Effect of physical, chemical and biological treatment on the removal of five pharmaceuticals from domestic wastewater in laboratory-scale reactors and a full-scale plant

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Abstract

Pharmaceuticals and their metabolites are known to enter the environment from the effluent of wastewater treatment plants. From statistical analysis on the usage of pharmaceuticals, and their effects on the environment, five pharmaceuticals were selected for this study (Metronidazole, Trimethoprim, Sulphamethoxazole, Paracetamol and Ranitidine). Trace concentrations of pharmaceuticals were determined using a sensitive analytical method, comprising solid phase extraction (SPE) and liquid chromatography with a mass spectrometry detector (LC-MS),operating in selected ion monitoring (SIM) mode. It was found that Metronidazole, Trimethoprim, Sulphamethoxazole, Paracetamol and Ranitidine were detected at the highest levels in the wastewater entering the Sulaibiya WWTP Kuwait, with concentrations of up to 58 $ng.L^{-1}$, 1814 $ng.L^{-1}$, 1669 $ng.L^{-1}$, 2086 $ng.L^{-1}$ and 2009 $ng.L^{-1}$, respectively. High removal efficiencies of these pharmaceuticals were found in the Sulaibiya WWTP. One year study was conducted to investigate the occurrence, persistence and fate of a range of these pharmaceuticals at different sampling points at the Sulaibiya WWTP. The treatment processes consisted of screening, grit removal and diffused air activated sludge treatment (primary and secondary treatment), followed by microfiltration (MF), reverse osmosis (RO), and chlorine oxidation (tertiary treatment). During primary and secondary treatment, Metronidazole, Trimethoprim, Sulphamethoxazole, Paracetamol and Ranitidine were removed efficiently with average removals efficiencies of 83.4%, 86.1%, 77.5%, 97.5% and 77.5%, respectively. The RO system lowered these pharmaceuticals further, giving overall removal efficiencies of 97%, 99%, 99%, 100% and 100% for Metronidazole, Trimethoprim, Sulphamethoxazole, Paracetamol and Ranitidine, respectively. All selected pharmaceuticals were tested in laboratory scale reactors to assess their removal by chlorination and ozonation, and results showed that 10 $mg.L^{-1}$ of chlorine removed these pharmaceuticals better than 15 $mg.L^{-1}$ of ozone.

Lab-scale aerobic reactors (2 L), seeded with activated sludge inoculum from the Sulaibiya WWTP and fed with different concentrations of pharmaceuticals (0.1, 1 and 10 $mg.L^{-1}$), spiked individually into a synthetic wastewater showed that the TOC could be removed efficiently without inhibition by these pharmaceuticals.

The fate of Metronidazole, Trimethoprim, Sulphamethoxazole, Paracetamol and Ranitidine was investigated in a membrane bioreactors (MBR), and a sequencing batch reactors (SBR), operating under strictly aerobic, and anoxic/aerobic conditions at different concentrations of a pharmaceutical mixture (PM) of the same pharmaceuticals (1 $\mu g.L^{-1}$, 1 $mg.L^{-1}$ and 10 $mg.L^{-1}$). The COD and TOC removal efficiency decreased when the PM concentration was increased to 10 $mg.L^{-1}$. The removal of Metronidazole and Trimethoprim was moderately effective, and similar in all the reactors. Sulphamethoxazole and Paracetamol were removed efficiently, but this decreased when the PM was increased to 10 $mg.L^{-1}$ for most of the reactors, whilst Ranitidine experienced high removal rates at all concentrations in all the reactors.

Analysis of the microbial diversity in laboratory reactors treating pharmaceuticals wastewater showed decreases in microbial community diversity when the PM concentration was increased. Pure cultures of bacteria isolated on selected pharmaceutical growth media were also detected in the microbial communities of reactor sludge by performing polymerase chain reaction–denaturing gradient gel electrophoresis (PCR-DGGE).

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ABBREVIATIONS

BOD	biological oxygen demand
BPA	bisphenol A
COD	chemical oxygen demand
CSTR	continuously stirred tank reactor
DO	dissolved oxygen
EC	electrical conductivity
EDCs	endocrine disrupting compounds or chemicals
GC	gas chromatography
GC-MS	gas chromatography with mass spectrometry
Hc	Henry's law constant
HPLC	high-performance liquid chromatography
HRT	hydraulic retention time
k_{bio}	biodegradation constant
kD	adsorption coefficient
LC-MS	Liquid chromatography–mass spectrometry
LOD	limits of detection
LOQ	limits of quantification
LP	low pressure
MBR	membrane bioreactor
MP	medium pressure
MF	microfiltration
MLSS	mixed liquor suspended solids
MLVSS	mixed liquor volatile suspended solids
MS	mass spectrometry
MWCO	molecular weight cut-off
NF	nanofiltration
PCR-DGGE	polymerase chain reaction–denaturing gradient gel electrophoresis
PM	pharmaceutical mixture

PVC	Polyvinyl chloride
RO	reverse osmosis
RSD	relative standard deviation
SBR	sequencing batch reactor
SDI	silt density index
SIM	selective ion monitoring
S/N	signal-to-noise ratio
SPE	solid phase extraction
SRT	solids retention time
TDS	Total dissolve solids
TOC	total organic carbon
TSS	total suspended solids
UF	ultrafiltration
UV	ultraviolet
VSS	volatile suspended solids
WWTPs	wastewater treatment plants

1. Introduction

During the last three decades, traces amount of different pharmaceutical have been discovered in the natural environment, primarily through anthropogenic sources (Kummerer, 2001; Kummerer, 2009). Pharmaceuticals have been released to the environment either as the parent compounds or their metabolites (Ternes et al., 2001; Celiz et al., 2009). They affect ecosystems through changes to physical and chemical behaviour which can cause a biological effect by interruption of the food chain (Halling-Sørensen et al., 1998; Liu et al., 2009). Pharmaceutical wastes eventually find their way into the aquatic environment such as rivers, lakes, seas and ground water, that may have an adverse effect on human health (Webb et al., 2003; Cunningham et al., 2010). Expired pharmaceuticals may transform into toxic compounds that may also affect human health. Thus, pharmaceutical wastes disposal has become an increasing concern over recent years as it is released into the environment following ingestion, subsequent excretion, and transport through the wastewater treatment network. Furthermore, the disposal of unused or expired pharmaceuticals can also contribute to the problem, because there are few rules for their collection disposal and treatment, as the amount of pharmaceutical waste disposed in the sewer systems is unknown. Veterinary pharmaceuticals for livestock treatment, aquaculture and fisheries are another source of pharmaceutical waste in the environment.

Modern wastewater treatment technologies are efficient biological and chemical systems for removal of the majority of organic compounds (BOD) and providing enhanced nutrient removal, thus preventing de-oxygenation and eutrophication of receiving water bodies (Jones et al., 1998; Randall and Sen, 1996; Rogalla et al., 2006; Sriwiriyarat and Randall, 2005; Tocchi et al., 2012). However these treatment systems face a greater modern challenge with the threat of new and persistent compounds entering our wastewaters through a number of different sources. Many trace compounds, such as pharmaceuticals and endocrine disrupting chemicals

(EDCs) are being increasingly used to reduce disease in humans and life stock, but their ability to be removed from these systems is poorly understood or optimised leading to their discharge into the aquatic environment with potential serious consequences on the health of the receiving biota, and further up the food chain including humans (Giger et al., 2003; Hirsch et al., 1999; Sacher et al., 2001; Luo et al., 2014).

Over the past decade water companies and regulators have grown increasingly concerned about reports of high concentrations of pharmaceuticals in wastewater, appearing in the aquatic environment, on a large scale, e.g.in streams, rivers, groundwater and drinking water (Ayscough et al., 2000; Hilton et al., 2004; Kanda et al., 2003; Thompson et al., 2005; Luo et al., 2014) and the potential implications of these chemicals. This has led to additional tertiary wastewater treatments, such as ozonation, granular activated carbon and chlorine dioxide treatment, designed and installed to provide an improved effluent quality free from these emerging contaminants. However, these systems come with additional burdens in that they are expensive to install and operate reducing the desire to implement these technologies. Subsequently, the optimisation of existing primary and secondary treatment technologies and assets is preferred to maximize the removal of pharmaceuticals and potential EDCs while minimising capital costs or increasing running/energy costs.

Secondary biological treatment processes have the most potential for optimisation as these have previously shown to have the most capacity for pharmaceutical removal from previous studies (Boyd et al., 2005; Carballa et al., 2005; Jones et al., 2005; Miao et al., 2005; Nakada et al., 2006; Perez et al., 2005; Ternes et al., 2004; Verenitch et al., 2006; Verlicchi et al., 2012).

Globally, the number and size of wastewater treatment plants (WWTP) has been increasing over the past two decades, and now widely use reverse osmosis (RO) technology to produce high quality recycled water (Ng et al., 2008). As the RO water production increases, the disposal also increases in the environment. Therefore, it is

essential that the fate of these micropollutants is more fully understood so that human health and the environment can be protected.

1.1 Aim and Objectives

The aim of this study was to investigate and detect the fate of five common pharmaceutical compounds in a full scale treatment at Sulaibiya wastewater treatment plant Kuwait, and to identify and optimise which treatment processes were most effective for the removal of pharmaceuticals using laboratory-scale simulations.

The objectives of the research were:

1. Develop analytical methods to analyse five selected pharmaceuticals in real and synthetic wastewaters at low concentrations.

2. Evaluate the removal of these pharmaceuticals from the different wastewater treatment processes at Sulaibiya WWTP.

3. Evaluate the capability of natural bacterial strains isolated from the biomass of the Sulaibiya WWTP to biodegrade the selected pharmaceutical compounds aerobically in laboratory-scale batch reactors.

4. Carry onto laboratory-scale experiments to evaluate the optimal conditions to enhance pharmaceutical degradation processes of full-scale WWTP.

5. Evaluate the relative effectiveness of two different designs of bioreactors, the sequencing batch reactor (SBR) and the membrane bioreactor (MBR), for pharmaceutical degradation efficiency using laboratory-scale reactors.

1.2 Thesis plan

The main aim of this study was to assess the removal of pharmaceuticals in Sulaibiya wastewater treatment plant, through investigations into the occurrence and fate of five major pharmaceuticals (Metronidazole, Trimethoprim, Sulphamethoxazole, Paracetamol and Ranitidine). These compounds were selected based on their previous detection in wastewater, combined with their anticipated health and environmental effects or bioaccumulation potential. The thesis has been divided into the following sections:

Chapter 2: a review of earlier literature exploring pharmaceutical wastes in the environment, the quantities of these pharmaceuticals present in the environment, and their removal mechanism and removal efficiency.

Chapter 3: a review the analytical methods used to detect those pharmaceuticals in previous research and developing analytical methods.

Chapter 4: Full details of experimental and analytical methods used in this research.

Chapter 5: a presentation of all results and discussion of the research under the following sections:

a. Investigation of the removal efficiency of the target pharmaceuticals during their treatment at Sulaibiya WWTP throughout one complete year of operation.

b. Evaluation of the removal efficiency of target pharmaceuticals using chemical oxidation processes (chlorination and ozonation) in laboratory experiments.

c. Evaluation of pharmaceutical removal efficiency, and the effect of pharmaceutical concentrations, in laboratory-scale stirrer tank bioreactors (CSTR).

d. Evaluation of pharmaceutical removal efficiency, and effect of pharmaceutical concentrations, in laboratory-scale continuous bioreactors:

i. Membrane bioreactors (MBR)
ii. Sequencing batch reactors (SBR)

e. Investigate changes in the microbial diversity of bacterial populations in the biomass of a laboratory-scale bioreactors operating under different pharmaceutical loading.

2. Literature Review

The presence of trace organic pollutants is of growing environmental concern (Halling-Sørensen et al., 1998; Kummerer, 2009). Pharmaceuticals are one such pollutant that has received great focus due to increasing use, with many routes to the environment, and the ability to treat these pollutants prior to reaching the environment is highly variable. These are becoming a major focus for environmental engineers for the next century.

2.1 Fate of pharmaceutical wastes in the environment

To date there has been significant research on the detection and fate of different pharmaceutical wastes in the environment. The occurrence of pharmaceutical residues in the environment are affected by the following main factors, the amount of pharmaceuticals and the fate of each compound in both the sewage treatment plants and the aquatic environment. The fate of the pharmaceutical waste in the environment may be related to the following three factors:

1- It may ultimately mineralise to carbon dioxide and water.
2- It may be retained in the sludge because is lipophilic and not readily degradable.
3- It may metabolise to a more persistent hydrophilic compound and pass through the WWTP, then discharge to water bodies and may affect the organisms if it is biologically active.

A first report about pharmaceutical in environment was done by Fielding et al. (1981) who discovered some pharmaceuticals and related compounds in a river and drinking water. Tetracycline and theophylline were the first reported pharmaceuticals in the environment; these were antibiotics found in a river in 1983 (Watts et. al, 1983) which were used to treat infection in fish farms. Fish farms expose the receiving waters to a large proportion of drugs because most of the antibiotics and chemotherapeutics used

are not consumed by the fish but fall through the cages and accumulate on the sea bed (Jacobsen & Berglind, 1988; Labella et al., 2013).Then they may affect the aquatic organisms on the sea bed, and subsequently bioaccumulate up the food chain. A study has shown that 80% of drugs used in a fish farms end up in the environment, and found drug concentrations with antibacterial activity in the sediment directly underneath the fish farms (Samuelsenet al., 1992; Snow et al., 2013).

Steroids have been discovered in the sewage effluent (Daughton & Ternes, 1999). Steroids are a physiologically active compounds such as dietary fat cholesterol, the sex hormones estradiol and testosterone and the anti-inflammatory drug dexamethasone.17a-ethynylestradiol for example detected in sewage effluent at low concentration (< 7 ng l^{-1}) (Ternes et al., 2002).In addition, the analgesic drug acetaminophen, the stimulant caffeine, and the non-steroidal anti-inflammatory agents ibuprofen and aspirin, have been found in municipal wastewater (Metcalfe et al., 2003; Boyd et al., 2003). The presence of pharmaceuticals in sewage is due to the drugs not being completely degraded in the human body and thus excreted from the human body either without any change in their chemical structure or transformed into more active compounds. It is has been found in European sewage effluents at concentrations up to 6 $\mu g.L^{-1}$ (Ternes, 1998) and up to 10 $\mu g.L^{-1}$ in USA natural waters (Kolpin, 2002). Metformin is an oral anti-diabetic drug for the treatment of type 2 diabetes. It is the most popular anti-diabetic drug in the world and one of the most prescribed drugs in the country overall. It is detected in surface water and ground water in the USA at maximum concentration of 0.15 $\mu g.L^{-1}$ (Kolpin et al., 2002).

Although the primary route for pharmaceuticals to enter the environment is through excretion and wastewater treatment, there are other routes that could potentially contribute significant quantities of pharmaceuticals to the environment.

The discovery of pharmaceuticals in wastewater is largely due to the excretion of pharmaceuticals by the human body into wastewater. However, there is also another source of pharmaceutical waste in the environment - disposal in landfill. Holm et al. (1995) report finding organic compounds from pharmaceutical industry waste at the

bottom of a landfill; wastes such as sulphonamides, propylphenazone and 5,5-Diallylbarbituric acid may have entered the surrounding aquifers (Holm et al., 1995).

A study on a landfill in Florida that received wastes between the period 1968 and 1969 from the naval base hospital, show the presence and persistence of pentobarbital, meprobamate and phensuximide in a nearby shallow ground water source (Eckel et al., 1993; Karthikeyan & Meyer, 2006). Study in Germany reported that tap water in Berlin is contaminated by clofibric acid which is a metabolite of a blood lipid regulator in human medical care (Stan et al., 1994). The study shows that all samples from tap water, surface water and several rivers in Germany are contaminated by clofibric acid in concentrations between 10 and 165 $ng.L^{-1}$.

Other sources of pharmaceuticals are from agricultural sources. Modern intensive agriculture for higher productivity has led to widespread use of different pharmaceuticals and there applications. This increase in usage has led to contamination of the natural environment. Chlortetracycline used in agricultural applications have been detected in soil surrounding these farms, when combined with poultry manure, antibiotics resistant bacteria (microorganisms) may develop (Warman & Thomas, 1981; Zielezny et al., 2006; Schauss et al., 2009). As a consequence, the pharmaceuticals used for animals as growth promoters may affect micro-organisms, and it may also be mineralized and reach the groundwater.

Pharmaceuticals consumption may change depending on the season. During the winter season the antibiotics load in the WWTP were twice as high as in the summer months, due either to the lower removal efficiency of pharmaceuticals in the WWTP; to lower biological activity during transport through the sewage system as being less efficient in winter, or because the input in winter is higher (McArdell et al., 2003).

2.2 Toxicology of pharmaceutical in the environment

The presence of these pharmaceutical in environment is a matter of major concern with largely unknown consequences (Daughton, 2005; Kümmerer, 2009). Usually concentrations of pharmaceuticals found in the environment are in the levels $ng.L^{-1}$ to $mg.L^{-1}$. Toxicology studies report that even at low concentrations may be cause for concern in certain tested mixtures (Parrott and Bennie, 2009; Pomati et al., 2006, 2008). Many antibiotics have been reported to have acute or chronic toxicity to the environment and the development of antibiotic resistance in pathogens which show a potential danger to human health.

Researchers reported that triclosan effect algal growth and develop bacterial resistance, where they observed that photodegradation of triclosan can form dioxin by-products which would increase dioxin-like activity (Orvos et al., 2002; Mezcua et al., 2004). Roh et al. (2009) speculated that *Nitrosomonas europaea* inactivation in the presence of the antimicrobials was either a result of toxic product formation or the antimicrobial effect of triclosan.

Study showed that levofloxacin and clarithromycin have high toxicity to microalgae as well as chronic toxicity to crustaceans (Yamashita et al. 2006). In another study, sulphamethoxazole hazard quotient, derived from the acute toxicity concentration on *Daphnia magna* and its predicted environment concentration (PEC) was reported to be 6.3 $\mu g.L^{-1}$, which suggests potential environmental concerns (Kim et al. 2007). This is in agreement with previous studies, which demonstrated that the photodegradation of some pharmaceuticals increases toxicity. Trovo et al. (2009) found that sulphamethoxazole irradiation increases *Daphnia magna* toxicity from 60% to 100%.

Pharmaceutical mixtures containing carbamazepine, ibuprofen, and clofibric acid have toxic effect on algae, which shows a correlation with their Log *D*lipw (Cleuvers, 2003; Caminada et al., 2006; Escher et al., 2005). In a test using membrane vesicles isolated from a photosynthetic bacterium, *Rhodobacter sphaeroides*, seven

pharmaceutical (i.e. clofibrate, acetaminophen, propranolol, diazepam, diclofenac, ethinyletradiol and ibuprofen) clearly exhibited baseline toxicity (Escher et al., 2002).

Furthermore, researchers found that Ranitidine and Lincomycin inhibited the ammonia degradation up to 78% in activated sludge wastewater lab-scale sequencing batch reactor (Carucci et al., 2006). Also naproxen mixed with non-steroidal anti-inflammatory drugs showed significant effect in both the *Daphnia* and algal tests (Cleuvers, 2004). Quinn et al. (2009) also observed an additive effect in *Hydra attenuata* following exposure to pharmaceutical mixtures (ibuprofen, naproxen, gemfibrozil, bezafibrate, carbamazepine, sulphapyridine, oxytetracycline, novobiocin, trimethoprim, sulphamethoxazole and caffeine) from various therapeutic classes.

Estrogenicity has been verified for many contaminants such as natural and synthetic hormones and alkylphenols commonly detected in wastewaters (Dagnino et al., 2010). Endocrine disruptors such as diethylstilbestrol and 17-α ethinylestradiol has been shown to have profound ecological impacts as they mimic a natural hormone, fooling the body or blocking the effects of a hormone from certain receptors. Study reported that a single dose of 2 $ng.L^{-1}$ 17-α ethinylestradiol in water can retard testes growth and development by 50% in maturing male trout (Tyler et al., 1998; Palace et al., 2009, Kidd et al., 2007). In other study, exposure to 50 $ng.L^{-1}$ of either 17-β ethinylestradiol or estrone in wastewater for 21 days induced vitellogenin (an egg yolk precursor protein that is normally produced only byadult females) synthesis and abnormal testicular growth in male fathead minnows (*Pimephales promelas*) (Panter et al., 2000; Martinovic et al., 2007).

2.3 Source of pharmaceuticals in the environment

Pharmaceuticals are released into the environment as a result of their use, alongside their use, any unused or expired pharmaceuticals that are incorrectly disposed off. The quantity and type of pharmaceuticals that are introduced into the environment is related to the quantity of pharmaceuticals produced, the dosage amount, the metabolism excretion efficiency and the biological transformation capability in the transferring or receiving environment. Figure 2.1 shows the primary routes of

pharmaceuticals entering into wastewaters and the aquatic environment. Studies have shown that humans are the main source of pharmaceuticals entering the aquatic environment via discharges from WWTPs (Alder et al., 2006).

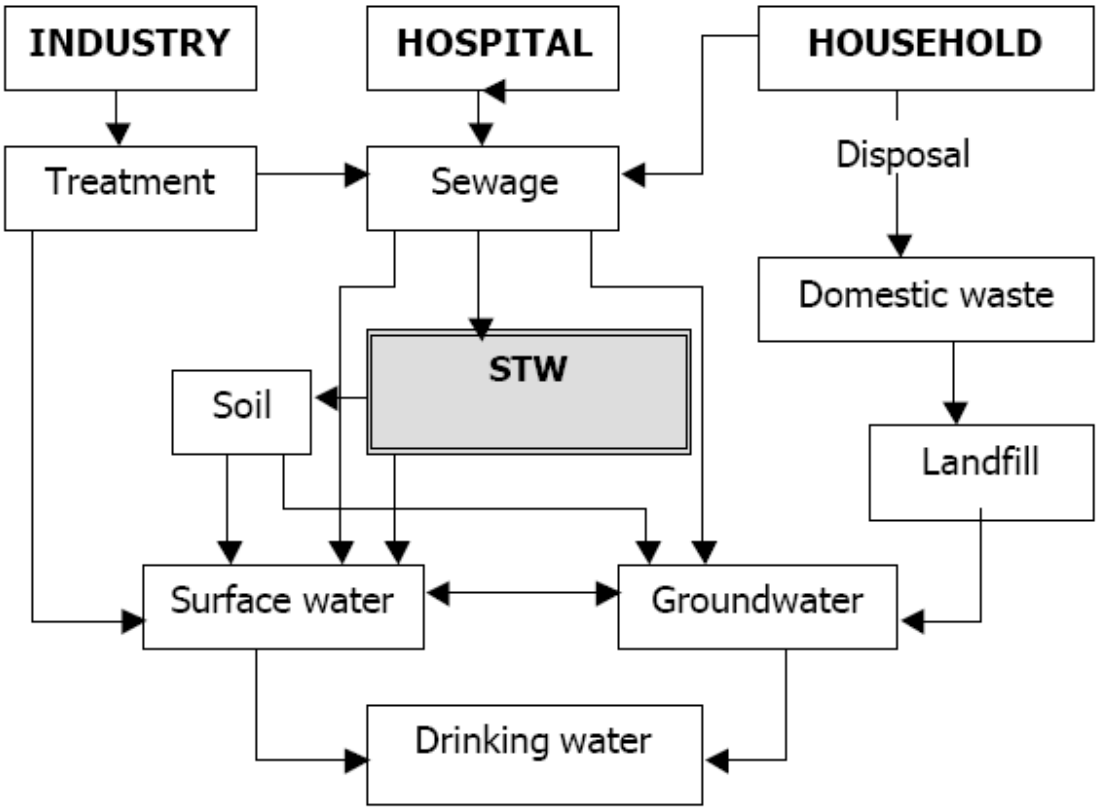


Figure 2.1 Overview of exposure routes of pharmaceuticals into wastewaters and the aquatic environment (Alder et al., 2006)

2.3.1 Pharmaceutical Industry

The pharmaceuticals industry can be considered a potential source of pharmaceuticals in the environment, where it may be present in solid waste or in wastewater effluent. Solid pharmaceuticals waste may be destroyed by incineration, whereas disposal in landfills may provide pharmaceuticals with access to aquifers through landfill leachate or in water drained from the landfill.

2.3.2 Household

Unwanted or unused pharmaceuticals are disposed of by incineration by most pharmaceutical companies and pharmacists following a regulated pathway. However, large quantities of pharmaceuticals which are not consumed are eventually disposed of through domestic household sewage (Daughton and Ternes, 1999; Ruhoy and Daughton, 2008). A study conducted in the Southeast of England indicates that 66% of people dispose of unwanted pharmaceuticals via their domestic municipal waste, 12% empty dispose of them via the toilet and 22% of people returned them to the pharmacy (Bound and Voulvoulis, 2005).

2.3.3 Hospital wastewater

Hospital wastewater also a major source of pharmaceuticals such as antibiotics, anti-cancer agents and iodinated contrast media containing individual pharmaceuticals at high concentrations (Alder et al., 2006). Several pharmaceuticals have been found in hospital wastewater effluent in relatively large concentrations (Table 2.1) (Kümmerer, 2001; Gómez et al., 2006). Hartmann et al. (1998) detected ciprofloxacin concentrations in the range of 3-87$\mu g.L^{-1}$ in hospital effluent. Heberer and Feldman (2005) found that 10% of the diclofenac and 15% of carbamazepine detected in wastewater treatment plants in Berlin, Germany was derived from local hospital wastewater. Typically most hospital effluent is directly connected to the municipal sewerage system without any additional treatment prior to the hospital discharge.

Table 2.1: Maximum pharmaceutical concentrations detected in hospital wastewater effluent (Gomez et al., 2006; McArdell et al., 2011).

Pharmaceutical	**Product group**	**Concentration µg.L^{-1}**
Paracetamol	Analgesic	29
Atenolol	Beta-blocker	122
Carbamazepine	Antiepileptic	0.07
Codeine	Analgesic	5.7
Diclofenac	Anti-inflammatory	1.9
Erythromycin	Antibiotic	0.03
Ibuprofen	Analgesic	151
Ketorolac	Anti-inflammatory	59.5
Metronidazole	Antibacterial	9.4
Propranolol	Beta-blocker	6.5
Ranitidine	H_2 antagonist	1.7
Trimethoprim	Antibiotic	0.037
Azithromycin	Antibiotic	0.11
Clarithromycin	Antibiotic	1.28
Sulfamethoxazole	Antibiotic	3.23
Sulfadiazine	Antibiotic	2.33
Sulfapyridine	Antibacterial	0.251
Ciprofloxacin	Antibiotic	15.7
Norfloxacin	Antibacterial	3.14
Clindamycin	Antibiotic	1.16

2.3.4 Wastewater Treatment plants (WWTP)

Wastewater treatment plant effluent is the main source of pharmaceuticals in the environment (Daughton and Ternes, 1999). In WWTPs there are wide ranges of processes such as primary screening and biological treatment, which may remove pharmaceuticals. Biodegradation (aerobic and anaerobic) and sorption of hydrophobic pharmaceuticals to activated sludge are examples of processes that may reduce concentrations present in the WWTP effluent. Sludge biosolids have a high organic content. The less polar or non-polar components of sludge are likely to sink, whereas polar substances are more likely to remain in aqueous phase. Some pharmaceuticals may be excreted as conjugates that will be broken down in wastewater treatment to release a less soluble compound. Other pharmaceuticals are not biodegradable and are hydrophilic, so there is incomplete elimination of these pharmaceuticals in WWTPs (Bendz et al., 2005).

Pharmaceuticals that are removed from wastewater by adsorption into sludge solids may enter the aquatic environment through sewage sludge when disposed of in landfill or agricultural application (Jones et al, 2005).

2.3.5 Leachate from Landfill

Landfills are the sources of a wide range of contaminate compounds that may effect the environmental, wildlife and human health (Eggen et al., 2012). Municipal landfills may generate leachate which contains significant amounts of dissolved organic matter, heavy metal and pharmaceutical (Li et al., 2009). Several kinds of contaminants such as hormones, pharmaceuticals and fire retardants detected in down gradient from the landfill (Buszka et al., 2009). Another study found similar observation that landfill in U.S has high concentration of pharmaceuticals and it is persistent in the groundwater (Barnes et al., 2004). Leachate contamination of the groundwater may occur by seepage of water from landfill. Three pharmaceuticals, namely propyphenazone, ibuprofen, and clofibric acid, were identified by Schwarzbauer et al. (2002) when they analyzed contaminated groundwater from seepage water of a domestic waste landfill in Germany.

2.3.6 Sewage Sludge Disposal

Sewage sludge is a by-product of the wastewater treatment process and it consists of organic and inorganic solids present in the influent as well significant quantities of the biomassformed during aerobic, anoxic, or anaerobic degradation processes, this biomass constitutes 40-80% of the total organic materials dependent on process (Schowanek et al., 2004). Typically sludge is used as an agricultural fertilizer and after concentration and pathogen treatment (typically with lime) is spread on the soil surface. It is spread for only a short time before cultivation as to avoid potential problems of odour, pest attraction and surface run-off. In EU countries the maximum allowable sludge disposal rate is 5 tonnes of dry matter/ha annually (Lucid et al., 2013). Soluble pharmaceuticals or metabolites of these pharmaceuticals have the potential to leach through the soil structure and enter the groundwater, especially problematic when rainfall occurs. Göbel et al. (2005) found five sulphonamides or macrolide antimicrobial's containing trimethoprim in samples of activated sludge taken from WWTPs in Germany and Switzerland. Kinney et al. (2006) detected 19 different pharmaceuticals in the sludge samples in nine different sludge products produced by WWTPs in seven different states in the USA.

2.4 Treatment of pharmaceuticals in wastewater treatment plants

Wastewater is water discharged from domestic homes, commercial properties, industry, and agriculture and can include a wide range of potential contaminants and concentrations. Wastewater is derived from human waste such as faeces, urine, washing water, and manufactured liquids from domestic sources such as drinks, cooking oil, pesticides, lubricating oil, paint, and cleaning liquids. Industrial effluent such as cooling waters which contain silt, sand, alkali, oil, and chemical residues; organic biodegradable waste from abattoirs, creameries, and ice cream manufacture; and organic non-biodegradable or difficult to treat waste such as that from pharmaceutical and pesticide manufacturing are also found in wastewater.

Most applications used in wastewater treatment plants, by removal the residual particulate matter in secondary treatment (e.g. activated sludge, trickling filter and membrane bioreactor) and even in tertiary treatment (filter technologies such as depth, surface and membrane filtrations), comply with the quality requirements for reuse of water.

Sorption of pharmaceuticals by the sludge in the treatment process can be present in two mechanisms, absorption and adsorption. Absorption is the hydrophobic interaction of the aliphatic or aromatics groups with the lipophilic cell membrane or with the lipid fraction of the sludge. Adsorption is the electrostatic interactions of positively charged groups of pharmaceuticals with negatively charged surfaces of the biomass (Schwarzenbach et al., 2003).

Stripping is an important removal mechanism for low molecular weight compounds and depends on the aeration intensity and the Henry's coefficient of a given compound. Stripping is not likely to be a practical removal mechanism for pharmaceuticals because the majority have a molecular mass above 250 $mg.mol^{-1}$ with a Henry coefficient below 0.005 (Larsen et al., 2004).

Water treatment plants for domestic and industrial consumption use unit processes such as coagulation, flocculation, sedimentation, filtration and disinfection. The objective of these processes are to provide drinking water free from pathogens, organic matter, neutralise taste compounds and remove any other chemical contaminants. Previously, focus has been primarily on pathogens, but with increasing concern over other chemical contaminants advanced processes are becoming more widespread.

Most pharmaceuticals in the aqueous phase are expected to be partially degraded and transformed by photo-transformative, physicochemical and biological degradation reactions. Removal of pharmaceuticals via adsorption processes typically uses activated carbon to adsorb the chemical in question. Activated carbon will adsorb a wide range of compounds, and so pharmaceutical adsorption has to compete with natural organic matter or other larger compounds present in the water. Snyder et al. (2003) found that when 10 to 20 $mg.L^{-1}$ of powdered activated carbon (PAC) is added

to distilled water containing seven antibiotics the removal efficiency was 50 to 99%, while in river water it decreased (10 to 20%). The removal efficiency of sulphonamides, trimethoprim, and carbadox in surface water samples containing 10.7 $mg.L^{-1}$ of dissolved organic matter by using 10 and 20 $mg.L^{-1}$ of PAC ranged from 49 to 73% and 65 to 100%, respectively (Adams et al., 2002). Westerhoff et al. (2005) conducted a batch study on sulphamethoxazole, trimethoprim, and erythromycin-H_2O at concentrations from 30 to 150 $ng.L^{-1}$ in natural water containing dissolved organic matter at 3.5 $mg.L^{-1}$ with PAC dose of 4 $mg.L^{-1}$ and a contact time of 4 h, finding that removal efficiency of sulphamethoxazole, trimethoprim, and erythromycin-H_2O to be 21%, 93%, and 65%, respectively.

Membrane filtration for water treatment is primarily undertaken for industrial applications when a high (chemical and microbiological) water quality is required. Processes such as reverse osmosis, nano filtration and ultrafiltration have been previously demonstrated to remove different pharmaceuticals efficiently (Kim et al., 2007 and Yoon et al., 2006). Although filtration processes provide a high quality pharmaceutical free effluent there are significant drawbacks to the process including cost, energy usage and importantly, the lack of degradation of these chemicals. Filtration removes the target chemicals, but in doing so produces a concentrated effluent high in suspended and dissolved constituents that still requires treatment before disposal (USEPA, 2005).

Chlorine is typically used in disinfectant a process which is primarily designed for the removal of pathogens. Chlorine disinfection takes place using a variety of different forms of chlorine; free chlorine and chloramines (Mono, di, tri), designed to provide effective bacterial kill and maintain a residual within the distribution network. Dissolving chlorine gas or hypochlorite into water produces free aqueous chlorine ($HOCl/OCl^-$), which reacts with ammonia to form chloramines. It is a strong oxidant, which reacts with many organic pollutants and produces chlorination by-products such as disinfection by-products (DBP) including harmful halogenated organics, mainly trihalomethanes (THM) and haloacetic acids (HAA). Chloramines are relatively weaker oxidants, which are expected to react much more slowly with organics (Rice & Gomez-Taylor, 1986). Rapid reactions happen when aliphatic amines react with HOCl to produce *N*-chloramines and reaction rates with chlorine

depend on the degree of nucleophilicity of amines (Abia et al., 1998). Further reaction present the *N*-chloro compounds with a hydrogen atom on the carbon α- to the amine to produce an imide, which subsequently hydrolyses, resulting in bond cleavage between the nitrogen and carbon atoms and removal of the α-carbon side-chain (Armesto et al., 1998). Aromatic amines tend to produce ring-substituted rather than *N*-chlorinated products (O'Connell et al., 2006). Phenol compounds react with free chlorine through a typical electrophilic substitution pathway (Doborde & Gunten, 2008). The phenolate anion reacts quite rapidly with HOCl because it has a higher electron density. In antibiotics, sulphonamides are subject to free chlorine attack because they contain an aromatic amine group. Fluoroquinolones, tetracyclines and macrolide antibiotics follow a different degradation pathway. These antibiotics contain aliphatic amine groups, which are likely to react with free chlorine to produce *N*-chloroamines that can then further degrade.

A study was conducted on the kinetics and reaction mechanisms of sulphamethoxazole, trimethoprim, and fluoroquinolone with free chlorine and chloramines at a lower disinfectant to analyte ratio (~10) (Dodd et al., 2005). It was shown that these antibiotics react slowly with chloramines and more rapidly with free chlorine. Sulphamethoxazole yields a *N*-chlorinated adduct, which rearranges to a ring chlorination product or leads to a break of the sulphonamide moiety to produce the main product *N*-chloro-p-benzoquinoneimine. The primary reaction of trimethoprim occurs on the molecule's trimethoxybenzyl moiety at a pH of less than 5, while a *N*-chlorinated intermediate is generated at a pH greater than or equal to 5. This may react further or rearrange to a number of stable substitution products. Free chlorine reacts very rapidly with Ciprofloxacin to produce a chloramine intermediate that spontaneously decays in water by piperazine fragmentation, whereas it reacts relatively slowly with enrofloxacin to produce a chlorammonium intermediate that can catalytically halogenate the parent compound in an aqueous solution. The oxidation processes of fluoroquinolones are not complete, which means they may not eliminate completely the biological effect of these compounds (Dodd et al., 2005).

However, the reaction of sulphamethoxazole with free chlorine produces substantial structures that may reduce the antimicrobial activities of sulphamethoxazole (Dodd & Huang, 2004). This is not such an issue in reality as sulphonamides have been shown

to be easily removed from drinking water at neutral pH, despite the proximity of barely affected by monochloramine (Chamberlain & Adams, 2006). Chlorination is unlikely to reduce the antimicrobial activities for trimethoprim because the reaction produces primarily stable and multiple-substituted compounds such as monochlorinated 3,4,5-trimethoxytolyl and dichlorinated 3,4,5-trimethoxytolyl(Dodd & Huang, 2004). Trimethoprim antibacterial activity is derived from its 2,4-diamino-5-methylpyrimidine moiety which blocks bacterial folate synthesis by occupying available dihydrofolate reductase enzymes (Walsh, 2003).

Ozone (O_3) is a strong oxidising agent, which oxidizes some organic compounds quicker than both chlorine and chlorine dioxide. It is used for disinfection in drinking water treatment plants to control colour, odour, iron and manganese concentrations, aid the deterioration of colloidal material to improve flocculation, remove disinfection by products precursors through oxidation, and further eliminate organic compounds (Haas, 1990). Ozone oxidation is a highly selective reaction and will interact with the double bonds, activated aromatic compounds, and amine groups, while the hydroxyl (OH) radicals generated from ozonation interact with the components of most of the water with nearly diffusion controlled rates (von Gunten, 2003).

A batch study was conducted to determine the degradation rate constants of several pharmaceuticals (carbamazepine, diclofenac, 17α-ethinylestradiol, sulphamethoxazole, and roxithromycin) with ozone and OH^- radicals. It was shown that under ozonation all three chemical were completely degraded (Huber et al., 2005). Removal of carbamazepine and sulphamethoxazole in a full-scale ozonation plant at a concentration of 2.4 and 9.7 $ng.L^{-1}$, were observed to below the detection limit (<1 $ng.L^{-1}$) (Snyder et al., 2003).

The mechanism for carbamazepine degradation under ozone involves the ozone reacting rapidly with the double bond in carbamazepine, with the formation of by-products containing quinazoline-based functional groups that can then be further oxidized by reaction with OH radicals (McDowell et al., 2005). In a pilot-scale study, when ozone was introduced to carbamazepine in the plant's source water, 66 to 96% reduction was observed (Hua et al., 2006).

The degradability of the pharmaceuticals by OH radical mediated reactions of advanced oxidation processes were observed, where 5 $mg.L^{-1}$ ozone and 1.8 $mg.L^{-1}$ hydrogen peroxide in river water was almost quantitatively degraded to 2.1% (clofibric acid), 0.6% (ibuprofen) and 0.1% (diclofenac) of the initial concentration of clofibric acid, ibuprofen and diclofenac, respectively (Zwiener & Frimmel, 2000). On the other hand, 2.5 $mg.L^{-1}$of ozone achieved greater than 70% removal of each in a pilot-scale plant (Snyder et al., 2003).

The reaction mechanisms of oxidation during ozonation have been widely studied (Deborde et al., 2005; Huber et al., 2005). During ozonation, micro-pollutants are oxidised through attack by either the ozone molecule itself or the creation of a hydroxyl radical, derived from direct ozone decomposition. Von Gunten (2003) hypothesised that ozone molecules react selectively with certain functional groups, but oxidation with hydroxyl radicals is indiscriminate. However, Nakada et al. (2007) suggests that molecular ozone attacks structures with high electron density, such as C=C bonds, activated aromatic systems, and non-protonated amines, but not aromatic rings with ethylene, amide or carboxylic groups. Currently, exact mechanisms on transformation products are still poorly understood.

The UV radiation process is widely used to disinfect and purify drinking water to remove biological pathogens. A small number of studies focusing on the degradation of pharmaceuticals through the process of UV treatment, in conjunction with hydrogen peroxide or ozone, found this process may actually transform these pharmaceuticals. In the United States, this technique is currently gaining greater importance because its use can reduce the dosage of chlorine to purify the final application and, thus, reduce the levels of disinfection by-products formed (Sharpless & Linden, 2001). UV can be used in advanced oxidation processes as an alternative to O_3 to remove disinfection by-product precursors and its use is attractive due to lower cost and lower potential for producing alternative chemical by-products.

Furthermore, degradation of pharmaceuticals can be obtained using direct photolysis and advanced oxidation processes. Accordingly, the absorption of light will cause the chemical to undergo transformation (Schwarzenbach et al., 1993).UV radiation can be generated using medium pressure (MP) lamps that emit a broadband ranging wavelength from 205 to above 500 nm, which was found to achieve a more effective degradation of bisphenol A, ethinylestradiol, and estradiol than direct photolysis using low pressure (LP) lamps that emit monochromatic light at 254 nm (Sharpless & Linden, 2003; Rosenfeldt & Linden, 2004).

A study on the kinetic degradation constant of carbamazepine and reaction intermediates formed using LP UV/H_2O_2 revealed an effective removal, whereas it leads to negligible degradation through direct photolysis in the absence of H_2O_2 (Vogna et al., 2004).

Degradation of paracetamol and diclofenac using ozonation and LP UV/H_2O_2 processes was found to be effective and achieved degrees of mineralization of approximately 30 and 40% for ozonation and H_2O_2 photolysis, respectively (Andreozzi et al., 2003; Vogna et al., 2004) .

Batch reactor experiments were conducted to evaluate LP and MP ultraviolet systems and to investigate the UV photolysis and UV/H_2O_2 oxidation of pharmaceuticals (carbamazepine, clofibric acid, iohexol, ciprofloxacin, naproxen, and ketoprofen) in the aquatic environment (Pereira et al., 2007). Pharmaceuticals' removal was very high under MP-UV photolysis and the MP-UV/H_2O_2 oxidation process, whereas it was well under LP-UV and was underestimated in the LP-UV/ H_2O_2. In general, MP lamps proved to be more degradation efficient in both UV photolysis and UV/H_2O_2 oxidation in the bench-scale experiments conducted.

A controlled laboratory scale photo-catalysis experiment achieved the reduction of carbamazepine, clofibric acid, iomeprol, and iopromide (Doll & Frimmel, 2005). This was accompanied by high photo-catalytic degradation of carbamazepine and clofibric acid with elimination of the model solution’s dissolved organic carbon showing that the xenobiotics were mineralized to some extent. On the other hand, the photo-

catalytic degradation of iomeprol was accompanied by formation of iodide as degradation products and intermediates.

2.5 Bioreactor

2.5.1 Sequencing Batch Reactor

A sequencing batch reactor (SBR) system consists of a wastewater fill-and-draw process. SBR is different to a conventional activated sludge system, because the processes of equalization, aeration and clarification are all achieved in the same tank at the same time (Morgenorth and Wilderer, 2000). SBR consists of five process steps carried out in sequence: (1) fill, (2) react (aeration), (3) settle (sedimentation/clarification), (4) draw (the effluent is decanted), and (5) idle (see Figure 2.2). Wastewater fills the tank, is treated and then discharged. Treated wastewater is usually drawn after the settling phase.

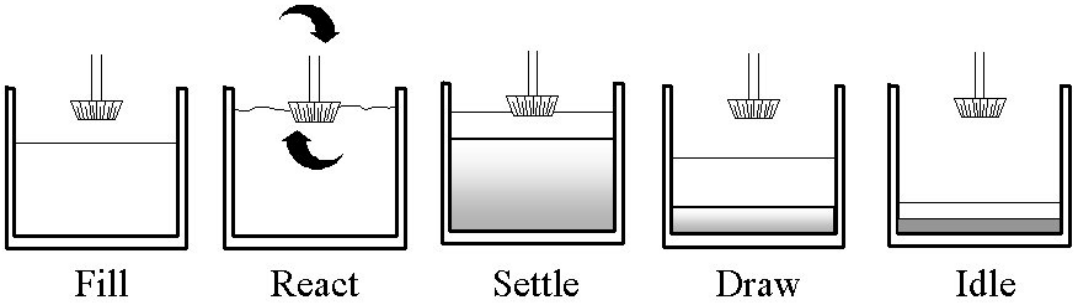


Figure 2.2: The processes operation of SBR during one cycle (http://www.lifesciences.napier.ac.uk/smaefiles/kinneil/kinneil.htm).

