français, soit respectivement 7,16 et 26,67 FNU. Cela est certainement due au caractéristique des différents sables, le sable du désert égyptien étant très fin et donc confère moins de porosité au lit filtrant et donc retient en grande quantité les matières en suspensions présentes dans l'eau quand bien même elles sont très petites.

Pour ce qui est du pH et de la conductivité, l'ensemble des échantillons analysés ont des valeurs du pH et de la conductivité qui ne varie presque pas. En générale elles tournent autour de 7,9 de pH et 400µs/m de conductivité. Ces valeurs sont néanmoins comprises entre les valeurs cibles minimales et les valeurs cibles maximales.

La DCO comparé aux autres paramètres à fait l'objet d'un suivi particulier car c'est un paramètre essentiel pour la détermination de la pollution organique d'une eau. Pour les

analyses de la DCO dans cette étude, les valeurs moyennes après la filtration sont comprises dans les intervalles des valeurs cibles soient 77,86 mg d'O2/L pour le sable du désert égyptien et 101mg d'O2/L pour le sable de piscine. Cette diminution de la DCO peut être attribuée à la présence du biofilm sur le lit filtrant et qui par conséquent réalise une dégradation de la matière organique par les bactéries présentes. En comparant ces valeurs de la demande chimique en oxygène avec la variation du débit, il à été démontré que lorsque le débit augmente, les valeurs d'abattement de la DCO diminuent et lorsque le débit baisse les valeurs d'abattement de la DCO augmentent.

Cela va de même pour le sable de piscine, le pourcentage d'abattement de la DCO augmente quand le débit baisse, sauf que les premier jours des analyse ça n'a pas été le cas. Cela est certainement dû à une absence de biofilm. En effet, le biofilm peut s'installer lentement sur le lit filtrant.

En comparant, les résultats des deux sables, on peut dire que le sable d'Egypte, est plus efficace car il a une valeur maximale d'abattement est de 70% et la valeur minimale de 47 % , contrairement aux valeurs d'abattement du sable de piscine qui varie entre 22% et 50%.

Quant aux analyses microbiologiques, elles montrent la présence en quantité non négligeable de micro-organismes tels qu'E. Coli, P.aeruginosa Entérocoques fécaux et la flore totale. Les ordres de grandeurs des valeurs cibles ne sont pas respectés dans nos échantillons. Bien que pour la flore totale et P.aeruginosa les valeurs cibles soient non déterminer, on note une quantité importante de ces bactéries dans les échantillons. L'ensemble de ces résultats souligne la nécessité de l'élimination des bactéries lors du traitement choisi pour le recyclage des eaux grises. Ceci étant les bactéries comme E.coli et la flore totale peuvent parfois se retrouver en abondance dans les eaux grises de salles de bain à causes des produits utilisé pour la douche (savon ou gel de douche) ou de leur développement à partir des matières organiques biodégradables. Ce procédé n'est pas efficace pour un traitement microbiologique car la quantité de bactéries avant filtration et après filtration ne baisse pas considérablement.

La quantité de bactéries ne diminue presque pas avant et après la filtration. En effet, la porosité des différents sables, ne permet pas une rétention des bactéries de diamètre très faible.

4. Effet de facteurs abiotiques sur la survie et la croissance *d'Escherichia coli* et *Pseudomonas aeruginosa* dans des eaux grises de salle de bain.

Cette étude a été menée pour examiner l'effet de divers types de facteurs abiotiques (non vivants, facteurs chimiques et physiques), qui permettra d'améliorer ou limiter la croissance des bactéries *Escherichia coli* (EC) et *Pseudomonas aeruginosa* (PA) dans les eaux grises de salle de bain. Les facteurs inclus, la température (8, 25 et 44 ° C); l'aérobie et l'anaérobie; la salinité (1,75% et de la salinité de 3,5%), la dilution des eaux grises (GW 50%: 50% DW) et la présence de nutriments avec utilisation de milieux de cultures riches et pauvres. La période de survie a été plus courte à 44 ° C qu'à 25 ° C et 8 ° C. La meilleure survie de *P. aeruginosa* a été mesurée dans des conditions aérobies et *E. coli* dans des conditions anaérobies. *P. aeruginosa* présente une meilleure survie en milieu de faible salinité qu'E. *coli* pour chaque concentration de l'étude. L'effet de la variation de concentration du substrat affecte non seulement la croissance mais aussi la survie et la taille des cellules ainsi que le potentiel zêta pour les deux espèces bactériennes étudiées.

Après cette étude, nous pouvons conclure que l'influence de différents facteurs abiotiques (température, l'aération des concentrations de sel, et la famine en éléments nutritifs) est importante et peu inhiber la croissance de certaines bactéries pathogènes (*E. coli et P. aeruginosa*) mais également agir sur leur survie. Nous concluons que: (1) la température et la salinité ont le plus grand impact sur la survie de ces bactéries, (2) si le milieu de culture a une faible concentration en nutriments la survie sera affectée; (3) le substrat et la concentration des nutriments dans le milieu affectent également la taille des cellules et le potentiel zêta. Ces divers paramètres sont importants pour l'élimination des micro-organismes par des procédés de filtration car ils font intervenir les phénomènes d''interaction entre les particules et le matériau de filtration tels que les membranes ou du sable. Notre étude met encore en évidence le fait que pour la réutilisation sans risque des eaux grises il est nécessaire d'éliminer la pollution biologique. L'élévation de la température est un facteur abiotique qui pourrait être utilisé comme voie de traitement simple et économique dans les zones arides et semi-arides afin de rendre l'utilisation sécuritaire des eaux grises pour l'irrigation. Cette recherche pourrait

être plus largement utilisée et appliquée dans les pays ensoleillés comme les pays de la zone aride et plus spécifiquement l'Egypte.

4. Inactivation d' *E. coli*, *P. aeruginosa* et du bactériophage MS2 dans des eaux grises par photolyse et photocatalyse (NF-TiO_2) sous diverses conditions d'irradiations.

Dans les dernières décennies, la plupart des pays du monde ont connu une pénurie d'eau et l'augmentation concomitante de sa consommation, ce qui a pour conséquence la nécessité vitale de trouver des voies alternatives a son utilisation excessive dans de nombreux aspects de la vie. Par conséquent, il est d'une grande importance à l'échelle mondiale de trouver des solutions de rechange pour faire face à cette pénurie, notamment en réduisant le gaspillage de cette ressource vitale. Les eaux grises (GW) représentent une ressource importante d'eau si le recyclage est considéré pour des usages ne nécessitant pas de qualité de l'eau potable. GW concernent l'eau domestique provenant des éviers, baignoires, lave-linge et lave-mains. En raison de son origine, cette eau peut contenir des bactéries, ainsi que des solides en suspension (TSS), des tensioactifs et des composés ayant une demande chimique en oxygène (DCO), y compris les micro-polluants et les contaminants préoccupants émergents. Les études précédentes ont montré que *E. coli* (EC) et *P. aeruginosa* (PA) peuvent survivre jusqu'à 29 jours dans GW à 25 ° C. Dans le but de réutilisation des GW il est indispensable de réduire la population microbienne. Une étape de désinfection possible pourrait être l'utilisation des UVC. Une alternative à faible coût sera l'utilisation de la lumière solaire, mais l'efficacité est moindre. L'utilisation d'un nouveau catalyseur comme NF-TiO_2 (Pelaez et al., 2010a), qui est développé pour l'utilisation de la lumière solaire, y compris une partie de la région visible, pourrait être un moyen intéressant pour améliorer l'inactivation microbienne.