SBR has good characteristics, by combining the reactor and the settling tank in the same container, which can easily control the performance of the reactor with respect to reaction time and sludge solids maintenance. The SBR process saves more than 60% of the expenses compared to the conventional activated sludge process (Chang et

al., 2000). SBR is an effective biological treatment system for the treatment of domestic wastewater, and for different industrial wastewaters such as landfill leachate, pulp and paper industry wastewater, dairy wastewater and chemical complex wastewater (Mace and Mata-Alvarez, 2002; Mohan et al., 2005; Tsang et al., 2007; Neczajet al., 2008; Elmolla and Chaudhuri, 2011).

2.5.2 Aerobic Membrane Bioreactor

A membrane bioreactor (MBR) is a combination of activated sludge processes and membrane filtration in one treatment process. Activated sludge is filtrated from the effluent by using an ultrafiltration or microfiltration membrane, which can be applied within the bioreactor by submergence or externally through recirculation. By using a membrane for separation of the suspended solid and colloidal material such as micro contaminants, bacteria and viruses from the effluent, the sludge concentration in the aeration tanks can be higher than in conventional systems (Ujang and Anderson, 2000; Trussell et al., 2005). Biological processes in a MBR are better than in conventional activated sludge systems, due to the long sludge ages, and nitrogen removal is more efficient because of the slow growing autotrophic bacteria (Ujang et. al., 2005).

A membrane is a barrier that separates two phases and restricts the transport of various particles in a selective manner (Paul and Yampol, 1994). The principle is that the semi-permeable membrane acts as a very specific filter that permits water to flow through permeate, while it retains suspended solids and other substances (retentate) (see Figure 2.3). A MBR is an integrated system consisting of the biological degradation of waste products and membrane filtration, where microorganisms responsible for biodegradation and suspended solids get separated from the treated water by membrane filtration (Jacques et al., 1996; Cicek, 2003).

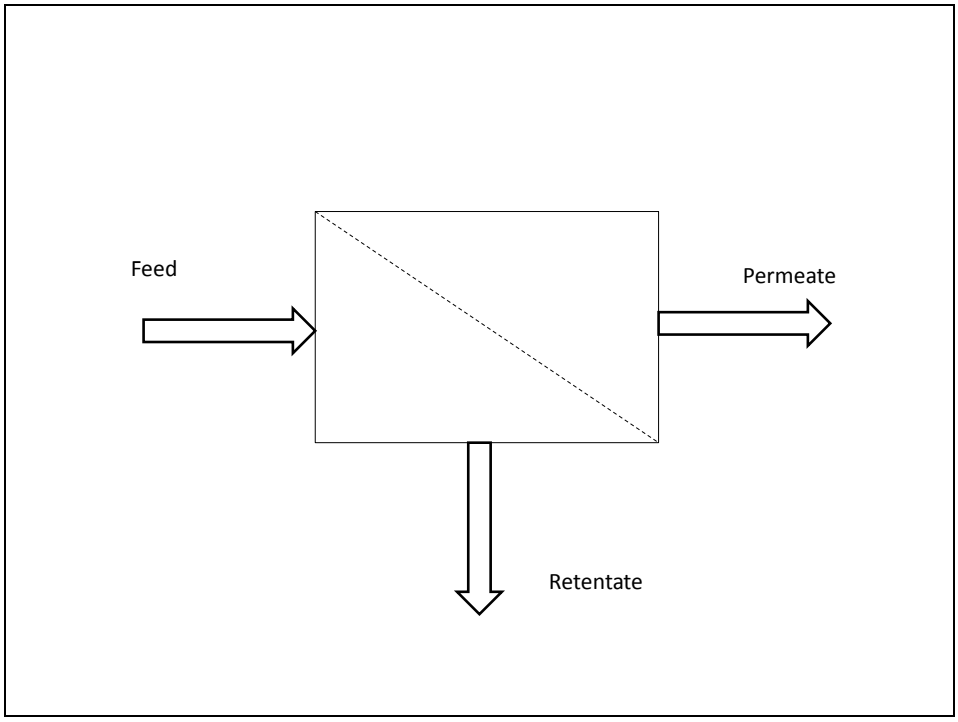


Figure 2.3. Scheme of membrane removal process

The MBR system can be classified according to the design (the separation principle employed and the aggregation state of the fluids contacting the membrane) and pore size (the largest particles that can permeate a membrane) of membrane modules. There are many membrane types according to whether the design is tubular, hollow fibre, rotary disk, plate and frame. The tubular membrane is commonly used to improve turbulent flow and mechanical cleaning. Hollow fibre has the highest membrane surface area of all the membrane module types and is considered as a self-supporting membrane, whereas the rotary disk membrane has an acceptable membrane surface area (Seung, 2004). The plate and frame shaped membranes are usually disposable and inexpensive. According to the pore sizes, there are four types of membrane, namely Microfiltration (MF), Ultrafiltration (UF), Nanofiltration (NF) and Reverse Osmosis (RO). MF removes particulate contaminants such as clay, algae, bacteria and microorganisms with less energy consumption than other types of membranes (Meier-Haack et al., 2003; Ujang et al., 2002).

Hydrophilicity refers to the chemical characteristics of the membranes which have the tendency to wet the membrane and form a water film or coating on their surface.

Hydrophobic means that the membranes have no tendency to absorb water, and water tends to stay on their surface. Many natural products are negatively charged, due to dipole or multiple chemical bonds in their structure, while particulates in aqueous media are hydrophobic and will attach to any material that is less hydrophilic than water. The precipitation of the particles on the membrane surface can be achieved by less exposure to hydrophobic particles. Hydrophobic membrane filtration tends to have more fouling than hydrophilic filtration, so membranes should preferably be hydrophilic for wastewater treatment (Fane et al., 1991; Belfort et al., 1994; Chang et al., 1999; Judd and Till, 2000; Choi et al., 2002; Hadidi & Zydney, 2014).

Membranes can be made from organics such as polyethylene, polyethersulfone, polysulfone, polyolefin, etc. and inorganic (ceramic) or metallic substances. Membrane materials should be inert and non-biodegradable, easily cleaned and able to withstand cleaning chemicals and high temperature and pressure. The surface charge of a membrane can attract or repel charged species in water. Moreover, the surface charge of the membrane should be neutral or negatively charged to avoid adsorption of microorganisms (Seung, 2004). The negative or neutral membrane surface charge is preferred to limit the adsorption of particles to the membrane, since the natural organic macromolecules in water and waste water are commonly negatively charged (Cardew and Lee, 1998).

The membrane separations depend on the membrane ability to permit one component from the feed mixture. The pore size of the membrane is large enough to allow some molecules to pass through, and too small to permit the others (Figure 2.4). The removal of trace contaminants in the Water Industry may be achieved by using RO or NF membranes in a water recycling plant. The combination of advanced water treatment systems such as ultrafiltration (UF) and microfiltration (MF), followed by reverse osmosis (RO), has become industry standard practice for the reclamation of municipal wastewater for industrial and indirect potable reuse applications.

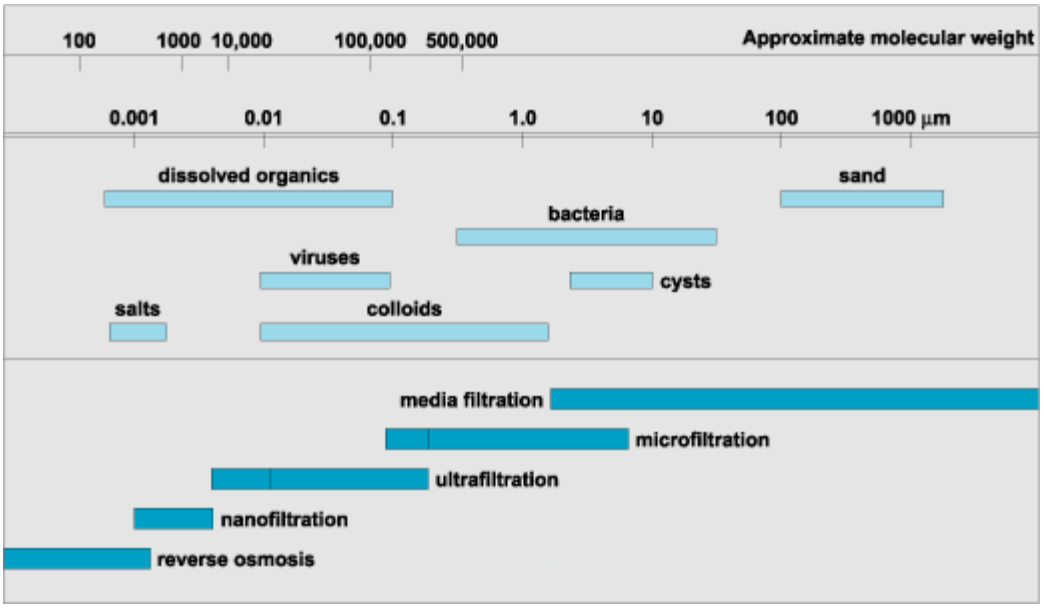


Figure 2.4: Pore sizes for different type of membranes relative to various water quality concerns. (USEPA, 2001)

Organic pollutants could be removed partially or totally in wastewater treatment plants; while it is not sufficient to remove all micro-contaminants, some treatment processes are clearly more effective than others at reducing the concentration of a broad range of trace contaminants. Research study has reported that the capability of NF/RO membranes to reject organic micropollutants such as endocrines, pharmaceuticals and others was incomplete (Golet et al., 2002; Kimura et al., 2003; Schäfer et al., 2003; Kimura et al., 2004; Nghiem et al., 2004).

The performance of membrane separation has been estimated in order to predict the mass balance through membranes (Williams et al., 1999; Bowen et al., 2002). The removal efficiencies for organic constituents is much more difficult than for inorganic compounds since the physico-chemical properties and interactions with the membrane properties significantly affect the compound's mass transfer (Williams et al., 1999; Van der Bruggen and Vandecasteele, 2002).

2.6 Kuwaiti pharmaceutical consumption and disposal

Most of the studies recorded on the detection of, and quantifying the fate of, pharmaceutical waste in the environment are done in cold or wet countries, but with very little undertaken in hot dry countries such as Kuwait.

Different factors may affect the pharmaceutical compounds; these factors could be physical or chemical, such as radiation, humidity and temperature. Photo-chemical (photolysis) reactions may transform the pharmaceuticals to a toxic form or degrade them into less harmful compounds, which this study aims to investigate.

The disposal method and quantity of unused or expired pharmaceuticals in Kuwait and other Arab countries are currently not known. They dispose of the pharmaceuticals together with other municipal waste in the same landfill. Inappropriate disposal methods may cause health problems (e.g. mixing dangerous pharmaceuticals such as those used in treating cancer with other volatile compounds).

The Kuwaiti government spent approximately 298 million US dollars on pharmacies in 2008. Government health centres provide for the majority of the population in Kuwait while private pharmacies provide for a smaller proportion of people. Because of the free medication provided by the government, the high proportion of public health system derived pharmaceuticals could be a reflection that this is where most patients obtain their pharmaceuticals.

A study on Arabian Gulf countries' households in 2001 established that 25% of pharmaceuticals are held in the home until they expire (Abu Auda, 2003). The same scenario may occur in Kuwait where the cost of expired pharmaceuticals can be estimated at around 74 million dollars annually. Thus, this increases the caution about the pharmaceutical waste in Kuwait, where pharmaceuticals expired with an average value of 25 dollar per person for 3 million populations. In the United Kingdom, a study in 1996 estimated that £37 million of unused pharmaceuticals were held in households (Hawksworth et al., 1996), where in the United States the value of unused pharmaceuticals was over $1 billion per year for mature patients alone (Morgan, 2001) and in Texas it is estimated that pharmaceutical waste is valued at $106 million per year (Garey et al., 2004).

A survey was conducted to determine the pharmaceuticals most commonly used annually in Kuwait collected from the Ministry of Health in November 2008; the study of the monthly consumption of commonly used pharmaceutical drugs during the

year 2008 in Kuwait and data was also collected from four different hospitals in Kuwait in April 2009. These results can help us to understand the usage behaviour of these drugs during the year and excitants in the environment.

In this study, pharmaceutical drugs were selected on the basis of the availability of eco-toxicity data of the most commonly used of these drugs in Kuwait (Table 2.2), such as Metformin, Paracetamol, Amoxicillin, Ranitidine HCL, Metronidazole and CO-Trimoxazole. Paracetamol is a common analgesic used by humans. Secondly, antibiotic drugs that are used in large quantities, such as Amoxicillin. It is used to treat many different types of infections caused by bacteria. Other antibiotic drugs used extensively for treating infections caused by anaerobic bacteria and protozoans such as Trichomonasvaginalis and Giardia lamblia are Metronidazole, where Co-trimoxazole is a Sulphonamide antibacterial combination of Trimethoprim and Sulphamethoxazole used in the treatment of a variety of bacterial infections. Most of these drugs are non-biodegradable or not easily degradable (Richardson and Bowron, 1985; Perez et al., 2005) nor soluble in water, so they are not typically removed during conventional sewage treatment meaning they are likely to accumulate in the aquatic environment (Kummerer, 2001).

Lastly, Ranitidine hydrochloride is a histamine H_2-receptor antagonist that inhibits stomach acid production. It is commonly used in the treatment of peptic ulcer disease and gastroesophageal reflux disease. Ranitidine is also used in other antihistamines for the treatment of skin conditions such as hives.

These pharmaceutical drugs have a toxic effect on humans and ecosystems; for example, Metronidazole along with other antibacterial and anticoccidial drugs with a Nitroimidazole ring structure are suspected of being carcinogens and mutagents (Revankar and Vedavathi, 2014). These drugs may be found in the wastewater due their excretion. Studies must be done to detect these drugs and enhance the degradation process in the wastewater biological treatment or physical chemical treatment.

A survey done to estimate the monthly use of the most common pharmaceuticals in Kuwait (Figure 2.5) revealed different consumption rates of pharmaceuticals during the year. Antibiotics are mostly used in the seasonal sick period, where Metronidazole is largely used in summer due to food poisoning. Paracetamol consumption substantially increases then stabilizes from May to the end of the year.

Table 2.2: Total of most pharmaceutical drug used in Kuwait*

Pharmaceutical	**Product group**	**Weight g**
Metformin	Diabetic 2	40748210
Paracetamol	Analgesic	38610781
Amoxicillin	Antibiotic	18209596
Ibuprofen	Anti-inflammatory	7149200
Cephalexin	Antibiotic	3622675
Ranitidine HCL	Antagonist	3213629.5
Acetyl salicylic acid (Aspirin)	Analgesic	2999300
Mefenamic acid	Anti-inflammatory	2456700
Metronidazole	Antibiotic	2218960
CO-Trimoxazole (Trimethoprim + Sulphamethoxazole)	Antibiotic	2190336
Bezafibrate	Hormones	1143090

* Taken from Ministry of Health (2009), Health & Vital Statistics Division, Department of Statistics & Medical Records.

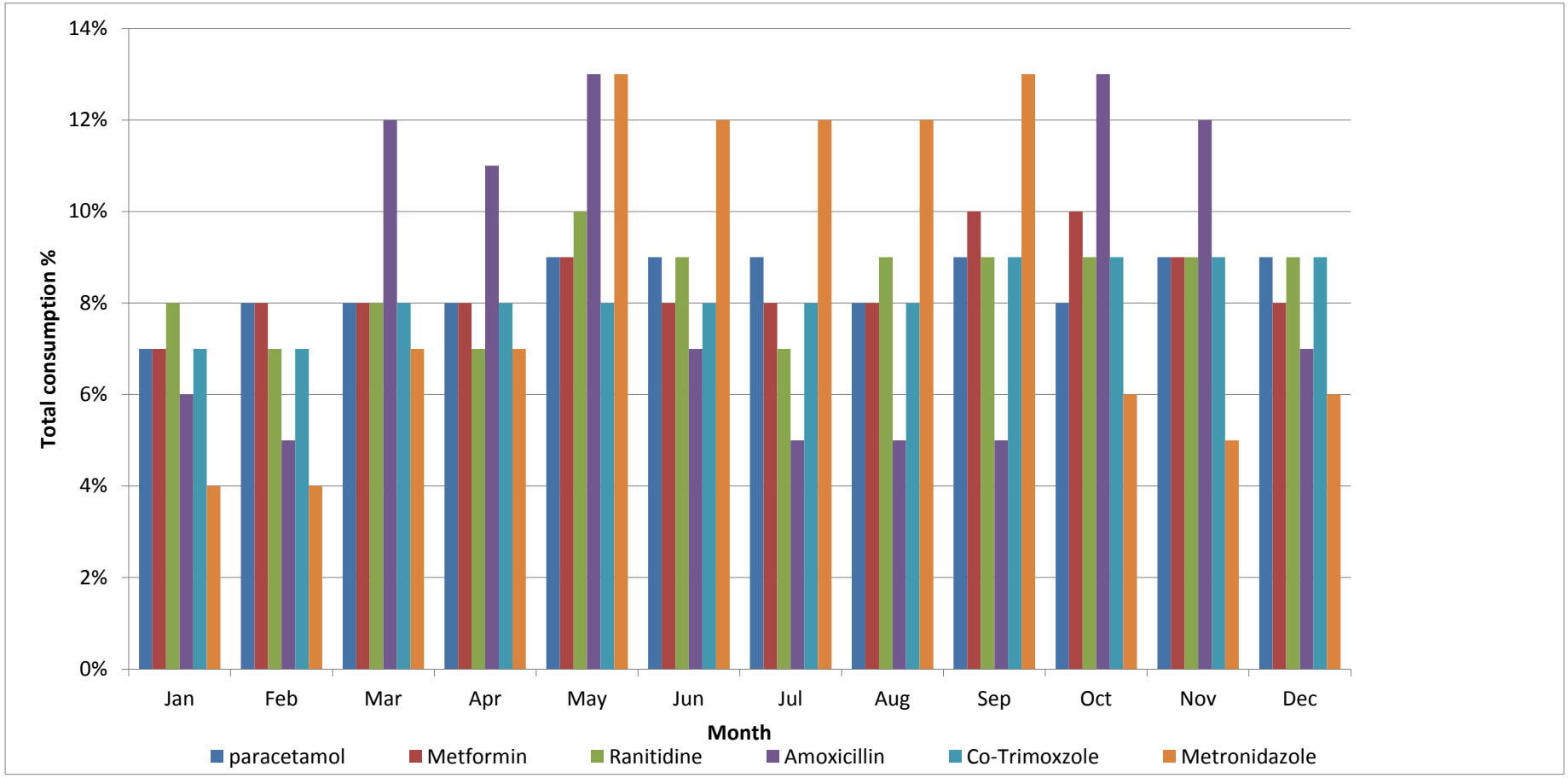


Figure 2.5: The consumption of commonly used pharmaceutical drugs during the year 2008 in Kuwait (Taken from Ministry of Health (2009), Health & Vital Statistics Division, Department of Statistics & Medical Records).

2.7 Sulaibiya wastewater treatment plant

2.7.1 Plant Description

The Sulaibiya wastewater treatment plant was opened in 2005 as the world's largest membrane-based water reclamation facility utilizing ultra-filtration (UF) and reverse osmosis (RO) systems. It serves a domestic population of approximately 1370650 with a capacity of 375,000 $m^3.d^{-1}$ (Alhumoud et al., 2010). As well as domestic wastewater, the plant receives effluent from 18 different hospitals with a patient capacity of 4744 beds.

Figure 2.6 shows that the preliminary treatment at Ardiya consists of particulate and grit removal, as well as fat, oil and grease removal. The wastewater is then pumped to Sulaibiya. Biological removal of nitrogen and phosphorus are the first stage in the Sulaibiya WWTP, where anoxic and aerobic systems are used in addition to secondary clarifiers with a nominal HRT of 14hours. The water reclamation facility (Figure 2.6) receives secondary-treated municipal wastewater, which is pre-filtered with micro-filters and then fed into the ultrafiltration (UF) system. UF permeate feeds a RO plant, and UF retentate is recycled to the WWTP. The UF system receives almost 100% of the effluent from the biological treatment plant since UF retentate is recycled. The RO plant is constructed for 85% water recovery and, therefore, the production rate is expected to be 318,750 $m^{3.}d^{-1}$.

Table 2.3 Statistics of hospitals in Kuwait describing the number of beds and patients*.

Hospitals	Beds	Inpatient	Outpatient	Emergency	Operations
Sabah	438	18631	112481	341865	3378
Amiri	394	11010	131287	153579	2009
Mubarak	437	17515	163240	283471	4487
Farwania	633	26263	318401	650701	6310
Al Razi	267	6107	162615	116434	6985
Phys. Med Rehab	78	339	50115	0	0
Maternity	375	20914	27034	17532	5678
Chest Diseases	131	6744	89930	0	1073
Infectious Diseases	151	3340	14285	17410	0
Psychological Med.	749	3058	55962	6610	0
Ibnsina	363	10918	244055	150824	13769
Kuwait Cancer Control Centre	112	2124	41460	0	1118
Kuwait Allergy Centre	128	1031	89212	0	0
Al Mowasat	88	6852	188452	0	4657
Hadi	101	11979	377996	0	7364
Dar–Al Shefa	88	12230	233595	0	5698
Al Rashid	84	3306	87000	0	2541
Al Salam	127	9355	186890	0	6723
Total	4744	171716	2574010	1738426	71790

*** Taken from Ministry of Health (2009), Health & Vital Statistics Division, Department of Statistics & Medical Records.**

2.7.2 Ultrafiltration System (UF)

Since RO systems require pre-treatment to protect the RO membranes from fouling, UF was selected to provide appropriate pre-treatment of the secondary-treated municipal effluent before being fed to the RO. The UF technology is robust, has favourable life cycle costs, and provides better quality water to the RO membranes (Alhumoud et al., 2010). The characteristics of the UF system used in this plant are presented in Table 2.4. In terms of its operation, each UF

unit can be operated individually. These units are regularly backwashed to ensure removal of suspended matter being retained and held by the membranes. The backwash water is pumped back upstream of the WWTP to receive appropriate treatment and achieve the maximum total water recovery for the plant. The influent to the UF first passes through a micro-filter and subsequently, a small amount of coagulant (ferric chloride at 1 – 2 $mg.L^{-1}$) is added to coagulate fine particulates and possibly allow some TOC removal to facilitate the operation of the plant. The silt density index (SDI) of the UF product is consistently below 2, which is the key standard for RO plant performance (Gagne, 2002).

2.7.3 Reverse Osmosis System (RO)

The characteristics of the RO system adopted in the Sulaibiya plant are shown in Table 2.4. This system is used to desalinate the wastewater effluent to 100 $mg.L^{-1}$ TDS and to provide an additional barrier to bacteria and viruses. The average salinity of the secondary-treated effluent is 1,280 $mg.L^{-1}$ TDS, with a maximum value of 3,014 $mg.L^{-1}$. The RO system modules are arranged in a train of 4:2:1 array, forming three stages of RO treatment. The first stage recovers 50%, the second stage recovers 50% and the third stage recovers 40% of the flow. The RO system is limited to recover 85% by the calcium phosphate precipitation, which can often be a limiting factor for the recovery of water in membrane desalination systems in municipal wastewater (Gagne, 2002). Reverse osmosis effluent passes through the stripper unit to remove the CO_2 and adjust the pH with a minimum addition of caustic soda prior to distribution, and then the product is chlorinated before leaving the plant. The system's brine is disposed of in the Arabian Gulf. In order to minimize fouling of the membranes they are regularly cleaned with CIP comprising surfactants, sulfuric acid, biocide and sodium hydroxide.

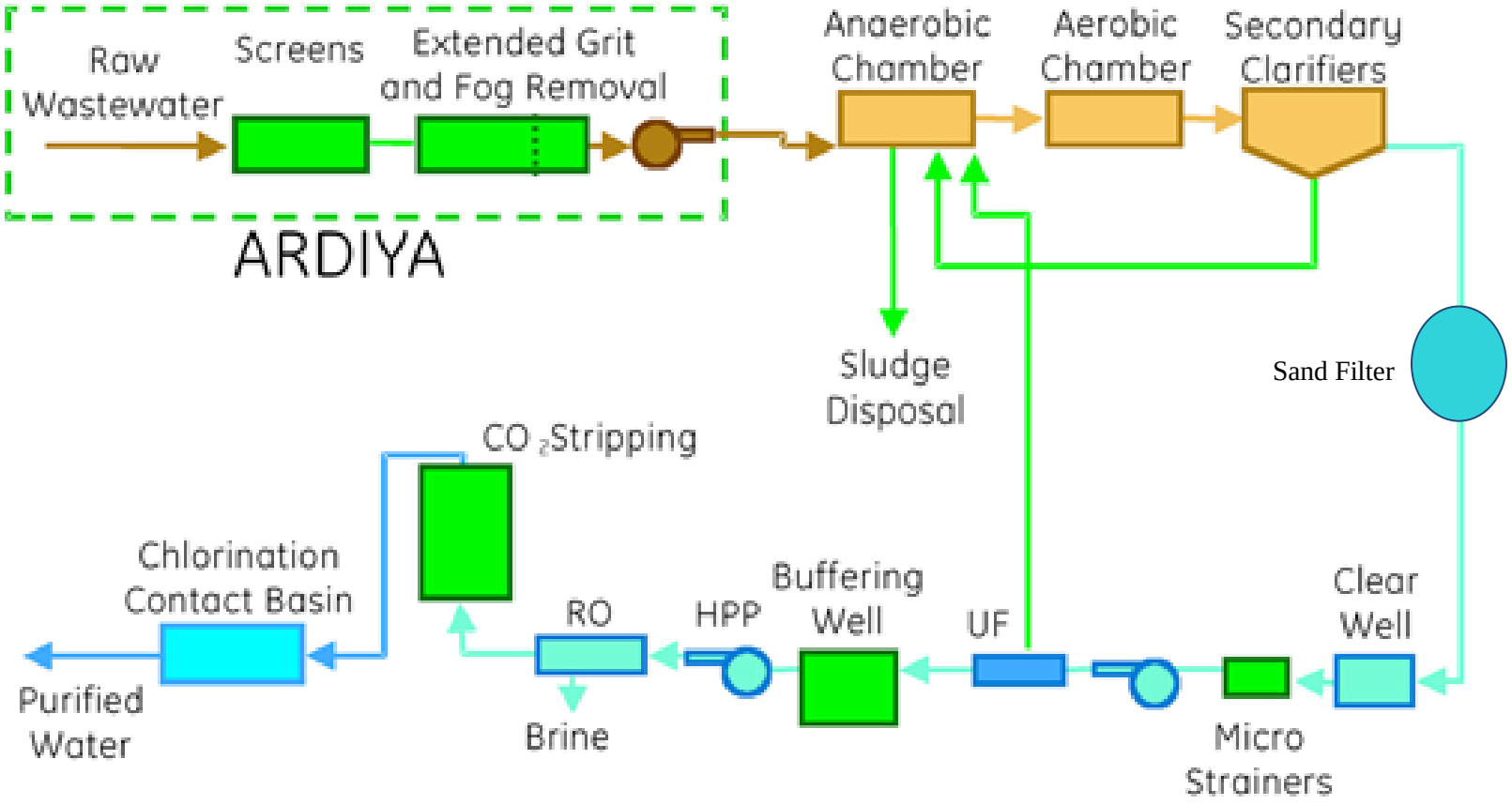


Figure 2.6: Flow diagram of treatment processes at Sulaibiya WWTP (Alhumoud et al., 2010)

Table 2.4. Characteristics of membrane systems employed at Sulaibiya water reclamation plant

Membrane System	Membrane Type	Membrane Configuration	Membrane Arrangement	Membrane Area
Ultrafiltration (UF)	Norit's X Flow Cross Flow, The Netherlands. (Model XIGA SXL-225). Polyvinyldene Fluoride	Capillary hydrophilic hollow fibers	Membranes are packed in 20 x 152 cm membrane elements (35 m^2 /element), 4 membrane elements are placed inside a membrane housing. There are 68 skids, each with 32 membrane housings for a total of 8,704 membrane elements (4 x 32 x 68).	8,704 x 35 m^2 = 304,640 m^2
Reverse Osmosis (RO)	Toray of America. (Model TML 20-400). Polyamide composite.	Spiral wound	Membrane modules of 42 identical skids in a 4:2:1 array (train) of modules. Each module contains about 504 RO elements (72 pressure vessels x 7 RO element/ vessel) for a total of 21,168 membrane elements (7 x 72 x 42).	21,168x 37 m^2 = 783,216 m^2

3. Development of Analytical Methods

Wastewater treatment plants (WWTPs) have been identified as a major point source of pharmaceuticals waste in the environment as they receive continuous inputs of these compounds either as the parent compound or as an array of metabolites (Daughton & Ternes, 1999). Pharmaceuticals are persistent contaminants that can be harmful to the environment, even at trace levels. Researchers are always attempting to develop more sophisticated analytical tools and sensitive techniques for their detection in water samples. The diversity of pharmaceuticals and the complexity of environmental matrices, as well as the ultra-trace detection limits required, make chemical analysis a difficult task for researchers.

For the purpose of this study, it was necessary to develop analytical methods which were accurate, precise and practical. Furthermore, such methods had to be quick, relatively inexpensive, require simple tools, and use the minimum of hazardous reagents.

3.1 Selection and development of pharmaceutical measurement method

There were two options for analytical methods to be used in this study. The first option was to use methods described in published papers, while the second option was to develop new analytical methods. Criteria for the selection of new analytical methods were as follows:

1. Simplicity: An analytical method required in this research should not be too complicated.
2. Availability of the instruments: So as to facilitate the sample analysis, the instruments required for sample preparation and analysis need to be available near the sample collections points. This criterion was set not only for making the analytical step as convenient as possible but also for minimising the cost and reducing sample change during transportation and storage.

3. Methodical detection limits: pharmaceuticals in the environment are commonly found at very low concentrations ($ng.L^{-1}$). Thus, the detection limits of a selected analytical method should meet the requirements.

4. Selectivity and sensitivity: these are the most important factors in the selection of analytical methods for the achievement of the desired detection limits.

3.2 Validation of Analytical Methods and Procedures

Method validation is the process used to confirm that the analytical procedure employed for a specific test is suitable for its intended use. Results from method validation can be used to judge the quality, reliability and consistency of the analytical method; it is an integral part of any good analytical practice.

The development of sensitive analytical techniques provides detailed information on the structure of metabolites and transformation products (TPs) of pharmaceuticals in extracts of environmental samples such as gas chromatography with mass spectrometry (GC-MS) or with tandem MS (GC-MS2) and Liquid chromatography–mass spectrometry (LC-MS) or (LC-MS2). They are many of the analytical techniques applied to the quantification of pharmaceuticals in samples of wastewater, which is a complex matrix containing many different organic molecules.

3.3 Application of GC-MS and LC-MS

Advanced analytical techniques (GC-MS, GC-MS2, LC-MS and LC-MS2) are common in pharmaceutical analysis, because they can provide compound confirmation and detailed information on the structure of the compounds analysed, and give separation and detection of compounds having the same molecular mass but different product ions.

As a result, there is growing interest in the application of these techniques, GC-MS2, LC-MS and LC-MS2, for the analysis of pharmaceuticals as they can provide low limits of detection (LODs) in wastewater and can be useful to obtain further information about new compounds, which can be metabolites or TPs of pharmaceuticals, which have not yet been identified.

After efforts to improve these technologies, progress has been made in recent years in equipment (Horimoto et al., 2002; Diaz-Cruz et al., 2005) as well as sample preparation, derivatization and clean-up procedures (Stumpf et al., 1999; Moder et al., 2000; Sacher et al., 2001; Jux et al., 2002; Andreozzi et al., 2003; Ollers et al., 2001; Renew et al., 2004; Balakrishnan et al., 2006). In order to obtain lower LODs the selective ion monitoring (SIM) mode of MS detection has been used for pharmaceuticals. On the other hand, the scan mode is also required for searching for the presence of unknown metabolites in samples for pharmaceutical analysis (Gomez et al., 2007; Zuccato et al., 2008; Pavlovic et al., 2007; Hao et al., 2007).

A significant disadvantage of GC-MS and GC-MS2 analysis is the requirement for derivatization of polar pharmaceuticals, which can affect the accuracy of the method, as losses of analytes can occur or the derivatization reaction can be incomplete. Thus, to avoid derivatization, LC-MS or LC-MS2 analysis is being used widely with good results (Castiglioni et al., 2005; Balakrishnan et al., 2006; Hao et al., 2007). However, analytical problems have also frequently occurred, especially during the analysis of wastewater, so that sensitivity has decreased (Fatta et al., 2007). In order to solve these analytical problems in both GC and LC analytical procedures, a clean-up step is added prior to analysis of the final extract.

The analytical methods considered in this study were selected to determine five pharmaceuticals in wastewater, namely Metronidazole, Trimethoprim, Sulphamethoxazole, Paracetamol and Ranitidine.

3.4 Development of liquid chromatography–mass spectrometry analysis

3.4.1 Chemicals and reagents

Methanol, acetonitrile, and water were HPLC grade obtained from Sigma–Aldrich (Steinham, Germany). Dichlorodimethylsilane, Sodium thiosulphate, and Toluene were also purchased from Sigma–Aldrich. Pharmaceutical standards were of a high purity ≥95%. Trimethoprim, Sulphamethoxazole, Metronidazole, Paracetamol, Ranitidine, and internal standard Metronidazole-$^{13}C_2$,$^{15}N_2$ were purchased from Sigma–Aldrich.

Stock solutions 100 mg.L^{-1} of each analyte were prepared in HPLC grade methanol and stored at 4 ◦C in the dark for increased stability. Supelco C18 (500 mg/3 mL) used for solid phase extraction was purchased from Phenomenex (United Kingdom). Sterile membrane filters 0.45 µm were obtained from Gelman Sciences, while glass microfibre filters (934-AH) were purchased from Whatman (United Kingdom).

3.4.2 Solid phase extraction

Supelco C18 (500 mg/3mL) was investigated for sample pretreatment and analyte preconcentration. Samples were first filtered through sterile membrane filters 0.45 µm to remove any microorganisms and solid particulates, and were adjusted to pH 4 using sulphuric acid. Samples were then divided into aliquots of 500 mL for solid phase extraction (SPE). A Phenomenex extraction manifold was used for SPE. Cartridges were first conditioned with 6 mL of methanol followed by 6 mL of water (HPLC grade). Following this, samples were percolated over the cartridge under vacuum at a pressure of −10 kPa. The sorbent was washed with 5 mL of water after sample addition and dried under vacuum for 30 min. The sorbent was then eluted with 10 mL of methanol, dried under a stream of nitrogen and reconstituted in 0.5 mL of methanol. This provided a measuring concentration factor of 10^3, from raw sample to methanol extract.

3.4.3 Instruments

The LC-MS analytical method was used to monitor selected mass ions of each pharmaceutical compound with high sensitivity. The significance of these methods is to evaluate good selectivity and sensitivity so as to permit fast analytical separation and achieve sensitive sample detection. Samples were prepared from different pharmaceuticals (Metronidazole, Trimothoprim, Sulphamethoxazole, Paracetamol, and Ranitidine) dissolved in methanol.

The LC analyses were performed using a Thermo LCQ fleet with Surveyor HPLC, APCI mode equipped with a SGE Wakosil C-18RS column (250mm × 4.6 mm) 5µm particle size. The HPLC pump (P4000, Spectra system, Thermo Finnigan, San Jose, CA, USA) was used to generate a gradient from two mobile phase solvents at a flow rate of 0.6 ml/min and temperature of 25°C. As a number of compounds were to be analysed by different separation methods, different elution gradient mixtures of the mobile phases, such as methanol and water with 0.1% formic acid, were examined in varying ratios as shown in Table 3.1.

The ion trap mass spectrometer (LCQ Fleet, Thermo Finnigan) was equipped with an atmospheric pressure chemical ionization (APCI) ion source, and the heated capillary temperature was set at 275°C. The MS parameters were optimised semi-automatically using LCQ internal software. The sheath and auxiliary nitrogen gas flows were 40 and 10 (arbitrary units), respectively, and the source voltage was maintained at a constant 3.10 kV for all analytes. The capillary voltage (36V) and the collision energy (for fragmentation in MS/MS) were individually optimised for each substance.

Metronidazole-$^{13}C_2$,$^{15}N_2$ internal standard (I.S.) was determined together with the different analytes. The time required to analyse one sample using this approach varied depending on the retention time. All the separated

pharmaceuticals compounds were detected using APCI -MS/MS in positive ion mode. Quantification was achieved using the internal standard calibration method. A five-point calibration curve was prepared for each substance, using values from approximately 1µg.$L^{-1}$to 100 µg.L^{-1}, based on analyte/I.S. peak area ratios. Levels of the internal standards used in native wastewater samples were monitored in order to avoid underestimation of analyte concentrations.

All the literature reported m/z-values of the selected pharmaceutical's parent and daughter ions were used for MS detection. For instance, the characteristic parent ions were Metronidazole (m/z 172), Trimethoprim (m/z 291), Sulphamethoxazole (m/z 254), Paracetamol (m/z 152), and Ranitidine (m/z 315), and daughter ions were Metronidazole (m/z 82.5, 111), Trimethoprim (m/z 261), Sulphamethoxazole (m/z 156), Paracetamol (m/z 107), and Ranitidine (m/z 176) (Hartig et al., 1999; Lindsey et al., 2001; Benotti, 2002; Cronly et al., 2009; Zeleny et al., 2009; Langford & Thomas 2009). Additional daughter ions of Metronidazole, Trimethoprim, Sulphamethoxazole, Paracetamol, and Ranitidine (m/z 128, 230, 188.0, 110, and 176), respectively, were also monitored (Benotti, 2002; Andreozzi et al., 2003; Lindberg et al., 2004) using the same instrument (Table 3.2.)

Table 3.1: Elution gradient used in the method of separation of different pharmaceutical in LC-MS

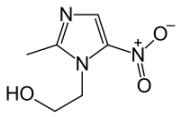
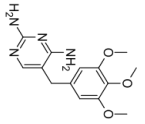
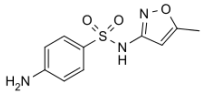
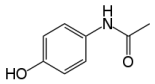
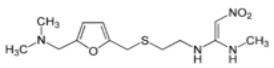
Compound	Gradient Programme				
Metronidazole 	Time (min)	Flow (ml/min)	Methanol (%)	H_2O (%)	
	0	0.4	30	70	
	14	0.4	90	10	
	16	0.4	30	70	
	20	0.4	30	70	
Trimethoprim 	Time (min)	Flow (ml/min)	Methanol (%)	H_2O (%)	
	0	1	60	40	
	6	1	60	40	
Sulphamethoxazole 	Time (min)	Flow (ml/min)	Methanol (%)	H_2O (%)	
	0	0.4	30	70	
	15	0.4	95	5	
	18	0.4	30	70	
	20	0.4	30	70	
Paracetamol 	Time (min)	Flow (ml/min)	Methanol (%)	H_2O (%)	
	0	0.4	40	60	
	13	0.4	90	10	
	15	0.4	40	60	
	20	0.4	40	60	
Ranitidine 	Time (min)	Flow (ml/min)	Methanol (%)	H_2O (%)	
	0	1.2	90	10	
	6	1.2	90	10	

Table 3.2: Monitored ions of pharmaceuticals and internal standards

Compound	*m/z* parent ion	Collision energy (%)	*m/z* daughter ion
Metronidazole	172	23	128
Trimethoprim	291	34	230.1
Sulphamethoxazole	254	27	188
Paracetamol	152	24	110
Ranitidine	315	28	270
Metronidazole-$^{13}C_2$,$^{15}N_2$ (I.S.)	176	23	132

3.4.4 Recovery

The extraction recovery process in each different sample was evaluated using internal standards. This was determined by spiking mixed pharmaceuticals in different samples (distilled water, tap water, and synthetic wastewater) at concentrations 1 µg.L^{-1}. Sodium thiosulphate was used at concentration 100 mg.L^{-1}, to remove the chlorine present in tap water. Calculation of the recovery of the pharmaceuticals was based on their standard concentrations.

3.4.5 Effect of chlorine on extraction

Chlorine is commonly used in the disinfection of drinking water and in the WWTP final step. The presence of chlorine may have a strong effect on the adsorption of organic compounds to adsorbents (Suffet & Wable, 1995; Gilloly et al., 1998). Therefore, the presence of residual chlorine may affect the adsorbent coating fiber of SPE. Furthermore, because chlorine is a strong oxidant, a reaction between chlorine and the analytes may also occur during the extraction process changing their chromatographic properties.

In this study, the effect of chlorine on the recovery using SPE analysis of the pharmaceuticals was investigated. Finally, to reduce the deleterious impact of chlorine during the SPE analysis, chlorine was removed to evaluate its effect on the extraction process. This experiment used different samples (distilled water, tap water, 1 $mg.L^{-1}$ of chlorine in wastewater, and wastewater). Sodium thiosulphate was used to remove the chlorine. Three different tests were conducted:

A- Sample with only pharmaceuticals at concentration 1 $\mu g.L^{-1}$.

B- Add pharmaceuticals at concentration 1 $\mu g.L^{-1}$ then after 5 min add Sodium thiosulphate at concentration 100 $mg.L^{-1}$.

C- Add Sodium thiosulphate at concentration 100 $mg.L^{-1}$ then after 5 min add pharmaceuticals at concentration 1 $\mu g.L^{-1}$.

3.5 Results and discussion

3.5.1 Standard stability

The stability of analytes standards during their storage is crucial when determining trace concentrations in wastewater samples, or any other matrix. Therefore, individual stock standard solutions of each analyte were stored in the

dark at 4°C, and tested over a period of three months. No significant degradation of any of the pharmaceuticals was observed during this period. However, in order to increase the rigour of the laboratory work, mixed standards of the analytes, in suitable solvents, were often made. Figure 3.1 shows the separation and detection peak of pharmaceuticals in the extracted sample from spiked wastewater.

3.5.2 Calibration and limits of quantification

Table 3.3 and Figure 3.1 presents data showing individual substance retention times, linearity of the calibration curves, limits of quantification, and recovery yields following extraction.

Calibration curves were prepared for each compound by plotting the average total ion peak area versus the analyte concentration. A sample with no analyte peaks was used as a blank for the calibration curves. Linearity was tested in the range 1–100 $\mu g.L^{-1}$ depending on the type of pharmaceutical, and all showed R^2 values >0.99, indicating a good linearity with high correlation. Variance of the method was investigated by determining the short-term and long-term relative standard deviations (RSDs) under identical conditions. The RSDs were obtained by analysing three replicates of samples at 10 μgL^{-1}. Intra- and inter-day variance were found to be lower than 10% for all compounds. Limits of detection (LODs) calculated as the signal-to-noise ratio (*S*/*N*) of 3:1, ranged from 1 to 5 $ng.L^{-1}$, depending on the compound spiked in the effluent wastewater (Table 3.3). The relative standard deviation (RSD) of the slope of the calibration curve was less than 10% for each of the individual substances, based on fresh calibration solutions that were made and injected on three different days. The limits of quantification (LOQ) were in the range of 7–10 $ng.L^{-1}$ depending on the analyte (pharmaceutical) injected. These values were determined by spiking pharmaceuticals in wastewater and extraction using SPE, then the extract was injected in LC-MS until the signal-to-noise ratio for any signal analyte reached a value of 10:1.

The SPE procedure was evaluated using standards prepared in distilled water, influent wastewater, synthetic wastewater, and tap water, with the studied compounds. Recoveries as evaluated in spiked distilled water, as well as in spiked real samples, are shown in Figure 3.2. The recoveries of the analytes from the samples spiked with pharmaceutical at a concentration of 100 $ng.L^{-1}$wasthe highest for distilled water and synthetic wastewater ranged from 87 to 99 %; with real wastewater samples giving moderate recoveries ranging from 77 to 94%. However, the lowest recoveries were for tap water (34 to 64%), due to the chlorine effect, except Ranitidine which showed the highest recovery level at 91%. This observation gives a high uncertainty in the quantification of pharmaceuticals at very low concentrations, but allows for their reliable detection, presence or absence, even at low concentrations.

Table 3.3: Limits of detection, limits of quantification and linearity of calibration curve of pharmaceutical compounds.

Compound	**R^2**	**Average R.S.D (%)**	**limit of Detection (LOD) $ng.L^{-1}$**	**limits of quantification (LOQ) $ng.L^{-1}$**
Metronidazole	0.9989	4.778	5	8
Trimothoprim	0.9993	2.24	5	7
Sulphamethoxazole	0.9989	6.838	1	10
Paracetamole	0.9993	4.63	5	10
Ranitidine	0.999	5.75	5	10

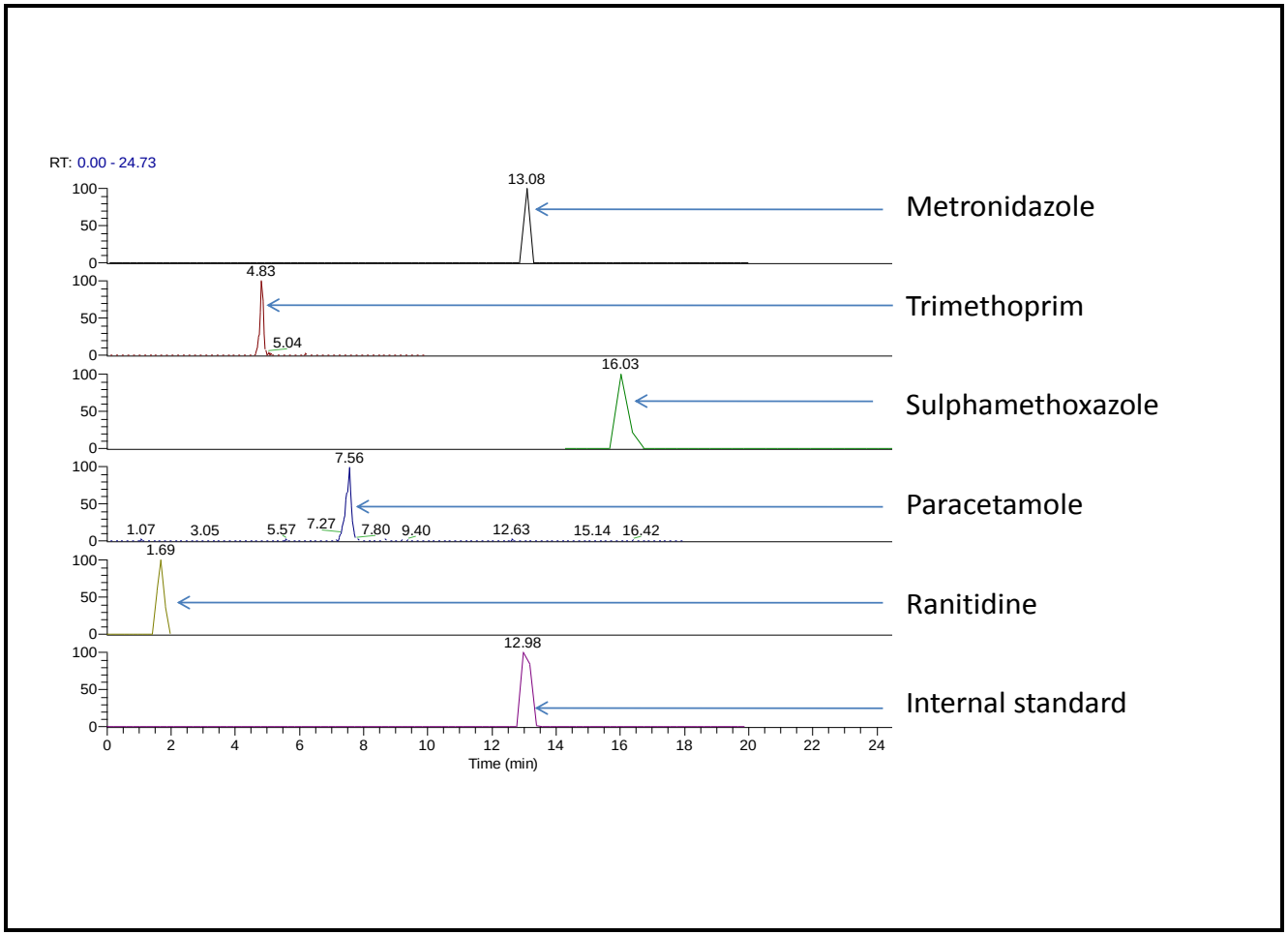


Figure 3.1: Chromatograms of standards: Metronidazole, Trimethoprim, Sulphamethoxazole, Paracetamole, Ranitidine, and IS on different LC-MS runs each compound at specific ion monitoring (SIM).

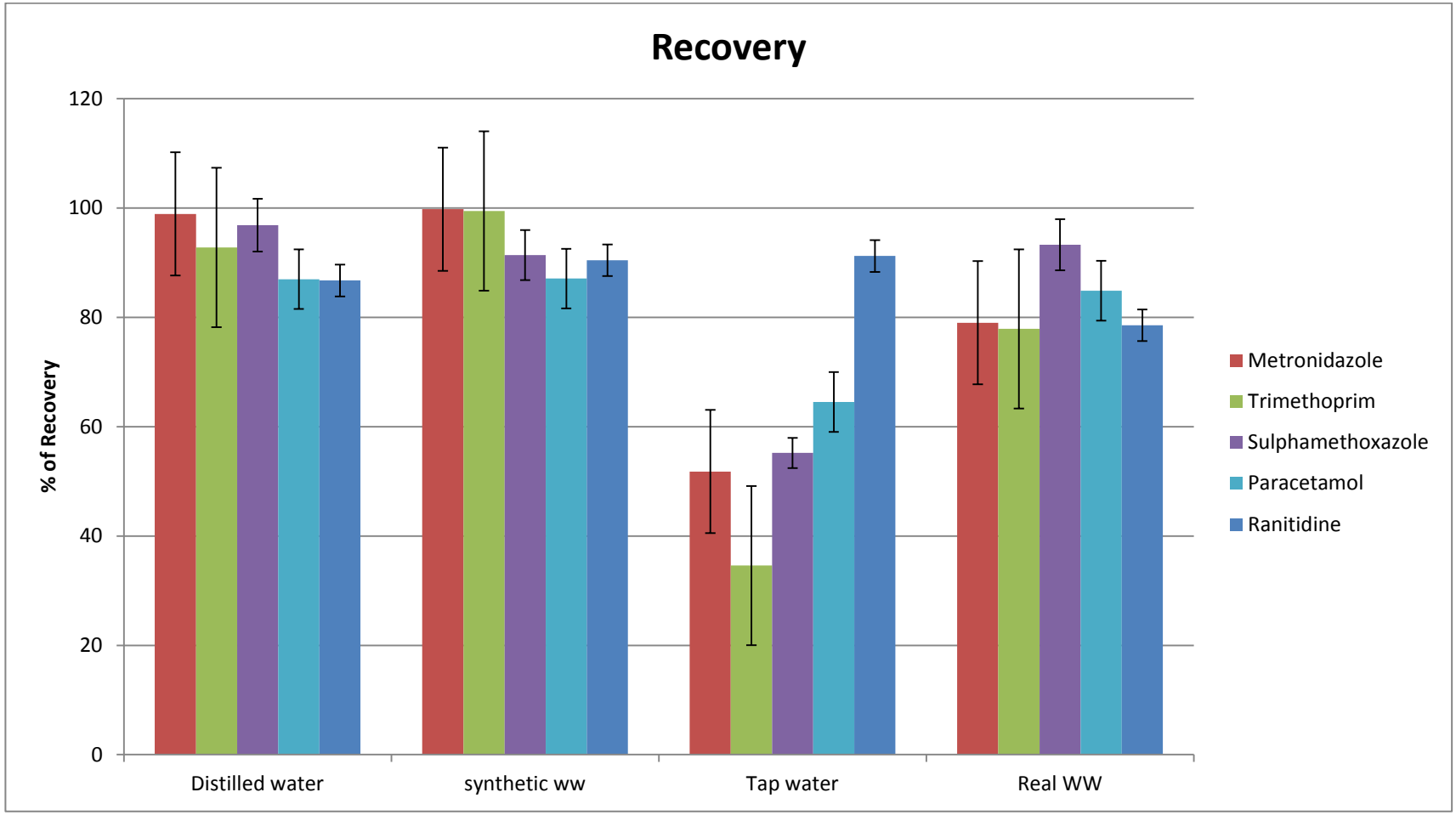


Figure 3.2: Recoveries of pharmaceutical compounds in spiked distilled water, tap water, synthetic wastewater, and real wastewater, with the standard solution (ST).

3.5.3 Effect of chlorine on pharmaceutical extraction efficiency

Figure 3.3 shows the analytical extraction results for Metronidazole, Trimothoprim, Sulphamethoxazole, Paracetamol, and Ranitidine using SPE. The relative concentrations (concentration compared to that without residual chlorine) of the laboratory samples at different free chlorine concentrations, are compared. The residual chlorine substantially reduced the observed concentrations of pharmaceuticals.

Depending on the pharmaceuticals and solvent nature, reductions of recovery between 10–80% were found. Reductions were found with tap water, whereas the lowest reductions were found with tab water and chlorinated wastewater. The effect of free chlorine in the chlorinated wastewater showed a reduction in the recovery of pharmaceuticals approaching 0%, as in the Ranitidine, whereas Trimethoprim had the lowest recovery in tap water due to the presence of free chlorine.

In chlorine removal experiments, samples with different pharmaceuticals were reacted with 1.0 $mg.L^{-1}$ of sodium hypochlorite. Sodium thiosulfate was then used to reduce any remaining free chlorine. As shown in Figure 3.3, the analytical results for the dechlorinated samples were very close to those without any free chlorine addition. A significant increase was observed in the recovery of pharmaceuticals from tap water and chlorinated wastewater when sodium thiosulphate was added prior to addition of the pharmaceuticals. These results confirm that the addition of sodium thiosulfate effectively eliminates the negative effect of chlorine on the extraction using SPE.

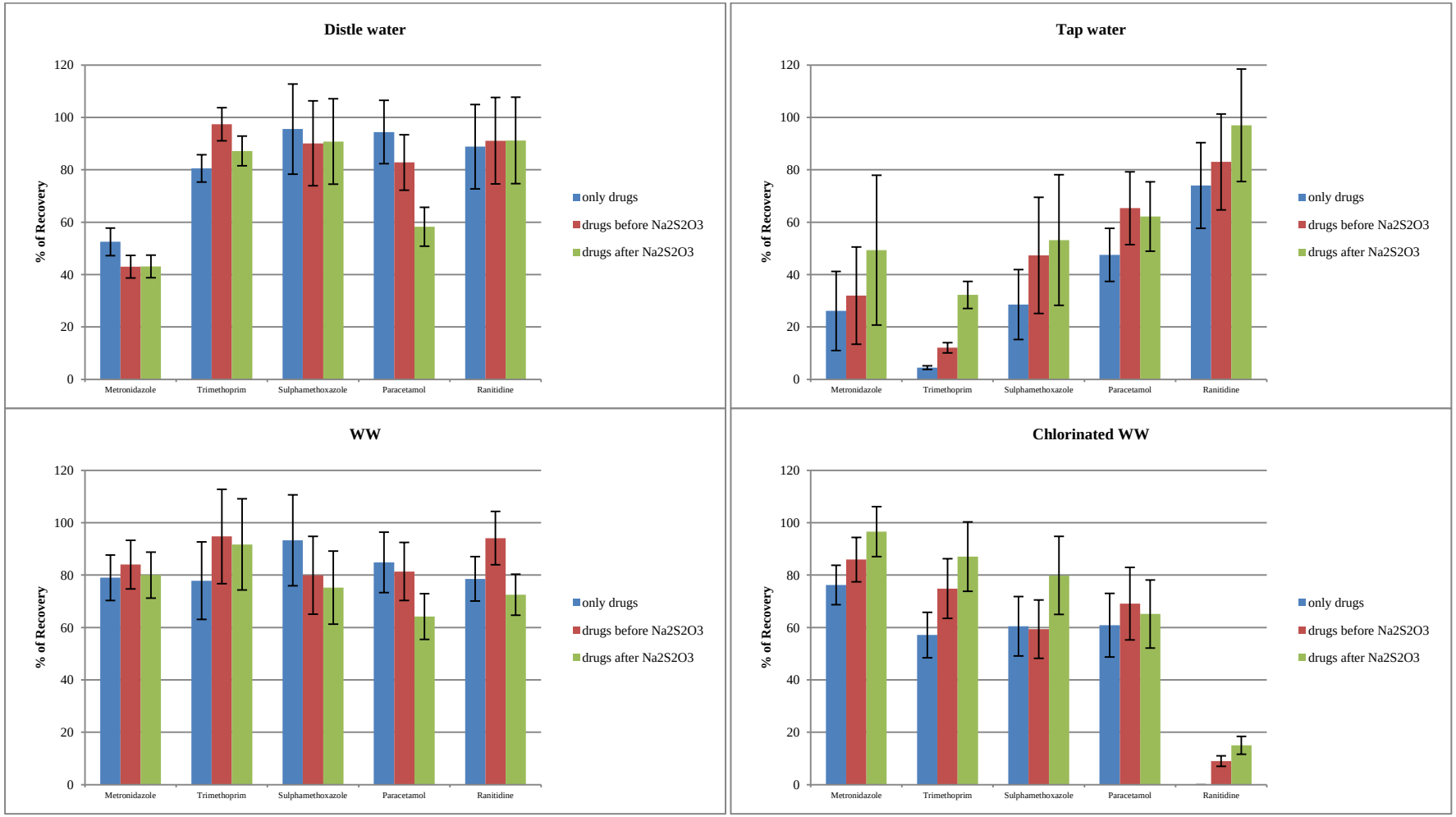


Figure 3.3: Effect of chlorine on the recovery of pharmaceutical compounds spiked in distilled water, tap water, wastewater, and chlorinated wastewater revealed by sodium thiosulphate treatment.

3.5.4 Matrix interferences

There are many studies reported in the literature on interferences between the co-elution of matrix components and the target analyte in LC-MS/MS, commonly referred to as ion suppression effects (Jemal et al., 2003; Schuhmacher et al., 2003).

These effects may influence both the precision and accuracy of a method. Here the precision was compared between analyte and internal standard ratios in both pure standard solutions and spiked extracts of distilled water, tap water, synthetic wastewater, and real wastewater. The recovery of repeated injections (n = 3) of a pure standard solution and a spiked extract (Figure 3.2) showed relatively small differences in recoveries were obtained between pure a standard solution and a spiked extract, irrespective of the matrix. The only exception was that widely lowering recoveries were observed for different pharmaceutical in tap water.

4. MATERIALS AND METHODS

4.1 Fate of pharmaceuticals in Sulaibiya wastewater treatment plant

4.1.1 Methodology

This study uses 12 months of complete monthly measurements of quantitative data from Sulaibiya WWTP for the period 1 September 2010 to 31 August 2011. A flow diagram with sampling points for analytical measurements is shown in Figure 4.1. Wastewater samples for analysis of pharmaceuticals were usually collected monthly in amber glass containers, which had been pre-cleaned with reagent water and organic solvents such as methanol and acetone. Both discrete and composite samples were sampled, but it is more appropriate to collect composite samples to evaluate the performance of a WWTP. UF backwash and RO brine were also sampled for investigation. To prevent any bacterial activity wastewater samples were filtered using 0.45 µm membrane filters (Gelman Sciences), sulphuric acid added to reduce the pH to 3, and stored up to 7 days at 4ºC until extraction.

4.1.2 Calculation of Concentration Factor

The concentration factor of a membrane filter is a ratio of the average concentration of a compound in the backwash and brine compared to the UF and RO feed respectively, which depends on the recovery of water and retention of individual compounds. It can be represented as shown in Equation 4.1.

$$F = \frac{Cc}{CF} \qquad (4.1)$$

where CF and Cc are the solute concentrations in the feed and in the backwash or brine, respectively.

The process variables analysed from the database of Sulaibiya WWTP were flow rate, temperature, total organic carbon (TOC) and physicochemical parameters (e.g., pH, electrical conductivity (EC), suspended solids (SS), total dissolved solids (TDS), chemical oxygen demand (COD), biological oxygen demand (BOD) and coliform bacteria (measured as total coliforms).

4.1.3 Trace of pharmaceuticals Analysis

The samples were filtered through sterile membrane filters 0.45 µm (Gelman Sciences), adjusted to pH 2-3, and extraction was carried out using SPE. Extracts were analysed by LC-MS-MS for detection of Metronidazole, Trimethoprim, Sulphamethoxazole, Paracetamol and Ranitidine (See section 3.4).

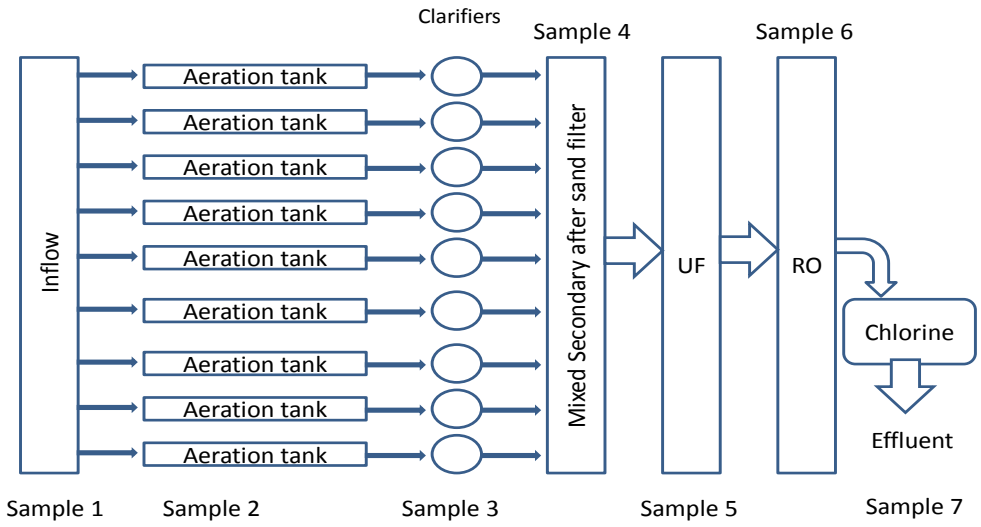


Figure 4.1: Diagram indicating the sampling points at the WWTP.

4.2 The removal of pharmaceuticals by chlorination and ozonation treatment processes

To identify any removal of pharmaceuticals by chlorine, a study was carried out on the chlorination of the pharmaceuticals at laboratory scale. In addition, other treatments also found in water reclamation plants such as ozonation were investigated. Several treatment processes such as chlorine and ozone concentration were tested to compare their removal efficiency of spiked pharmaceuticals at concentration 100 $\mu g.L^{-1}$ in different sample media such as synthetic wastewater, real wastewater and tap water. Chlorination and ozonation test of the five pharmaceuticals were performed in order to assess their degradability. A bench scale experiment was conducted using a 500 ml sample in a stirred beaker spiked with pharmaceuticals at concentration 100 $\mu g.L^{-1}$ treated for 30 minutes with 5 and 10 $mg.L^{-1}$ chlorine from commercial Clorox sodium hypochlorite bleach and 5, 10 and 15 $mg.L^{-1}$ ozone by ozone generator (Reef Scientific Ozoniser, UK).

4.3 Fate of pharmaceuticals in batch experiments

4.3.1 Methodology

Batch tests were carried out using the return activated sludge (RAS) taken directly from the aeration tank of the WWTP. This batch study was divided into two parts. One was a batch test to determine the effect of pharmaceutical concentration on organic removal, and the other was to examine the kinetic rates of organic removal in the activated sludge process. Batch experiments were performed using a continuously stirred tank reactor (CSTR) (Figure 4.2.) Each tank CSTR contained 2L of wastewater, and spiked pharmaceuticals (Metronidazole, Trimethoprim, Sulphamethoxazole, Paracetamol, and Ranitidine) in separate experiments with initial concentrations of 0.1, 1, and 10 $mg.L^{-1}$. The synthetic wastewater was added

as the sole organic carbon source at TOC 400 $mg.L^{-1}$. These batch experiments were conducted with activated sludge inoculums at pH 6.8, 25 °C, and activated sludge concentration of ≈2.56 $g_{MLSS}.L^{-1}$.

Three CSTR investigated different concentrations of pharmaceuticals, while the fourth was used as a control (without pharmaceutical). Air diffusers were installed at the bottom of the reactor giving a flow of 96 $L.h^{-1}$, so that the dissolved oxygen (DO) concentration was maintained above 2 $mg.L^{-1}$ in each reactor. Each batch experiment was run for five days.

Figure 4.2: Photograph of the continuously stirred tank reactors.

4.3.2 Batch analysis test

10 ml samples were collected from each batch in order to evaluate batch performance. Parameters analysed in this study were pH, temperature, DO, MLSS, TOC, and individual pharmaceutical (according to Section 4.5).

4.3.3 Isolation of bacteria from a CSTR

The stock minimal media solutions of 1M, 20X fold phosphate buffer at pH 7.2 (86.6 $g.L^{-1}$ Na_2HPO_4 and53 $g.L^{-1}$ KH_2PO_4), 54 $g.L^{-1}$ NH_4Cl, 204 $g.L^{-1}$ $MgSO_4$, and 44 $g.L^{-1}$ $CaCl_2$ were separately prepared and sterilized by autoclaving. 1000X fold trace elements at pH 6.7 (1.5 $g.L^{-1}$ $FeCl_2.4H_2O$ (dissolve in conc. HCl), 70 $mg.L^{-1}$ $ZnCl_2$, 100 $mg.L^{-1}$ $MnCl_2.4H_2O$, 200$mg.L^{-1}$ $CoCl_2.6H_2O$, 20 $mg.L^{-1}$ $CuCl_2.2H_2O$, 20 $mg.L^{-1}$ $NiCl_2.6H_2O$, 40 $mg.L^{-1}$ $Na_2MoO_4.2H_2O$ and 20 $mg.L^{-1}$ H_3BO_3), and 1000X fold vitamins (100 $mg.L^{-1}$ Cyanocobalamine (B_{12}), 300 $mg.L^{-1}$ Pyridoxamine-2HCl (B_6), 100 $mg.L^{-1}$ Ca-D (+)Pantothenate, 200 $mg.L^{-1}$ Thiamine dichloride (B_1), 200 $mg.L^{-1}$ Nicotinic acid, 160 $mg.L^{-1}$ 4-Aminobenzoic acid and 20 $mg.L^{-1}$ D (+) Biotin), were sterilized by filtration using 0.22 µm (Nalgene) and kept protected from light (Gilbert et al., 1998).

For preparation of 1 L of working minimal media, the following solutions were mixed together: **(**50 ml of potassium phosphate buffer (1M), 10 ml NH_4Cl (1M), 1 ml $MgCl_2$ (1M), 1 ml $CaCl_2$ (0.3 M), 1 ml of trace elements, and 1 ml of vitamins, then completed to 1L by distilled H_2O**)**. The pH of the medium was adjusted to be 7.4.

Activated biomass was collected from a CSTR that contained pharmaceuticals at concentration 1 $mg.L^{-1}$ after each run. One litre of suspended sludge was collected, and allowed to settle. The supernatant was discarded and the biomass was pooled. 1

g of aliquots sample were collected in sterile 50 ml tubes with 10 ml of wastewater, and preserved at 4 ºC. The sample was centrifuged and the biomass suspended in a sterile 10 ml phosphate buffer (8 g NaCl, 0.2 g KCl, 1.44 g Na_2HPO_4 and 0.24 g KH_2PO_4, in 1 L of distilled water, pH 7.4). 1 ml of the suspension was inoculated in a flask containing 100 ml of minimal media, 10 $mg.L^{-1}$ yeast extract and 1 $mg.L^{-1}$ pharmaceutical. The flask was incubated at 37 ºC under agitation (120 rpm) for a period of 3 d. Inoculations on 10 % LB agar plates containing minimal media, 10 $mg.L^{-1}$ yeast extract and 1 mgL^{-1} pharmaceutical. The plates were incubated at 30 ºC for 3 d and the colonies were picked at random, and sub-cultured three times with LB agar plates. Randomly selected isolates colonies were used for DNA isolation using Genomic DNA Purification Kit (Promega), according to the method described in Appendix B.

4.4 Fate of pharmaceuticals in continuous flow bioreactors

This section describes details of the experiments conducted using a bench-scale membrane bioreactor (MBR) and sequencing bioreactor (SBR), including the study of the effects of the concentration of pharmaceuticals on the removal efficiency. Details of the experimental setup, reactor start- up procedure and characteristics of the synthetic wastewater used in the experiment are given below.

4.4.1 Membrane bioreactor (MBR)

MBRs were constructed from PVC flexible pipes (1.5m x 0.3m internal diameter), coated with rubber and equipped with submerged A4 Kubota membranes (cartridge type 203), made from chlorinated polyethylene with a nominal pore size of 0.45 μm and an effective surface of 0.3 m^2. They had a working volume of 8 L Figure 4.3a. The MBRs were operated in a sequencing batch mode, comprising periods of Fill, React, and Draw (Table 4.1). The MBRs were fed with wastewater by peristaltic pump (MANOSTAT - division of Barnant Company, Simon varistaltic pump, USA)

with a 3 $L.h^{-1}$ flow rate, controlled by a timer switch and a level control switch to ensure that no spillage occurred during the fill step. Oxygen required for both aerobic bacteria and membrane surface scouring, was provided by compressed air via air diffusers controlled by timer switch. Membrane permeate was drawn out by peristaltic pumps (MANOSTAT - division of Barnant Company, Simon varistaltic pump, USA) with a flow rate 1.5 $L.h^{-1}$, controlled by a timer switch (Figure 4.4). Each MBR was seeded with activated sludge inoculums at pH 6.8, 25 °C, to achieve a starting concentration of ≈2.56 g_{MLSS} $.L^{-1}$. The MBR system operation is described fully in Table 4.1.

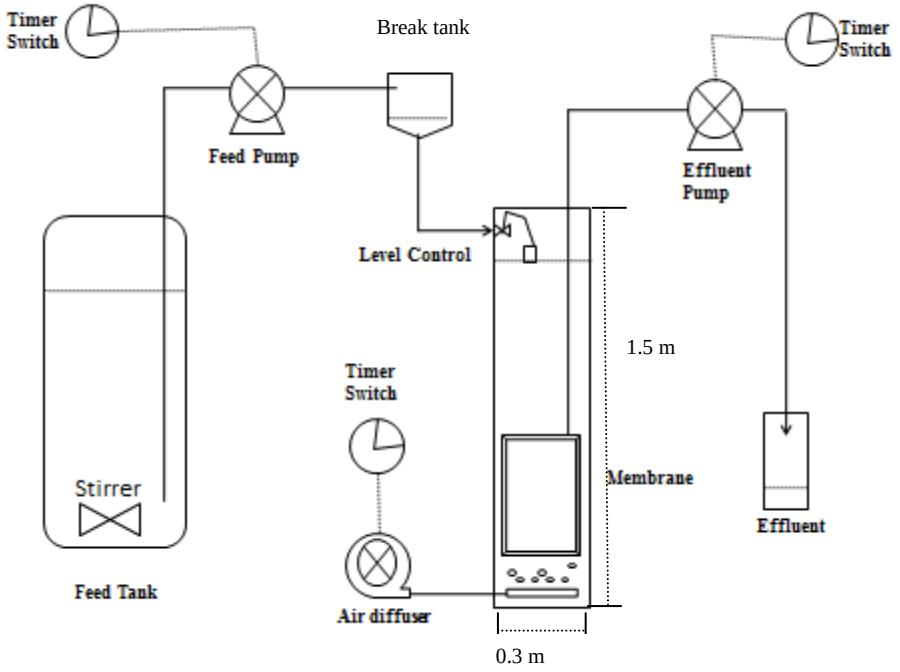
4.4.2 Sequencing bioreactor

The SBR system was built from rigid PVC pipes (1.5m x 0.2m internal diameter) and a working volume of 8 L. The SBR was operated according to a conventional laboratory-scale cycle, comprising periods of fill, react, settle and draw (Table 4.1). The reactor was fed with wastewater by peristaltic pump (MANOSTAT - division of Barnant Company, Simon varistaltic pump, USA) with a 3 $L.h^{-1}$ flow rate, controlled by a timer switch and a level control switch (Figure 4.3b), to ensure that no spillage occurred during the fill step. Oxygen required for both aerobic bacteria and membrane surface scouring, was provided by compressed air via air diffusers controlled by a timer switch. Treated wastewater was drawn out by solenoid valve with flow rate 6 $L.h^{-1}$, controlled by a timer switch (Figure 4.4). Each SBR was seeded with activated sludge inoculums at pH 6.8, 25 °C, to achieve a starting concentration of ≈2.56 g_{MLSS} $.L^{-1}$. The SBR system operation is described fully in Table 4.1.

Table 4.1: Summery operating conditions for the MBR and SBR

Reactor	Process phase order				Duration	Pharmaceutical concentration	Duration
		Fill	Aeration	Draw			
MBR (control)	Fill Aerobic Draw	on off off	off on off	off off on	2 h 18 h 4 h	No addition of pharmaceutical	63 day
SBR (control)	Fill Aerobic Settle Draw	on off off off	off on off off	off off off on	2 h 18 h 2 h 2 h	No addition of pharmaceutical	63 day
MBR1	Fill Aerobic Draw	on off off	off on off	off off on	2 h 18 h 4 h	No addition 1µg.L^{-1} in synthetic WW 1mg.L^{-1} in synthetic WW 10mg.L^{-1} in synthetic WW 1mg.L^{-1} in real WW	10 day 29 day 10 day 7 day 9 day
MBR2	Fill Anoxic Aerobic Draw	on off off off	off off on off	off off off on	2 h 2 h 16 h 4 h	No addition 1µg.L^{-1} in synthetic WW 1mg.L^{-1} in synthetic WW 10mg.L^{-1} in synthetic WW 1mg.L^{-1} in real WW	10 day 29 day 10 day 7 day 9 day
SBR1	Fill Aerobic Settle Draw	on off off off	off on off off	off off off on	2 h 18 h 2 h 2 h	No addition 1µg.L^{-1} in synthetic WW 1mg.L^{-1} in synthetic WW 10mg.L^{-1} in synthetic WW 1mg.L^{-1} in real WW	10 day 29 day 10 day 7 day 9 day
SBR2	Fill Anoxic Aerobic Settle Draw	on off off off off	off off on off off	off off off off on	2 h 2 h 18 h 2 h 2 h	No addition 1µg.L^{-1} in synthetic WW 1mg.L^{-1} in synthetic WW 10mg.L^{-1} in synthetic WW 1mg.L^{-1} in real WW	10 day 29 day 10 day 7 day 9 day

(a) MBR



(b) SBR

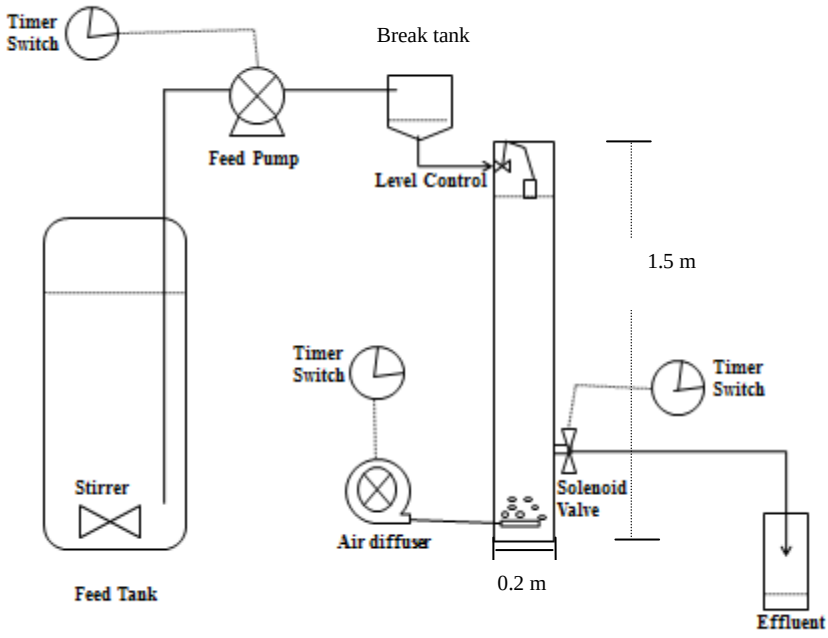


Figure 4.3: Schematic diagram of the reactor setup and reactor dimensions (a) MBR (1.5m x 0.3m internal diameter) and (b) SBR (1.5m x 0.2m internal diameter).

Figure 4.4: Photograph of the MBR (right) and SBR (left) reactors showing floor-standing reactors, suspended tanks and peristaltic pumps.

4.5 Preparation of synthetic wastewater

The synthetic wastewater used in this study was prepared by dissolving the following substances in 1 litre of distilled water to make a stock concentrate.

Peptone	16g
Meat Extract	11g
Urea	3g

Sodium Chloride (NaCl)	0.7g
Calcium Chloride dihydrate ($CaCl_2.2H_2O$)	0.4g
Magnesium Sulphate Heptahydrate ($MgSO_4.7H_2O$)	0.2g
di-Potassium Hydrogen Phosphate (K_2HPO_4)	2.8g

This stock synthetic wastewater is a 100-fold concentrate of that described in the OECD, 1976, but with the addition of di-potassium hydrogen phosphate for improved buffering. Immediately after preparation, the stock synthetic wastewater was autoclaved at 120ºC for 15min and then stored in the dark at 4ºC for no longer than one week. Before each use, the stock solution was diluted 40 times with tap water, to yield approximately 600-700 $mg_{COD}.L^{-1}$, which is equivalent to a medium strength synthetic wastewater in terms of COD (Metcalf and Eddy, 2003).

4.6 Analytical methods

Performance parameters were pH, dissolved oxygen (DO), total organic carbon (TOC), mixed liquor suspended solids (MLSS), mixed liquor volatile suspended solids (MLVSS), and chemical oxygen demand (COD).

4.6.1 pH

Samples were measured for pH using the glass electrode pH meter (JENWAY 3310, Jenway Limited, Essex, U.K.) according to standard methods $4500H^+$ (APHA, 1998).

4.6.2 Dissolved oxygen (DO)

DO of the reactor content was detected by the microprocessor logging dissolved oxygen meter (HANNA instruments, HI 91410). This meter utilised the membrane probe and could measure temperature to automatically compensate for temperature changes. The DO was taken from the laboratory bioreactors by reading directly from the screen according to standard methods 4500-O G (APHA, 1998).

4.6.3 MLSS and MLVSS

To measure the MLSS, a suitable volume of sample was filtered through glass microfiber filters (934-AH) (Whatman, England), which was subsequently dried at 105^0C in an oven for one hour. This filter paper was weighed using an analytical balance after cooling in the desiccator. To determine the MLVSS, the same filter paper was then ignited at 550ºC in a muffle furnace for 15 minutes and cooled in a desiccator before being weighed using an analytical balance. MLSS and MLVSS of samples were calculated using the following equations (4.2 and 4.3).

$$\text{MLSS (mg/l)} = \frac{W_{fo} - W_i}{V} * 1000 \qquad (4.2)$$

where: W_{fo} = weight of the filter paper after being dried at 104ºC in an oven (mg)

W_i = weight of the filter paper with 105^0 residue (mg)

V = volume of sample (ml)

$$\text{MLVSS (mg/l)} = \frac{W_{fo} - W_{ff}}{V} * 1000 \qquad (4.3)$$

where: W_{ff} = weight of the filter paper with residue after ignition at 550ºC in a furnace (mg)

4.6.4 Total organic carbon (TOC)

Samples were filtered using the glass microfiber filter papers (934-AH) before 50 µL was injected in triplicate into the total organic carbon analyzer (TOC-5050A, SHIMADZU, Japan), and the average result was taken, the sample was diluted if necessary to be calculated in the calibration curve range. The instrument was calibrated with standard solutions of hydrogen potassium phthalate with three point concentrations between 1-100 $mg.L^{-1}$. Firstly CO_2 produced was detected by the analyzer when phosphoric acid was added into a portion of the sample giving the inorganic carbon (IC) value. Then, the total carbon (TC) was measured from the

amount of CO_2 produced when another portion of the same sample was ignited. TOC measurement of a sample was the result of IC subtracted from TC, which was directly given by the analyzer.

4.6.5 Chemical oxygen demand (COD)

COD was measured by using the standard method involving potassium-dichromate oxidation for the analysis; standard test tubes (Lovibond) were used at two ranges, low range (0-150 $mg.L^{-1}$) and medium range (0-1500 $mg.L^{-1}$) from Orbeco-Hellige. 1ml from the filtered sample was added to the standard reagent test tubes, and then was heated at 150^0C for 2 hours by the COD digester (Lovibond, ET 108). The COD values were measured after cooling to room temperature with a COD photometer (Lovibond PCCheckIt) at wave length 254 nm. Blank filtered distilled water samples were also treated similar to the unknown sample to reset the photometer to zero value.

4.7 Pharmaceutical concentration analysis

The samples were collected and filtered through sterile membrane filters 0.45 µm (Gelman Sciences), adjusted to pH 2-3 with sulphuric acid, and the extraction was carried out using SPE to follow the degradation of the pharmaceutical. Extracts were analysed by LC-MS-MS for detection of Metronidazole, Trimethoprim, Sulphamethoxazole, Paracetamol and Ranitidine. Full details of the experimental method have been described previously in Section (3.4).

4.8 Removal rate and the specific removal rate

To evaluate and compare the performance of different bioreactors during different operating conditions in the same reactor, the removal rate and the specific removal rate were determined. The removal efficiency was defined as a percentage of the ratio of the removed substance compared to the initial amount of substance, and was calculated using Equation 4.4.

$$\text{Removal efficiency} = \frac{C_t - C_{t+\Delta t}}{C_t} \times 100 \quad (4.4)$$

where: C_t = concentration of a parameter at time t (mg/l)

$C_{t+\Delta t}$ = concentration of a parameter at time t+Δt (mg/l)

The specific removal rate is a relatively important parameter because it shows the relationship between removal rate and mass unit of microorganisms.

$$\text{Specific removal rate (g removed.g MLVSS}^{-1}\text{.d}^{-1}) = \frac{(C_t - C_{t+\Delta t}) \times Q_i}{V \times MLVSS} \quad (4.5)$$

where: Q_i = influent flow rate (m^3/d)

V = reactor volume (m^3)

4.9 Microbial diversity study

Microscopic analysis was used to study major physiological changes of the microbial communities at different pharmaceutical concentration conditions. To study the microbial diversity in more detail, the molecular technique, polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE), was utilised.

4.9.1 Microscopic analysis

MLSS samples were taken directly from the bioreactor at the end of the reaction phase to ensure that the contents inside the bioreactor were completely mixed and the sample was representative of the bioreactor biomass. After the sludge was allowed to settle, one drop of the settled sludge was placed directly on a microscope

slide, and covered with a cover slip before being viewed (×100 magnification) under an Olympus BX51 microscope (Olympus Optical Co, Ltd., Tokyo, Japan). Four fields of view per slide were observed and pictures were taken using a digital camera Olympus DP12.

4.9.2 PCR-DGGE

Sludge samples for the MBRs and SBRs in experiment 4.3 were fixed to retain the morphological integrity of microorganism cells before the DNA extraction was carried out. 5 ml of the MLSS sample was taken and transferred to the sterile plastic universal bottle, which was pre-filled with 5 ml absolute ethanol. The sample was stored at -20ºC before conducting the DNA extraction (see Appendix C).

The obtained DGGE band patterns were analysed using the Bionumerics software version 3.5 (Applied Maths, Sint-Martens-Latem, Belgium). This software comprises steps which allow the defining of different lanes, background subtraction, and marker assisted normalisation, which includes compensating for intensity difference between the lanes, and assigning the different bands in each lane. The DGGE compares the DNA bands of isolated pure culture bacteria in experiment 4.2 with the DNA bands of MBRs and SBRs in experiment 4.3.

4.10 Statistical analysis

The data was processed qualitatively and quantitatively by using computer analytical programmes such as Microsoft Excel 2007. The data was analysed statically using the ANOVA MINITAP. In each comparison, the confidence level is indicated where results are presented, to minimise the effects of the possibly unequal variances of each population in the one-way ANOVA analysis.

4.1.1 Analysis of Variance (ANOVA)

The implications of each of the independent factors and their interactions on the responses were analyzed using ANOVA which was carried out with the aids of MINITAB (Version 16). As a result of such analyses is the *p*-value is determined which refers to the significance of the factor in affecting the response. A smaller *p*-value refers to a higher significance level of the factor affecting the response. It can determine the level of confidence through the application of equation (3):

$$\text{Confidence level} = (1 - p\text{-value}) \times 100 \quad (3)$$

4.1.2 Correlation Analysis

The correlation between the pharmaceutical concentration and performance parameters was determined. The responses were selected based on how significant is the effect of pharmaceutical concentration. These parameters are TOC and COD removal, TOC and COD biodegradation rate, MLSS concentration, pharmaceutical removals, and pharmaceutical degradation rate. The regression test was chosen to determine the correlation keeping in mind that the correlation coefficient R^2 value higher than 90% indicates high correlation. Figures show the correlation between the concentration and each of the selected responses. All of these quantitative correlations corresponded with the qualitative correlations between the pharmaceutical concentration and each of biomass concentrations and removal efficiencies.

5. Results and Discussion

5.1 Fate of pharmaceutical waste in Sulaibiya wastewater treatment plant

5.1.1 Physicochemical Characterisation of Influent

The inflow of the wastewater was monitored during the period between September 2010 and August 2011. This data showed that the inflow of the wastewater ranged between 271000 and 298400 m^3/day. The physicochemical parameters of the influent during the sampling period exhibited large variations. The wastewater temperature values varied from 23 °C in winter to 35 °C in summer. The values of pH, nitrogen, phosphorous, chemical oxygen demand (COD), electrical conductivity (EC) and suspended solids (SS) fluctuated during the sampling period (see Appendix D).

5.1.2 Performance of wastewater treatment processes of Sulaibiya WWTP

The physicochemical properties of the effluent are presented in Appendix D. The total phosphorus content decreased from 6.3 $mg.L^{-1}$ in the influent to 1.6 $mg.L^{-1}$ in the effluent and the total Kjeldahl nitrogen content of the influent 43.5 $mg.L^{-1}$ decreased to 2.5 $mg.L^{-1}$ in the effluent. Phosphorus and nitrogen are normally known as limiting nutrients for eutrophication in the natural balance of aquatic ecosystems; therefore, careful management of their discharge is important to prevent excessive algal growth (Andersen et al., 2006). The primary and secondary treatments of the wastewater effectively reduced the phosphorus and nitrogen by 75% and 94%, respectively compared to influent levels. The final effluent had a better quality with regard to the nitrogen and organic content due to the efficiency of the activated sludge process in the WWTP where average COD removals were 93%. The suspended solids in the secondary effluent were much lower than the influent

and showed typically 95% removal. During the primary and secondary treatments, the cation concentrations did not change significantly, which can be seen by only a small change in the electrical conductivity between the influent and effluent, with an average reduction of 17%.

5.1.3 Occurrence of pharmaceuticals in wastewater influents

The concentrations of the target pharmaceuticals in the influent over the year-long sampling period at Sulaibiya are summarized in Figure 5.1.1. Trimethoprim, Sulphamethoxazole, Paracetamol and Ranitidine were always found in influent samples, whereas Metronidazole was not detected in October and November. Metronidazole detection ranged between 4 $ng.L^{-1}$ in December and 58 $ng.L^{-1}$ in April, these both being lower than other reported values (Rosal et al., 2010). Trimethoprim and Sulphamethoxazole were found in the influent within the range of 61 - 1814 and 11 - 1669 $ng.L^{-1}$, respectively. The highest concentration of Trimethoprim was found in August, and the lowest was found in April, whereas the highest concentration of Sulphamethoxazole was found in October, and the lowest in February.