Cette partie du travail se concentre sur l'étude de l'efficacité de la désinfection à la lumière visible en utilisant le nouveau catalyseur à base d'un film de NF-TiO_2 fabriqué par un procédé sol-gel pour l'inactivation de EC et PA dans des eaux grises de salle de bain et dans de l'eau désionisée (DW) servant de référence. Les expériences de photocatalyses ont été réalisées à l'aide d'un photoréacteur de 1 L avec agitation, alimenté en air, et contrôlé en température. L'irradiation a été fournie par une lampe au xénon (450 W) et en utilisant des

miroirs dichroïques permettant la sélection d'une gamme de longueur d'onde appropriée: 280-400 nm et 350-630 nm. Un filtre AM1.5 fixe sur la sortie du faisceau a permis d'obtenir le spectre solaire. Le taux de décroissance constante k (h^{-1}) pour chaque condition a été calculé conformément à la loi de Chick qui est décrite par la relation :

$$-kt = (Nt / N0)$$

avec N0 et Nt sont, respectivement, les concentrations en nombre de bactéries au temps zéro (début de l'irradiation) et au temps t mesuré en heures. Le temps nécessaire pour inactiver 90% de la population initiale est définie comme T_{90}, et est représentée par

$$T_{90} = - t / \log (N / N0)$$

Pour EC et PA la condition la plus efficace était le traitement par irradiation UV. Néanmoins, la présence du catalyseur NF-TiO_2 dans la lumière visible augmente considérablement l'effet bactéricide. Les valeurs extrapolées pour k et T_{90} ont permis la classification suivante en termes d'efficacité d'inactivation bactérienne pour toutes les conditions étudiées:

UV> lumière visible + catalyseur> lumière visible> sombre

La comparaison entre les expériences réalisées dans DW et GW a montré une augmentation d'un facteur 2 des valeurs T_{90} pour GW. Par exemple, valeurs T_{90} ont augmenté de 0,052 à 0,12 h pour PA dans DW et GW, respectivement. Cette différence pourrait être attribuée à la présence des molécules organiques dissoutes dans les GW. La lumière visible seul (sans catalyseur) n'a causé que peu d'effet d'inactivation bactérienne, T_{90} est de 1,47 h et 2,15 h dans l'obscurité pour EC dans GW. En présence de lumière UV, les groupes fonctionnels des acides nucléiques ou des protéines de cellules bactériennes ont été directement endommagés lors de l'irradiation. Dans le cas de la présence du catalyseur TiO_2 NF sous la lumière visible, le mécanisme proposé peut être lié à la formation d'espèces réactives de l'oxygène comme l'anion radical superoxyde, l'oxygène singulet, et / ou le radical hydroxyle. Après leurs formations, ces espèces réactives de l'oxygène peuvent endommager les constituants cellulaires. L'inactivation du coliphage MS2 peut être due à des changements ou des dommages aux structures de surfaces, tels que la protéine d'enveloppe (capside) ou sur les

spicules, sites de fixation nécessaires pour l'infection des cellules hôtes (*E. coli* Hfr spécifiques pour les coliphages MS2). Le nombre de coliphage MS2 en fonction du temps au cours des traitements photocatalytiques mettent clairement en avant un effet du catalyseur sous irradiation en lumière visible. Le temps nécessaire à l'inactivation des coliphages MS2 a été d'environ 60 minutes sous une lumière UV, tandis que plus de 180 minutes ont été nécessaires pour l'inactivation sous la lumière visible en présence du catalyseur.

Les propriétés bactéricides de la lumière UV et de la lumière visible avec le catalyseur NF-TiO2 ont été démontrées par leur capacité à inactiver les bactéries à Gram négatif *E. coli, P. aeruginosa* et le coliphageMS2. La cinétique d'inactivation photocatalytique est plus élevée pour les cellules de *E. coli*. L'utilisation du catalyseur NF-TiO2 dans l'eau déminéralisée a été trouvée pour être plus efficace que son utilisation dans les eaux grises dans les mêmes conditions expérimentales. La désinfection photocatalytique peut être une technologie prometteuse. Avec de nouvelles recherches sur l'aspect pratique pour le traitement de l'eau, il peut devenir un processus de substitution possible à la chloration.

5. Conclusion générale et perspectives

Ce travail a abordé une question importante qui occupe beaucoup de chercheurs à travers le monde, à savoir la question du manque d'eau et la réutilisation des eaux grises. Il a été noté que le défi le plus important du traitement est la présence d'agents pathogènes microbiens dans les eaux grises, qui conduisent à l'apparition de la maladie et, parfois, pourrait entraîner la mort.

Le premier traitement utilisé dans ce travail est la filtration lente sur filtre à sable avec deux types de sable : le sable du désert égyptien et du sable pour filtre de piscines. Une caractérisation de sable a été réalisé par mesure de la distribution taille des grains de sable par observation MEB et analyse élémentaire par fluorescence X. La porosité ainsi que la distribution du temps de séjour des lits de sable a également été déterminée. Les résultats montrent des différences entre les deux sables utilisés. Les caractéristiques des eaux grises ont été mesurées, tels que la demande chimique en oxygène (DCO), la conductivité et la turbidité et la microbiologie. Dans une deuxième section, les capacités de traitement des eaux grises de douche par un biofiltre constitué de lits de sable ont été étudiées. Il est observé un diminution de la DCO avec temps de filtration et la formation de biofilm. Le sable du désert égyptien

semble présenter une meilleure efficacité de traitement. La valeur de la turbidité varie selon le produit d'hygiène utilisé (gel douche ou savon). L'impact du procédé filtration lente sur sable sur la survie des micro-organismes a également été étudié et un très faible effet a été observée. La concentration microbienne restante après traitement était trop élevée pour une réutilisation directe de l'eau.

La deuxième partie de ce travail a été consacrée à l'étude de l'effet des facteurs abiotiques qui pourraient affecter la survie des micro-organismes et coliphageMS2. Par exemple, l'indicateur de la survie des bactéries pathogènes à 6 ± 2, 23 ± 2 et 42 ± 2 ° C, coliphage MS2 survie à 6 ± 2, 23 ± 2, 39 ± 2 et 63 ± 2 ° C a été observée. L'effet de la concentration en sel sur la survie des bactéries et du coliphge MS2 à 1,75% et 3,5% de NaCl, montre un effet négatif sur la survie dans une concentration de sel de 3,5% de NaCl. Par ailleurs, l'oxygène disponible et indisponible (aérobie et anaérobie) affecte la survie des bactéries. Une carence en éléments nutritifs (médias riches ou pauvres) a été étudiée afin de mieux comprendre l'influence du milieu de croissance sur la taille et la charge électrique de surface des micro-organismes. Les résultats mettent clairement en évidence que les milieux pauvres et des eaux grises permettre la survie de *E. coli et P. aeruginosa* mais avec une réduction de la taille des cellules jusqu'à un facteur 2. L'élévation de la température a pour effet une plus grande inactivation des bactéries indicatrices de même pour le coliphageMS2 (42 ± 2 ° C et 65 ° C respectivement).

Enfin, l'irradiation par diverse sources de lumière (visible et UV) avec ou sans photocatalyseur ont été utilisés pour l'inactivation des bactéries pathogènes et du coliphage MS2 dans les eaux grises. La lumière visible et ultraviolette en présence d'un catalyseur conduire à l'inactivation des bactéries et des coliphages. En général, l'inactivation prend plus de temps dans les eaux grises que dans de l'eau déminéralisée.