Trimethoprim was reported at 290 $ng.L^{-1}$ in raw influent wastewater in Switzerland (Goebel et al., 2005), and at relatively high concentrations 2100 – 7900 $ng.L^{-1}$ in the USA (Batt et al., 2007). On the other hand, Sulphamethoxazole has been previously reported at a high concentration of 6000 $ng.L^{-1}$ (Giger et al., 2003), but a concentration of 1669 $ng.L^{-1}$ was the upper limit in the current study. Paracetamol and Ranitidine were found in the influent at concentrations substantially higher than the other target drugs, all of which were among the top ten pharmaceuticals dispensed in Kuwait. Paracetamol was detected in all the wastewater samples at concentrations ranging from 101 - 2086 $ng.L^{-1}$ with the highest concentration in November 2010 and the lowest concentration in February 2011. These concentrations were, to some extent, lower than those reported previously (Pham and Proulx, 1997; Ternes, 1998; Blanchard et al., 2004). On the other hand, Ranitidine

ranged from 365 - 2009 ng.L^{-1}, so is fairly consistent with other studies which have reported 580 ng.L^{-1} (Kolpin et al., 2002) and 1700 ng.L^{-1} (Gomez et al., 2006).

The temperature fluctuated during the sampling between summer and winter. This fact might indicate that the concentrations of pharmaceuticals in the influent may be related to higher consumption during the winter periods of the year when more seasonal illnesses occur.

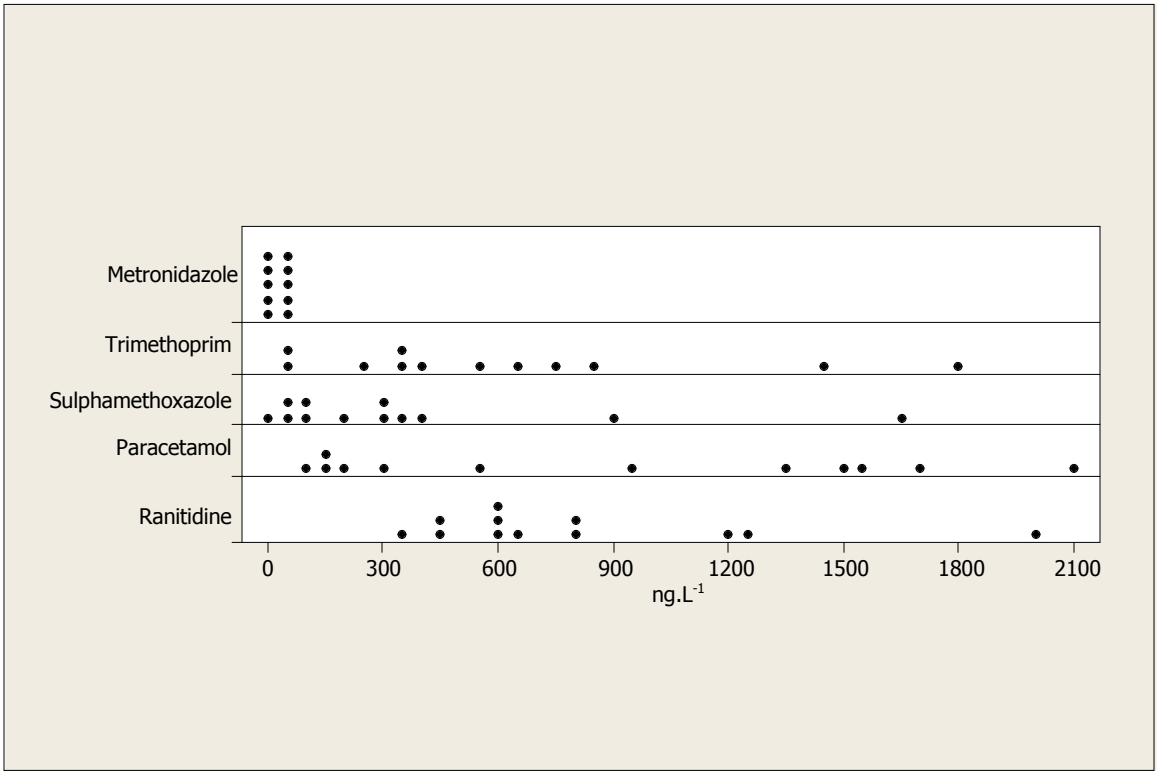


Figure 5.1.1: Variation of concentration of various target compounds (ng.L^{-1}) in the influent, with each point representing one monthly detected sample.

5.1.4 Removal of pharmaceuticals during the primary and secondary treatment at Sulaibiya WWTP

The removal rates of pharmaceuticals during the sampling period are shown in Figure 5.1.2 (a,b,c,d,e). Paracetamol was removed efficiently by the secondary

treatment, at an average of 97.5%, with the highest removal reaching 99.9%, and the lowest removal being 86.1%. Trimethoprim was removed less effectively than Paracetamol, with an average removal of 86.1%, where the highest removal was 96.1%, and the lowest removal was 63%. Removal efficiency of Metronidazole during secondary treatment was at an average of 83.4%, with the highest removal at 93.9%, and the lowest removal at 59.4%. Sulphamethoxazole and Ranitidine showed the lowest removal efficiencies with an average of 77.5% removal, where the highest removal of Sulphamethoxazole was 98.7%, and the lowest removal was 31.3%, while the highest removal of Ranitidine was 99.2%, and the lowest removal was 47.4%.

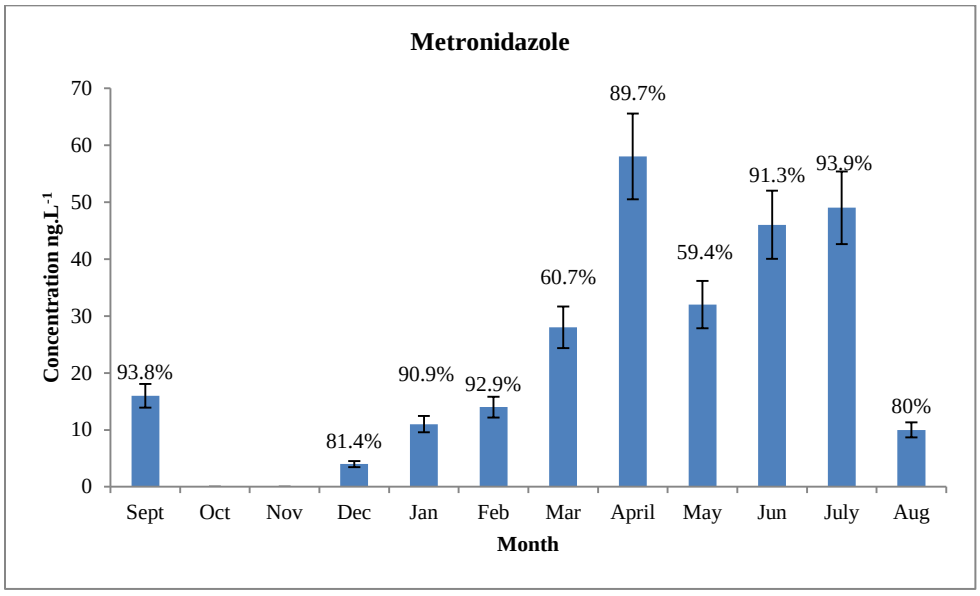


Figure 5.1.2.a: Concentration of Metronidazole (ng.L^{-1}) in the influent and the removal percentage after secondary treatment processes of Sulaibiya WWTP between 2010 -2011.

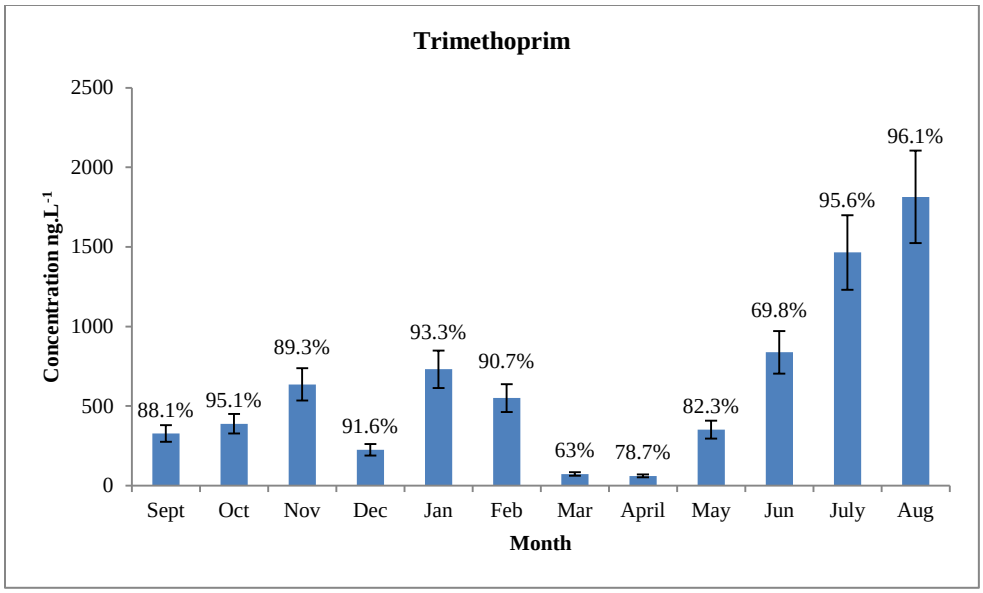


Figure 5.1.2.b: Concentration of Trimethoprim (ng.L^{-1}) in the influent and the removal percentage after secondary treatment processes of Sulaibiya WWTP between 2010 - 2011.

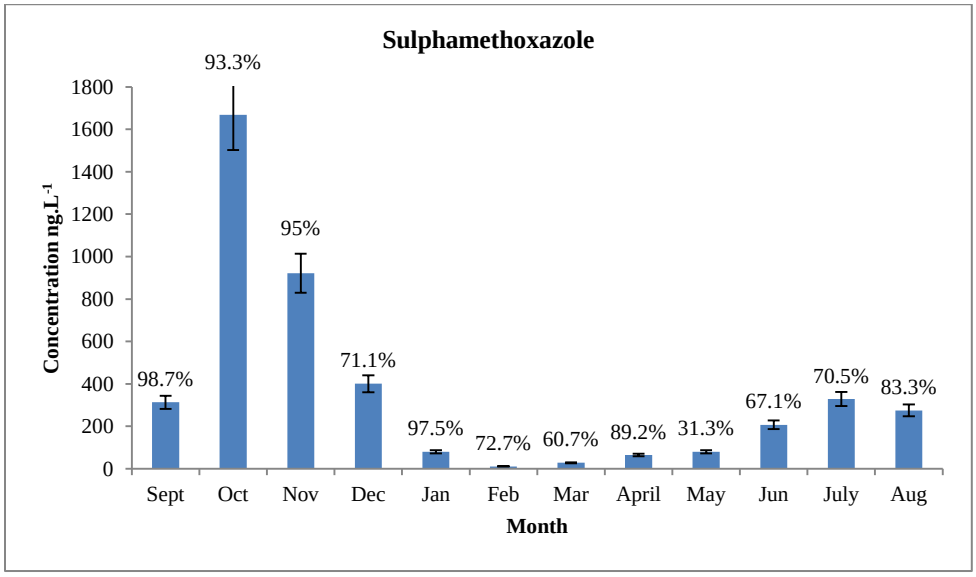


Figure 5.1.2.c: Concentration of Sulphamethoxazole (ng.L^{-1}) in the influent and the removal percentage after secondary treatment processes of Sulaibiya WWTP between 2010 - 2011.

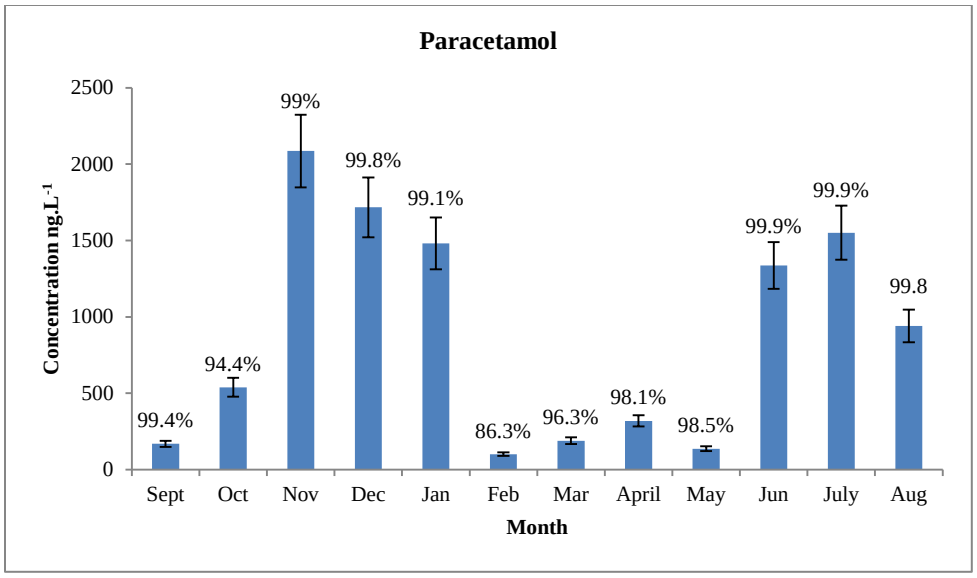


Figure 5.1.2.d: Concentration of Paracetamol (ng.L^{-1}) in the influent and the removal percentage after secondary treatment processes of Sulaibiya WWTP between 2010 - 2011.

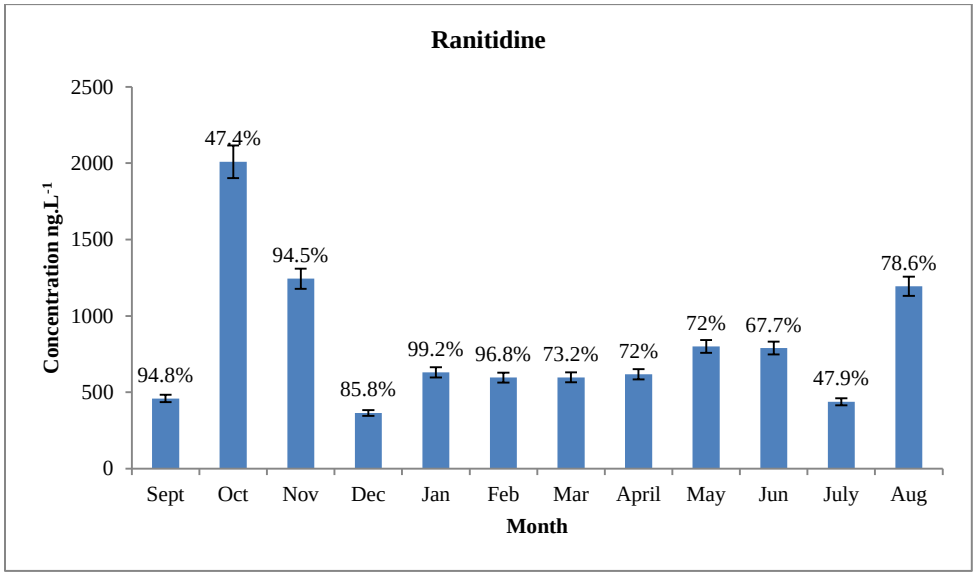


Figure 5.1.2.e: Concentration of Ranitidine (ng.L^{-1}) in the influent and the removal percentage after secondary treatment processes of Sulaibiya WWTP between 2010 - 2011.

In general, the removal efficiencies found in this study were consistent with other WWTPs using primary treatment and secondary treatment with activated sludge. For example, a 75% removal rate was observed for Diclofenac in Germany (Ternes, 1998; Stumpf et al., 1999), up to 90% removal of Ibuprofen being reported in Spain (Santos et al., 2007), and greater than 90% removal efficiency was observed for Aspirin, Ibuprofen, and Thymol in Japan (Nakada et al., 2006). The removal efficiency for a single compound can vary greatly from one WWTP to another depending on the type of treatment (e.g. biological and physicochemical) and the residence time of wastewater in the primary sedimentation tank (Santos et al., 2007).

Removal efficiency of Metronidazole has been reported with a large variability range of 65 - 80% in Spain (Gros et al., 2010). On the other hand, Trimethoprim has been reported to show incomplete removal during conventional treatment by several studies (Gobel et al., 2007; Jelic et al., 2011), while Gros et al. (2010) reports 65 - 80% removal efficiency in treatment plants with longer hydraulic retention times. Similar observations were made by other researchers for the removal efficiency of Sulphamethoxazole and Ranitidine with reported removal efficiencies of 30 - 92% and 50 - 98%, respectively (Gros et al., 2010). In Germany, Paracetamol was found to be removed efficiently at 95% due to its biodegradability, and was detected in less than 10% of all WWTP effluent (Ternes et al., 1998; Kolpin et al., 2004; Roberts and Thomas, 2006).

Concentrations of the target pharmaceuticals detected in the WWTP effluent were in the range 1 - 1000 $ng.L^{-1}$, and are presented in Figure 5.1.3 (a, b, c, d, e). This is in agreement with Ternes et al. (1998), who reported that many pharmaceuticals were

detected in the effluents and measured at high concentrations due to incomplete removal in German sewage treatment plants.

The efficiency of modern wastewater treatments has increased the removal of pharmaceuticals from the influent with the introduction of the activated sludge process. Elimination of pharmaceuticals in the activated sludge process occurs due to several processes, adsorption, biological or chemical degradation and biotransformation. Ternes et al. (1998) suggested that the activated sludge process removes higher amounts of pharmaceuticals than other treatments, most likely due to the bacterial activity in the activated sludge. The results of the current study showed that there was incomplete elimination of trace levels of pharmaceuticals in the effluent. Therefore, implementing other technologies such as membrane systems would be necessary for more complete removal of these trace quantities.

5.1.4.1 Effect of temperature on the removal efficiencies of secondary treatment

Although the total concentrations of target compounds in the influent samples fluctuated throughout the year-long sampling period, the removal process in the WWTP appeared to work as efficiently during the summer months than during the winter months; therefore, the effect of temperature was analysed statistically using ANOVA. The increase in temperature was correlated to an increase in the removal of COD, BOD, organic nitrogen, TKN, MLVSS, and target pharmaceuticals, and was highly significant ($p < 0.05$). This conclusion agrees with other researchers who have found that the removal processes in wastewater treatment plants were higher in summer than in winter (Vieno et al., 2005). They suggested that the reason was the lower biodegradation in the plant because of the low temperatures in winter.

5.1.4.2 The correlation of pharmaceutical concentrations with the removal efficiencies of WWTP

The correlation of pharmaceutical concentrations with the removal parameters of the WWTP (COD, BOD, organic nitrogen, TKN, MLVSS and pharmaceutical) was highly significant ($p < 0.05$), except for Sulphamethoxazole, which had a significant correlation with COD, BOD, organic nitrogen, and TKN removal ($p< 0.1$), and had highly significant correlation with MLVSS and Sulphamethoxazole removal efficiency ($p< 0.05$).

The primary and secondary wastewater treatment stages gave moderate to high removal efficiencies for all pharmaceuticals. However, the secondary effluent still had considerable concentrations of some pharmaceuticals in the range 1 - 1000 $ng.L^{-1}$, with most pharmaceuticals present in the influent being found in the effluent, which indicates the need for further treatment stages for complete removal of these pollutant compounds.

5.1.5 Physical removal of pharmaceuticals at Sulaibiya WWTP

5.1.5.1 Physicochemical Characteristics

The physicochemical characteristics of the feed and permeate from the ultrafiltration process at Sulaibiya WWTP during the sampling period are presented in Appendix D. The average value of the physicochemical characteristics at the inlet of the ultrafiltration stage was pH (7.04), TSS (8.68 $mg.L^{-1}$), TDS (437.1 $mg.L^{-1}$), COD (24 $mg.L^{-1}$), BOD (4.07 $mg.L^{-1}$), total iron (1.38 $mg.L^{-1}$), and total coliforms (426261 CFU/100ml).

The average removal efficiencies for the TSS, total iron and total coliforms by the ultrafiltration process were 98%, 95% and 99%, respectively, while there was no significant change in the TDS measurements (Appendix D). The concentrations of

COD were measured for ultrafiltration feed and permeate. Results did not show high removal of trace organic contaminants through the filtration processes, indicated by an average removal of COD of 42% while the BOD was 72%. Thus, the ultrafiltration process provides an essential pre-treatment for the RO by removing particulate and colloidal material from the feed (such as iron precipitates), but the removal is limited to particles larger than the membrane pore size (Van der Bruggen et al., 2003).

The average removal efficiencies of the RO process for the TSS and total coliforms measurements were 68% and 99%, while there was a highly significant removal in the TDS measurements with an average removal of 96% (Appendix D). Furthermore, the concentration of BOD in the RO feed and the permeate shows high removal of trace organic contaminants through the RO filtration processes with an average removal of 90%.

Trace organic compounds including pharmaceuticals, may be completely or partially degraded in the WWTP, with degradation taking place mostly in the activated sludge process. Pharmaceuticals fluctuate in their degradation in various wastewater treatment processes; however, remaining pharmaceuticals may be removed by ultrafiltration to RO systems.

Pharmaceutical compounds were detected in the RO feed that was derived from the WWTP. Variations in concentration were a result of annual fluctuations of compounds in the raw wastewater, in addition to other processes involved in wastewater treatment. Most of the pharmaceuticals, namely Metronidazole, Trimethoprim, Sulphamethoxazole, Paracetamol, and Ranitidine, were found in all samples from the RO inlets during the sampling year. The average concentrations of these compounds found in the RO inlets were 4 $ng.L^{-1}$, 61 $ng.L^{-1}$, 47 $ng.L^{-1}$, 8 $ng.L^{-1}$,

and 210 ng.L^{-1}for Metronidazole, Trimethoprim, Sulphamethoxazole, Paracetamol, and Ranitidine respectively (Figure 5.1.3 (a,b,c,d,e)). The highest removal efficiency of these compounds was 97% for Ranitidine, 92% for Sulphamethoxazole and Paracetamol, and 86% for Trimethoprim. Lastly, the lowest removal efficiency of 56% found for Metronidazole was probably due to the low starting concentration found in the RO inlets.

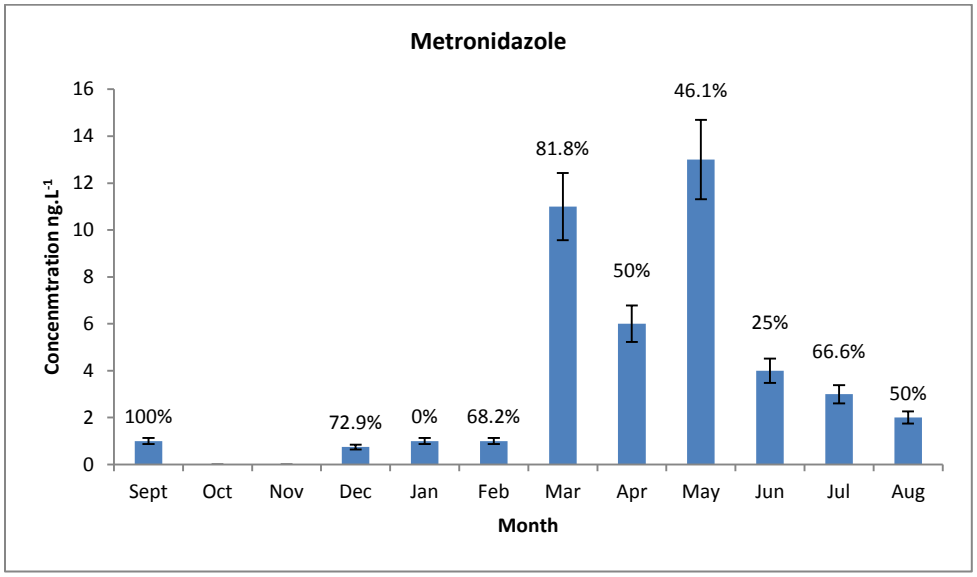


Figure 5.13a: Concentrations of Metronidazole (ng.L^{-1}) detected in the RO inlet, and the removal percentage by RO process in the Sulaibiya WWTP during the year.

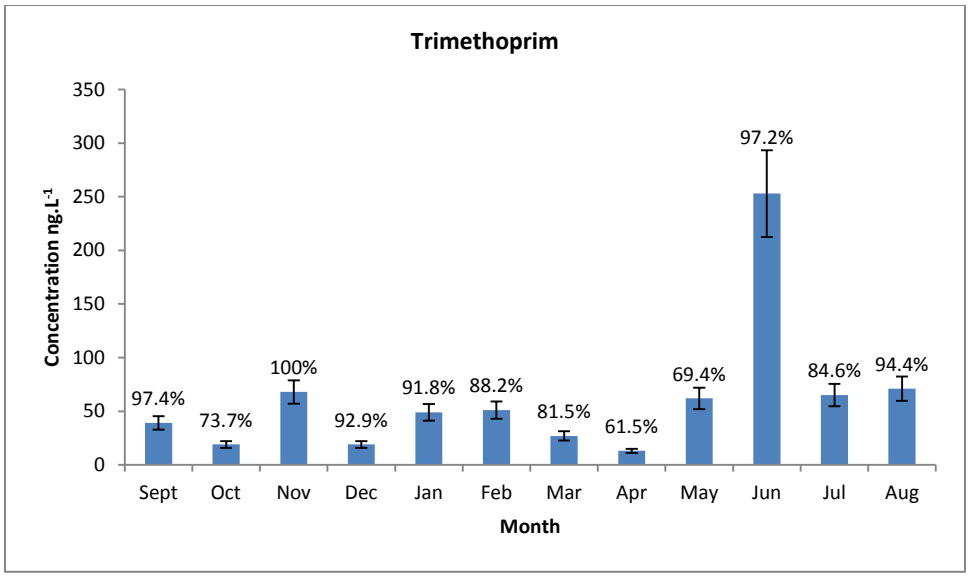


Figure 5.1.3b:Concentrations of Trimethoprim (ng.L^{-1}) detected in the RO inlet, and the removal percentage by RO process in the Sulaibiya WWTP during the year.

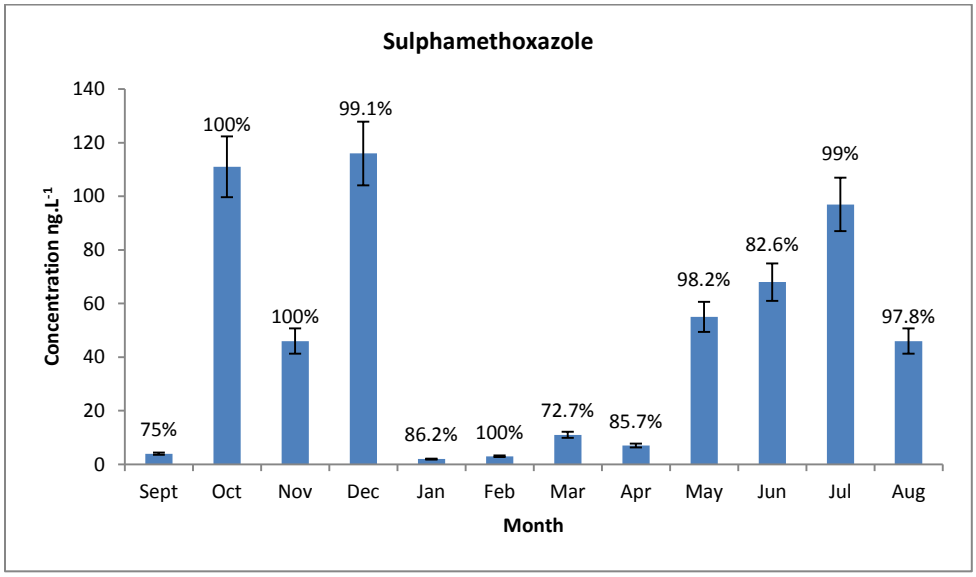


Figure 5.1.3c: Concentrations of Sulphamethoxazole (ng.L^{-1}) detected in the RO inlet, and the removal percentage by RO process in the Sulaibiya WWTP during the year.

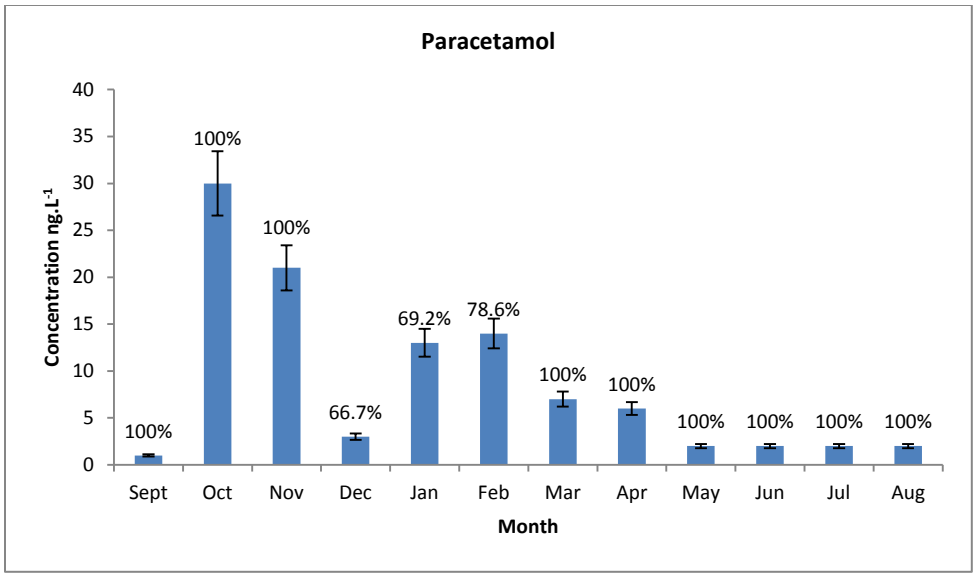


Figure 5.1.3d: Concentrations of Paracetamol (ng.L^{-1}) detected in the RO inlet, and the removal percentage by RO process in the Sulaibiya WWTP during the year.

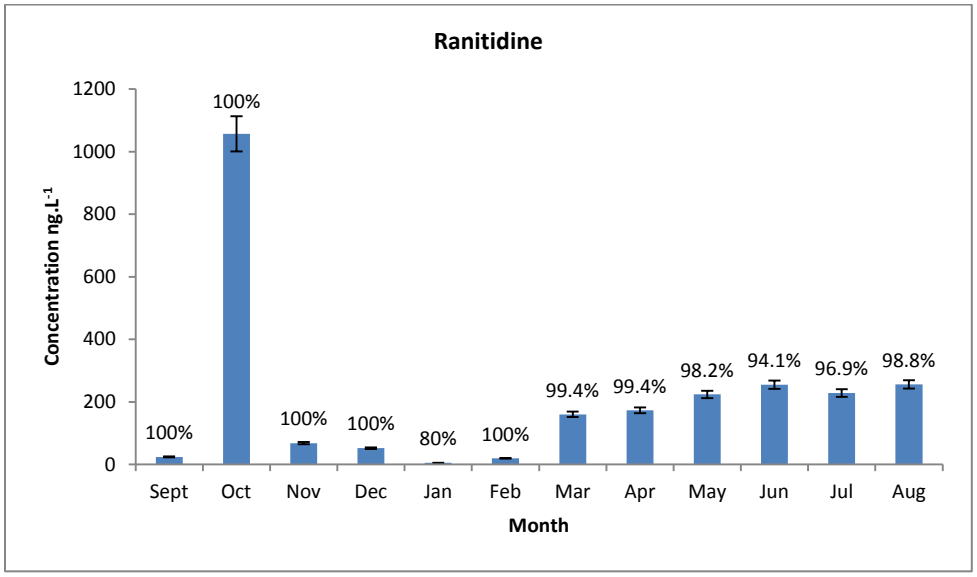


Figure 5.1.3e: Concentrations of Ranitidine (ng.L^{-1}) detected in the RO inlet, and the removal percentage by RO process in the Sulaibiya WWTP during the year.

All five pharmaceuticals were found in all the RO feeds, permeate and brine (Figure 5.1.4). The average concentrations of these pharmaceuticals found in the RO brine were up to 6, 22, 109, 5, and 35 ng.L^{-1}for Metronidazole, Trimethoprim, Sulphamethoxazole, Paracetamol, and Ranitidine, respectively which shows that they were concentrated in the brine by limited permeability through the RO

membrane. In general, concentrations of these compounds in the brine were 16 times that in the feed for Metronidazole; twice for Trimethoprim, Sulphamethoxazole and Paracetamol; but it was totally removed from the feed and no traces found in the permeate for Ranitidine, and the concentration factor was found very small in the brine. Different concentration factors were found in the chosen pharmaceuticals, the reason behind that could be explained due to the different physical and chemical properties of these pharmaceuticals.

The solubility of these pharmaceuticals varies; some are moderately soluble such as Sulphamethoxazole and Trimethoprim where the solubility was 281 $mg.L^{-1}$ and 400 $mg.L^{-1}$, respectively; some are highly soluble like Ranitidine, Paracetamol and Metronidazole where the solubilities were 24.7 $g.L^{-1}$, 14 $g.L^{-1}$and 10 $g.L^{-1}$, respectively. Log K_{ow} values of these pharmaceuticals ranged between -0.02 and 0.92. The correlation of log K_{ow} with removal efficiency is shown in Figure 5.1.5, the solubility and log K_{ow} did not correlate with the behaviour of these pharmaceuticals in the RO stage; however, Tolls, (2001) has suggested that log K_{ow} may not be a good indicator of the behaviour of pharmaceuticals in the environment. It has been reported that the removal efficiency of solutes by ultrafiltration and RO can be affected by different parameters such as pH, solute charge, molecular weight and geometry, polarity and hydrophobicity, as well as the membrane surface charge (Van der Bruggen et al., 1998; Van der Bruggen et al., 1999; Kiso et al., 2000; Kiso et al., 2001; Ozaki and Li, 2002; Kimura et al., 2003b; Kimura et al., 2004).

Investigations carried out previously on the removal efficiency of RO compared to other types of membranes showed a great advantage in using RO in producing high quality recycled water. According to Lopez-Ramirez et al. (2006), reclaimed wastewater with RO membranes widely exceed the drinking water standards and RO membranes achieve highly reduced levels of pollutants in the permeate. Furthermore, microorganisms were removed from the RO permeate, which would allow safe reuse of water in agriculture.

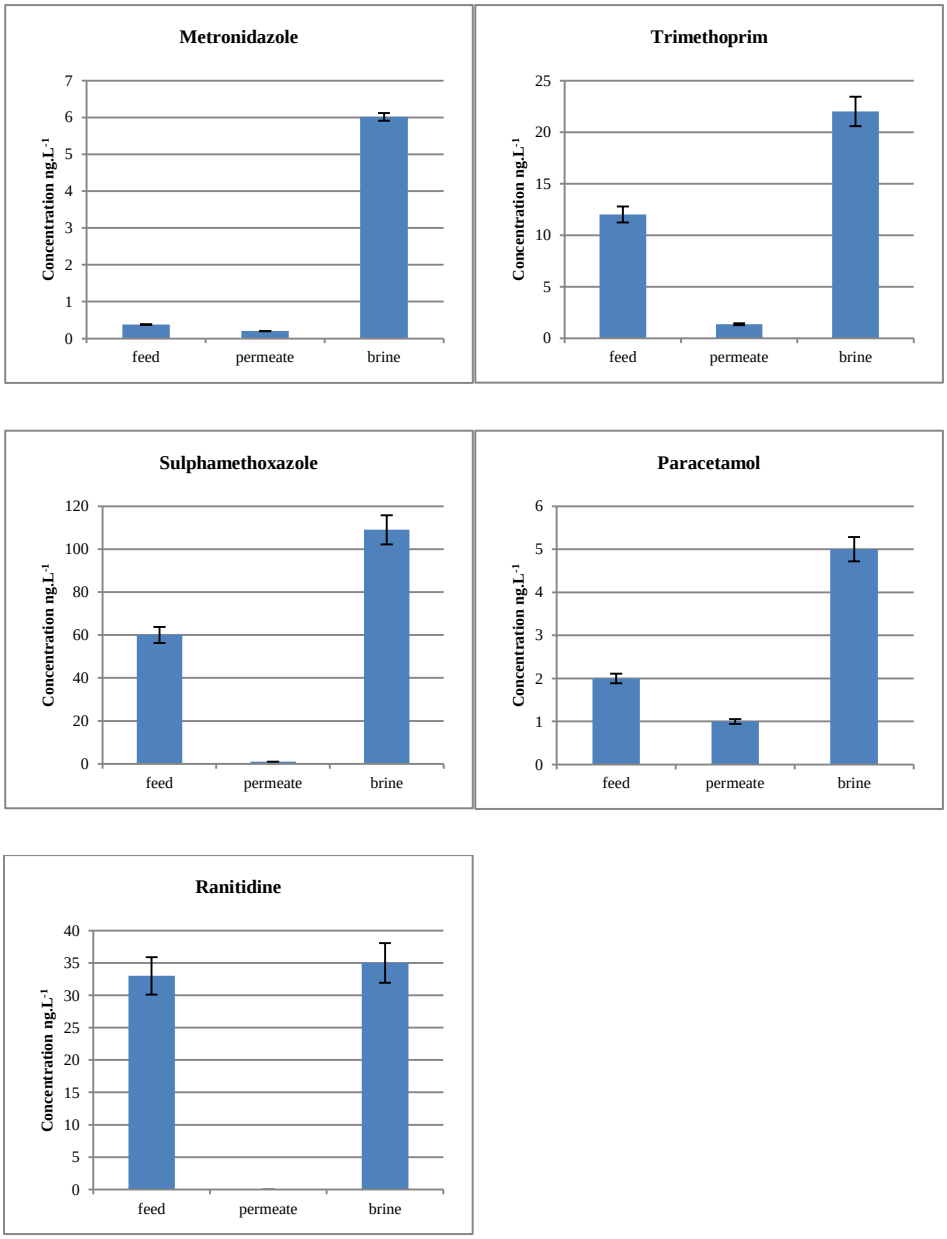


Figure 5.1.4:Concentration of Metronidazole, Trimethoprim, Sulphamethoxazole, Paracetamol and Ranitidine (ng.L^{-1}) in RO feeds, permeate and brine for December 2010.

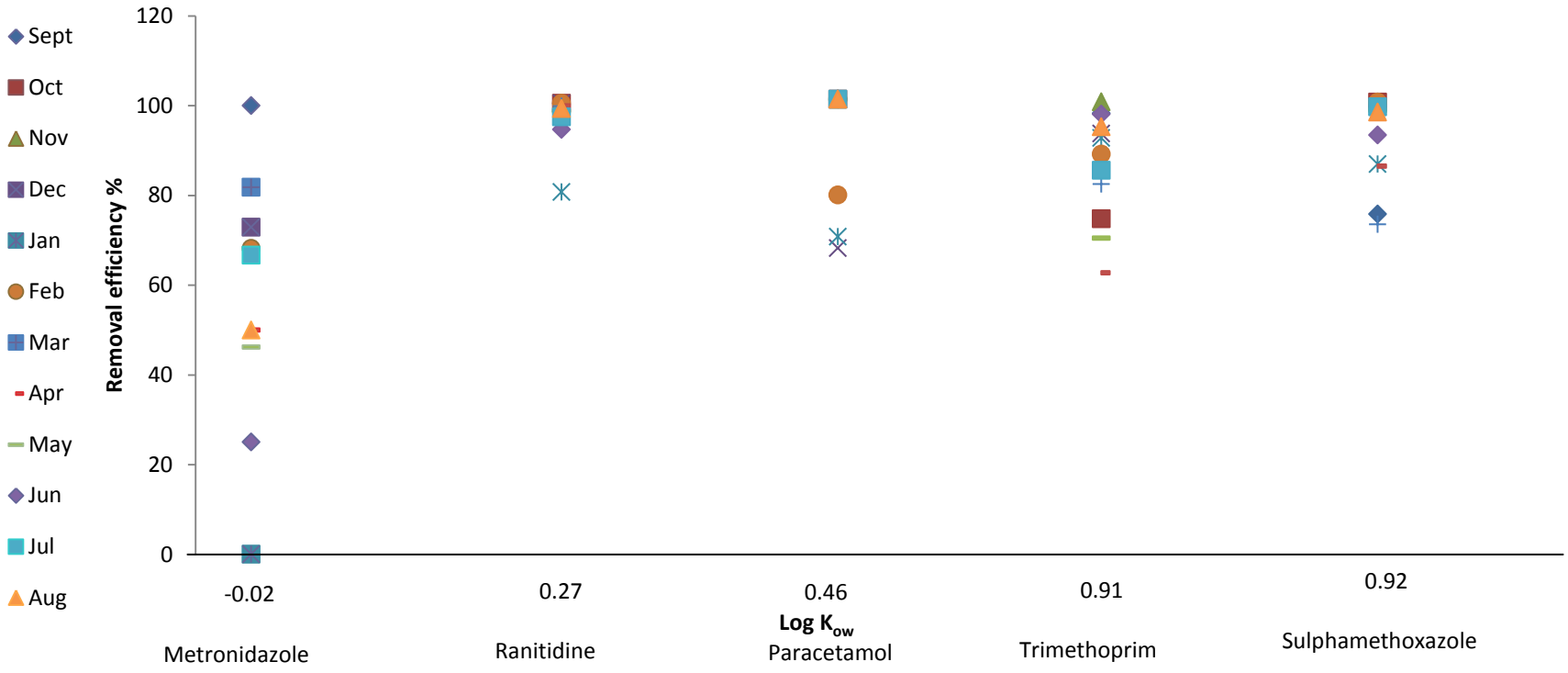


Figure 5.1.5:Plot of the octanol water coefficient (log K_{ow}) of pharmaceuticals against permeate removal efficiency.

5.1.5.2 Effect of temperature and pH on removal efficiencies by the RO process

As with biological treatment, temperature affected the removal processes in the RO system. The concentrations of target pharmaceuticals in the RO inlet samples during the sampling year fluctuated during the summer and winter months. Therefore, the effect of temperature and pH was also analysed statistically using ANOVA. The correlation of temperature and pH with the removal of BOD, TSS, TDS, total coliforms and all target pharmaceuticals was highly significant ($p< 0.05$).

In this study, comparison between the removal of pharmaceuticals and the other removal parameters tested, such as TSS, TDS, BOD, and total coliforms in the RO streams were tested, found that regression analysis did not show any correlation between the removal efficiency of pharmaceuticals with the removal of TSS, TDS, BOD, and total coliforms. This might be due to the complexity of the RO feed in WWTP, and to a broad range of rejection of the RO membrane. Therefore, it is very difficult to associate these operating parameters with the removal rates of the pharmaceuticals.

Due to the wide range of variability, and limitations in the data, it was not possible to determine any relationship between the removal of pharmaceuticals and their molecular weight and molecular size. According to Kimura et al. (2003) there was a linear relationship between molecular weight of the non-charged compounds and their removal. However, in the current study, there was a relationship between the molecular weight and the removal of Metronidazole, Trimethoprim and Ranitidine, as observed by the linear regression analysis, but for Sulphamethoxazole and Paracetamol there was no relationship (Figure 5.1.6). The physicochemical characteristics of the pharmaceuticals tested in this study differ from each other. Thus, a relationship between any of the removal trends could possibly be described by different physicochemical characteristics such the charge, shape and polarity of the compounds. Steric hindrance is the main removal mechanism of RO membranes, as well as electrostatic interaction and hydrophobic interaction between compounds and the membrane (Bellona et al., 2004). The removal efficiency of RO has been investigated by many researchers, and suggests that the removal may be influenced

by the dipole moment of compounds, and the hydrophobicity of compounds as represented by $K_{ow,}$and molecular size (Ozaki and Li, 2002; Van der Bruggen et al., 2003). Positive correlation between hydrophobicity of non-phenolic compounds (log K_{ow}) and their removal by nanofiltration was reported by Kiso et al., 2000. On the other hand, hydrophilic compounds tended not to adsorb to the membrane polymeric matrix (Alturki et al., 2010). According to Snyder and co-workers (2007), some compounds are able to pass through the RO membrane, thus no clear and consistent relationship between molecular structure and membrane permeability has been established. Penetration of molecules through the RO membrane could be as a result of diffusion into and through the membrane, short-circuiting of the membrane, or supporting media failure. The removal of micropollutants by RO is influenced by complex interactions of electrostatic and other physical forces acting between the specific solute, the solution and the membrane. Furthermore, electrostatic attraction or repulsion forces can affect the removal of some micropollutants by RO membranes due to their negative surface charge (e.g. repulsion of Sulphamethoxazole due their negative charge) (Bellona et al., 2005).

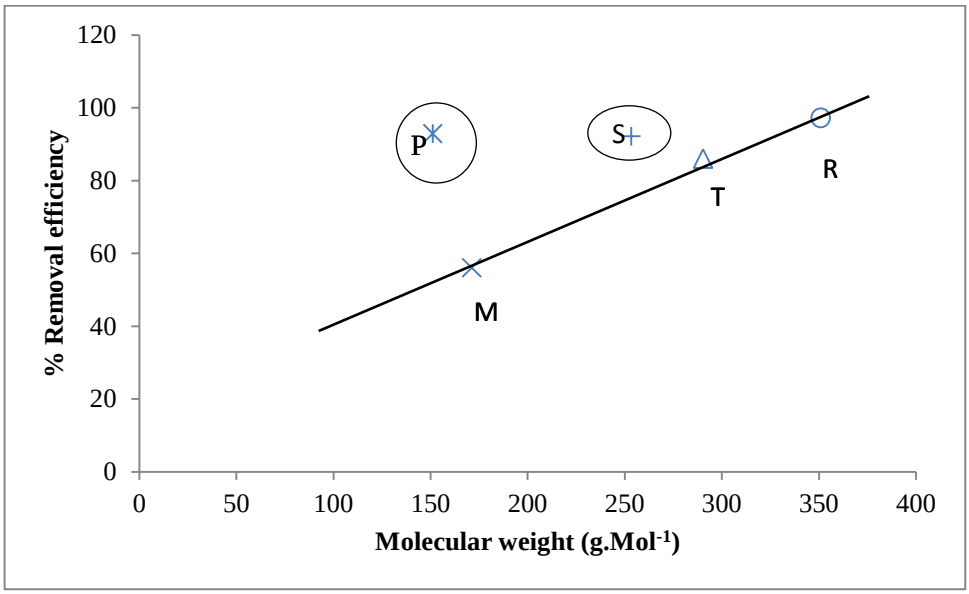


Figure 5.1.6: The relationship between the molecular weight and the removal efficiencies of Metronidazole (M), Trimethoprim (T), Sulphamethoxazole (S), Paracetamol (P) and Ranitidine (R) by the RO process.

The concentrations of the pharmaceuticals were reduced as they passed through the ultrafiltration systems in the water reclamation plant so that Metronidazole, Trimethoprim, Sulphamethoxazole, Paracetamol and Ranitidine were detected in the effluent at maximum concentrations of 13, 190, 73, 15, and 236 $ng.L^{-1}$, respectively.

Other researchers have found high levels of pharmaceuticals in the effluents of WWTPs such as analgesics/anti-inflammatories at a concentration of 57 $\mu g.L^{-1}$ (Nakada et al., 2006; Roberts and Thomas, 2006; Gomez et al., 2007; Santos et al., 2007). Therefore, the removal of pharmaceuticals is more effective in an advanced treatment plant using RO systems than in conventional treatment plants (Snyder et al., 2007).

However, removal rates with RO membrane observed in the current study were high, which is in agreement with results obtained by other researchers (Alturki et al., 2010; Radjenovic et al., 2008; Reznik et al., 2011; Snyder et al., 2003; Dolar et al., 2012). In a pilot scale experiment using a MBR with RO membranes Dolar et al. (2012), observed that the majority of compounds present in the influent were completely removed in the permeate. Joss et al. (2011) reported that most organic micropollutants were removed or retained by RO to below their detection limit. Carbamazepine, Sulphamethoxazole, Metoprolol and Sotalol were removed with high removal rates (>98%) using RO membranes (Radenovic et al., 2008 and Gur-Reznik et al., 2011).

5.1.6 Effect of chlorination on pharmaceuticals during treatment at Sulaibiya WWTP

The effluents of the RO process at Sulaibiya were treated further by chlorine oxidation before discharge. Most of the pharmaceuticals were able to pass through the RO system to some extent, so that trace levels were always detected in the RO effluent. The maximum concentration detected was 19 $ng.L^{-1}$ and 15 $ng.L^{-1}$ for Trimethoprim and Ranitidine, 7 $ng.L^{-1}$, 5 $ng.L^{-1}$, and 4 $ng.L^{-1}$ for Metronidazole,

Sulphamethoxazole and Paracetamol, respectively. On the other hand, the lowest concentration detected in the RO effluents for Metronidazole, Trimethoprim, Sulphamethoxazole, Paracetamol, and Ranitidine were 0.2 $ng.L^{-1}$, 1 $ng.L^{-1}$, 0.2 $ng.L^{-1}$, 1 $ng.L^{-1}$, and 1 $ng.L^{-1}$, respectively. The incomplete removal of these pharmaceuticals at wastewater treatment plants have permitted their spread extensively in surface waters (Boyd et al., 2003; Carballa et al., 2004; Kim et al., 2007; Metcalfe et al., 2003; Okuda et al., 2008; Paxeus, 2004; Reemtsma et al., 2006; Tauxe-Wuersch et al., 2005; Ternes et al., 1998), many of which are used as a source of raw water for drinking water.

Sulaibiya WWTP was designed to treat the product water with chlorine before discharge. Samples were taken to follow the fate of these pharmaceuticals when treated with chlorine. Chlorine treatment allowed between 0 to 100% removal or transformation of these pharmaceuticals (Figure 5.1.7). However, most of the detected pharmaceuticals in the final effluent after chlorination were below 4 $ng.L^{-1}$ or completely removed below detection limits, indicating highly effective oxidation of the investigated pharmaceuticals in the presence of the free chlorine residual.

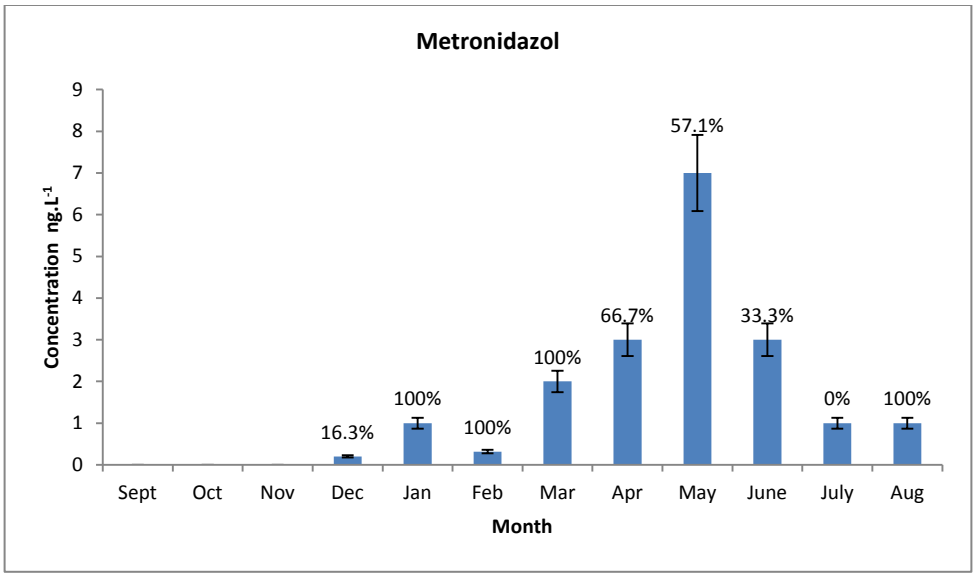


Figure 5.1.7.a: Seasonal concentrations of Metronidazole (ng.L^{-1}) detected in the RO outlet, and the removal percentage by chlorination process at Sulaibiya WWTP.

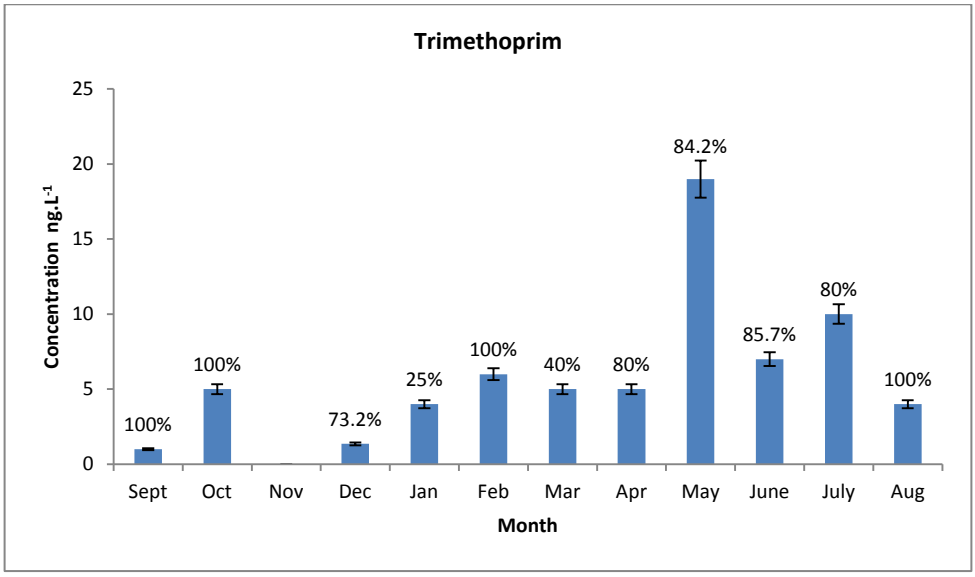


Figure 5.1.7.b: Seasonal concentrations of Trimethoprim (ng.L^{-1}) detected in the RO outlet, and the removal percentage by chlorination process at Sulaibiya WWTP.

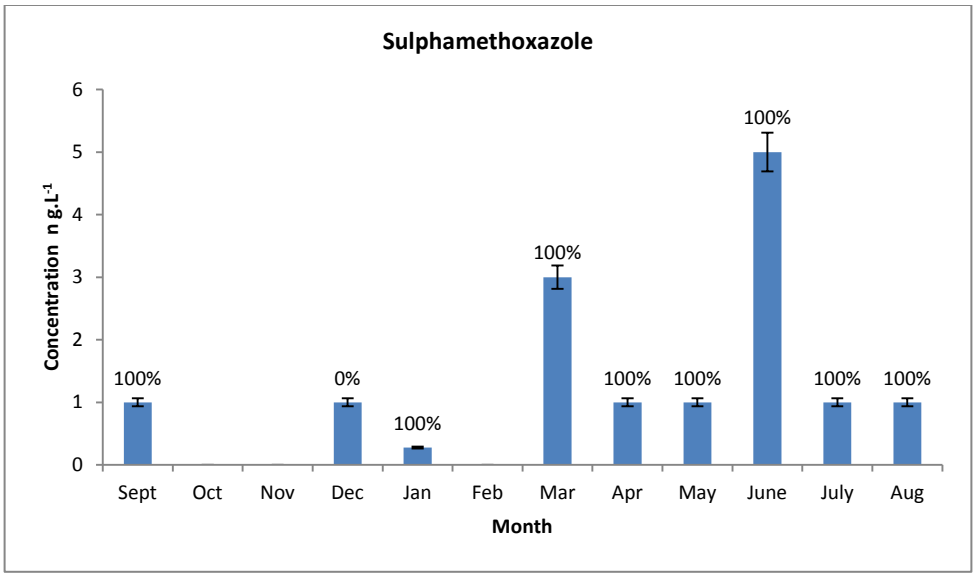


Figure 5.1.7.c: Seasonal concentrations of Sulphamethoxazole (ng.L^{-1}) detected in the RO outlet, and the removal percentage by chlorination process at Sulaibiya WWTP.

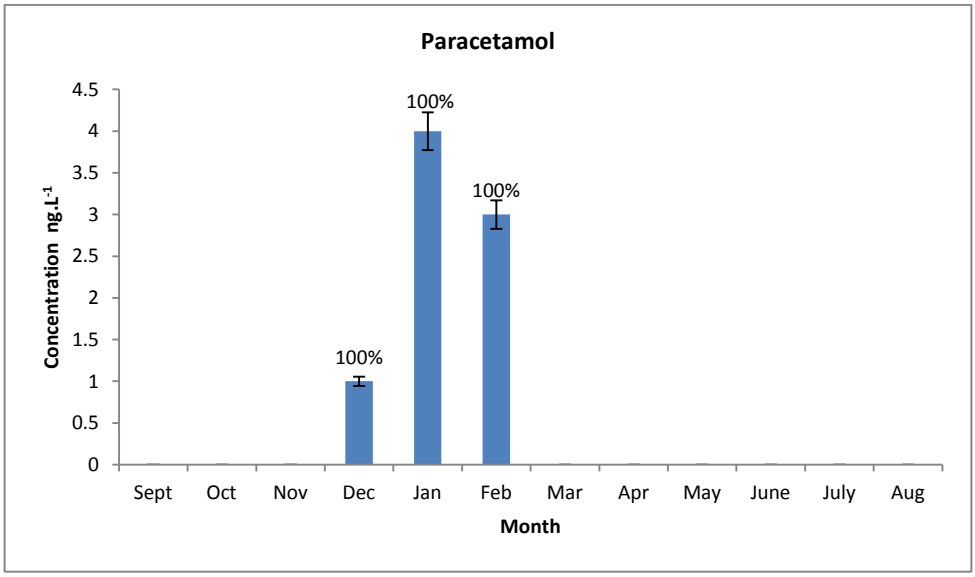


Figure 5.1.7.d: Seasonal concentrations of Paracetamol (ng.L^{-1}) detected in the RO outlet, and the removal percentage by chlorination process at Sulaibiya WWTP.

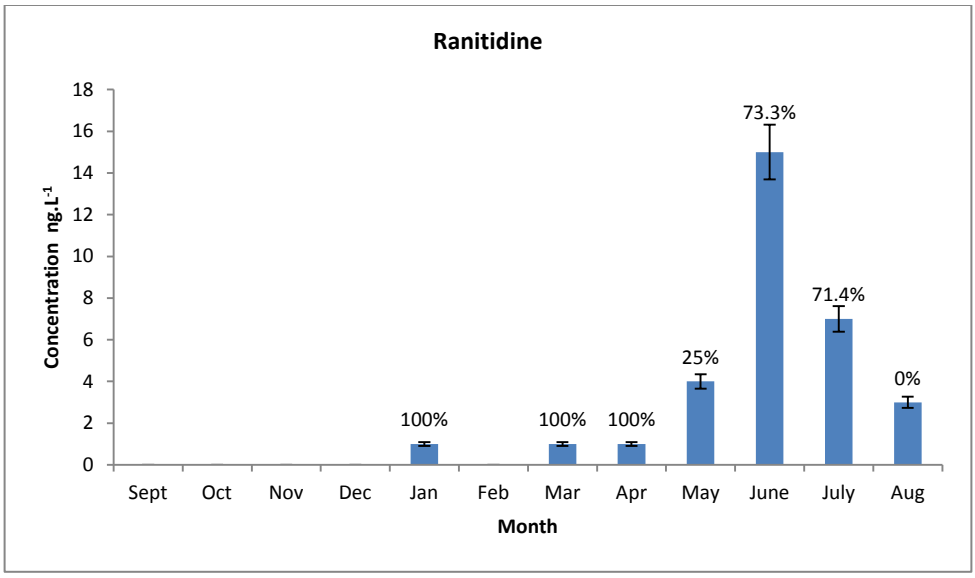


Figure 5.17.e: Seasonal concentrations of Ranitidine (ng.L^{-1}) detected in the RO outlet, and the removal percentage by chlorination process at Sulaibiya WWTP.

Previous studies have found that pharmaceuticals can be degraded under chlorination treatment (Pinkston and Sedlak, 2004; Westerhoff et al., 2005). Efficient removal of the pharmaceuticals after chlorination was expected since 90% removal has been reported previously for most Sulfonamides and for Trimethoprim in river water at a chlorine dose of 1 mg.L^{-1} (Adams et al., 2002). Some of the pharmaceuticals still remained at a noticeable level after RO (from 1 to 19 ng.L^{-1}) in the product water, so the discharge of this water could cause potential ecological risks and/or the proliferation of bacterial resistance in downstream environments. However, chlorination almost completely removed the target pharmaceuticals from the recycled water, but complete removal of any toxicity of these pharmaceuticals was not confirmed since the formation of chlorinated by-products (DBP) may have occurred, and there is evidence here these may be more harmful than their parent compound, as shown for antibiotics (Von Gunten et al., 2006). Researchers found that the residual of chlorine in tap water can react with organic pollutants and produce by-products (Canosa et al., 2006; Negreira et al., 2008).

Oxidation process can yield numerous intermediates and end-products, especially from heterocycles which can create complex products of new chemical structures

include DBPs. Most products are more polar and of lower molecular weight than the parent pharmaceutical (halogenated, hydroxylated, cleaved rings), and many of which are isomeric and more persistent (Daughton, 2010). Other factors adding further complexity include the potential for certain reaction products to revert back to the original pharmaceutical, as reported for the N-chlorinated intermediate from sulphamethoxazole when free chlorine is insufficient (Dodd and Huang, 2004).

The reaction intermediates and end products can sometimes express combined toxicity greater than the parent pharmaceutical (Radjenovic et al., 2009). The halogenated DBPs (those containing chlorine, bromine, or iodine) are of particular toxicological concern as many halogenated DBPs in finished drinking water occur at concentrations well above 1 $\mu g.L^{-1}$ (Krasner et al., 2006).

5.1.7 Overall removal of pharmaceuticals during wastewater treatment

Modern WWTPs can effectively remove micro-pollutants, as well as microbial pollution. These WWTPs receive a large number of different trace organic polluting compounds, among them pharmaceuticals; however, conventional treatments have not been specifically designed to remove these pharmaceuticals (Suárez et al., 2008). Therefore, pharmaceuticals often occur in effluents because either they do not have the tendency to adsorb onto activated sludge, or their biodegradation is not possible within the hydraulic retention time. However, using further treatment such as filtration (ultrafiltration, nanofiltration and RO) and chemical disinfection (chlorine and ozone), further to conventional treatment, increased removal efficiency can be achieved by the WWTP.

Pharmaceuticals were followed through the overall treatment process to evaluate the removal process (see Methods Section 4.1). Box plots have been used to show levels of pharmaceuticals found in influent and the effluent of each process in the Sulaibiya WWTP (Figure 5.1.8).

5.1.7.1 Biological removal

Metronidazole concentration in the influent raw sewage was low compared to other pharmaceuticals, and at all sampling times the concentration was highly variable, with a median concentration of 15 $ng.L^{-1}$ and a mean of 22.33 $ng.L^{-1}$ (Figure 5.1.8). Statistical analysis of the data showed that biological removal of Metronidazole was highly significant, the median and mean concentration after biological treatment being 3 $ng.L^{-1}$ and 6.28 $ng.L^{-1}$, respectively. On the other hand, Trimethoprim concentration in the influent was high, and also highly variable with one point outside the box plot (Figure 5.1.8). The median concentration was 470 $ng.L^{-1}$ and the mean concentration was 622 $ng.L^{-1}$. The biological removal of Trimethoprim was also statistically highly significant, the median and mean concentration of the secondary effluent being the same at 107.5 $ng.L^{-1}$. Similarly, Sulphamethoxazole concentration in the influent was high and moderately variable, with two points outside the box plot, and a median concentration of 241 $ng.L^{-1}$ and a mean concentration of 365 $ng.L^{-1}$. Highly significant biological removal of Sulphamethoxazole gave a median and mean concentration in the secondary effluent of 66.5 $ng.L^{-1}$and 134.1 $ng.L^{-1}$, respectively, with two points outside the box plot. Paracetamol concentration in the influent was also high and highly variable, with a median concentration of 740 $ng.L^{-1}$ and a mean of 881 $ng.L^{-1}$ (Figure 5.1.8). Paracetamol was very effectively removed by the activated sludge process with near 100% efficiency, and statistically high significance; the median and mean concentration of the effluent was 10.5 $ng.L^{-1}$ and 15.25 $ng.L^{-1}$, respectively. Lastly, Ranitidine concentration in the influent was high and also highly variable, one point was outside the box plot, with a median concentration of 624 $ng.L^{-1}$ and a mean concentration of 812 $ng.L^{-1}$ (Figure 5.1.8). The biological removal of Ranitidine was

also statistically highly significant, with the median and mean concentration of the secondary effluent being 199 ng.L^{-1} and 320 ng.L^{-1}, respectively, with one point outside the box plot.

The treatment of secondary effluent at Sulaibiya WWTP with sand filtration is intended to remove suspended solids and turbidity that persists after clarification. Pharmaceutical degradation can also occur in these systems by further biological degradation by biofilms that develop on the filter media (Gobel et al., 2007). Adsorption to the filter solids is also possible.

Metronidazole was removed by sand filtration during the sampling period by 50% where the mean and median concentrations in the sand filtration effluent were 3.56 ng.L^{-1} and 1.5 ng.L^{-1}, respectively (Figure 5.1.8), with one point outside the box plot (13 ng.L^{-1}). Similarly, Trimethoprim was reduced by 53% by sand filtration, where the mean concentration was 61.3 ng.L^{-1} and the median concentration was 50 ng.L^{-1}, with one point outside the box plot (253 ng.L^{-1}). In contrast, Sulphamethoxazole was removed by only 31% with nearly constant concentrations in the effluent with a mean 47.2 ng.L^{-1} and median 46 ng.L^{-1} (Figure 5.1.8). Paracetamol was removed by 57%, with mean and median concentrations in the sand filtration effluent of 8.58 ng.$L^{-1}$and 4.5 ng.L^{-1}, respectively (Figure 5.1.8). However, Ranitidine showed minimal removal by sand filtration, with only a 16% reduction, and the mean and median concentrations in the effluent were 210.1 ng.L^{-1} and 166.5 ng.L^{-1}, respectively (Figure 5.1.8), with one point outside the box plot (1057 ng.L^{-1}), and the residual concentration being relatively high compared to the other pharmaceuticals.

In summary, Metronidazole, Trimethoprim and Paracetamol reveal removal efficiencies ≥50%, while Sulphamethoxazole and Ranitidine removal efficiencies were 31% and 16%, respectively, when sand filtration is employed. Removal by sand

filters is attributable to biological activity or adsorption, and from the structural and physical properties of the pharmaceuticals it can be predicted which will be more susceptible to treatment. Although from the observations of sorption tendencies (i.e., correlation to K_{ow} values), the highest removal efficiencies were obtained for pharmaceuticals previously identified as being efficiently removed during the active sludge process. Moreover, there are some influences of operational variables such as hydraulic residence time, hydraulic loading rate and bulk water quality characteristics on pharmaceutical removal during sand filtration. Gobel et al. (2007) found significant differences in the removal of Trimethoprim (15% versus 74%) in two sand filters with comparable hydraulic retention times and hydraulic loading rates (per biofilm surface area) in each case. Furthermore, Nakada et al. (2007) suggested the removal of 24 different pharmaceuticals during sand filtration. Similarly, Gobel et al. (2007) observed that some of the pharmaceuticals were eliminated to the greatest extent during the sand filtration stage. Therefore, sand filter could remove pharmaceuticals as it is reduced during the treatment.

5.1.7.2 Pharmaceutical removal by membrane filtration

Results show that pharmaceutical removal by UF and RO processes in wastewater treatment is more efficient compared to secondary treatment. The concentration of pharmaceuticals was low in the UF influent, with median concentrations 1.5, 50, 46, 4.5 and 166.5 $ng.L^{-1}$ for Metronidazole, Trimethoprim, Sulphamethoxazole, Paracetamol and Ranitidine, respectively. The median removal by UF was low to moderate 0%, 44%, 64%, 77% and 74% for Metronidazole, Trimethoprim, Sulphamethoxazole, Paracetamol and Ranitidine, respectively. In contrast, pharmaceutical removal in the RO process was moderate to high compared to the UF process, with removal rates of 33%, 82%, 94%, 100% and 97% for Metronidazole, Trimethoprim, Sulphamethoxazole, Paracetamol and Ranitidine, respectively. RO therefore showed very high pharmaceutical removal efficiencies. However, some pharmaceuticals have been detected in RO permeate (Figure, 5.1.8) and their breakthrough cannot be rationalized by their physicochemical properties.

5.1.7.3 Pharmaceutical removal by chlorination

Box plot analysis reveals that some of the pharmaceuticals can break through the RO membrane, and were detected at median concentrations ranging between 0.667 and 5 $ng.L^{-1}$ (Figure, 5.1.8). Chlorination of all effluents achieved further pharmaceutical removal greater than 86%, while the highest concentrations were 3, 3, 1, and 4 $ng.L^{-1}$ for Metronidazole, Trimethoprim, Sulphamethoxazole and Ranitidine, respectively in chlorinated RO filtrate. Laboratory investigations by other researchers using model systems have demonstrated convincingly that chlorination of common pharmaceuticals can lead to the formation of known toxicants and probable carcinogens. Dodd and Huang (2007) found that chlorination reacted with Trimethoprim, and the products were predominantly multi-chlorinated and hydroxylated. Bedner and MacCrehan (2006) demonstrated that free chlorine doses typically used in water treatment could react with Paracetamol and led to the production of several products.

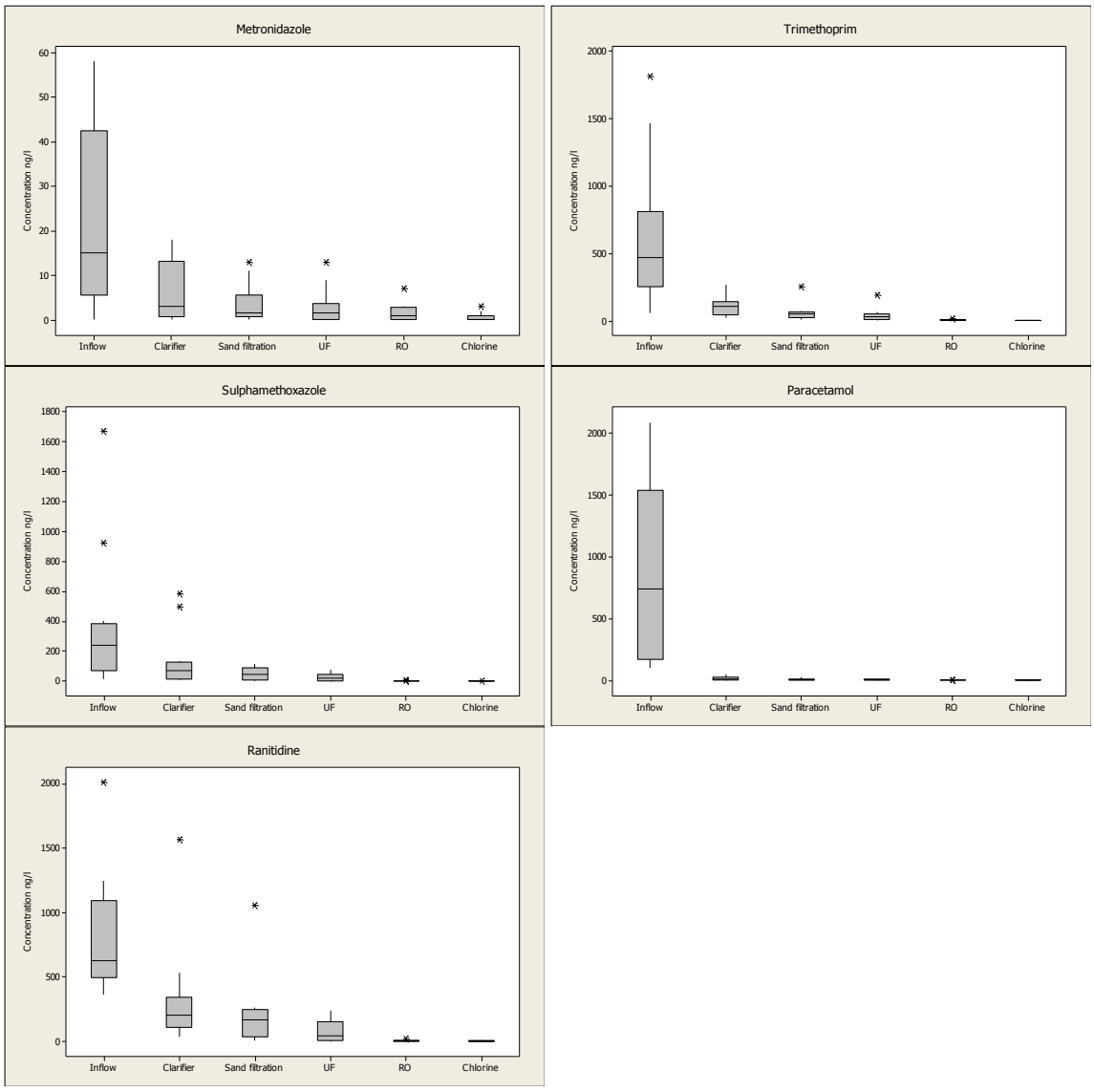


Figure 5.1.8: Box plot showing pharmaceutical removal efficiencies in Sulaibiya WWTP. Plots show the distribution of removal efficiencies, expressed in terms of fraction of pharmaceuticals remaining after each treatment compared to all before the treatment stage. The solid line in each box represents the median.

5.2 Removal of pharmaceuticals by chlorination and ozonation in laboratory investigations

5.2.1 Effect of chlorination and ozonation on pharmaceuticals in laboratory investigations

The removal percentage of each pharmaceutical at the end of a laboratory conducted experiment is shown in Figure 5.2.1 (a,b,c,d,e) and showed that removal varied: Metronidazole had the lowest removal and Ranitidine the highest by both chlorination and ozonation.

Results showed that Metronidazole was affected by the doses of chlorine and ozone oxidants in different media such as synthetic wastewater, tap water and real wastewater. The removal gradually increased with the increase in oxidant dose. The highest removal was 96% in tap water that contained a chlorine dose of 10 $mg.L^{-1}$ while, in synthetic wastewater and real wastewater with the same doses of chlorine, 59 and 58% removal was observed, respectively. On the other hand, the effect of ozone doses were similar, with the highest removal of Metronidazole occurring at 15 $mg.L^{-1}$ of ozone in synthetic wastewater 55%, and 51% being observed in tap water and real wastewater.

Trimethoprim almost completely disappeared in tap water and real wastewater at the 10 mgL^{-1} dose of chlorine, with 99% removal for both, while the removal rate at the same doses in synthetic wastewater gave 92% removal. On the other hand, the removal rate of Trimethoprim by ozonation was similar in synthetic wastewater, tap water and real water, with a removal rate >84% at the 15$mg.L^{-1}$ ozone dose.

Sulphamethoxazole was almost completely removed in the synthetic wastewater, tap water and real wastewater at all doses of chlorine, whereas the highest removal of 95% was observed at 15 $mg.L^{-1}$ dose of ozone. The removal of Sulphamethoxazole

in synthetic wastewater, tap water and real wastewater by 15 $mg.L^{-1}$ of ozone was comparable, with removal rates of 90%, 92% and 95%, respectively.

Paracetamol was removed from the synthetic wastewater, tap water and real wastewater at similar rates for the different doses of chlorine and ozone. The maximum removal was found at the 10 $mg.L^{-1}$ chlorine dose, with a rate of >96%, while the removal rate at the 15 $mg.L^{-1}$ ozone dose was 91%.

Ranitidine was removed more effectively than the other pharmaceuticals, being almost completely removed at different doses of chlorine and ozone, except for the 5 $mg.L^{-1}$ ozone dose where the removal rate was 71%, 73% and 75% for synthetic wastewater, tap water and real wastewater, respectively.

Chlorine and ozone are oxidation processes and have the potential to transform pharmaceuticals. Other researchers reported that Sulphamethoxazole, Trimethoprim and Paracetamol were efficiently removed by chlorination (Alum et al., 2004; Westerhoff et al., 2005). Ozone is known as a strong oxidant and is very effective in the transformation of Sulphamethoxazole, Roxithroymcin, Diclofenac, Naproxen and Steroids that can be oxidized at 90 - 99% (Ternes et al., 2002; Alum et al., 2004; Westerhoff et al., 2005; Huber et al., 2005). But in the current study it was found that chlorine is more effective than ozone for the chosen pharmaceuticals.

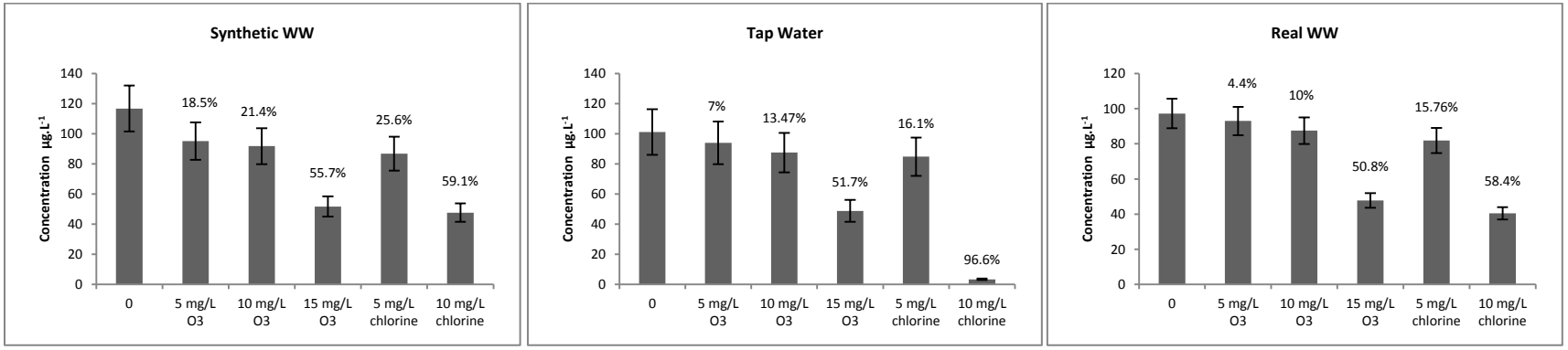


Figure 5.2.1.a: Effect of the initial chlorine and ozone dose on the Metronidazole removal or transformation in laboratory experiments performed at different doses (5mg.L^{-1} O_3, 10mg.L^{-1} O_3, 15mg.L^{-1} O_3, 5mg.L^{-1} Cl_2, and 10mg.L^{-1} Cl_2) with synthetic wastewater, tap water and real wastewater.

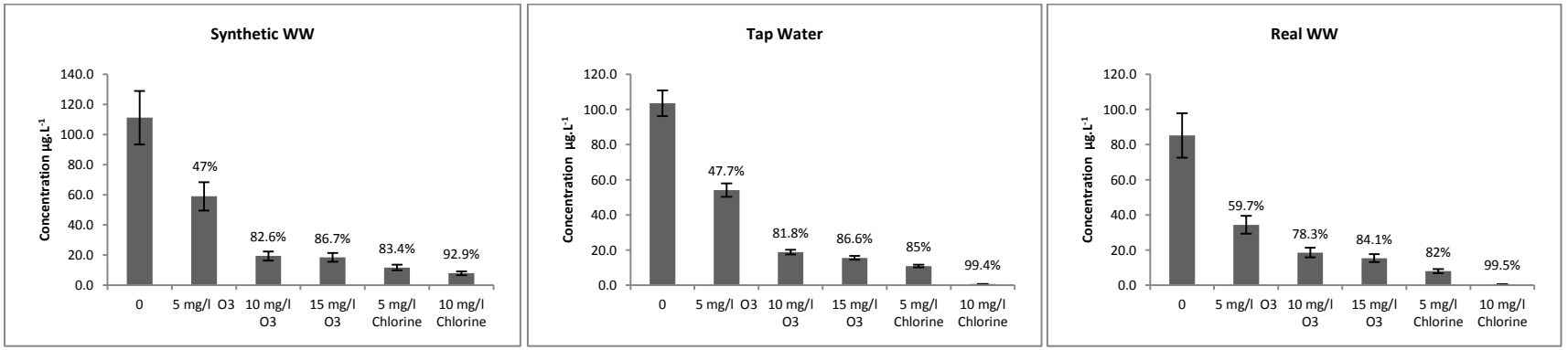


Figure 5.2.1.b: Effect of the initial chlorine and ozone dose on the Trimethoprim removal or transformation in laboratory experiments performed at different doses (5mg.L^{-1} O_3, 10mg.L^{-1} O_3, 15mg.L^{-1} O_3, 5mg.L^{-1} Cl_2, and 10mg.L^{-1} Cl_2) with synthetic wastewater, tap water and real wastewater.

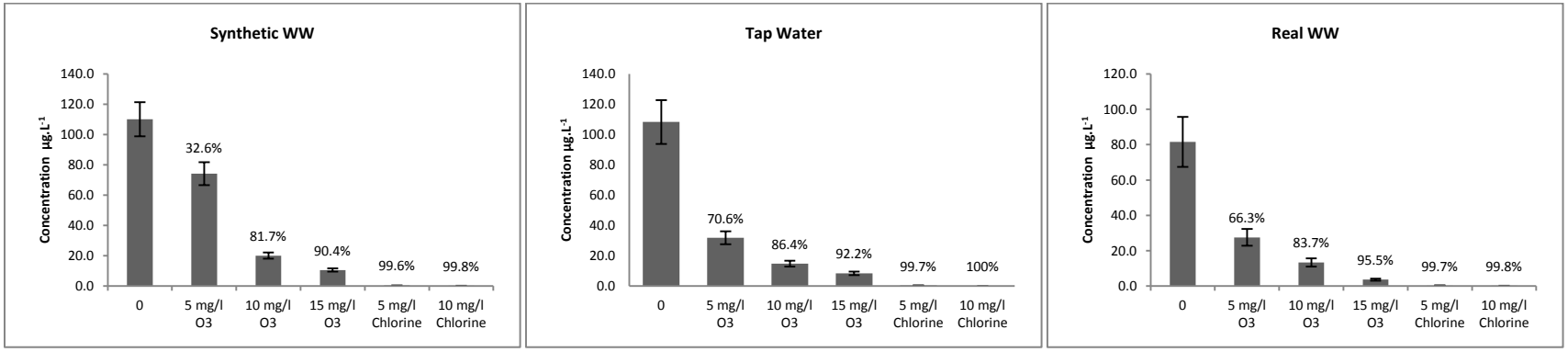


Figure 5.2.1.c: Effect of the initial chlorine and ozone dose on the Sulphamethoxazole removal or transformation in laboratory experiments performed at different doses (5mg.L^{-1} O_3, 10mg.L^{-1} O_3, 15mg.L^{-1} O_3, 5mg.L^{-1} Cl_2, and 10mg.L^{-1} Cl_2) with synthetic wastewater, tap water and real wastewater.

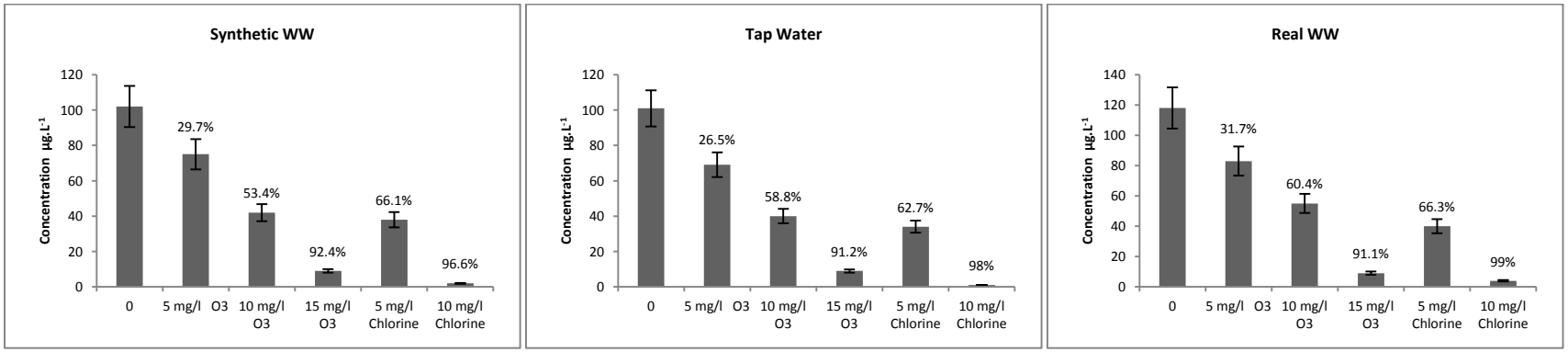


Figure 5.2.1.d: Effect of the initial chlorine and ozone dose on the Paracetamol removal or transformation in laboratory experiments performed at different doses (5mg.L^{-1} O_3, 10mg.L^{-1} O_3, 15mg.L^{-1} O_3, 5mg.L^{-1} Cl_2, and 10mg.L^{-1} Cl_2) with synthetic wastewater, tap water and real wastewater.

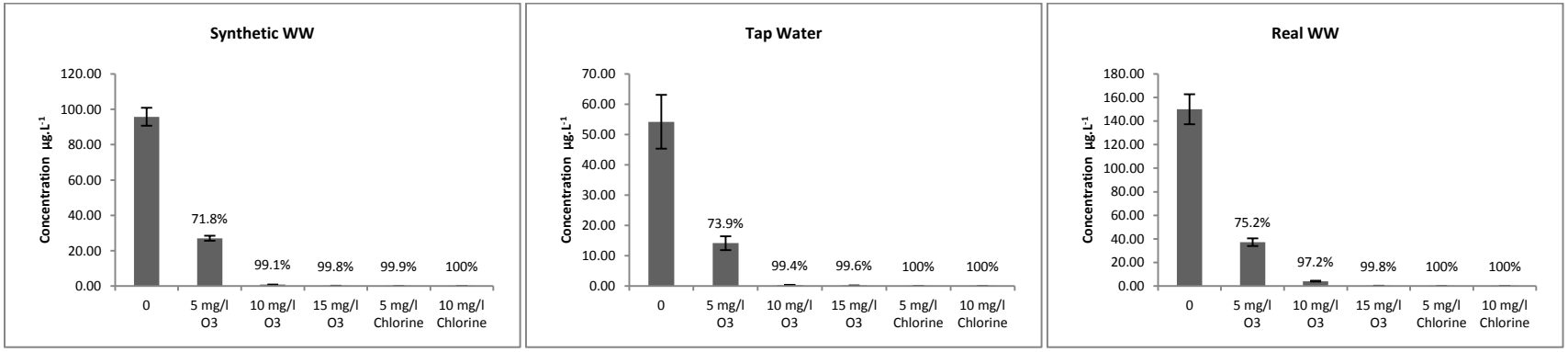


Figure 5.2.1.e: Effect of the initial chlorine and ozone dose on the Ranitidine removal or transformation in laboratory experiments performed at different doses (5mg.L^{-1} O_3, 10mg.L^{-1} O_3, 15mg.L^{-1} O_3, 5mg.L^{-1} Cl_2, and 10mg.L^{-1} Cl_2) with synthetic wastewater, tap water and real wastewater.

5.2.2 The degradation pathway of Chlorination

The effective removal of pharmaceuticals from water by chlorination requires sufficient free chlorine concentration and contact time. Chlorination can degrade or transform chemical compounds via one of two pathways; firstly, by chlorine substitution or addition reactions, which may alter active functional groups; and secondly, chlorine radicals may oxidize (break down) the target compounds, such as pharmaceuticals, into smaller molecules, which may or may not possess the active properties (Crain and Gottlieb, 1935).

A study by Gibs et al. (2007) on the effect of free chlorine on the transformation of some pharmaceutical compounds in drinking water during distribution, found 50%-80% removal for sulphonamides and 42% for Trimethoprim after one day, with complete removal after 10 days. At a concentration of 3.5 - 3.8 $mg.L^{-1}$ of free chlorine, 90% to 99% removal was achieved for Sulphamethoxazole and Trimethoprim in river water after 24 h contact time (Westerhoff et al., 2005). HOCl and ClO_2 oxidise Sulphamethoxazole at specific functional groups with high electron densities, such as neutral tertiary amines and aniline (Huber et al., 2005). On the other hand, rapid and substantial transformation of Trimethoprim to a wide range of chlorinated and hydroxylated products is expected to occur under typical conditions of wastewater and drinking water chlorination (Dodd and Huang, 2007). Therefore, chlorine appears to be effective at oxidising pharmaceuticals in the treated wastewater, but the formation of oxidative by-products appears to be likely toxic.