Maintenant, le défi est d'améliorer le procédé de biofiltration lent avec un filtre à sable afin de stabiliser l'efficacité du traitement et de choisir la meilleure façon de réduire la teneur microbienne des eaux grises produites. En outre, la faisabilité économique ainsi que le concept de développement durable grâce à la réutilisation des eaux grises dans diverses applications telles que l'irrigation dans l'agriculture et la chasse d'eau doit également être étudiée.

Avenir et perspectives

La recherche présentée dans cette thèse semble avoir soulevé plus de questions qu'elle n'a apportée de réponses. En effet, ce type d'étude aborde de nombreux domaines, tels que la photocatalyse, les traitements et la réutilisation des eaux grises dans l'agriculture et la microbiologie. Les défis qui nous attendent sont important dans le contexte d'une application en zone aride et semi-aride. Par exemple, l'Egypte fait face à une croissance démographique élevée et donc une demande croissante en eau. Compte tenu des quantités limitées de ressources en eau douce renouvelables en Égypte, ces ressources doivent être protégés et sauvegardés pour l'eau potable. Par conséquent, le secteur agricole doit être préparé à dépendre de plus en plus de ressources en eau marginales telles que des eaux grises traitées.

1 - Perspectives

- Perspectives pour la photocatalyse et filtre à sable lent

Utilisez largement dans la désinfection l'Ag comme la catalyse peut être ajouté dans le photocatalyseur parce qu'il a un d'effet potentiellement plus important sur les bactéries. Des études sur la production par photocatalyse de radicaux libres etl'étude de leurs effets sur la paroi cellulaire et le cytoplasme peuvent être envisagées. En général, il pourrait être l'utilisation direct du soleil avec la présence de catalyseurs. Pour le traitement des eaux grises par filtre à sable lent il est important de mener des expérimentations avec des eaux grises réelles pendant une longue période.

- Perspective pour les eaux grises réutilisation dans l'agriculture:

La réutilisation des eaux grises traitées pour l'irrigation doit être étudiée en tenant compte de l'impact sanitaire et la faisabilité économique. Les eaux grises ont une composition qui peut rendre le sol un peu plus basique. Les plantes qui préfèrent la croissance et le sol basique sont les olives, l'acacia, de figuiers et d'arbres de bois de sorte qu'il est possible d'imaginer l'irrigation de ces cultures pour augmenter la production des arbres.

2 - Les projets: un projet pilote dans la South Valley University

Le projet consiste à recycler les eaux grises par la collecte en unités de traitement et de transport par les eaux grises traitées pour irriguer les espaces par des conduites. Le projet comprend également l'utilisation d'un procédé présentant de faible investissement et des coûts

d'exploitation. En conséquence de quoi il est envisagé de réaliser un traitement par filtre à sable avec désinfection par utilisation de catalyseur et du spectre solaire, avant la réutilisation des eaux grises pour l'irrigation.

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Annex

Annex 1 : Tracing fluorescein

Diamter column(CM)	Flowrate (ml/min)	injection(10-3) volume(ML)	Mass sand(kg)	porosity	RTD(min)	HTD(min)	SPEED SUPERF(m/s)
80	10	2.5	5	0.337	219 .6	101 .6	5.89E -04

Egyptian Desert Sand

N° de tube	temps (min)	A à 490,5 nm	A(t)dt	tA(t)dt	t^2A(t)Dt	C/C0	exp	J RAC	piston dispersion
1	30	0.025	0.75	22.5	0.75	0.00854421	E(t)	E(t)	E(t)
2	60	0.022	0.66	22.5	0.088	0.0075189	7.30162E-05	3.96417E-19	3.78185E-23
3	90	0.021	0.63	39.6	0.189	0.00717713	6.42542E-05	4.23591E-07	9.542E-07
4	100	0.025	0.25	56.7	0.4	0.00854421	6.13336E-05	4.56733E-03	0.005482409
5	110	0.034	0.34	25	0.85	0.01162012	7.30162E-05	1.49888E-02	0.014175783
6	120	0.032	0.32	37.4	1.152	0.01093659	9.9302E-05	2.58537E-02	0.021642316
7	130	1.483	14.83	38.4	72.667	0.50684237	9.34607E-05	2.62190E-02	0.022264426
8	140	1.942	19.42	1927.9	124.288	0.66371401	0.004331318	1.70222E-02	0.016910735
9	150	1.953	19.53	2718.8	158.193	0.66747346	0.005671895	7.55893E-03	0.010122553
10	160	1.954	19.54	2929.5	195.4	0.66781523	0.005704022	2.41953E-03	0.005009088
11	170	1.947	19.47	3126.4	235.587	0.66542285	0.005706942	5.82364E-04	0.002123888
12	180	1.936	19.36	3309.9	278.784	0.6616634	0.005686498	1.09112E-04	0.000793054
13	190	1.891	18.91	3484.8	319.579	0.64628383	0.005654371	1.63759E-05	0.000266389
14	200	1.884	18.84	3592.9	369.264	0.64389145	0.005522942	2.01661E-06	8.18591E-05
15	210	1.814	18.14	3768	408.15	0.61996767	0.005502497	2.07939E-07	2.33231E-05
16	220	1.777	17.77	3809.4	454.912	0.60732224	0.005298052	1.82673E-08	6.22859E-06
17	230	1.684	16.84	3909.4	486.676	0.57553779	0.005189988	1.38777E-09	1.57303E-06
18	240	1.582	15.82	3873.2	512.568	0.54067743	0.004918368	9.23574E-11	3.78452E-07
19	250	1.43	14.3	3796.8	516.23	0.48872865	0.004620462	5.44546E-12	8.72697E-08
20	260	1.304	13.04	3575	521.6	0.44566584	0.004176524	2.87277E-13	1.93875E-08
21	270	1.237	12.37	3390.4	545.517	0.42276737	0.003808522	1.36791E-14	4.16744E-09
22	280	1.204	12.04	3339.9	582.736	0.41148902	0.003612839	5.92476E-16	8.69975E-10
23	290	1.17	11.7	3371.2	618.93	0.39986889	0.003516458	2.35033E-17	1.76932E-10
24	300	1.141	11.41	3393	657.216	0.38995761	0.003417156	8.59234E-19	3.51524E-11
25	310	1.009	10.09	3423	630.625	0.3448442	0.003332457	2.91088E-20	6.83867E-12
26	320	1	10	3127.9	676	0.34176829	0.002946932	9.18412E-22	1.30542E-12
27	330	0.727	7.27	3200	529.983	0.24846554	0.002920646	2.71090E-23	2.44947E-13
28	340	0.562	5.62	2399.1	440.608	0.19207378	0.00212331	7.51677E-25	4.525E-14
29	350	0.322	3.22	1910.8	270.802	0.11004939	0.001641403	1.96524E-26	8.24129E-15
30	360	0.296	2.96	1127	266.4	0.10116341	0.000940448	4.86120E-28	1.48162E-15
31	370	0.18	1.8	1065.6	172.98	0.06151829	0.000864511	1.14123E-29	2.63219E-16
32	380	0.131	1.31	666	134.144	0.04477165	0.000525716	2.55005E-31	4.62551E-17
33	390	0.11	1.1	497.8	119.79	0.03759451	0.000382605	5.43768E-33	8.04716E-18
34	400	0.061	0.61	429	70.516	0.02084787	0.000321271	1.10923E-34	1.38709E-18
35	410	0.052	0.52	244	63.7	0.01777195	0.000178159	2.16947E-36	2.37057E-19
36	420	0.051	0.51	213.2	66.096	0.01743018	0.000151874	4.07667E-38	4.0194E-20
37	430	0.039	0.39	214.2	53.391	0.01332896	0.000148953	7.37425E-40	6.76517E-21
38	440	0.04	0.4	167.7	57.76	0.01367073	0.000113905	1.28638E-41	1.13092E-21
39	450	0.031	0.31	176	47.151	0.01059482	0.000116826	2.16763E-43	1.87855E-22
SOMME			342.39	76419.9			9.054E-05	3.53377E-45	3.10198E-23