5.2.3 The degradation pathway of ozonation

Other researchers have reported the effective use of ozonation for the removal of pharmaceuticals in water and wastewater effluents (Adams et al., 2002; Ternes et al.,

2003; Huber et al., 2005). Adams et al. (2002) found that ozonation removed more than 95% of several sulphonamides and Trimethoprim from river water within 1.3 min contact time at an ozone dose of 7.1 $mg.L^{-1}$. Huber et al. (2005) also observed that at doses >2 $mg.L^{-1}$ of ozone, sulphonamides were oxidised 90% to > 99% in secondary wastewater effluents.

Oxidative degradation of organic chemicals by ozone treatment can occur either by direct reaction with molecular ozone (O_3) or indirectly via hydroxyl radicals (Staehelin and Hoigne, 1985). Dodd et al. (2006), through ozonation of wastewater, showed that many pharmaceuticals were predominantly transformed via direct reaction with the ozone. Furthermore, the oxidation reaction depended on the ratio of molecular ozone and hydroxyl radicals, the corresponding reaction kinetics, and presence of organic matter (Elovitz et al., 2000; von Gunten, 2003). Ozone and/or hydroxyl radicals deactivate the bactericidal properties of antibiotics by attacking or modulating their pharmaceutically active functional groups, such as aniline moieties of sulphonamides (Huber et al., 2005), and the phenol ring of Trimethoprim (Dodd et al., 2009). Highly effective removal (>90%) by ozonation was observed for those compounds with electron-rich aromatic systems, such as hydroxyl, amino (e.g. Sulphamethoxazole), acylamino, alkoxy and alkyl aromatic compounds, as well as those compounds with deprotonated amines (e.g. Trimethoprim) and nonaromatic alkene groups since these key structural moieties are highly vulnerable to oxidative attack (Dickenson et al., 2009).

Adams et al. (2002) found more than a 95% conversion of Trimethoprim by ozonation in a pre-filtered river water sample spiked with this antibiotic at an initial concentration of 50 $mg.L^{-1}$. Similar reactivity of Trimethoprim and Sulphamethoxazole by ozonation has been found in wastewater (Ternes et al., 2003). Ternes et al. (2003) confirmed that 5 mgL^{-1} of applied ozone could completely remove 0.62 $mg.L^{-1}$ Sulphamethoxazole present in biologically treated municipal

wastewater. Similar results were also reported elsewhere (Huber et al., 2003, 2005). Paracetamol was effectively degraded by ozone, which could degrade 0.8 $g.L^{-1}$ Paracetamol in 30 min with an ozone flow rate of about 72 $g.h^{-1}$ (Andreozzi et al., 2003). A number of degradation intermediates were found during the ozone treatment; these follow typical phenol ozonation pathways, such as hydroxylation of the phenol ring, anomalous ozonation to cleave the aromatic ring of hydroquinone, and decarboxylation by hydroxyl radicals (Andreozzi et al., 2003).

5.3 Fate of pharmaceuticals in batch experiments

5.3.1 The effect of pharmaceutical concentration on TOC removal

The effect of pharmaceutical concentration on the removal rate of organic substrates, as expressed by total organic carbon (TOC) and pharmaceuticals was investigated. Figure 5.2.1(a, b, c, d and e) shows that the decrease in TOC concentration with time was high during the first 10 hours, and gradually dropped between 10 to 25 hours before levelling off. This trend was observed in all cases, indicating a first-order kinetic model (Figure 5.3.2). Pharmaceutical concentration had a small effect on the degradation rate of TOC in the case of Metronidazole, Trimethoprim, and Sulphamethoxazole, but for Paracetamol and Ranitidine, the reduction in TOC concentration occurred more slowly for high drug concentrations compared to low drug concentrations. The effect of pharmaceutical concentration on TOC removal efficiency during batch experiments was highly significant for all pharmaceuticals ($p<0.05$, one-way ANOVA at a 95% confidence level).

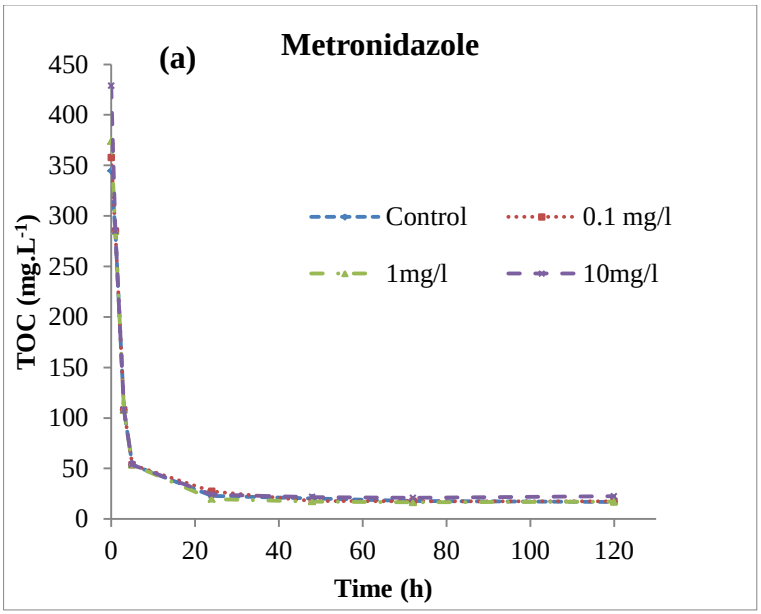


Figure 5.3.1(a): TOC removal in a batch experiment with Metronidazole present at concentrations of 0.1mg.L^{-1}, 1mg.L^{-1}, 10mg.L^{-1}, and control (0mg.L^{-1}).

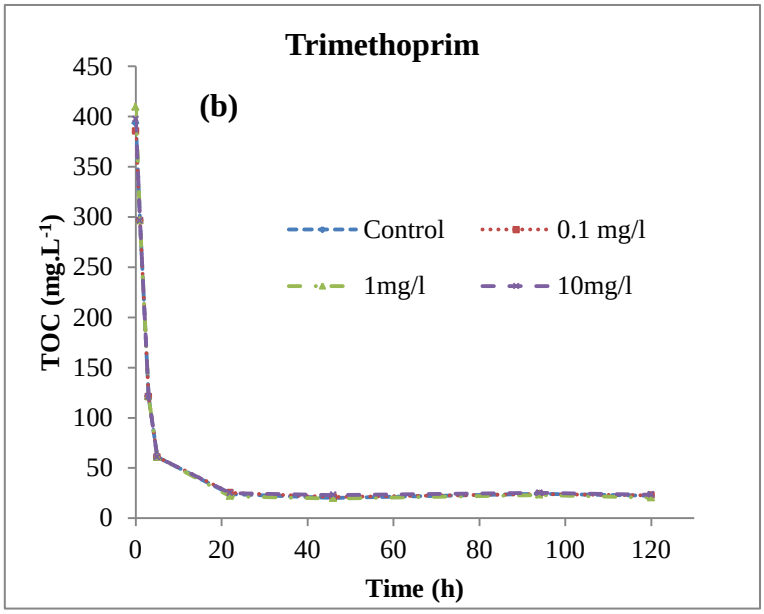


Figure 5.3.1(b): TOC removal in a batch experiment with Trimethoprim present at concentrations of 0.1mg.L^{-1}, 1mg.L^{-1}, 10mg.L^{-1}, and control (0mg.L^{-1}).

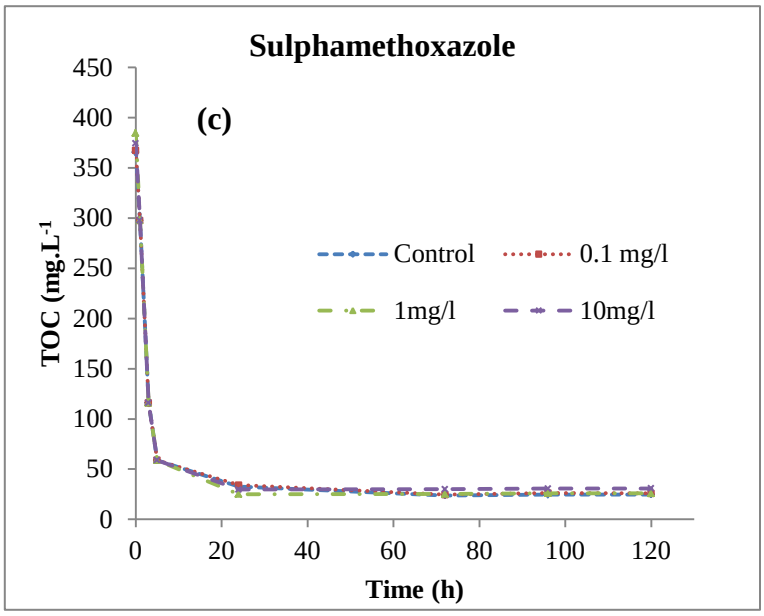


Figure 5.3.1(c): TOC removal in a batch experiment with Sulphamethoxazole present at concentrations of 0.1mg.L^{-1}, 1mg.L^{-1}, 10mg.L^{-1}, and control (0mg.L^{-1}).

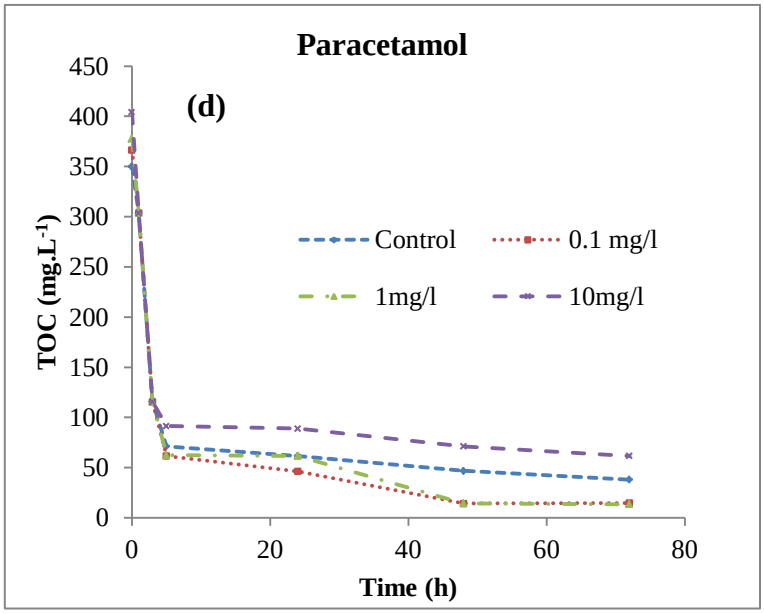


Figure 5.3.1(d): TOC removal in a batch experiment with Paracetamol present at concentrations of 0.1mg.L^{-1}, 1mg.L^{-1}, 10mg.L^{-1}, and control (0mg.L^{-1}).

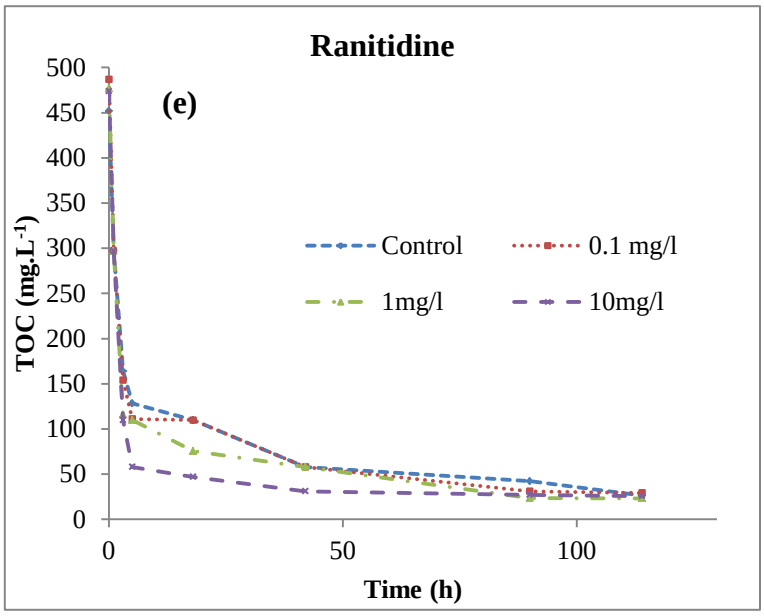


Figure 5.3.1(e): TOC removal in a batch experiment with Ranitidine present at concentrations of 0.1mg.L^{-1}, 1mg.L^{-1}, 10mg.L^{-1}, and control (0mg.L^{-1}).

The removal of TOC in the activated sludge process can be presented by a pseudo-first-order kinetic model as observed in Figure 5.3.1. Other researchers using batch experiments with activated sludge have reported that the removal of pharmaceuticals and other organic substances in the water phase can be described by a pseudo-first-order reaction, as given in Equation (1) (Layton et al., 2000; Shi et al., 2004; Li et al., 2005).

$$\frac{dC}{dt} = -kC \qquad (1)$$

where C is the concentration of the target substance in the water phase (mg.L^{-1}), k is the first-order-rate constant (h^{-1}) and t is the reaction time (h). Integration and rearrangement of equation (1), gives the following equation

$$\frac{C}{C_0} = e^{-kt} \qquad (2)$$

where C_0 is the initial concentration of the target substance in the water phase (mg.L^{-1}). This equation suggests that a plot of ln(C/C_0) versus t yields a straight line and the

first-order rate constant k is obtained from the slope of the straight line. The term $\ln(C/C_0)$ could be converted to $\log(C/C_0)$ to give k to the base 10.

The concentration profiles of TOC and pharmaceuticals as substrates in the first series of batch experiments plotted in the form of $\log(C/C_0)$ versus t are shown in Figure 5.3.2(a, b, c, d, and e). Linear relationships between $\log(C/C_0)$ and t were observed in all experiments, with a high correlation coefficient, confirming that the removal of organic substances in the current study followed the first-order kinetic model in Equation (1). The TOC removal rate constants obtained in the presence of each pharmaceutical at a concentration $1mg.L^{-1}$ over first 5 hours of reaction in Figure 5.3.2, were found to be 0.177, 0.166, 0.173, 0.170 and 0.176 (h^{-1}) for Metronidazole, Trimethoprim, Sulphamethoxazole, Paracetamol, and Ranitidine, respectively.

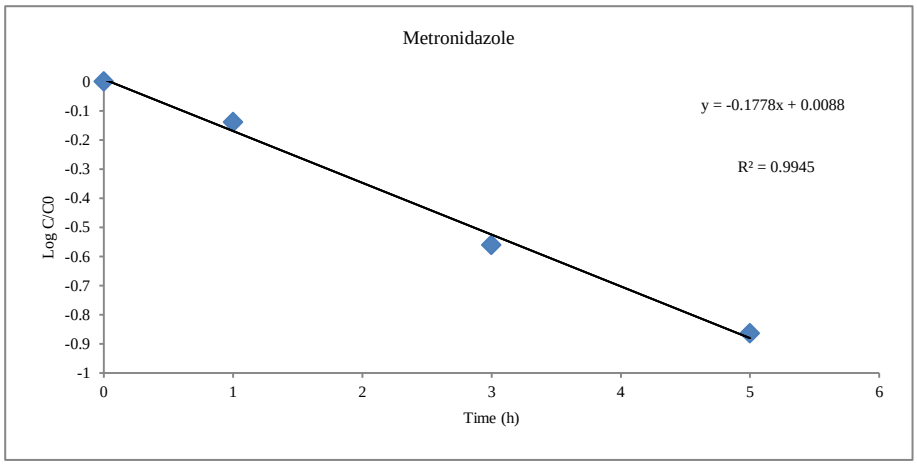


Figure 5.3.2 (a): Kinetic plots of TOC reduction verses time during batch experiments for Metronidazole at concentration 1mg.L^{-1}.

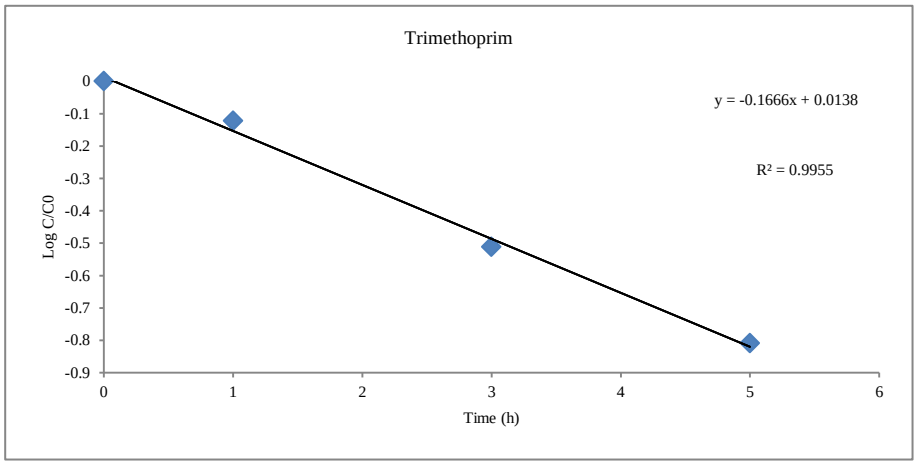


Figure 5.3.2 (b): Kinetic plots of TOC reduction verses time during batch experiments for Trimethoprim at concentration 1mg.L^{-1}.

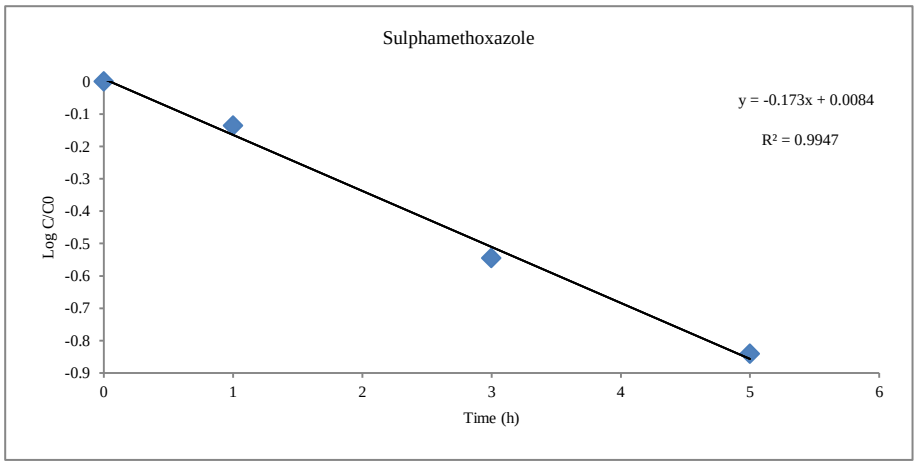


Figure 5.3.2 (c): Kinetic plots of TOC reduction verses time during batch experiments for Sulphamethoxazole at concentration 1mg.L^{-1}.

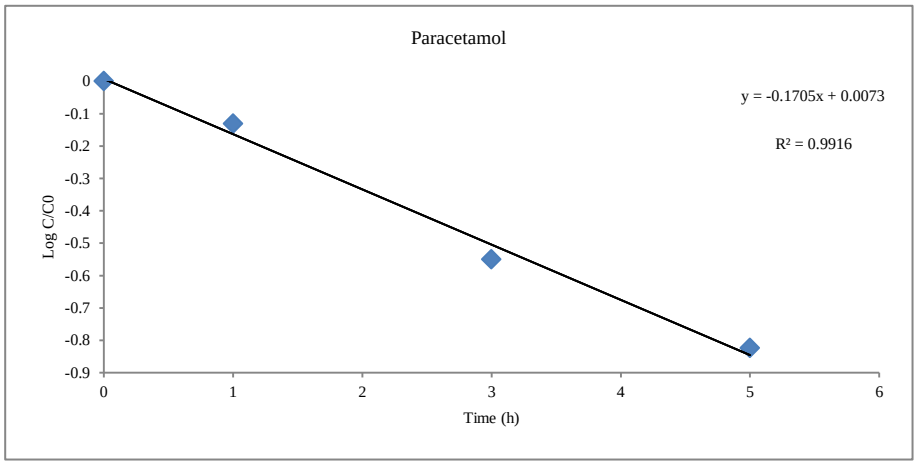


Figure 5.3.2 (d): Kinetic plots of TOC reduction verses time during batch experiments for Paracetamol at concentration 1mg.L^{-1}.

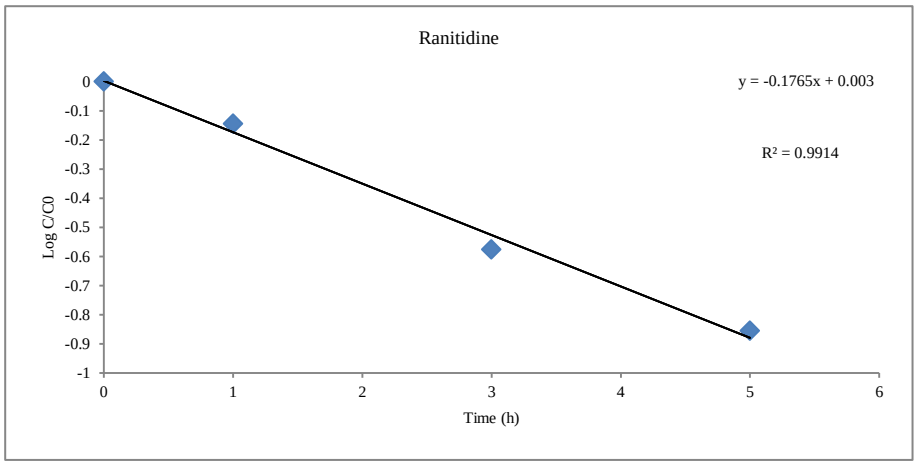


Figure 5.3.2 (e): Kinetic plots of TOC reduction verses time during batch experiments for Ranitidine at concentration 1mg.L^{-1}.

5.3.2 The Effect of Pharmaceutical Concentration on biomass growth (MLSS)

The air flow rate to each reactor was kept constant at 96 L.h^{-1}. This was sufficient to maintain DO concentration in each reactor above the 2 mg.L^{-1} required for aerobic biodegradation. The MLSS growth was increased steadily and rapidly for all pharmaceuticals at all concentrations in the first period; then, the MLSS concentration became steady, except for Ranitidine where MLSS decreased later for all concentrations as shown in Figure 5.3.3 (a, b, c, d and e).

There was no clear effect of Metronidazole, Trimethoprim, Sulphamethoxazole, and Paracetamol concentration on the biomass concentration and biodegradation rate at different concentrations (0.1, 1 and 10 mg.L^{-1}) (p>0.2 ANOVA). Since MLSS growth decreased with the increase of the Ranitidine concentration that may indicate an inhibitory effect on the microorganisms in the MLSS (p=0.11 ANOVA). It can be seen that MLSS concentration has a great effect on the biodegradation rates (Figure 5.3.3) with the increase of MLSS concentration being attributed to the growth of microorganisms in the activated sludge, which then improved organic degradation. Furthermore, the increase in MLSS with the increase of pharmaceutical concentration could be explained by the utilisation of such pharmaceutical by

microorganisms as the sole carbon source. This might also contribute to the increase in the degradation rates of the pharmaceuticals (Table 5.3.1) with the increase of MLSS concentration.

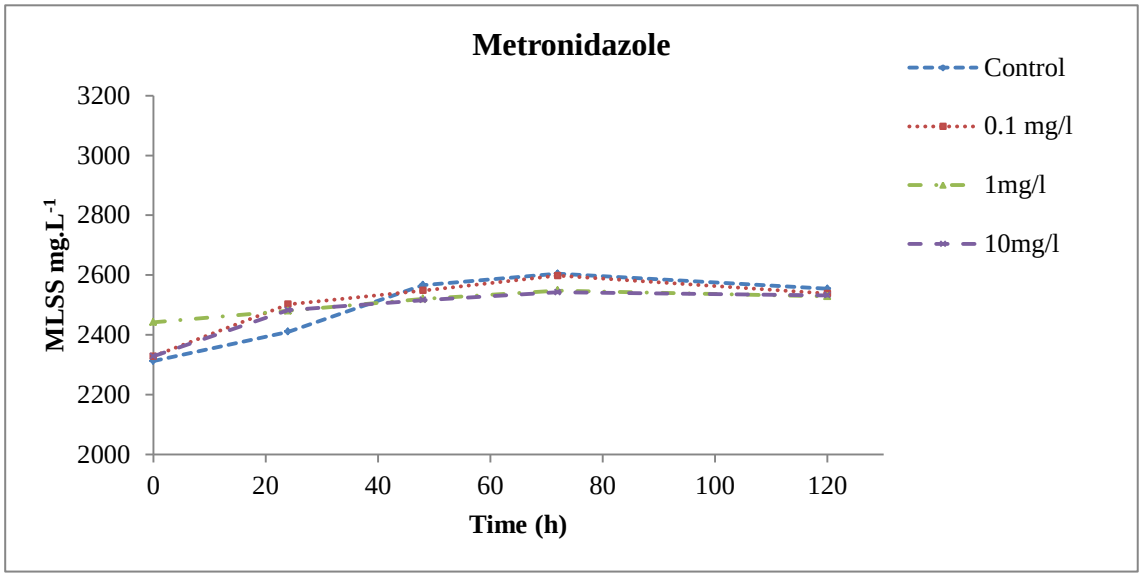


Figure 5.3.3 (a): The effect of Metronidazole concentration on MLSS growth in batch experiment.

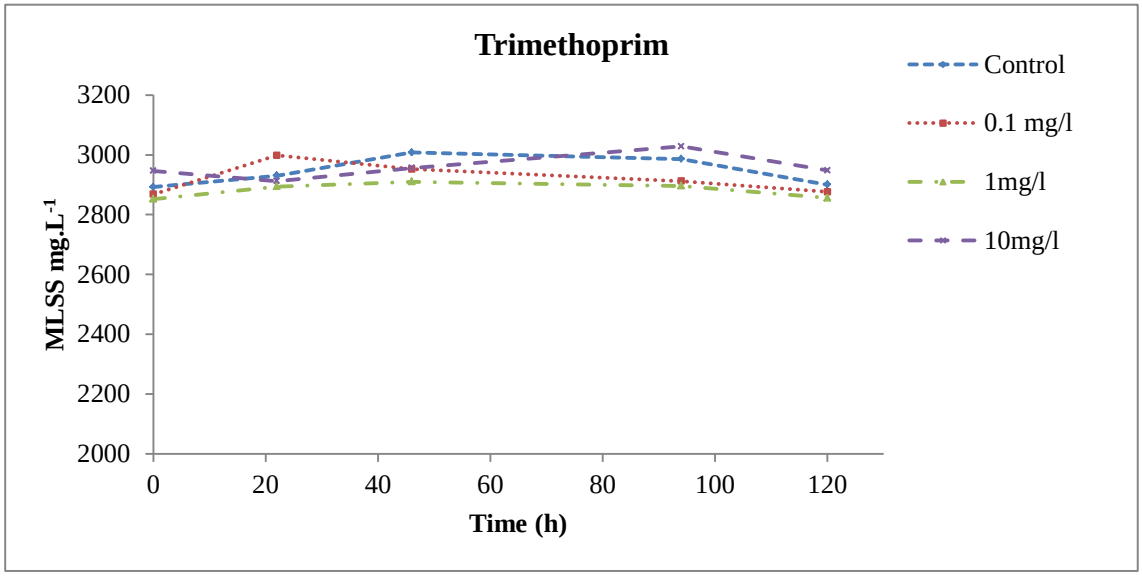


Figure 5.3.3 (b): The effect of Trimethoprim concentration on MLSS growth in batch experiment.

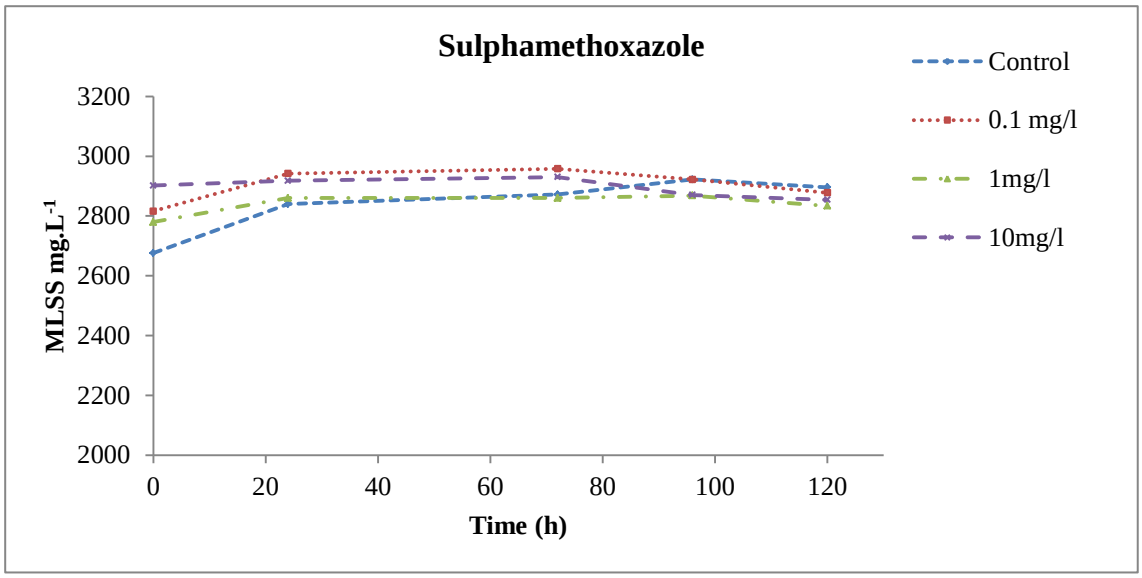


Figure 5.3.3 (c): The effect of Sulphamethoxazole concentration on MLSS growth in batch experiment.

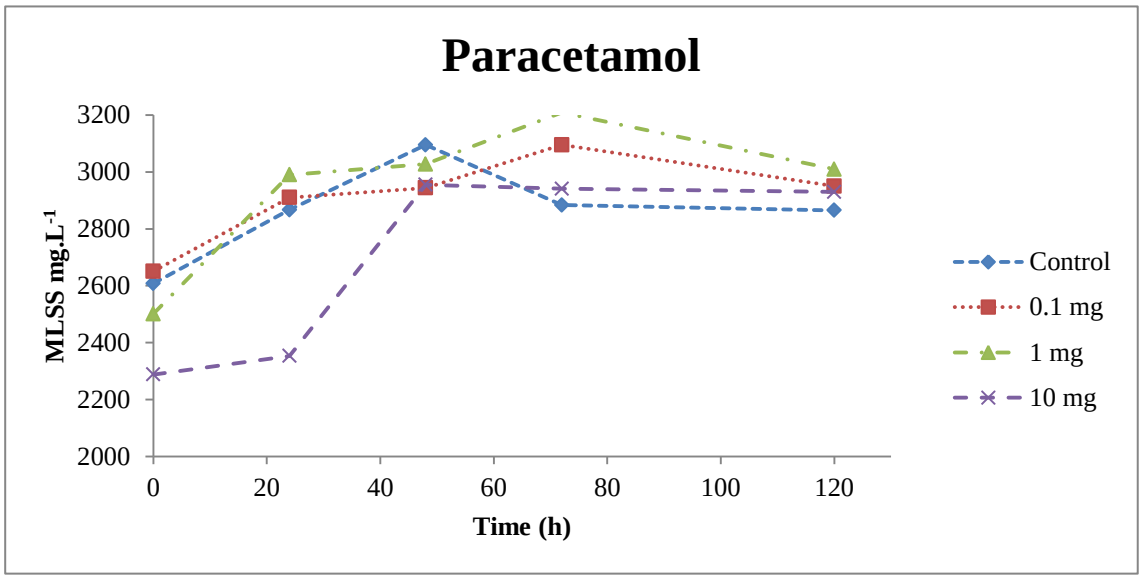


Figure 5.3.3 (d): The effect of Paracetamol concentration on MLSS growth in batch experiment.

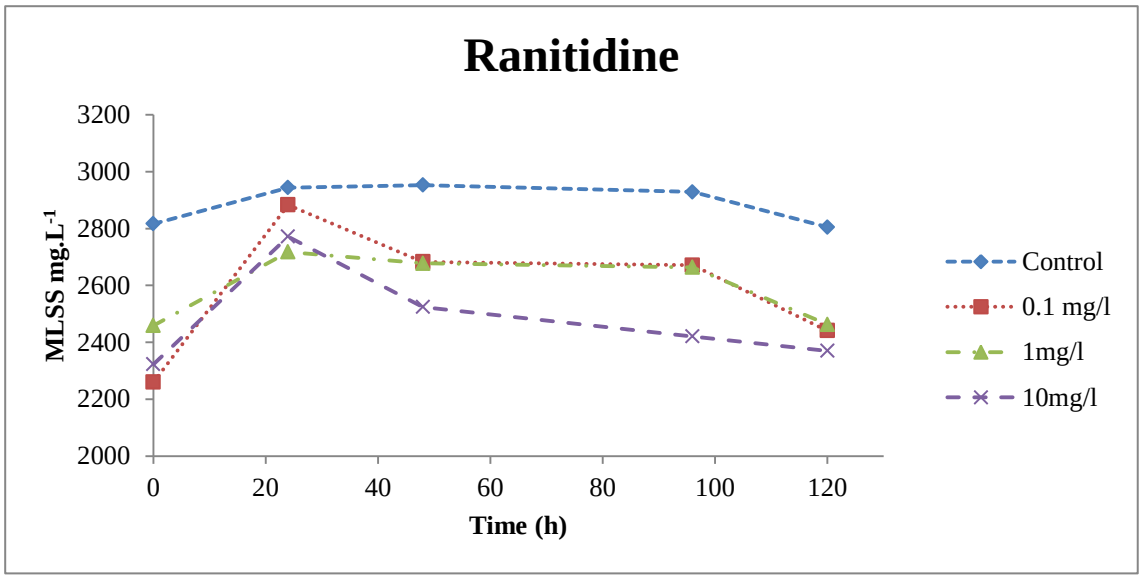


Figure 5.3.3 (e): The effect of Ranitidine concentration on MLSS growth in batch experiment.

5.3.3 Variations in pH

At 25 °C approximately all pH values at the starting time for all batch reactors were within the range of 6 – 7, then pH decreased in relation to time to reach a minimum of pH 5.5 as shown in Figure 5.3.4 (a, b, c, d and e). The pH values were the same at all concentrations of each pharmaceutical compared with the control. Nitrobacter and other types of bacteria may have been enriched causing a nitrification process in the batch reactor. Consequently, due to the nitrification process (release of H+), pH values decreased to less than 7 during the last 5 days of operation in all reactors, where the biomass concentrations stay steady (Figure 5.3.3).

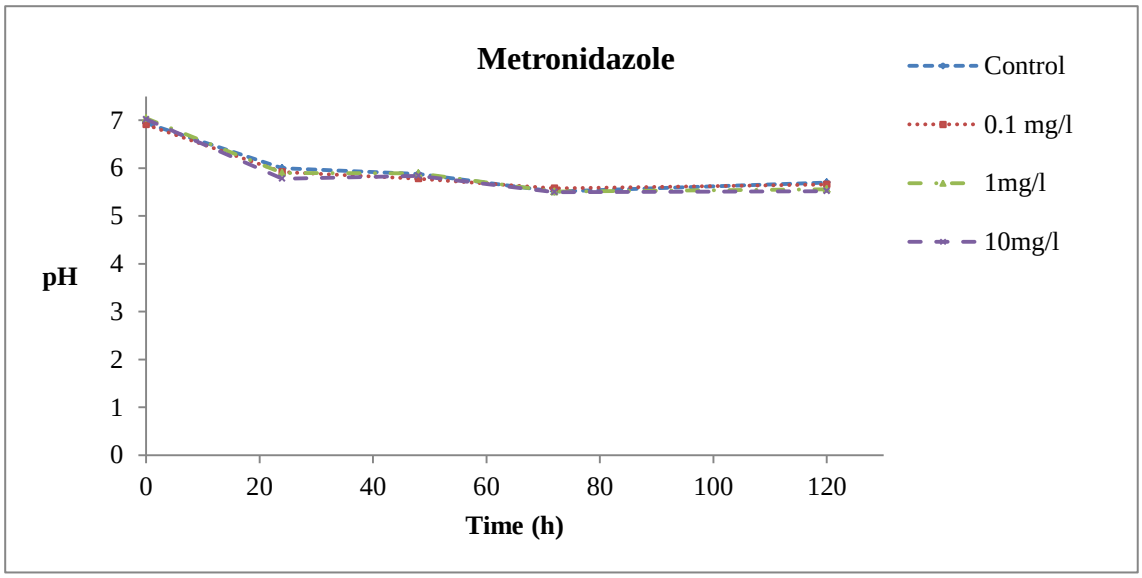


Figure 5.3.4 (a): pH measurement during the batch experiment of Metronidazole at different concentrations.

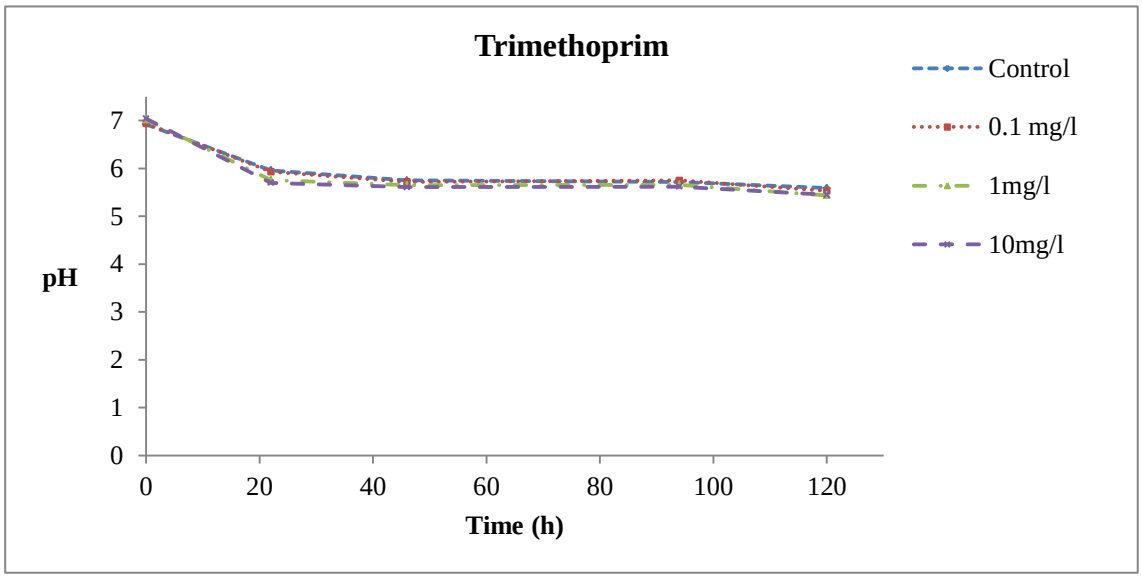


Figure 5.3.4 (b): pH measurement during the batch experiment of Trimethoprim at different concentrations.

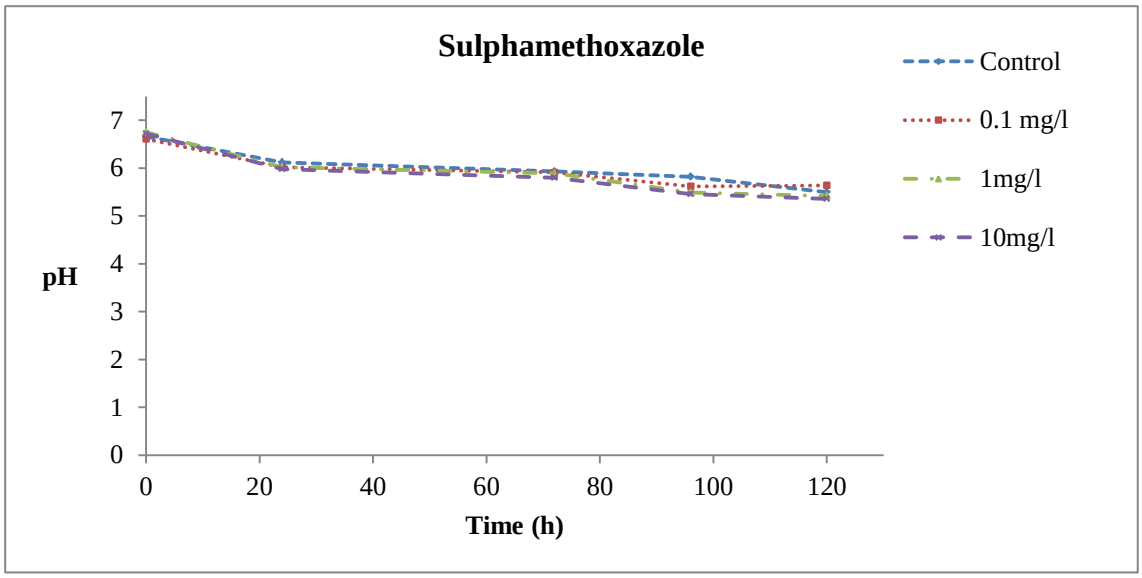


Figure 5.3.4 (c): pH measurement during the batch experiment of Sulphamethoxazole at different concentrations.

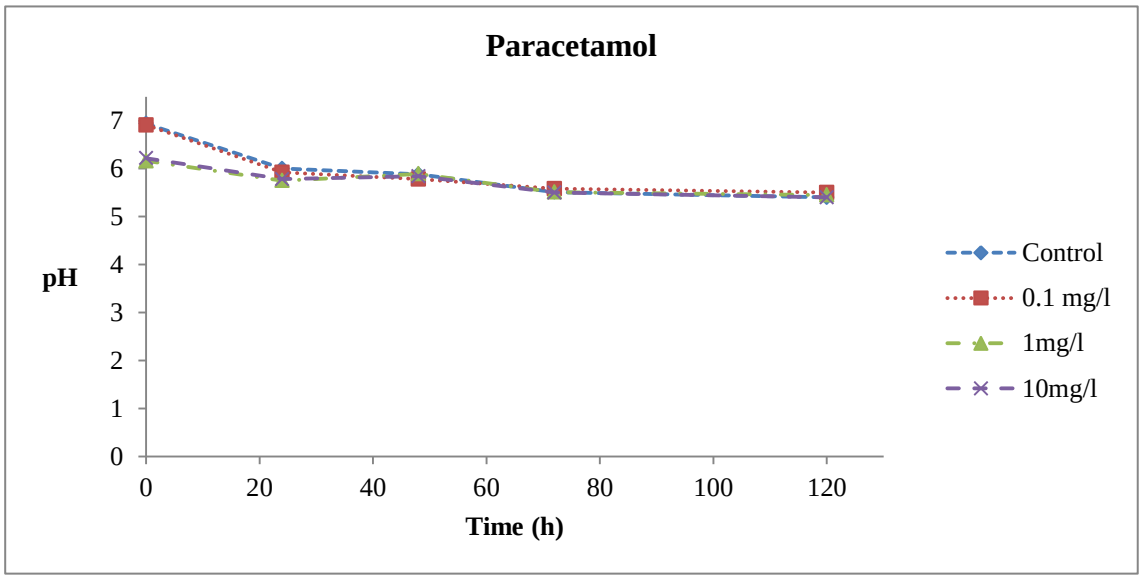


Figure 5.3.4 (d): pH measurement during the batch experiment of Paracetamol at different concentrations.

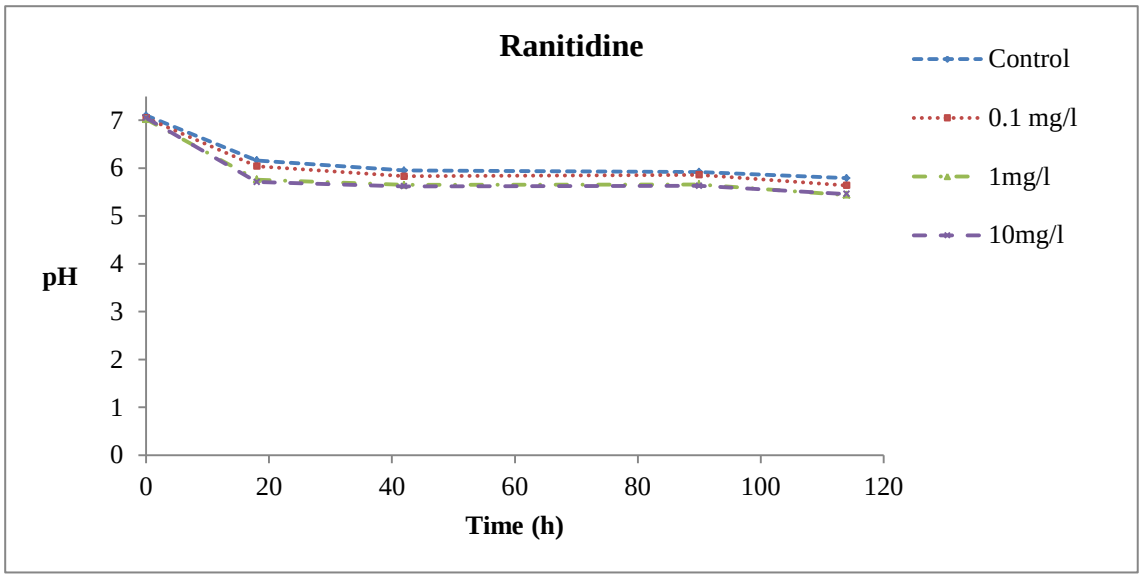


Figure 5.3.4 (e): pH measurement during the batch experiment of Ranitidine at different concentrations.