ANNEXE :

Diamter column(CM)	Flowrate (ml/min)	injection(10-3) volume(ML)	Mass sand(kg)	porosity	RTD(min)	HTD(min)	SPEED SUPERF(m/s)
80	10	2.5	4.8	0.338	223 .19	1117 .02	5.89E -04

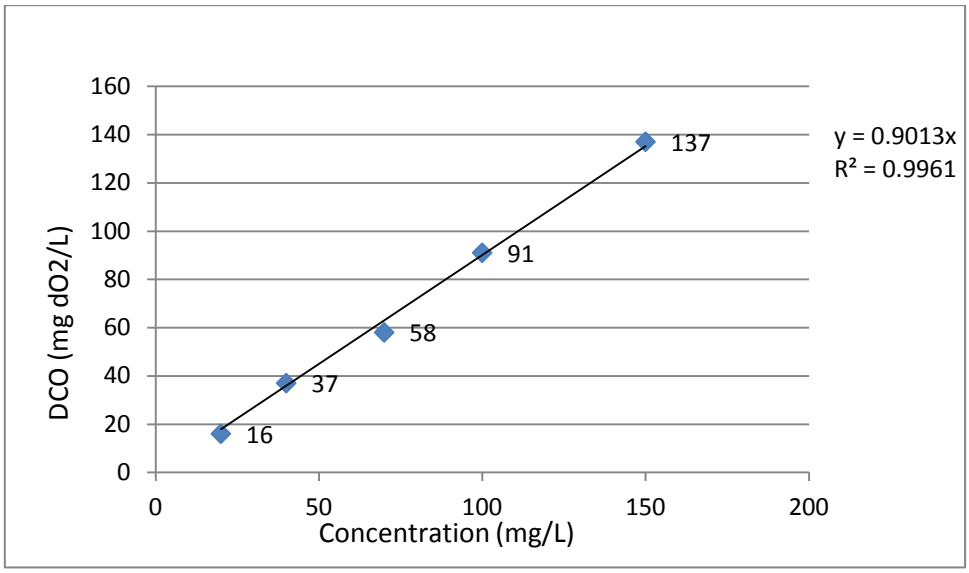
Swimming Pool Sand

N° Ech	temps sec	t/tp	A à 490,5 nm	A(t)dt	tA(t)dt	t^2A(t)Dt		exp	J RAC	piston dispersion
1	30	0.2951687	0.013	0.39	11.7	351	C/C0	E(t)	E(t)	E(t)
2	60	0.5903373	0.014	0.42	11.7	1512	0.00365367	3.59483E-05	3.02718E-16	1.73451E-18
3	90	0.885506	0.128	3.84	25.2	31104	0.00393473	3.87136E-05	2.59777E-05	5.71475E-05
4	100	0.9838955	1.06	10.6	345.6	106000	0.03597465	0.000353953	2.24948E-02	0.020054512
5	110	1.0822851	1.9	19	1060	229900	0.29791503	0.002931173	3.18500E-02	0.02638852
6	120	1.1806747	1.933	19.33	2090	278352	0.53399865	0.005253989	2.37022E-02	0.02198683
7	130	1.2790642	1.925	19.25	2319.6	325325	0.54327336	0.005345242	1.03706E-02	0.013008702
8	140	1.3774538	1.886	18.86	2502.5	369656	0.54102494	0.00532312	2.90487E-03	0.005916516
9	150	1.4758433	1.852	18.52	2640.4	416700	0.53006392	0.005215275	5.56536E-04	0.002188963
10	160	1.5742329	1.809	18.09	2778	463104	0.52050815	0.005121257	7.68576E-05	0.000686693
11	170	1.6726224	1.78	17.8	2894.4	514420	0.50842292	0.00500235	7.98128E-06	0.000188425
12	180	1.771012	1.705	17.05	3026	552420	0.50027242	0.004922158	6.45167E-07	4.63104E-05
13	190	1.8694015	1.665	16.65	3069	601065	0.47919352	0.004714764	4.17762E-08	1.03849E-05
14	200	1.9677911	1.492	14.92	3163.5	596800	0.46795145	0.004604153	2.21956E-09	2.15592E-06
15	210	2.0661806	1.409	14.09	2984	621369	0.41932946	0.004125764	9.87422E-11	4.19204E-07
16	220	2.1645702	1.305	13.05	2958.9	631620	0.39600215	0.003896248	3.74253E-12	7.70674E-08
17	230	2.2629597	1.263	12.63	2871	668127	0.36677275	0.003608661	1.22667E-13	1.34993E-08
18	240	2.3613493	1.179	11.79	2904.9	679104	0.35496857	0.00349252	3.52214E-15	2.2673E-09
19	250	2.4597389	1.08	10.8	2829.6	675000	0.33136021	0.003260238	8.95967E-17	3.67081E-10
20	260	2.5581284	1	10	2700	676000	0.30353607	0.002986478	2.03930E-18	5.75436E-11
21	270	2.656518	0.931	9.31	2600	678699	0.28105192	0.002765257	4.18949E-20	8.76694E-12
22	280	2.7549075	0.858	8.58	2513.7	672672	0.26165934	0.002574455	7.82881E-22	1.30227E-12
23	290	2.8532971	0.784	7.84	2402.4	659344	0.24114255	0.002372591	1.33991E-23	1.89125E-13
24	300	2.9516866	0.743	7.43	2273.6	668700	0.2203447	0.002167962	2.11340E-25	2.69162E-14
25	310	3.0500762	0.714	7.14	2229	686154	0.20882158	0.002054586	3.08899E-27	3.76168E-15
26	320	3.1484657	0.65	6.5	2213.4	665600	0.20067107	0.001974394	4.20487E-29	5.17162E-16
27	330	3.2468553	0.585	5.85	2080	637065	0.18268375	0.001797417	5.35489E-31	7.00524E-17
28	340	3.3452448	0.527	5.27	1930.5	609212	0.16441537	0.001617676	6.40606E-33	9.36191E-18
29	350	3.4436344	0.51	5.1	1791.8	624750	0.14811436	0.001457291	7.22598E-35	1.23587E-18
30	360	3.542024	0.49	4.9	1785	635040	0.14333648	0.001410281	7.71167E-37	1.61328E-19
31	370	3.6404135	0.478	4.78	1764	654382	0.13771544	0.001354976	7.81090E-39	2.08444E-20
32	380	3.7388031	0.37	3.7	1768.6	534280	0.13434282	0.001321793	7.53007E-41	2.66793E-21
33	390	3.8371926	0.34	3.4	1406	517140	0.10398921	0.001023145	6.92765E-43	3.38529E-22
34	400	3.9355822	0.271	2.71	1326	433600	0.09555765	0.000940187	6.09702E-45	4.26132E-23
35	410	4.0339717	0.269	2.69	1084	452189	0.07616507	0.000749385	5.14482E-47	5.32456E-24
36	420	4.1323613	0.24	2.4	2016	423360	0.07560297	0.000743854	4.17104E-49	6.60776E-25
37	430	4.2307508	0.235	2.35	2021	434515	0.06745246	0.000663662	3.25522E-51	8.14836E-26
38	440	4.3291404	0.22	2.2	1936	425920	0.0660472	0.000649835	2.44993E-53	9.98911E-27
39	460	4.5259195	0.12	2.4	1104	507840	0.06183142	0.000608357	1.78111E-55	1.21787E-27
SOMME				361.63	79431		0.03372623	0.000331831	8.53721E-60	1.78334E-29

Annex 2: Standard curves for COD and turbidity

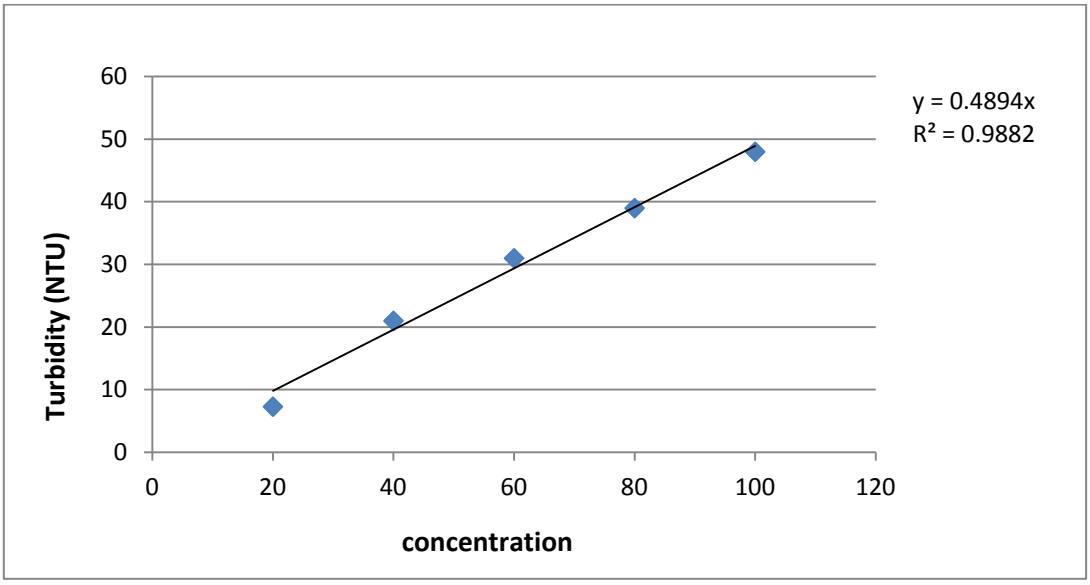
1- COD standard curve

Concentration mg/L	20	40	70	100	150
COD (mg d'O2/L)	16	37	58	91	137



2- Turbidity standard curve

solution	20	40	60	80	100	S (GW)
turb. (NTU)	7,3	21	31	39	48	38



Annex 3 : The Sand elemental composition

Group : Solides_VAC (mylar)

Sample : EDS

Layer Info	Analyte	Result		(Std. Dev.) Proc.-Calc.	Line	Intensity
1 Layer1						
1 Layer		6.000	um	(------) Fix	--------	--------
1 Elem.	C10H804	100.000	%	(------) Fix	--------	--------
B Base						
2 Elem.	Si02	87.406	%	(0.120) Quant.-FP	SiKa	11.3551
2 Elem.	Al203	5.873	%	(0.061) Quant.-FP	AlKa	0.2904
2 Elem.	S03	2.441	%	(0.018) Quant.-FP	S Ka	0.6806
2 Elem.	P205	1.255	%	(0.026) Quant.-FP	P Ka	0.1545
2 Elem.	K20	0.913	%	(0.008) Quant.-FP	K Ka	0.1300
2 Elem.	Ca0	0.871	%	(0.006) Quant.-FP	CaKa	0.4990
2 Elem.	Fe203	0.867	%	(0.006) Quant.-FP	FeKa	8.4483
2 Elem.	Ti02	0.338	%	(0.010) Quant.-FP	TiKa	0.7971
2 Elem.	Zn0	0.014	%	(0.001) Quant.-FP	ZnKa	0.3421
2 Elem.	Zr02	0.013	%	(0.000) Quant.-FP	ZrKa	1.1697
2 Elem.	Sr0	0.010	%	(0.000) Quant.-FP	SrKa	0.7875

Sample : SPS

Layer Info	Analyte	Result		(Std. Dev.) Proc.-Calc.	Line	Intensity
1 Layer1						
1 Layer		6.000	um	(------) Fix	--------	--------
1 Elem.	C10H804	100.000	%	(------) Fix	--------	--------
B Base						
2 Elem.	Si02	93.015	%	(0.115) Quant.-FP	SiKa	12.1987
2 Elem.	S03	2.743	%	(0.018) Quant.-FP	S Ka	0.7237
2 Elem.	Al203	2.275	%	(0.052) Quant.-FP	AlKa	0.1097
2 Elem.	P205	1.530	%	(0.027) Quant.-FP	P Ka	0.1786
2 Elem.	Ca0	0.284	%	(0.004) Quant.-FP	CaKa	0.1581
2 Elem.	Fe203	0.075	%	(0.002) Quant.-FP	FeKa	0.7375
2 Elem.	K20	0.050	%	(0.003) Quant.-FP	K Ka	0.0067
2 Elem.	Zn0	0.027	%	(0.001) Quant.-FP	ZnKa	0.6889

Annex 4 : Tables of physico-chemical and microbiological measurements of SSF

Annexe4. 1 : Table of physico-chemical and microbiological Egyptian desert sand

Hygien production		Shower Gel				soap		
Work days		EX.24/4/12	EX.10/5/12	EX.14/5/12	EX.21/5/12	EX.23/5/12	EX.29/5/12	EX.31/5/12
paramètre physico-chimique	turbidity	281	40	4,7	23	91	69	21
		1,07	1,73	0,81	1,47	3,5	7,7	4,9
		1,24	2,6	0,85	2,8	11,8	14,4	6,5
		1,1	1,3	0,67	2,6	18	16,1	4,7
		1,5	1,95	0,87	2,2	19,7	17,2	4,2
		1,93	3,1	1,37	2,9	21,1	17	2,7
	pH	7,4	7,48	7,5	7,59	7,7	7,56	7,37
		8,2	7,85	7,79	7,56	7,44	7,9	7,41
		8,1	7,84	7,81	7,54	7,6	7,7	7,7
		8,2	7,88	7,82	7,5	7,65	7,8	7,6
		8,1	7,82	7,82	7,63	7,6	7,8	7,64
		7,8	7,79	7,94	7,66	7,61	7,8	6,8
	conductivity	410	387	385	390	380	426	358
		463	484	443	444	444	478	451
		490	405	409	450	397	477	396
		445	410	411	413	389	479	381
		446	409	413	413	387	481	391
		446	410	409	412	389	485	387
	COD	298	271	69	285	220	214	190
		50	104	66	34	98	89	67
		103	94	64	35	82	78	52
		193	94	55	45	74	77	62
		152	85	50	41	70	79	58
		146	83	73	98	71	98	78
		129	87	36	73	52	106	62

ANNEXE :