5.3.4 The removal rate of pharmaceuticals during batch experiments

In wastewater, pharmaceuticals exist at extremely low concentrations ($ng.L^{-1}$), and many other organic compounds are present at much higher concentrations ($mg.L^{-1}$). In this study, the initial concentrations of pharmaceuticals ranged from 0.1 to 10 $mg.L^{-1}$. The removal of pharmaceuticals during the batch experiments was high, and decreased with increasing concentration, except for Paracetamol where the removal % did not decrease (Figure 5.3.5). The effect of the pharmaceuticals' concentration on its removal efficiency was highly significant ($p<0.05$, ANOVA). Pharmaceuticals could be immediately removed from the water phase to the sludge phase by sorption as a first step, and then further removal of the pharmaceutical progresses gradually with biodegradation.

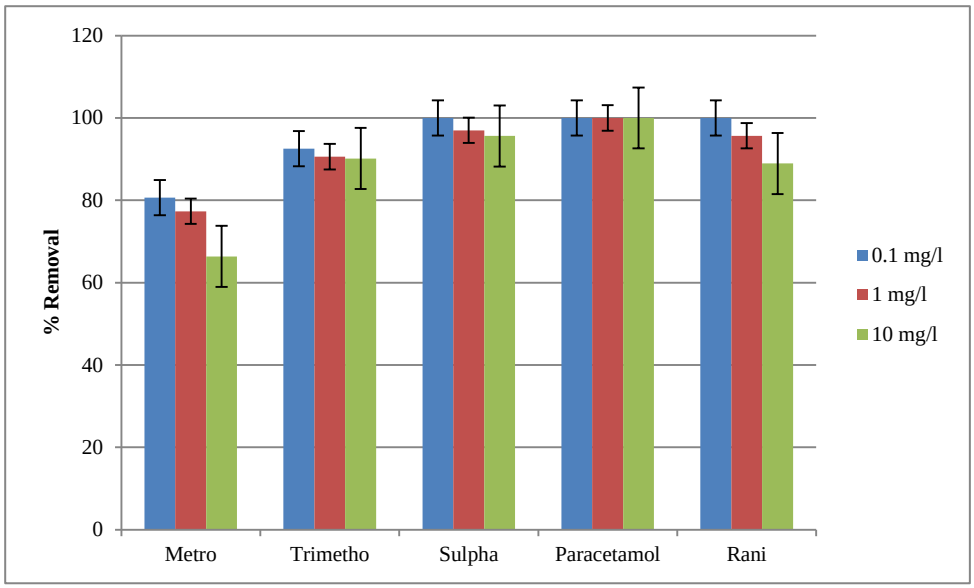


Figure 5.3.5: Removal efficiency of pharmaceuticals after 5 days at concentration 0.1 mg.L^{-1}, 1 mg.L^{-1} and 10 mg.L^{-1}in batch experiments.

The % degradation of pharmaceuticals by microorganisms over 5 days depends on the initial concentration of the pharmaceutical. This is shown by a clear trend that generally exists between % degradation over 5 days of Metronidazole, Trimethoprim, Sulphamethoxazole, Paracetamol, and Ranitidine and increases in the initial concentration of pharmaceutical in the range from 0.1 to 10 mg.L^{-1} (Table 5.3.1). The biodegradation of pharmaceuticals increased with an increase in the initial concentration of these pharmaceuticals and the regression line having an R^2 value of > 0.99, except for Trimethoprim (R^2 = 0.61), indicating no apparent inhibition of pharmaceuticals on their biodegradation (Figure 5.3.6). These results suggest that the higher the initial pharmaceutical concentrations the greater the stimulatory effect on the microorganisms degrading them in activated sludge; there was no inhibitory effect at higher concentrations (p>0.2 ANOVA). The TOC biodegradation rate also increased with the increase of the initial concentration of pharmaceuticals (Table 5.3.1), indicating that the bacteria could utilize TOC more efficiently at higher initial concentrations of pharmaceuticals. Generally, the results show pharmaceuticals are readily degraded over a range of initial concentrations from 0.1 to 10 mg.L^{-1}.

Table 5.3.1: Pharmaceutical concentrations and their effect on the specific TOC biodegradation rate and on the specific drug biodegradation rate.

Drug	Pharmaceutical Concentration ($mg.L^{-1}$)	Specific TOC Biodegradation rate $mg_{TOC}.mg_{MLSS}^{-1}.\ d^{-1}$	Specific pharmaceutical Biodegradation rate $mg_{Ph}.mg_{MLSS}^{-1}.\ d^{-1}$
Metronidazole	Control	0.136	-
	0.1	0.136	$2.56x10^{-5}$
	1	0.146	$13.7x10^{-5}$
	10	0.168	$71.6x10^{-5}$
Trimethoprim	Control	0.125	-
	0.1	0.122	$1.3x10^{-5}$
	1	0.136	$22.9x10^{-5}$
	10	0.127	$30x10^{-5}$
Sulphamethoxazole	Control	0.120	-
	0.1	0.118	$3.52x10^{-5}$
	1	0.124	$15.8x10^{-5}$
	10	0.127	$79.3x10^{-5}$
Paracetamol	Control	0.101	-
	0.1	0.115	$3.6x10^{-5}$
	1	0.114	$36.4x10^{-5}$
	10	0.134	$430.9x10^{-5}$
Ranitidine	Control	0.118	-
	0.1	0.146	$3.75x10^{-5}$
	1	0.155	$34x10^{-5}$
	10	0.167	$261.6x10^{-5}$

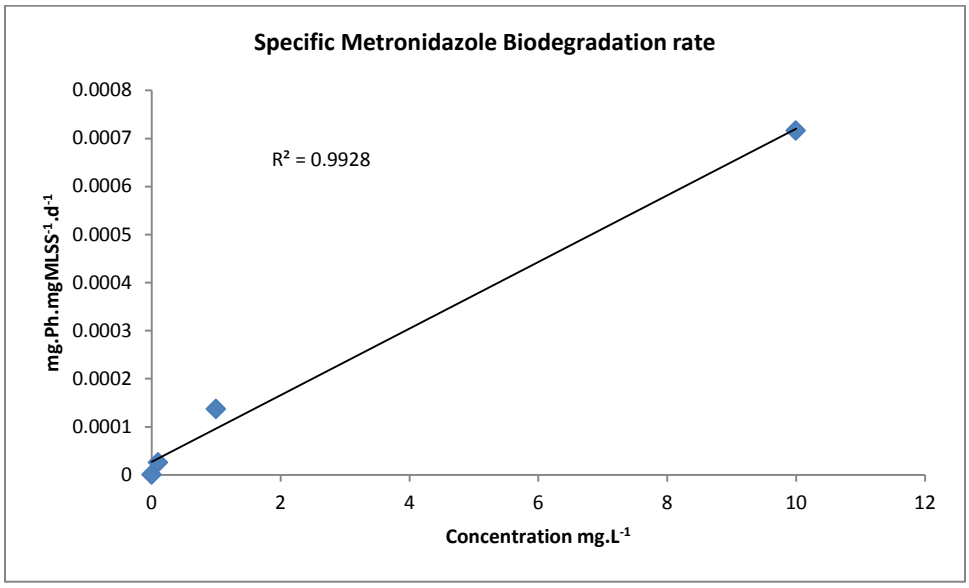


Figure 5.3.6 (a): The regression line comparing the Metronidazole concentration and the specific Metronidazole biodegradation rate ($mg_{Ph}.mg_{MLSS}^{-1}.d^{-1}$).

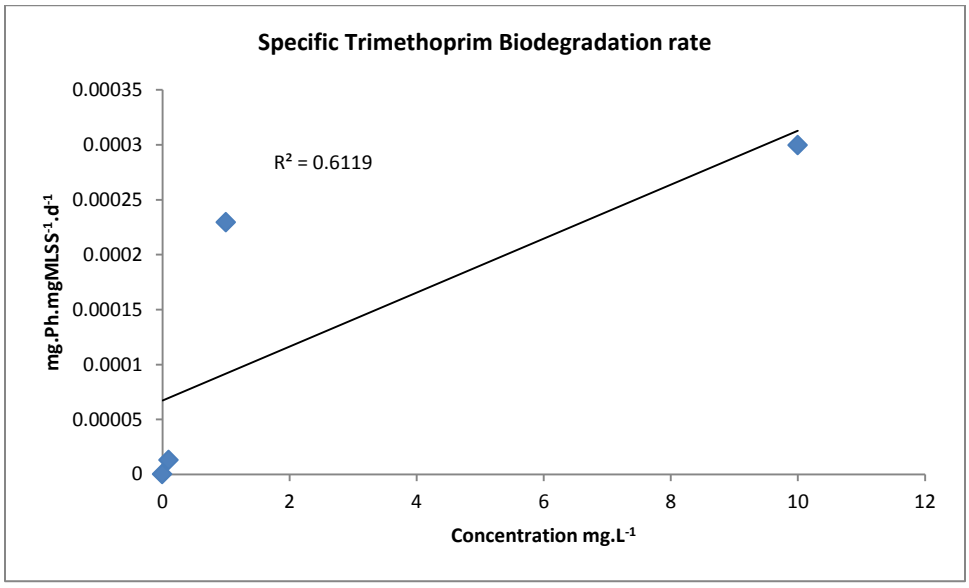


Figure 5.3.6 (b): The regression line comparing the Trimethoprim concentration and the specific Trimethoprim biodegradation rate ($mg_{Ph}.mg_{MLSS}^{-1}.d^{-1}$).

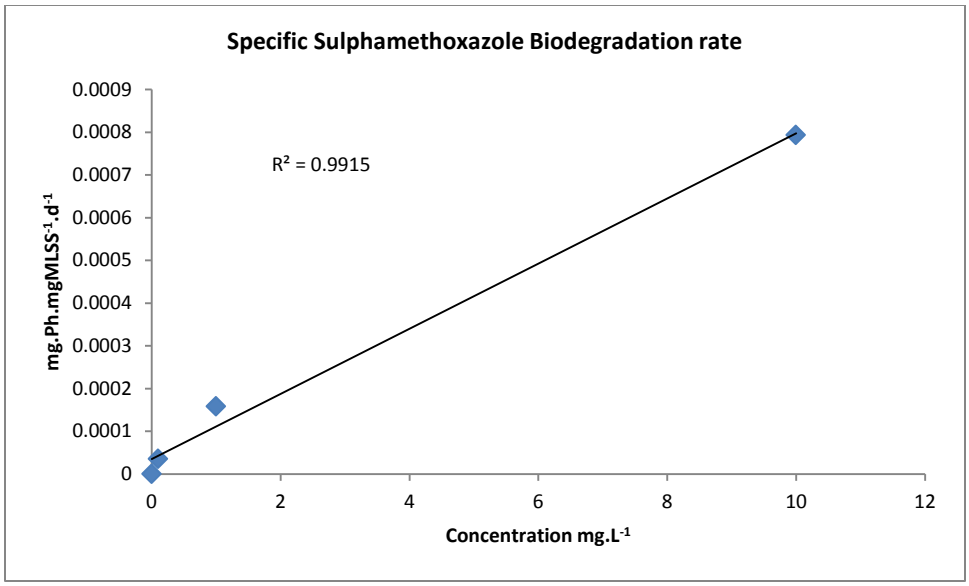


Figure 5.3.6 (c): The regression line comparing the Sulphamethoxazole concentration and the specific Sulphamethoxazole biodegradation rate ($mg_{Ph}.mg_{MLSS}^{-1}.d^{-1}$).

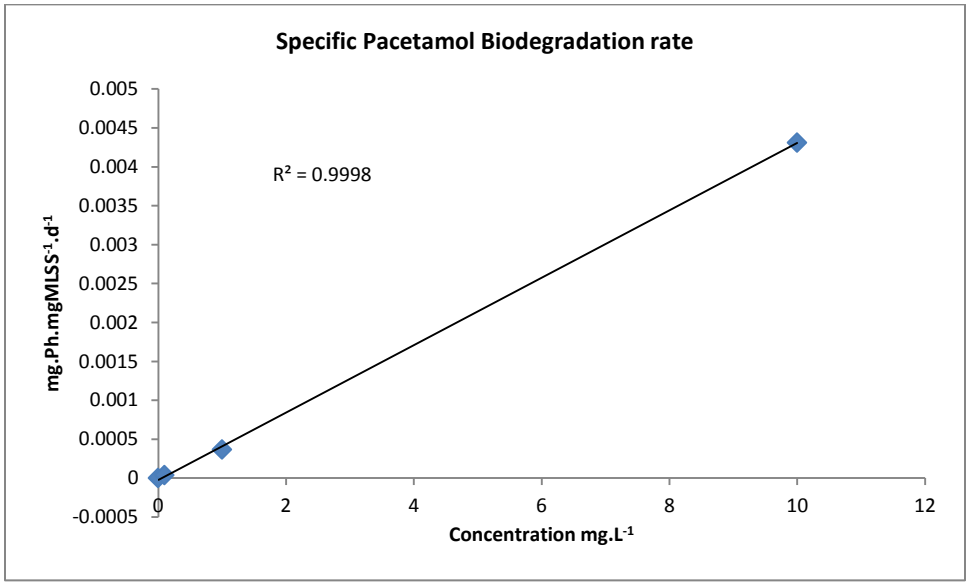


Figure 5.3.6 (d): The regression line comparing the Paracetamol concentration and the specific Paracetamol biodegradation rate ($mg_{Ph}.mg_{MLSS}^{-1}.d^{-1}$).

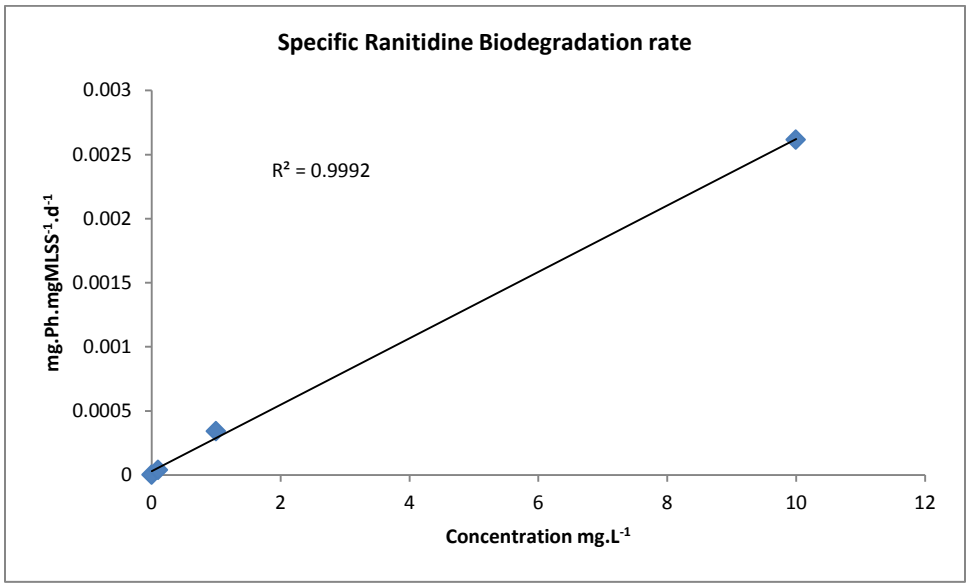


Figure 5.3.6 (e): The regression line comparing the Ranitidine concentration and the specific Ranitidine biodegradation rate ($mg_{Ph}.mg_{MLSS}^{-1}.\ d^{-1}$).

5.3.5 Discussion

The effect of Metronidazole concentration within the range 0.1 to 10 $mg.L^{-1}$ on the TOC removal efficiency was negligible with an average TOC removal of 95% over 5 hours (Figure 5.3.1a). Furthermore, the MLSS was similar at all concentrations which reveals that there are similar trends of microbial growth at all Metronidazole concentrations, while the Metronidazole removal efficiency decreased with an increased Metronidazole concentration with an average removal of 80%. On the other hand, the specific TOC biodegradation rate ($mg_{TOC}.mg_{MLSS}^{-1}.d^{-1}$) and the specific Metronidazole biodegradation rate ($mg_{Metronidazole}.mg_{MLSS}^{-1}.d^{-1}$) increased linearly with the increase of drug concentration with correlation coefficient (R^2) of 0.94 and 0.99 respectively. In contrast, Ingerslev et al. (2001) reported that the biological treatment to remove Metronidazole required long periods of treatment, and the Metronidazole removal efficiencies obtained were usually very low. A study by Kummerer et al. (2000) revealed that Metronidazole was not eliminated during batch experiments. On the other hand, Metronidazole reduction seems to be faster than reported by Ingerslev et al. (2001).

Trimethoprim concentration within the range 0.1 to 10 $mg.L^{-1}$ had little effect on the TOC removal efficiencies with an average removal of 94% over 5 hours (Figure 5.3.1b). During the batch experiment, the MLSS concentration was almost similar at all Trimethoprim concentrations; however, the MLSS growth rate (Figure 5.3.7) increased with an increase in drug concentration with high correlation ($R^2 = 0.93$). Furthermore, the Trimethoprim removal efficiency decreased with an increase in Trimethoprim concentration, achieving a maximum 92% removal after 24 hours. On the other hand, the specific TOC biodegradation rate ($mg_{TOC}.mg_{MLSS}^{-1}.d^{-1}$) remained constant with the increase in Trimethoprim concentration, while the specific Trimethoprim biodegradation rate ($mg_{Trimethoprim}.mg_{MLSS}^{-1}.d^{-1}$) increased sharply between 0.1 and 1 $mg.L^{-1}$ and then, slowly increased up to the 10 $mg.L^{-1}$ dose concentration. This is in agreement with results reported by Celiz et al. (2009) where

Trimethoprim was effectively eliminated by biological treatment at up to 97%. This compound has been observed to have a high biodegradation rate in conventional activated sludge systems, which involve a nitrification process (Batt et al., 2006). The removal of Trimethoprim by sludge adsorption can be considered to be negligible because of its high water solubility and very low log K_{ow}. Consequently, it is likely that the high removal efficiency of Trimethoprim can be attributed to biodegradation. On the other hand, Pérez et al. (2005) and Yu et al. (2011) reported that Trimethoprim exhibited high adsorptivity and low biodegradability in the activated sludge process. They concluded that more than 40% of the substance was removed from the aqueous phase by bio-sorption or sorption, while there was only 27% removal via biodegradation.

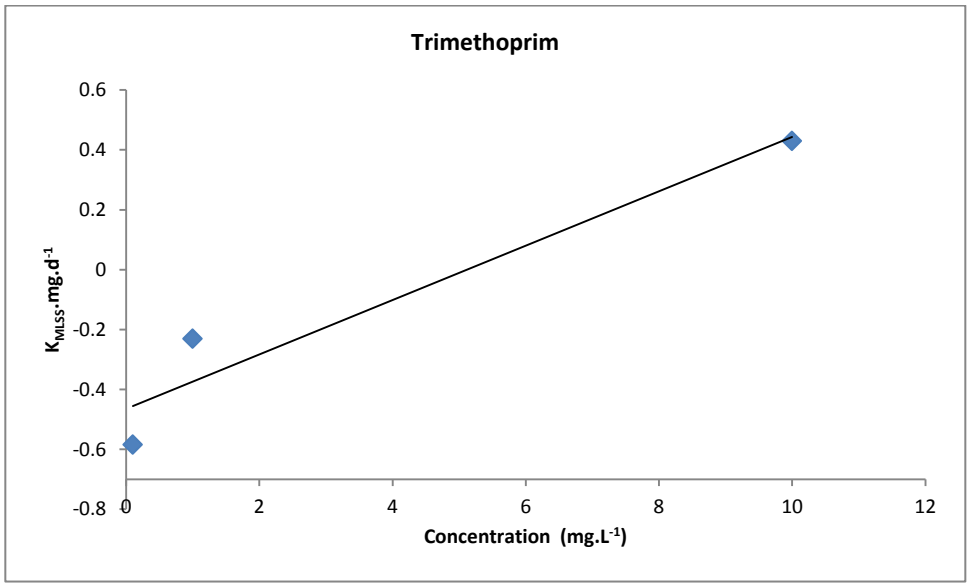


Figure 5.3.7: regression line comparing the Trimethoprim concentration and MLSS growth rate (k).

The effect of increases in Sulphamethoxazole concentration within the range 0.1 to 10 mg.L^{-1} on the TOC removal efficiency was negligible, and the average TOC removal was 92% over 5 hours (Figure 5.3.1c). During the batch experiment, the

MLSS concentration was almost the same at all concentrations which reveals no effect on increasing Sulphamethoxazole concentration, while at a dose concentration of 0.1 $mg.L^{-1}$ the Sulphamethoxazole was removed to an undetectable concentration. However, the Sulphamethoxazole removal efficiencies remained high even with the increase in Sulphamethoxazole concentration, achieving 95% removal at the 10 $mg.L^{-1}$ dose. On the other hand, the specific TOC biodegradation rates ($mg_{TOC}.mg_{MLSS}^{-1}.d^{-1}$) sharply increased between 0.1 and 1 $mg.L^{-1}$, then gradually increased to 10 $mg.L^{-1}$ Sulphamethoxazole concentration; whereas, the specific Sulphamethoxazole biodegradation rates ($mg_{Sulphamethoxazole}.mg_{MLSS}^{-1}.d^{-1}$) were increased linearly with an increase in Sulphamethoxazole concentration, with a R^2correlation value of 0.99. These results are in agreement with those of Drillia et al. (2005) who found that Sulphamethoxazole removal efficiency was very high, even when Sulphamethoxazole concentration in the feed was increased up to 383 $mg.L^{-1}$. Several studies reported that biodegradation of Sulphamethoxazole was the dominant removal mechanism in biological treatment systems (Batt et al., 2007; Abegglen et al., 2009; Wu et al., 2009; Li and Zhang, 2010). On the other hand, other researchers reported that Sulphamethoxazole was fairly well biodegraded and weakly sorbed to the bio-carriers and the removal efficiency via biodegradation of Sulphamethoxazole was 59% where the removal efficiencies via bio-sorption was 31% (Yu et al., 2011).

Paracetamol concentration within the range 0.1 to 10 $mg.L^{-1}$ exhibited a decreasing TOC removal efficiency with a minimum of 84% removal at 10 $mg.L^{-1}$ dose over 5 hours (Figure 5.3.1d). During the batch experiment, the MLSS growth rate gradually increased with an increase in Paracetamol concentration (correlation R^2 = 0.99). Furthermore, the Paracetamol was removed effectively to undetectable levels at all concentrations studied. The removal efficiencies remained high, even with an increase in Paracetamol concentration, achieving almost 100% removal at all Paracetamol concentrations. On the other hand, the specific TOC biodegradation rates ($mg_{TOC}.mg_{MLSS}^{-1}.d^{-1}$) increased linearly with an increase in Paracetamol concentration (correlation R^2 = 0.99), while the specific Paracetamol biodegradation

rate ($mg_{Paracetamol}.mg_{MLSS}^{-1}.d^{-1}$) increased with an increase in Paracetamol concentration (correlation $R^2 = 1$). Joss et al. (2006) reported similar results from batch biodegradation experiments, showing that biological degradation of Paracetamol removed the drug at 90% efficiency, and a kinetic biodegradation constant was greater than10 $L.g_{ss}^{-1}.d^{-1}$. Furthermore, Jones et al. (2006) found no adsorption of Paracetamol on activated sludge. The removal results of Paracetamol obtained in this study agree with those reported by Ivshina et al. (2006) and Takenaka et al. (2003).

The effect of an increase in Ranitidine concentration on reactor performance was negligible within the range 0.1 to 10 $mg.L^{-1}$. The TOC removal results were almost constant for all concentrations studied and reached 94% over 5 hours (Figure 5.3.1e). The MLSS concentration was similar at all concentrations of Ranitidine, which showed no effect of higher Ranitidine concentrations on MLSS concentrations, although the growth rate decreased gradually with an increase in concentrations (correlation $R^2 = 0.87$). Furthermore, the removal efficiency of Ranitidine decreased from 100% to 88% with the increase in Ranitidine concentration (correlation $R^2 = 0.90$); whereas, the specific TOC biodegradation rates ($mg_{TOC}.mg_{MLSS}^{-1}.d^{-1}$) increased slightly with an increase in Ranitidine concentration, while the specific Ranitidine biodegradation rate ($mg_{Ranitidine}.mg_{MLSS}^{-1}.d^{-1}$) gradually increased with increased Ranitidine concentration (correlation $R^2 = 0.99$). Carucci et al., (2006) conducted SBR experiments operated with different sludge ages (8 and 14 days) to determine the removal kinetics of Ranitidine at several influent concentrations (2, 3 and 5 $mg.L^{-1}$) and the tests showed generally low removal efficiencies (17–26%), and a chronic inhibition on nitrification, whereas Barceló and Petrovic (2007) found that Ranitidine was rapidly eliminated (95% removal).

5.4 Fate of pharmaceuticals mixture in continuous flow bioreactors

This phase of the study was conducted to compare the performance of membrane bioreactors (MBR) and sequencing batch reactors (SBR) treating synthetic wastewater containing a pharmaceuticals mixture (PM) of Metronidazole, Trimethoprim, Sulphamethoxazole, Paracetamol and Ranitidine in a continuous flow system to simulate full-scale wastewater treatment plants. Control MBR and SBR units were also operated in parallel to examine system performance during the treatment of synthetic wastewater in the absence of pharmaceuticals. The effect of PM concentration on the performance of the bioreactors, and the COD, TOC and PM removal efficiency, was examined. The influence of anoxic conditions on the removal of pharmaceuticals by MBR and SBR systems was also investigated. Details of the experimental conditions are described in Section 4.3.

5.4.1 Performance of control MBR and SBR systems

5.4.1.1 Variations in dissolved oxygen (DO)

Figure 5.4.1 shows that the DO profile of the control MBR and SBR showed a similar response. The DO concentration dropped gradually at the beginning of the MBR operation, and remained fairly constant from day 10 until the end of the experiment. Similar effects were observed in the SBR. The operation of both systems was strictly aerobic since the DO concentration never dropped below 2 $mg.L^{-1}$.

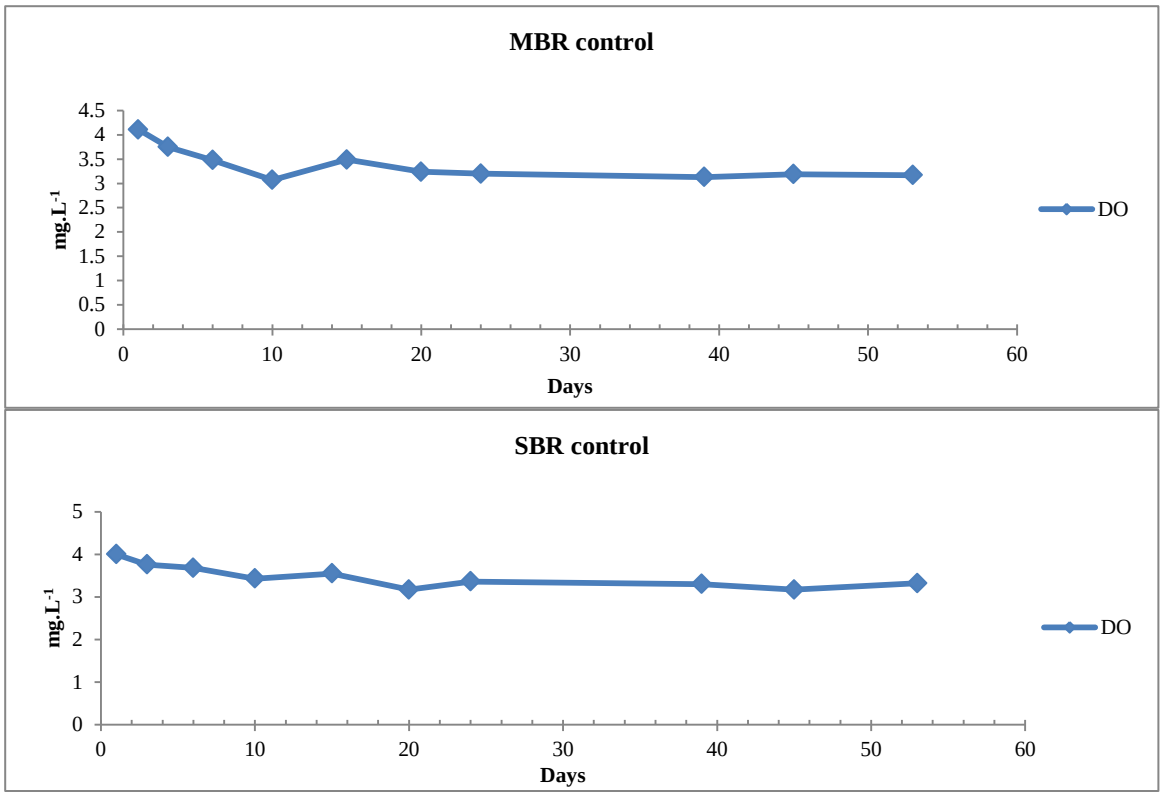


Figure 5.4.1: Changes in DO concentration in the control MBR and SBR.

5.4.1.2 Variation of pH

The average pH of the influent to both reactors was 7.77 (±0.33) and the average temperature was 22 (±3) °C (Figure 5.4.2). The effluent pH in the control MBR was stable with an average value of pH 5.96 (±0.24), while the average pH in the SBR was 5.74 (±0.32). The effluent pH values were similar for both MBR and SBR during the first 5 days of operation, decreasing slightly each day. During the next 10 days the pH values remained at similar values with the lowest value of pH 5.55 for the MBR. The pH remained stable at around pH 6 (± 0.23) from day 24 onwards. pH values for SBR also remained stable from day 24 to day 44 at an average of pH 5.93 (±0.04), but after day 50 the pH started to decrease to reach the lowest pH value of 5.14.

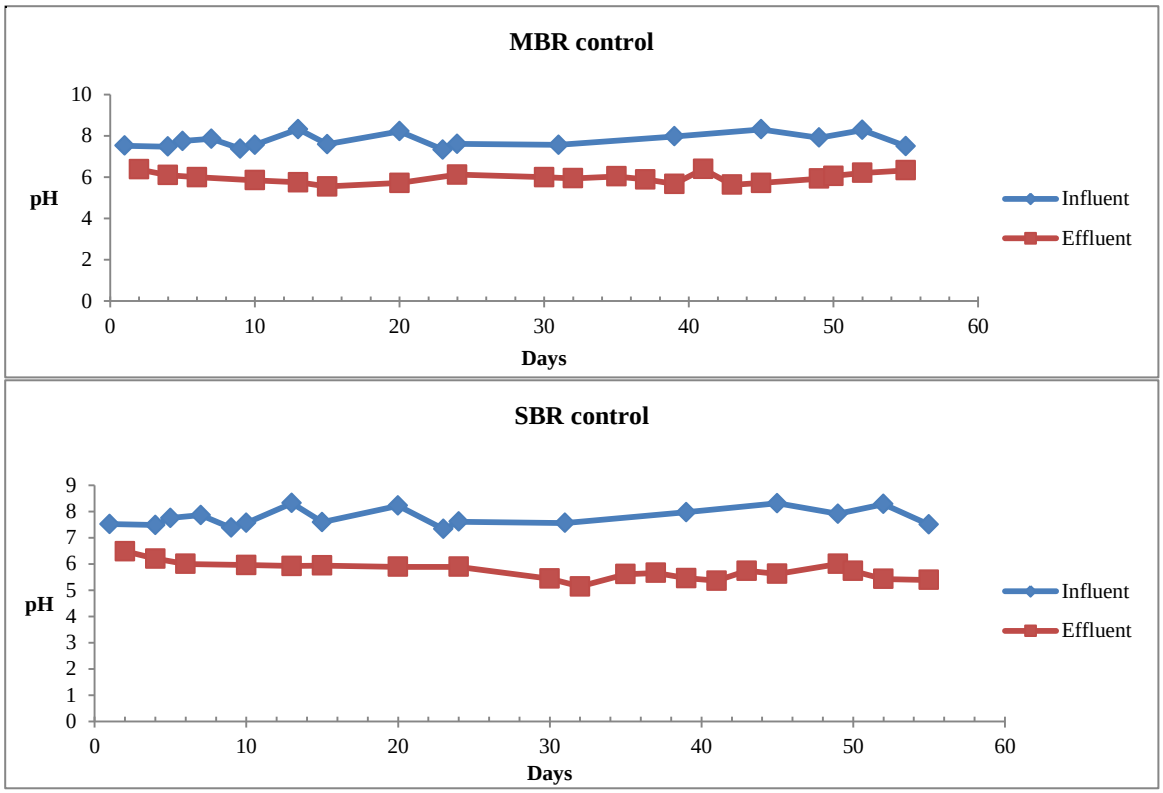


Figure 5.4.2: pH of influent and effluent synthetic wastewater in the control MBR and SBR.

5.4.1.3 Variation of mixed liquid suspended solids and mixed liquid volatile suspended solids

MLSS and MLVSS of the control MBR and SBR are presented in Figure 5.4.3. During the acclimation stage when the MBR was fed by the synthetic wastewater, the MLSS and MLVSS increased immediately (day 0 – day 4); however, MLSS and MLVSS then decreased gradually to reach a steady state from day 15 onwards, with 3057 ($\pm$100) mg.L^{-1} and 2207($\pm$89) mg.L^{-1}, respectively. Furthermore, the MLSS and MLVSS in the control SBR followed a similar pattern, increasing in the first 5 days then decreasing to reach a steady state after 10 days, at levels of 2926($\pm$69) mg.L^{-1} and 2527($\pm$66) mg.L^{-1}, respectively.

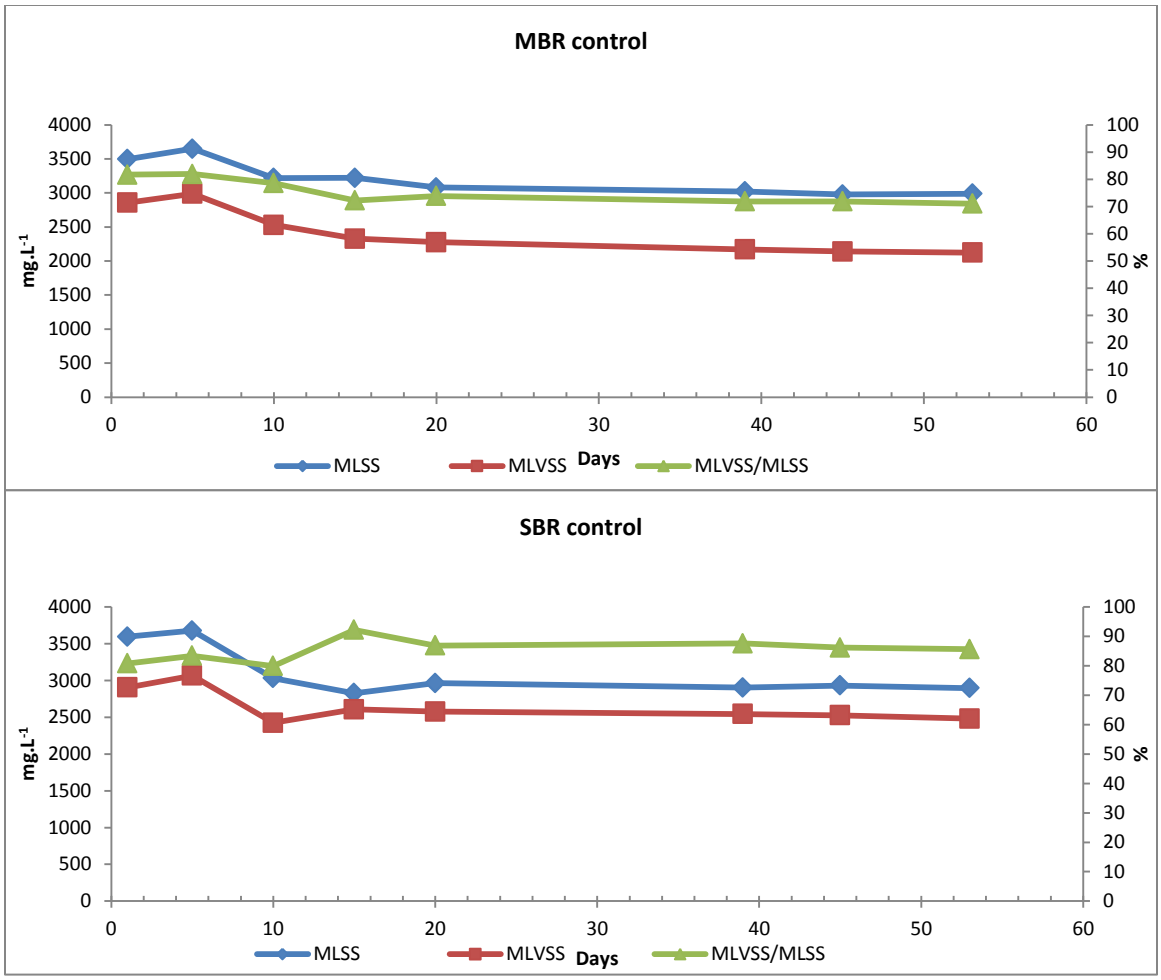


Figure 5.4.3: Variation of MLSS and MLVSS in the control MBR and SBR with synthetics wastewater.

5.4.1.4 Removal of COD and TOC in control MBR and SBR

During operation of the control MBR and SBR systems, influent TOC was kept at 360 (±45) $mg.L^{-1}$, and influent COD at 674 (±34) $mg.L^{-1}$. After the first 10 d of the acclimation stage, TOC removal in the MBR remained at a stable level of more than 90% (Figure 5.4.4). Steady and high levels of TOC removal were also observed in the SBR. The COD showed a similar pattern to the TOC in both the MBR and the SBR. The COD and TOC removal, and the specific utilisation rates, are presented in Figure 5.4.5. Overall removal efficiency of the COD and TOC for the MBR and the SBR was maintained at greater than 90% throughout each experiment. The specific COD utilisation rate for the MBR and the SBR were in the same range (Figure 5.4.5). The high COD and TOC removal efficiencies mean that the heterotrophic

bacteria and microorganisms responsible for decomposition of organic carbon were active under the operating conditions used in the control experiments.

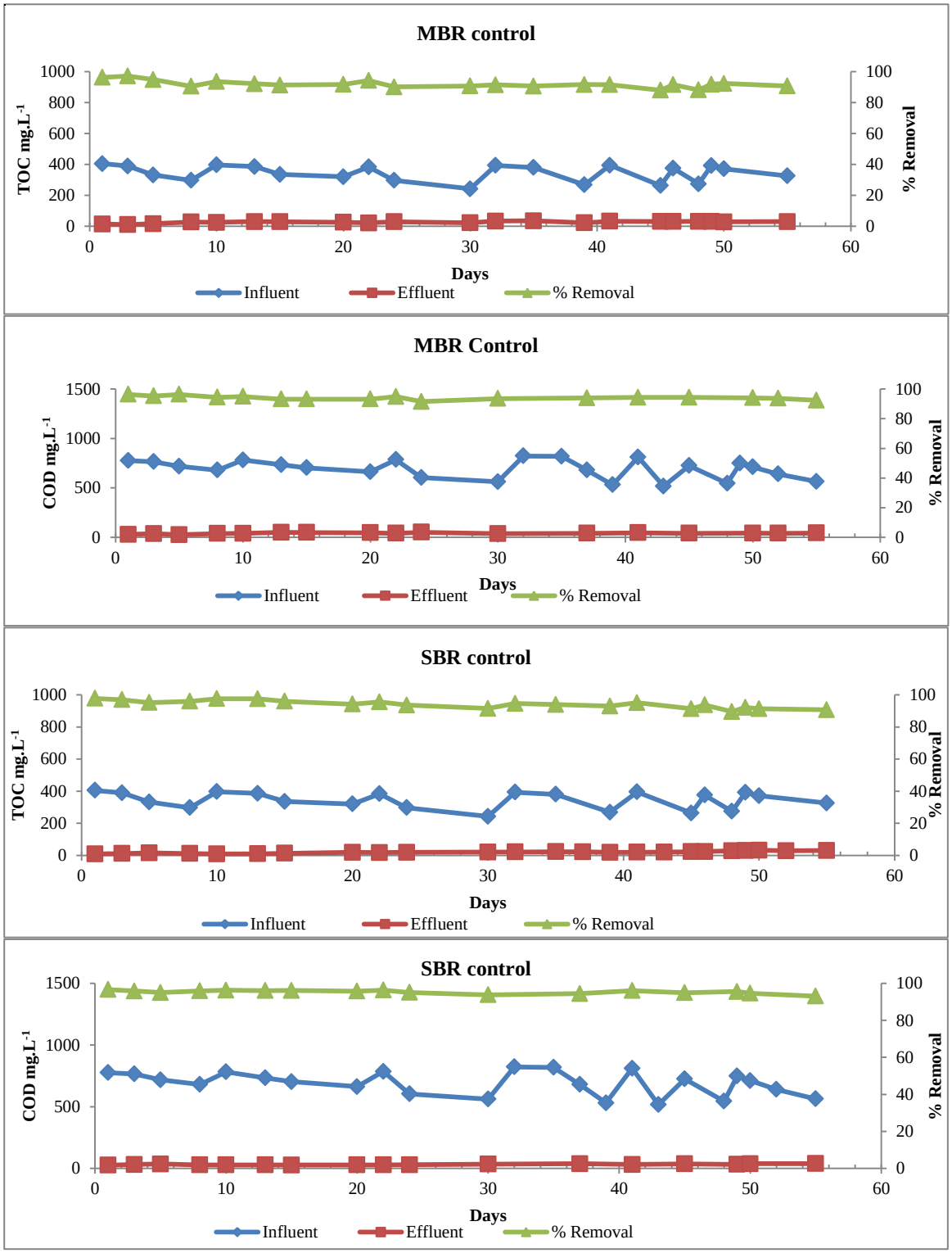
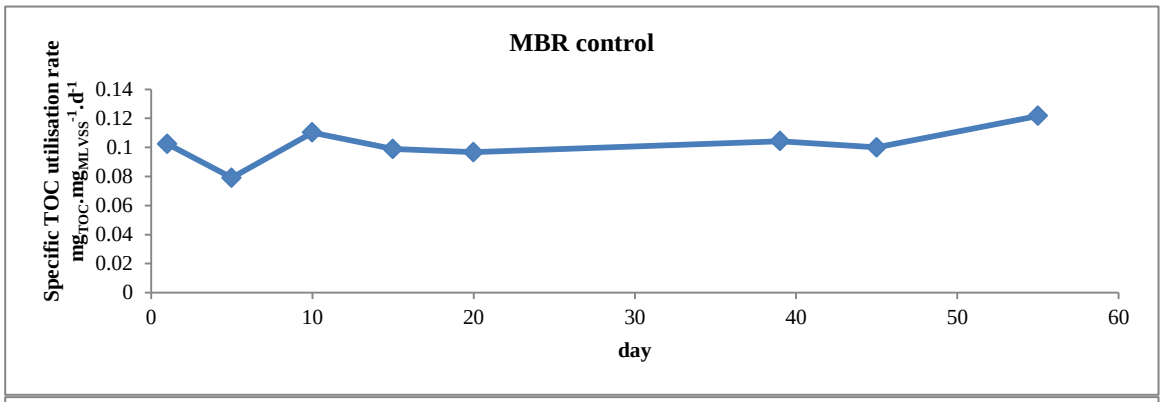
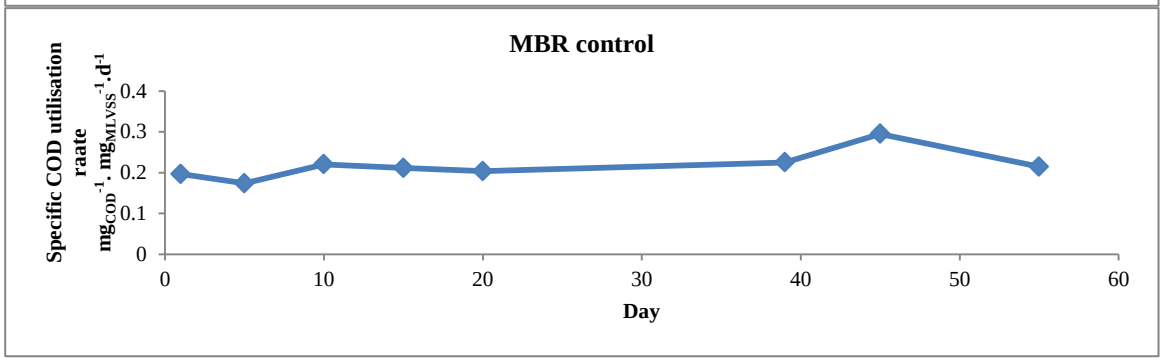
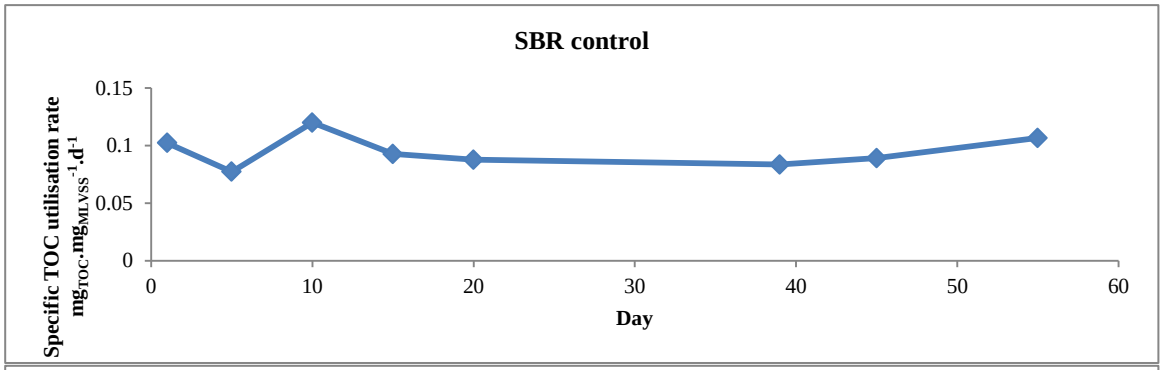


Figure 5.4.4: TOC and COD concentrations and removal in the control MBR and SBR.