Microbiologic parameters	***E.Coli(CFU/ml)***	4,00E+04	1,36E+05	3,14E+05	3,64E+03	8,61E+03	3,84E+04	1,34E+04
		2,00E+02	1,24E+04	1,11E+05	4,76E+03	4,29E+03	3,31E+04	3,33E+03
		7,00E+02	7,00E+02	5,80E+04	3,58E+03	2,63E+03	2,53E+04	5,11E+03
		2,00E+02	8,20E+02	2,77E+03	1,33E+03	2,93E+03	2,58E+04	4,75E+03
		1,70E+02	1,70E+02	1,90E+03	1,24E+03	2,43E+03	3,67E+04	6,40E+03
		2,00E+02	2,00E+01	2,00E+03	3,00E+01	1,91E+03	2,21E+04	4,80E+03
	pseudomonas	2,40E+05	3,00E+04	4,37E+05	1,91E+03	2,25E+03	5,43E+03	2,75E+03
		4,00E+05	4,02E+04	6,98E+04	1,76E+03	1,28E+03	5,58E+03	1,78E+03
		2,89E+06	4,70E+04	4,40E+03	7,08E+03	6,60E+02	3,45E+03	1,57E+03
		7,20E+06	4,50E+04	3,30E+02	1,93E+03	9,10E+02	2,45E+03	1,43E+03
		4,00E+05	4,00E+03	5,00E+02	1,20E+03	1,79E+03	2,80E+03	1,85E+03
		0,00E+00	0,00E+00	3,90E+02	9,50E+01	2,12E+03	1,60E+03	1,60E+03
	Entérocoques	6,10	7,35E+02	1,40E+02	1,50E+02	5,00E+01	5,00E+01	3,10E+02
		2,00E+01	2,00E+02	2,00E+01	5,00E+01	0,00E+00	0,00E+00	0,00E+00
		0,00E+00	2,00E+01	1,00E+01	2,00E+01	0,00E+00	0,00E+00	0,00E+00
		0,00E+00	0,00E+00	0,00E+00	0,00E+00	0,00E+00	0,00E+00	0,00E+00
		0,00E+00	0,00E+00	0,00E+00	0,00E+00	0,00E+00	0,00E+00	0,00E+00
		0,00E+00	0,00E+00	0,00E+00	0,00E+00	0,00E+00	0,00E+00	0,00E+00
	flore totale	4,80E+05	5,30E+04	4,13E+04	9,45E+04	1,64E+05	4,75E+05	1,23E+05
		1,00E+04	3,25E+04	1,11E+04	2,55E+04	3,40E+03	3,80E+04	2,56E+05
		2,10E+04	4,40E+04	1,07E+04	1,45E+04	3,12E+04	1,32E+04	1,32E+05
		3,70E+04	1,79E+04	4,17E+04	3,04E+04	2,61E+04	6,95E+04	9,66E+04
		0,00E+00	1,84E+04	9,90E+03	4,25E+04	4,20E+04	5,75E+04	5,37E+04
		0,00E+00	1,36E+04	7,50E+03	4,67E+03	0,00E+00	5,00E+04	2,31E+04

Annexe4. 2 : Table of physico-chemical and microbiological swimming pool sand

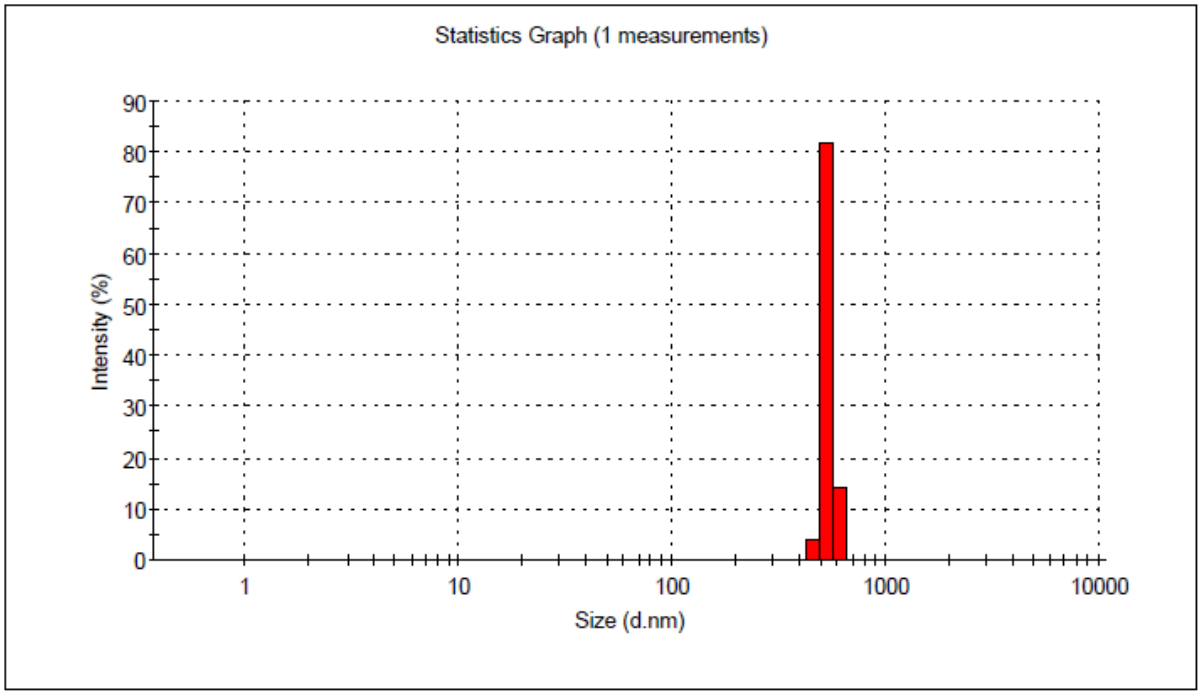
Hygien production		Shower Gel				soap		
Work days		EX.24/4/12	EX.10/5/12	EX.14/5/12	EX.21/5/12	EX.23/5/12	EX.29/5/12	EX.31/5/12
physico-chimique parameters	**turbidity**	281	40	4,7	23	91	69	21
		1,1	9,4	5,1	47	5,7	5,4	15,4
		5,6	19,2	2,4	6,7	34	38	13
		6	13	1,7	8,9	69	48	11
		7,6	21	1,76	10,3	71	54	9,7
		8,2	24,8	3,3	11,9	75	56	7,5
	pH	7,4	7,42	7,5	7,59	7,7	7,56	7,31
		7,9	7,88	7,43	7,44	7,88	7,6	7,36
		7,8	7,36	7,44	7,45	7,62	7,6	7,51
		7,5	7,33	7,35	7,3	7,77	7,7	7,57
		7,7	7,3	7,46	7,36	7,8	7,8	7,6
		7,4	7,3	7,59	7,6	7,82	7,8	7,64
	conductivity	410	387	385	390	380	426	385
		463	425	391	416	405	382	420
		425	377	387	397	375	396	386
		408	374	380	393	372	388	388
		406	374	381	387	378	394	390
		412	376	382	392	378	397	385
	COD	298	271	69	285	220	214	290
		26	123	101	36	91	69	86
		147	133	81	35	135	158	78
		193	139	78	97	132	194	117
		196	140	52	197	130	194	115
		200	157	71	146	115	186	138
		158	88	34	151	106	67	103

ANNEXE :

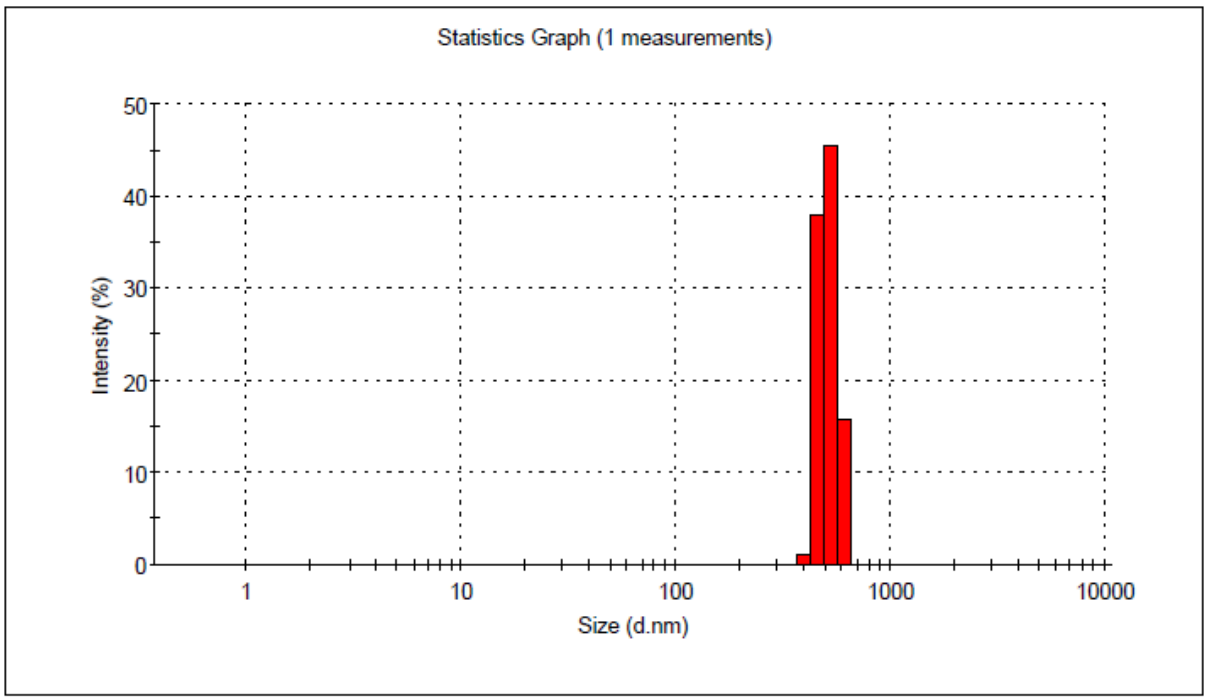
Microbiologice parameter								
	E.Coli (CFU/ml)	4,00E+04	2,10E+05	3,14E+05	3,64E+03	8,61E+03	3,84E+04	1,34E+04
		2,00E+02	3,05E+04	1,69E+05	1,20E+03	5,27E+03	1,59E+04	9,15E+03
		4,00E+03	3,79E+04	6,50E+04	1,35E+03	4,13E+03	2,26E+04	5,35E+03
		2,00E+05	1,32E+04	1,13E+04	2,95E+03	2,83E+03	3,76E+04	6,80E+03
		1,45E+04	3,08E+04	8,30E+03	1,41E+03	4,62E+03	4,71E+04	6,10E+03
		1,30E+04	0,00E+00	7,50E+02	6,60E+02	3,01E+03	1,09E+04	4,80E+03
	P.aeroginosa	2,40E+05	3,35E+04	4,37E+05	1,91E+03	2,25E+03	5,43E+03	2,03E+04
		4,00E+04	1,55E+04	5,92E+04	1,02E+03	1,54E+03	4,76E+03	2,92E+04
		1,80E+04	1,80E+04	2,80E+04	2,55E+02	9,60E+02	5,25E+03	1,70E+04
		3,20E+03	3,20E+03	3,93E+03	4,25E+02	1,01E+03	4,30E+03	2,20E+04
		0,00E+00	0,00E+00	4,00E+03	4,55E+02	1,90E+03	7,40E+03	1,60E+04
		0,00E+00	0,00E+00	3,50E+02	2,55E+02	1,80E+03	3,80E+03	1,41E+04
	Enterococcus	6,00E+01	3,50E+02	4,10E+02	1,50E+02	5,00E+01	5,00E+01	5,00E+01
		4,00E+01	1,50E+02	3,00E+01	8,00E+01	0,00E+00	0,00E+00	0,00E+00
		2,00E+02	3,00E+01	1,00E+01	4,00E+01	0,00E+00	0,00E+00	0,00E+00
		1,70E+02	0,00E+00	0,00E+00	0,00E+00	0,00E+00	0,00E+00	0,00E+00
		1,00E+01	0,00E+00	0,00E+00	0,00E+00	0,00E+00	0,00E+00	0,00E+00
		0,00E+00	0,00E+00	0,00E+00	0,00E+00	0,00E+00	0,00E+00	0,00E+00
	flore totale	4,80E+05	5,30E+04	4,13E+04	2,90E+05	1,64E+05	4,75E+05	1,23E+05
		1,00E+04	2,62E+04	4,59E+04	1,95E+05	2,35E+05	1,54E+05	1,75E+05
		2,10E+04	4,24E+04	3,31E+04	3,50E+05	3,40E+05	2,37E+04	9,47E+04
		3,70E+04	5,38E+04	2,75E+04	6,65E+05	9,20E+04	4,30E+04	5,60E+04
		0,00E+00	1,05E+04	4,15E+03	7,03E+05	5,34E+05	8,30E+04	4,46E+04
		0,00E+00	1,36E+04	4,63E+03	4,63E+03	4,55E+05	7,52E+04	3,32E+04

Annex 5: Cell size measurements

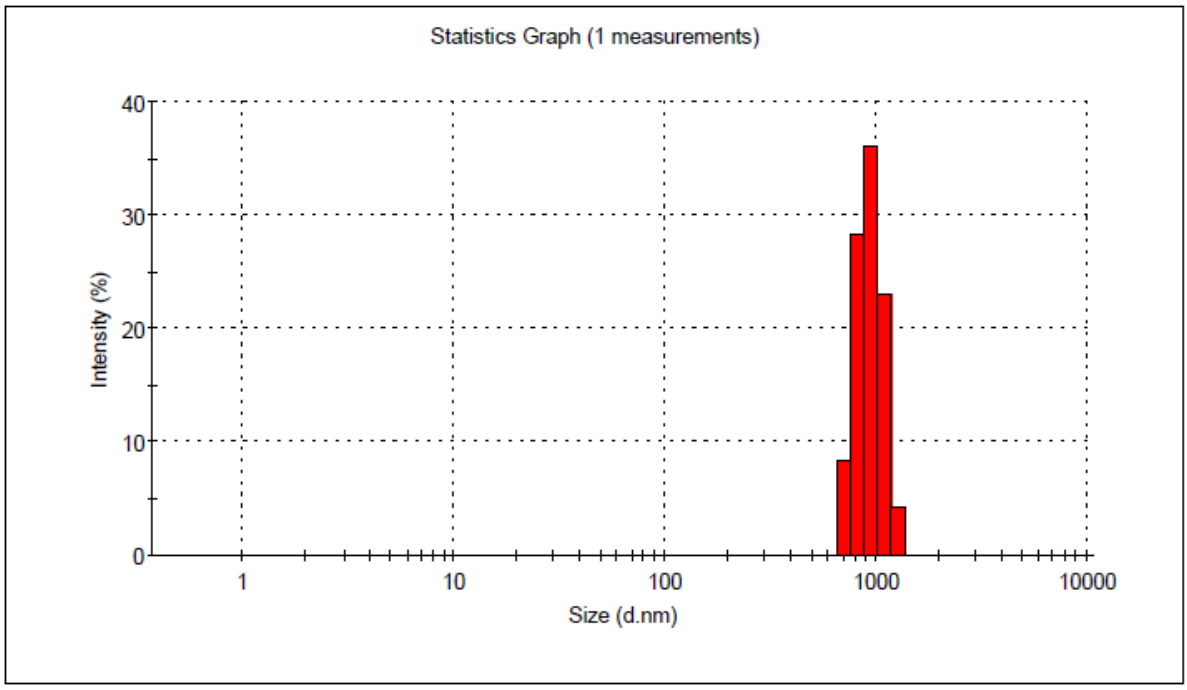
Effect of Poor media(GW) on *E.coli* cell size



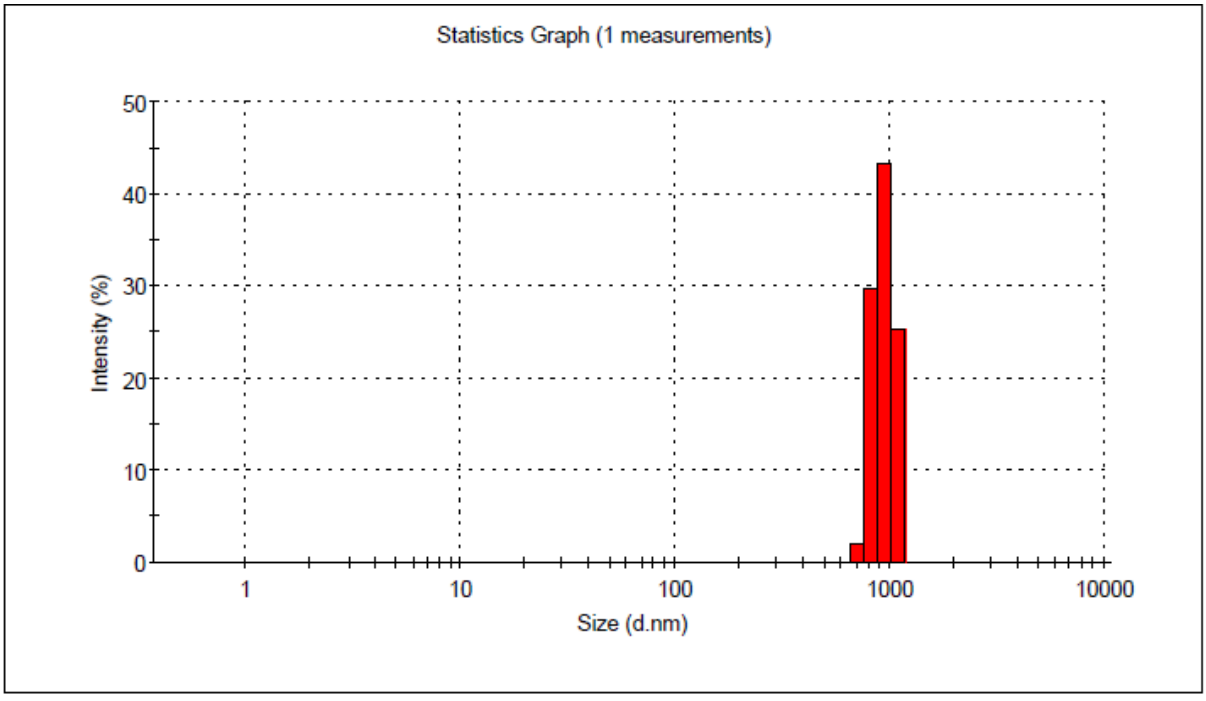
Effect of Poor media(GW) on *P.aeruginosa* cell size



Effect of rich media (NB) on *P.aeruginosa* cell size



Effect of rich media(NB) on *E.coli* cell size



Rafat KHALAPHALLAH

Traitement des eaux grises par filtration lente pour leur réutilisation : étude de la survie des micro-organismes pathogènes et des bactériophages
Greywater treatment for reuse by slow sand filtration: study of pathogenic microorganisms and phage survival

Résumé

Dans les dernières décennies, la plupart des pays du monde ont connu une pénurie d'eau et l'augmentation du taux de consommation. Aujourd'hui, tous les pays dans le monde essayent de trouver des alternatives pour remédier à cette pénurie. Une solution consiste en la réutilisation des eaux grises (GW) pour l'irrigation après traitement. Les GW correspondent aux eaux usées générée dans une maison à l'exception de l'eau des toilettes. Les risques associés à la réutilisation de ces eaux est la présence de microorganismes pathogènes qui peuvent infecter les humains, les animaux et les plantes. Dans cette thèse centrée sur l'étude de la survie des représentants d'agents pathogènes, comme *E. coli, P. aeruginosa*, et le bactériophage MS2 qui sont trouvés dans les eaux grises. Il a été étudié l'effet de quelques facteurs physico-chimiques tels que; température (6 ± 2,23 ± 2 et 42 ± 2 ° C), la salinité (1,75 and 3.5% de NaCl), de l'oxygène (aérobie et anaérobie), des éléments nutritifs (milieu riche et de milieux pauvres), la lumière avec la photocatalyse (lampes UV et visible) et filtre à sable lent (sable du désert égyptien et le sable piscine). Une combinaison de la température, la lumière du soleil et de haute photocatlysis sont principalement responsables de la baisse rapide des bactéries et du coliphage MS2. Le filtre à sable lent a une influence nettement moindre sur la survie des bactéries dans les eaux grises, mais il est efficace pour diminuer la turbidité et de la DCO.

Mots clés

Filtration lente, filtre à sable, eaux grises, réutilisation, survie microbienne *(E. coli, P. aeruginosa*, coliphage MS2), facteurs abiotiques, photocatalyse, UV.

Abstract

In recent decades, most countries of the world have experienced a shortage of water and increase its rate of consumption. Today, every country in the world are interested in this problem by trying to find alternatives to address this shortage. One solution is reuse greywater (GW) for irrigation after treatment. GW is all water generated from Household except toilet water. The risks associated with the reuse of these waters are the presence of pathogens that can infect humans, animals and plants. In this thesis focused on studying treatment by slow sand filtration and the survival of representatives of pathogens, such as *E. Coli, P. aeruginosa , E. Faecalis* and Bacteriophage MS2 which could be found in the greywater. Te study factors was a physico-chemicals factors such as; temperature (6±2,23±2,42±2°c), salinity (1.75 and 3.5% Nacl), oxygen (aerobic and anaerobic condition), nutrient (rich media , 50%: 50% salt and poor media), light with photocatalysis (UV and Visible lights) and slow sand filter (Egyptian desert sand and swimming pool sand). A combination of high temperature, sunlight and photocatlysis are mainly responsible for the rapid decline of bacteria and MS2 coliphage. Slow sand filter have clearly less influence on the survival of bacteria in the greywater, but it effective to decline turbidity and COD for short times.

Key Words

Slow sand filtration, Greywater, Reuse, Abiotic factors, Microbial survival (*E.coli, P. aeruginosa*, coliphage MS2), Photocatalyse, UV.

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