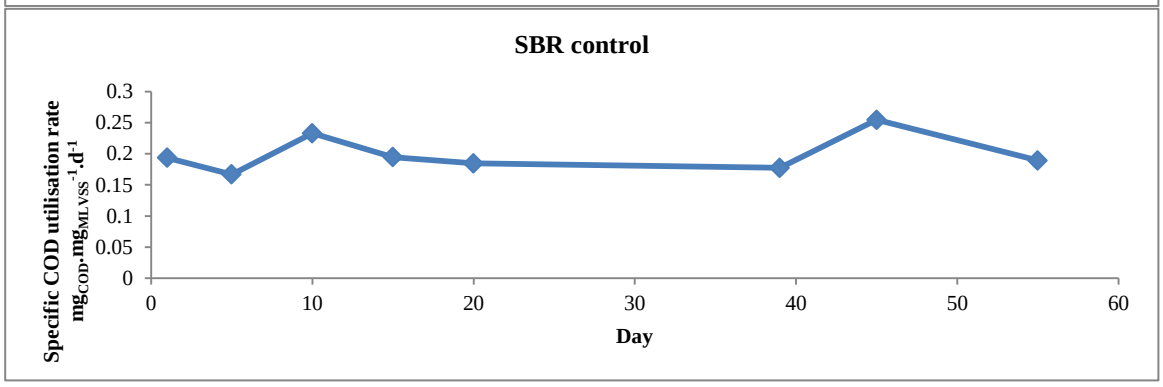


Figure 5.4.5: Specific TOC and COD utilization rate of the control MBR and SBR.

5.4.2 Performance of MBR1 treating PM at three different concentrations under strictly aerobic condition

The fate of PM was investigated under strictly aerobic conditions at different concentrations (Table 4.1) (see Section 4.3). The concentrations of PM treated in this experiment were 1 $\mu g.L^{-1}$, 1 $mg.L^{-1}$, and 10 $mg.L^{-1}$. The MBR1 was operated at a hydraulic retention time (HRT) of 18 hours and a solids retention time (SRT) of 63 d.

5.4.2.1 Variation of dissolved oxygen (DO)

Figure 5.4.6 shows the variation of DO in MBR1 at different pharmaceutical concentrations. The DO decreased from 4 $mg.L^{-1}$ initially to 3.67 $mg.L^{-1}$ at day 10 before spiking with the pharmaceutical. After spiking 1 $\mu g.L^{-1}$ of PM, DO decreased to 3.19 $mg.L^{-1}$. The DO continued to decrease when the PM concentration was increased to 1 $mg.L^{-1}$, and further decreased to reach 2.93 $mg.L^{-1}$ when the PM concentration was increased to10 $mg.L^{-1}$ on day 53.

The DO concentration decreased (DO consumption) corresponded with the biomass growth, which increased more smoothly for the different PM concentrations than in the control. On the other hand, the DO consumption increased with the biomass reduction (Figure 5.4.8) until the end of first phase (1 $\mu g.L^{-1}$). Then, the DO consumption became steady and started to increase at the beginning of the second phase (1 $mg.L^{-1}$) until the last phase (10 $mg.L^{-1}$) where changes were highly significant (ANOVA, $p<0.05$). This change was presumably due to the biomass adaptation to the high concentration of PM which took a longer time compared to the control.

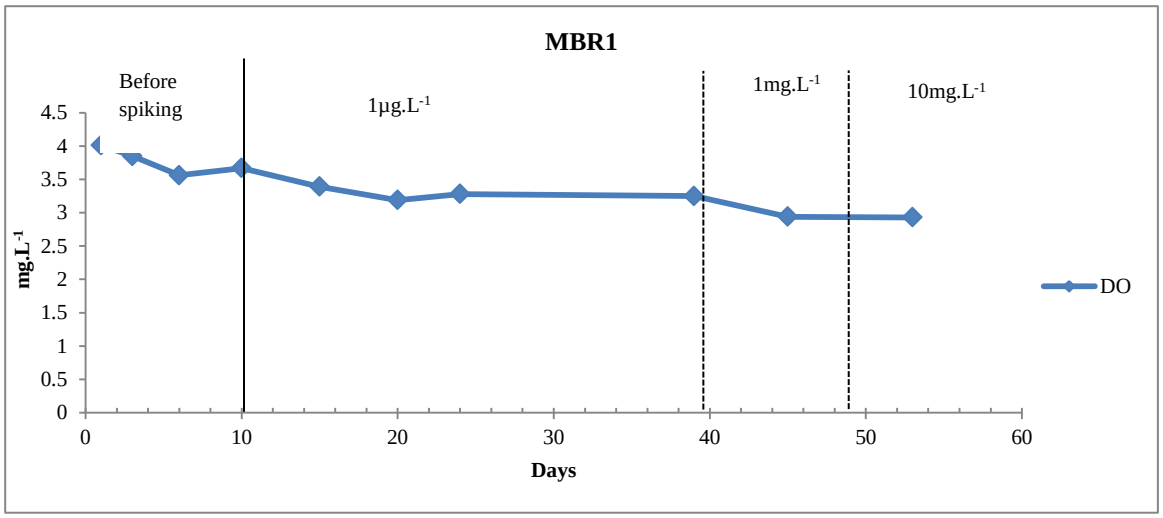


Figure 5.4.6: Changes in DO concentration with different PM concentrations in MBR1.

5.4.2.2 Variation of pH

As shown in Figure 5.4.7, the influent of MBR1 was at pH 7.56 (±0.22) throughout the first 10 d of experiment while the effluent pH dropped to 6.19 (±0.22). Influent pH after spiking the PM at concentration 1 µg.L^{-1} was 7.8 (±0.32) and effluent pH was 6 (±0.22) for 30 d. On the other hand, influent pH increased to 8.27 (±0.35) and effluent pH 6.03 (±0.44) at a PM concentration of 1 mg.L^{-1}, whereas, at a concentration of 10 mg.L^{-1}, the influent pH was 7.97 (±0.28) and effluent pH was 6.37 (±0.11). These pH values were very similar at all concentrations, and also similar to the control.

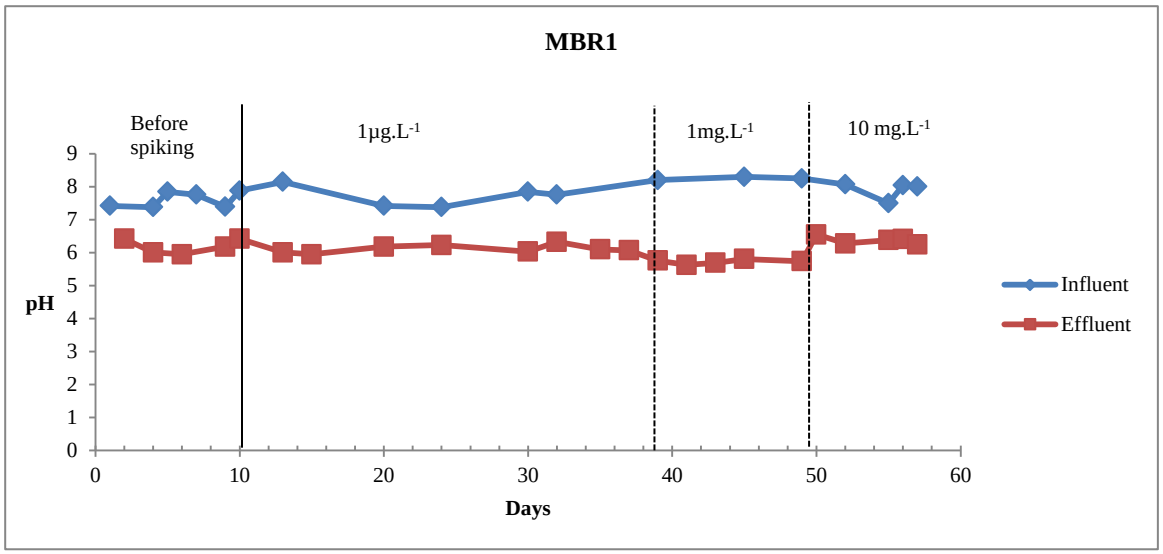


Figure 5.4.7: pH value of the influent and effluent of synthetic wastewater containing different PM concentrations in MBR1.

5.4.2.3 Variation of mixed liquid suspended solids (MLSS) and mixed liquid volatile suspended solids (MLVSS)

Figure 5.4.8 shows biomass growth as solids changed inside MBR1 during a period of 63 days. Before spiking with PM, MLSS increased from 2660 $mg.L^{-1}$ initially to 2750 $mg.L^{-1}$ at day 5 and 2866 $mg.L^{-1}$ at day 10. When the PM was spiked at concentration 1 $\mu g.L^{-1}$ from day 10, MLSS gradually decreased to 2383 $mg.L^{-1}$ at day 20, then MLSS become steady to the end of day 39 with MLSS 2198 $mg.L^{1}$. The MLVSS varied similarly to MLSS, except the MLVSS reduced further from 2000 $mg.L^{-1}$ on day 39 to 1437 $mg.L^{-1}$ at the end of day 53, which increased the gap between MLSS and MLVSS. This could have been affected by the high concentration of PM at 10 $mg.L^{-1}$. The ratios of MLVSS/MLSS were similar at concentrations 1 $\mu g.L^{-1}$ and 1 $mg.L^{-1}$, but they were much lower at concentration 10 $mg.L^{-1}$. The effects of an increase in the PM concentration on MLVSS growth were highly significant (ANOVA, $p<0.05$). Since the MLVSS expresses the microbial biomass more accurately than MLSS, it could indicate that the biomass concentration reduced at a PM concentration of 10 $mg.L^{-1}$ probably due to the partial inhibition of bacterial growth.

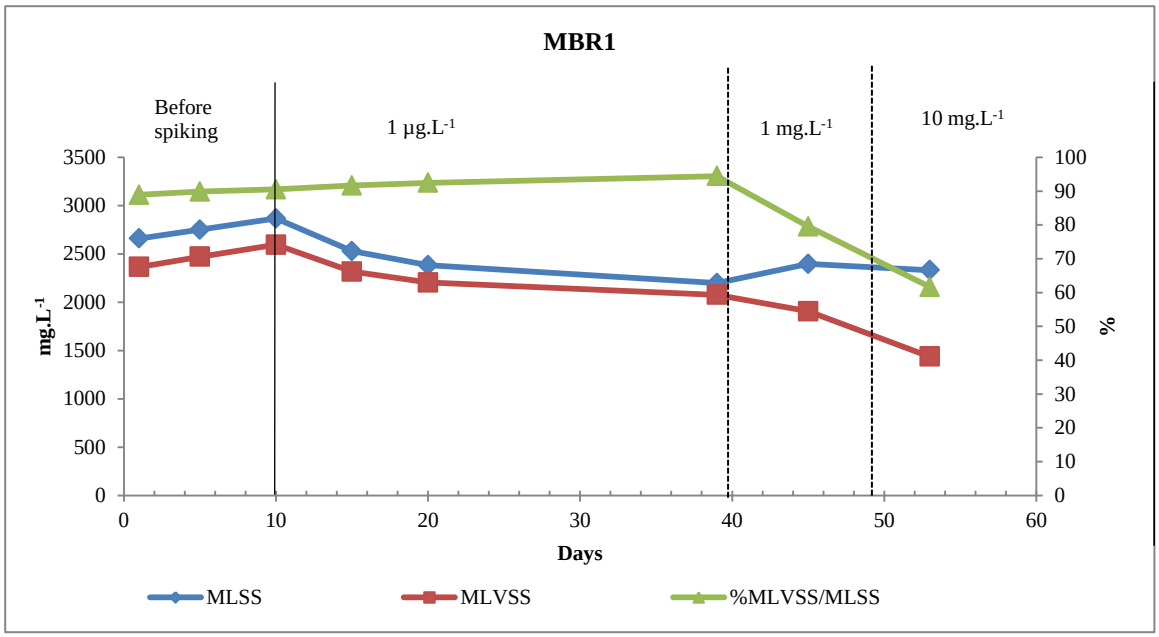


Figure 5.4.8: Variation of MLSS and MLVSS with change of PM concentration in MBR1.

5.4.2.4 Removal of COD and TOC in MBR1 at different PM concentrations

The COD removal efficiencies in MBR1 during the first 10 days before the PM addition were high at 95 % (±1). However, when MBR1 was operated at different PM concentrations, namely 1 µg.L^{-1}, 1 mg.L^{-1}, and 10 mg.L^{-1}, COD removal efficiencies decreased significantly to 92% (±1), 89% (±3), and 87% (±3), respectively ($p<0.05$, ANOVA) (Figure 5.4.9). The TOC removal efficiencies followed a similar pattern to that of the COD, since 95% (±3) TOC removal was observed during the first 10 days without the PM (Figure 5.4.9), with significantly decreasing TOC removal efficiencies, namely 90% (±1), 84% (±5), and 83% (±2) at PM concentrations of 1 µg.L^{-1}, 1 mg.L^{-1}, and 10 mg.L^{-1}, respectively ($p<0.05$, ANOVA).

The specific COD utilisation rates did not appear to change following the PM addition at concentrations 1 µg.L^{-1}, 1 mg.L^{-1}, and 10 mg.L^{-1} (ANOVA, $p>0.2$) (Figure 5.4.10). The specific TOC utilisation rates were relatively steady at PM concentrations of 1 µg.L^{-1} and 1 mg.L^{-1}, whereas it appeared to increase at a PM

concentration of 10 mg.L^{-1} ($p<0.05$, ANOVA) (Figure 5.4.10). The highly significant effect of the PM concentration on COD and TOC removal efficiencies needs to be explained. The removal efficiencies of COD and TOC appear to be affected by the MLVSS reduction (Figure 5.4.8) as a result of PM concentration increase, while the specific COD utilisation rates do not change significantly with the change in PM concentration. The specific TOC utilisation rates were less than 50% of the specific COD utilisation rates for all PM concentrations except for concentration 10 mg.L^{-1} where it increased to 66% of specific COD utilisation rates. It is to be noted that TOC expresses the concentration of the organic carbon whereas COD determines not only organic carbon but also other elements (e.g. N, P, S etc) in the substrate as gross parameters.

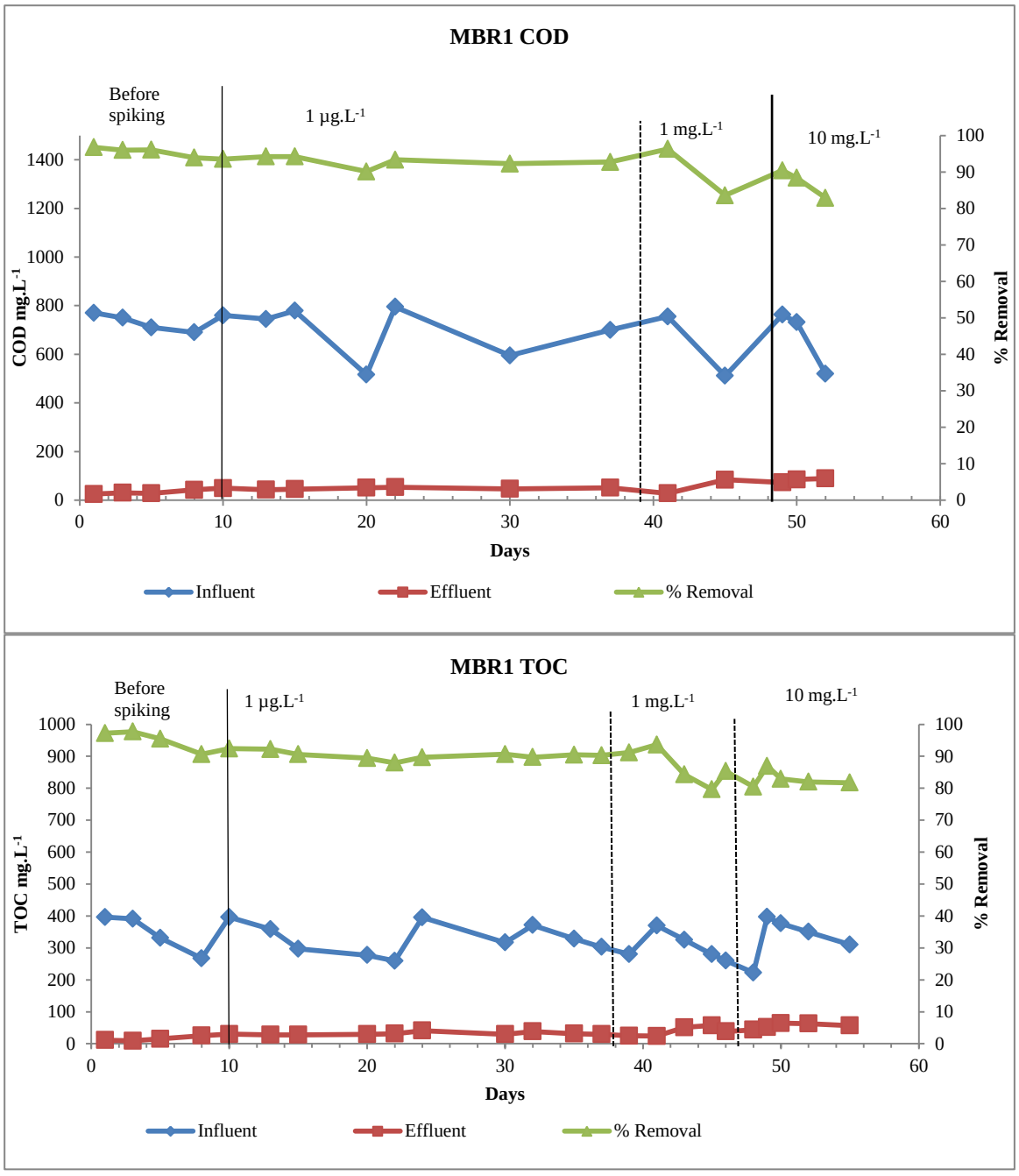


Figure 5.4.9: TOC and COD concentration and removal in MBR1 at different PM concentrations.

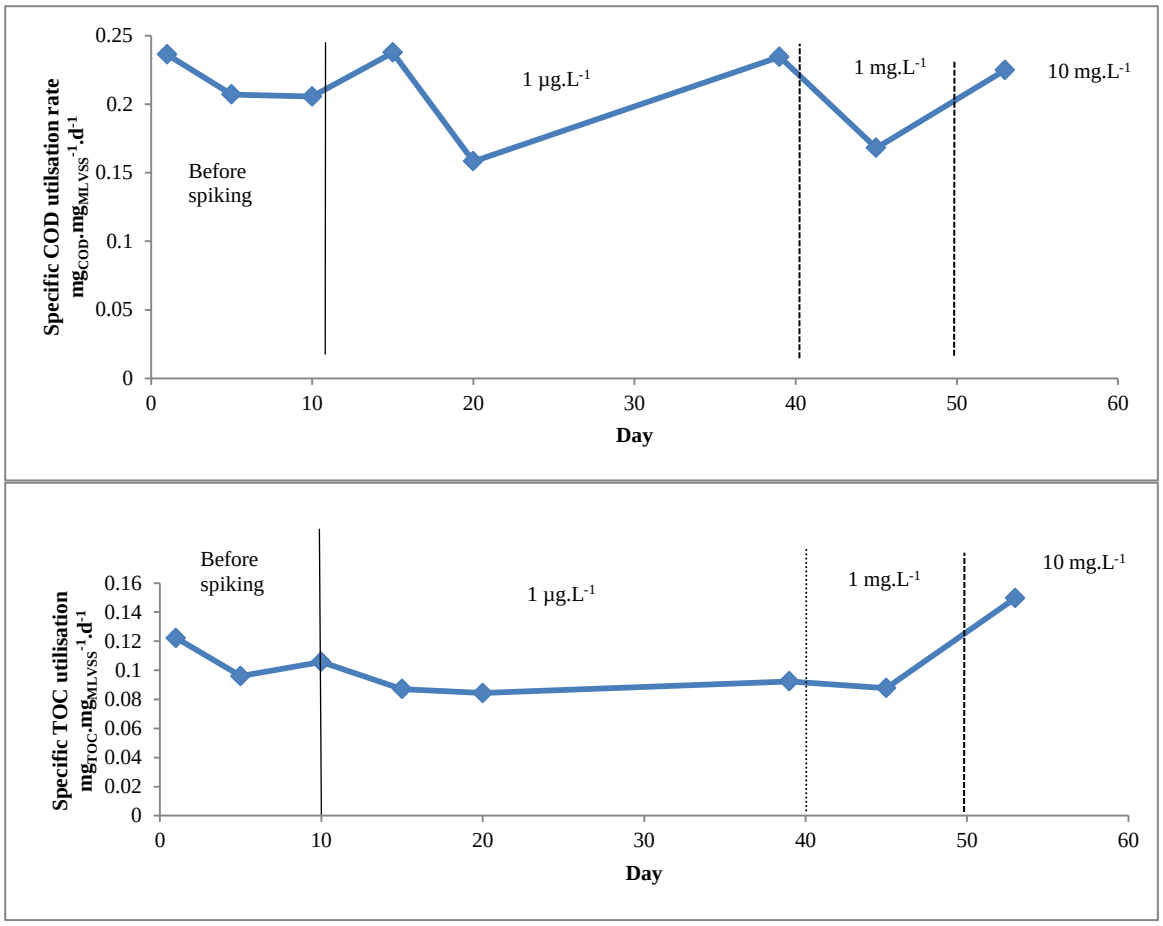


Figure 5.4.10: Specific TOC and COD utilisation rates for MBR1 at different PM concentrations.

5.4.2.5 PM removal efficiency in MBR1 at different concentrations

Figure 5.4.11 shows the removal of five pharmaceuticals (Metronidazole, Trimethoprim, Sulphamethoxazole, Paracetamol and Ranitidine) spiked into MBR1 at different concentrations of 1 µg.L^{-1}, 1 mg.L^{-1}, and 10 mg.L^{-1} over a period of 53 days. The removal efficiency of Metronidazole was 60% at a concentration of 1µg.L^{-1}, then increased to 83% at a concentration of 1 mg.L^{-1} but decreased to 59% at a concentration of 10 mg.L^{-1} ($p>0.2$, ANOVA) (Figure 5.4.11). Low removal efficiencies for Trimethoprim decreased with increasing concentration, so that 67%, 47, and 42% were removed at concentrations of 1 µg.L^{-1}, 1 mg.L^{-1}, and 10 mg.L^{-1}, respectively ($p = 0.108$, ANOVA) (Figure 5.4.11). Sulphamethoxazole was removed efficiency in MBR1, decreased with increasing concentration, so that 95%, 94% and

88% were removed at concentrations of at concentration 1 µg.L^{-1}, 1 mg.L^{-1}, and 10 mg.L^{-1}, respectively ($p>0.2$, ANOVA) (Figure 5.4.11). Paracetamol was also removed efficiency in MBR1, decreased with increasing concentration, so that 88%, 88% and 59%were removed at concentrations of at concentration 1 µg.L^{-1}, 1 mg.L^{-1}, and 10 mg.L^{-1}, respectively ($p>0.2$, ANOVA) (Figure 5.4.11). Ranitidine was also removed efficiency in MBR1, increased with increasing concentration, so that 96%, 99% and 99%were removed at concentrations of at concentration 1 µg.L^{-1}, 1 mg.L^{-1}, and 10 mg.L^{-1}, respectively ($p<0.05$, ANOVA) (Figure 5.4.11).

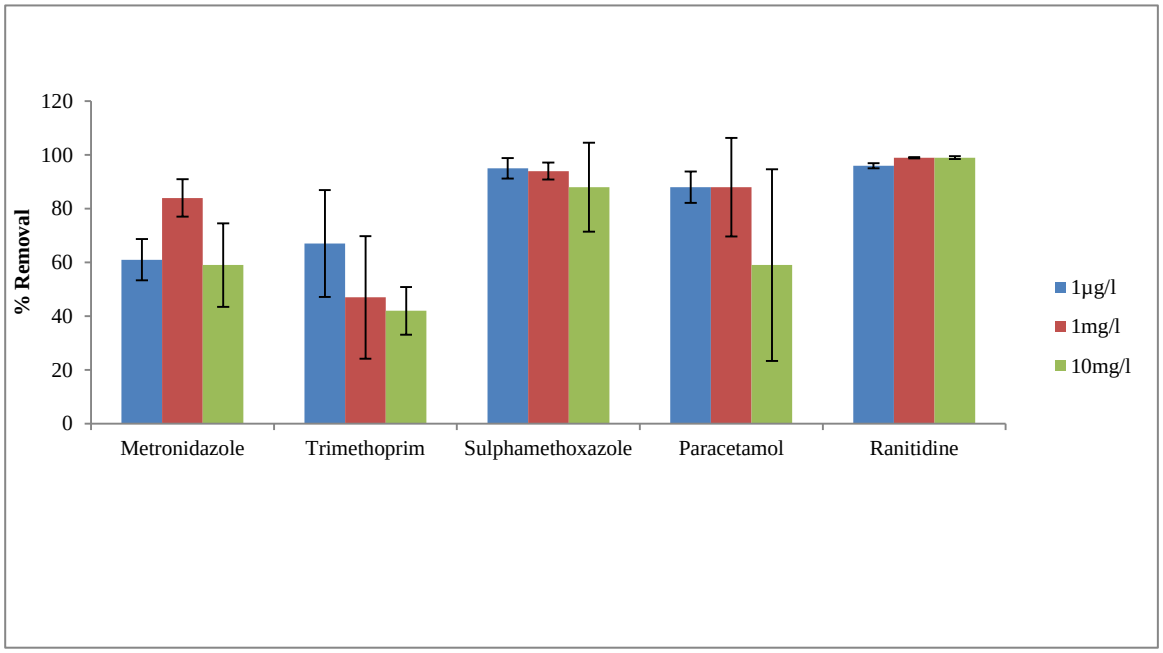


Figure 5.4.11: The removal efficiencies of pharmaceuticals in MBR1 at different PM concentrations.

The specific pharmaceutical utilisation rate was dependent on the initial concentration of the PM. A clear trend was observed showing the specific utilisation rates of Metronidazole, Trimethoprim, Sulphamethoxazole, Paracetamol, and Ranitidine generally increased with increased initial concentration over the range 1 µg.L^{-1}, 1 mg.L^{-1}, and 10 mgL^{-1} (Figure 5.4.12). This suggests that higher initial substrate concentration has a greater stimulatory effect on the microorganisms in activated sludge, and any inhibitory effect was unnoticeable. Despite the decrease in PM removal efficiency with the increase in PM concentration, the mass of

pharmaceuticals removed in the MBR1 divided by the mass of microorganism (i.e. the specific pharmaceutical utilisation rate) was higher. To describe the specific utilisation rate of Metronidazole, Trimethoprim, Sulphamethoxazole, Paracetamol, and Ranitidine at different initial concentrations, kinetic plots were determined and the data was found to fit the regression line $R^2 > 0.99$ (Figure 5.4.12). The highest specific pharmaceutical utilisation rate was found in Ranitidine followed by Sulphamethoxazole, then Metronidazole and Paracetamol, and the lowest was Trimethoprim. The specific pharmaceutical utilisation rates ($mg_{ph}.mg_{MLVSS}^{-1}.d^{-1}$) increased linearly with the increase of PM concentration at correlation coefficients (R^2) of 1, 0.99, 0.99, 0.99, and 0.99 for Metronidazole, Trimethoprim, Sulphamethoxazole, Paracetamol, and Ranitidine, respectively ($p<0.05$, ANOVA) (Figure 5.4.12).

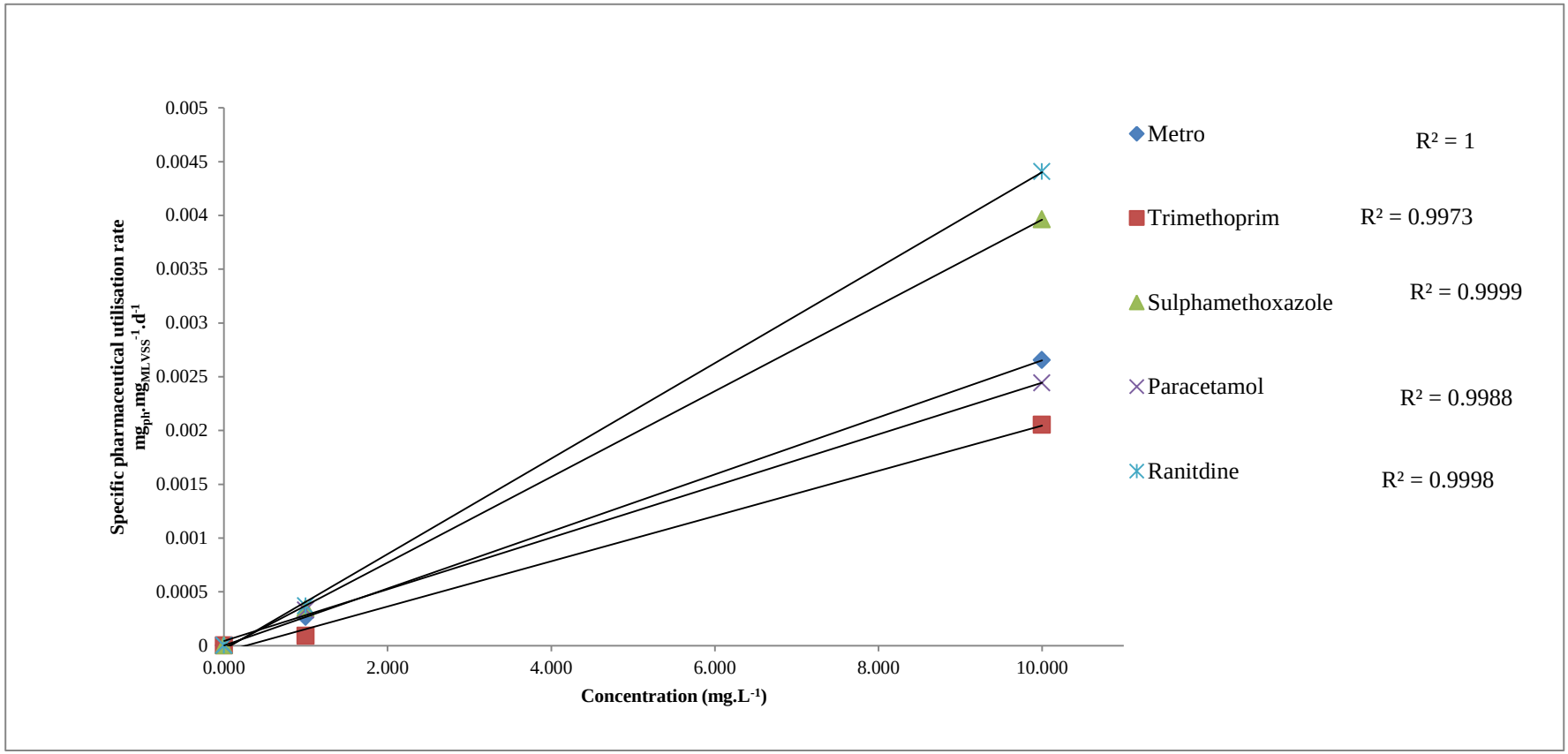


Figure 5.4.12: Specific pharmaceutical utilisation rates at different PM concentrations in MBR1 with regression analysis.

5.4.3 Performance of MBR2 treating PM at three different concentrations under anoxic and aerobic conditions

In this section the fate of the PM was investigated under the combination of anoxic and aerobic conditions using the operational schedule of MBR2 (Table 4.1) (see Section 4.3). The effect of the PM concentration was studied with an anoxic/aerobic cycle (anoxic period 2 hours, aerobic period 16 hours), a hydraulic retention time (HRT) of 18 hours, and a solids retention time (SRT) of 53 d. The PM concentrations used in this experiment were 1 $\mu g.L^{-1}$, 1 $mg.L^{-1}$, and 10 $mg.L^{-1}$.

5.4.3.1 Variation of dissolved oxygen (DO)

The variation of DO in MBR2 under different PM concentrations is presented in Figure 5.4.13. The DO decreased from 4.1 $mg.L^{-1}$ initially to 3.6 $mg.L^{-1}$ at day 10 before spiking with the PM. After spiking 1 $\mu g.L^{-1}$ of PM the DO decreased to 2.99 $mg.L^{-1}$, but increased to 3.61 $mg.L^{-1}$ when the PM concentration was increased to 1 $mg.L^{-1}$ and then decreased to 3 $mg.L^{-1}$ at 10 $mg.L^{-1}$ on day 53. The DO consumption increased correspondingly with the biomass growth, which increased in a similar pattern observed with MBR1. On the other hand, the DO concentration became steady and started to increase higher than that in MBR1 at the beginning of the second phase (1 $mg.L^{-1}$).

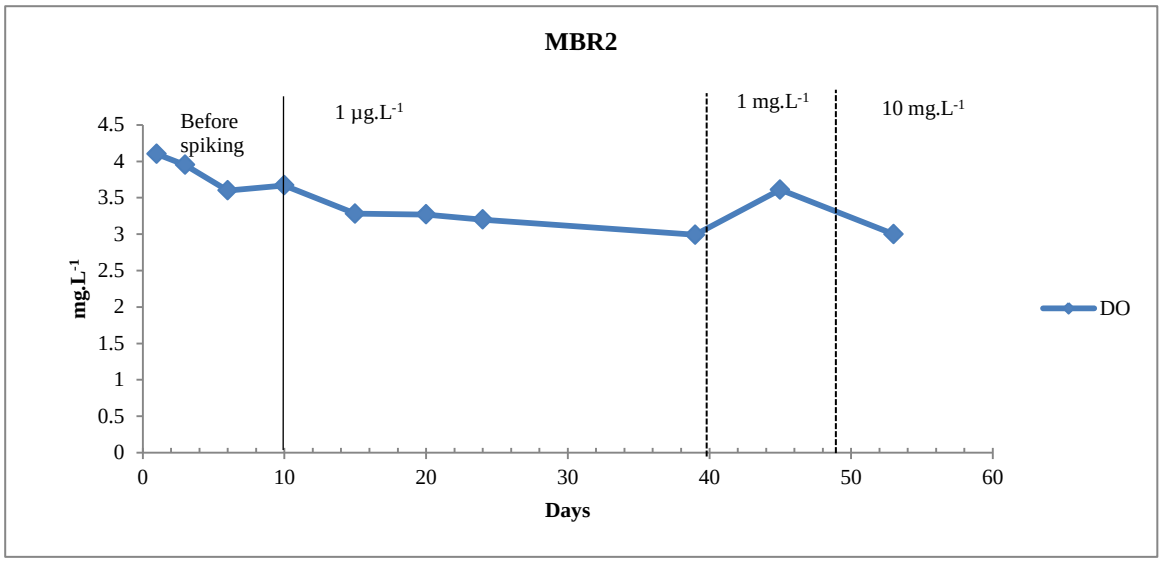


Figure 5.4.13: Changes in DO concentration with different PM concentrations in MBR2.

5.4.3.2 Variation of pH

As shown in Figure 5.4.14, the influent pH of MBR2 was similar to the influent of MBR1 as they feed from the same tank. In the first 10 d of experiment the effluent pH dropped to 6.39 (±0.35). The effluent pH after spiking with PM at concentration 1 µg.L^{-1} was 5.63 (±0.24) for 30 d. On the other hand, the effluent pH increased to 6.12 (±0.66) at PM concentration of 1 mg.L^{-1} whereas, at concentration 10 mg.L^{-1}, the effluent pH was 7.38 (±0.32). The pH values were almost the same compared with the control and MBR1 at all concentrations except for 10 mg.L^{-1} which showed high pH values compared to other ranges of PM concentrations and similar values of influent pH.

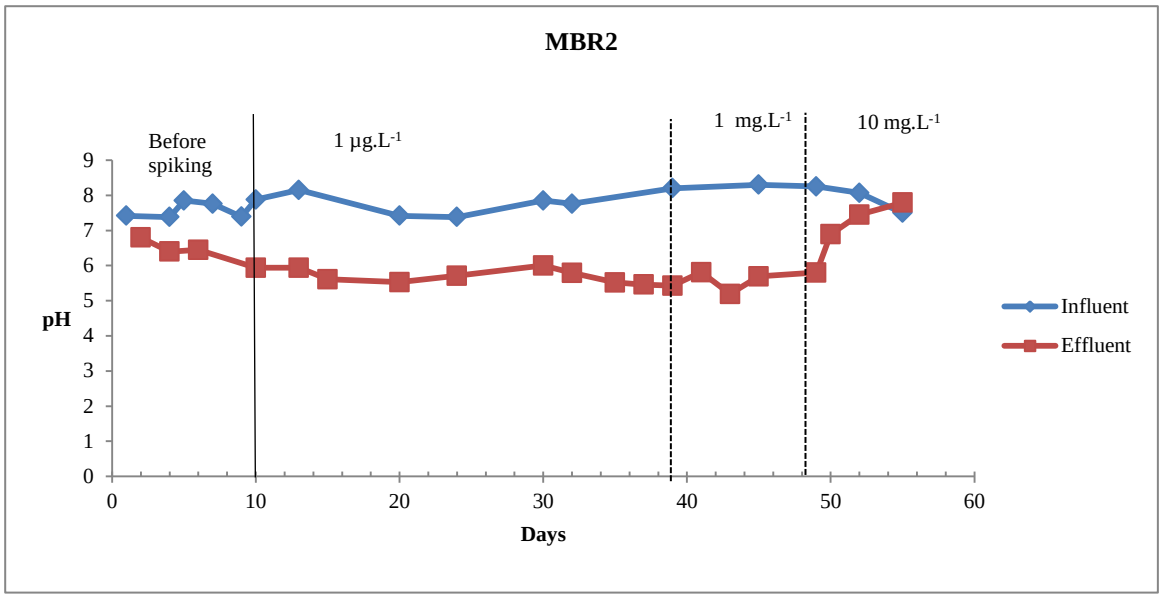


Figure 5.4.14: pH value of the influent and effluent of synthetic wastewater containing different PM concentrations in MBR2.

5.4.3.3 Variation of mixed liquid suspended solids (MLSS) and mixed liquid volatile suspended solids (MLVSS)

The biomass growth rate of MLSS in MBR2 during a period of 53 days changed differently to that in MBR1. As can be seen in Figure 5.4.15, MLSS increased before spiking the PM from 3655 $mg.L^{-1}$initially to 3730 $mg.L^{-1}$ at day 5 and decreased to 3551 $mg.L^{-1}$ at day 10. When the PM was spiked at concentration 1 $\mu g.L^{-1}$ from day 10 to day 41, MLSS gradually decreased to 2870 $mg.L^{-1}$ at the end of day 39 with no signs of achieving MLSS relative stability. However, the MLVSS concentration was different than that of MLSS where MLVSS was steady from day 15 at concentration 1 $\mu g.L^{-1}$ to day 39. MLVSS start decreasing from day 39 to 1532 $mg.L^{-1}$ at the end of day 53. The concentration of MLVSS growth in MBR2 were significant changed with PM concentrations (ANOVA, p=0.086).

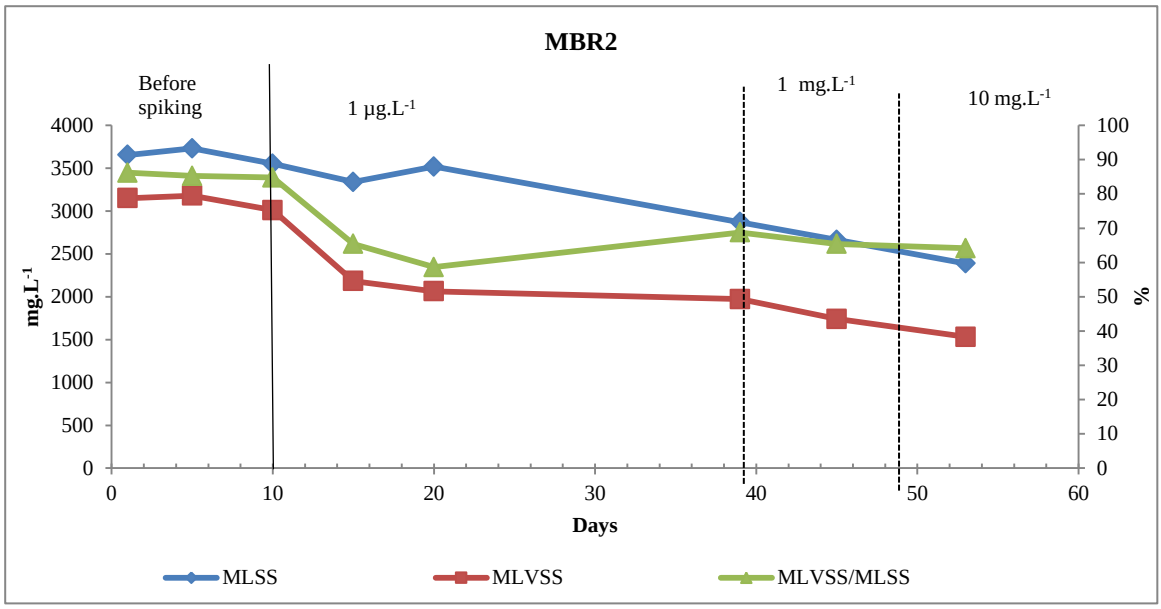


Figure 5.4.15: Variation of MLSS and MLVSS with change of PM concentration in MBR2.

5.4.3.4 Removal of COD and TOC in MBR2 at different PM concentrations

The COD removal efficiencies in MBR2 were similar to MBR1 at different ranges of PM concentrations. The COD removal efficiency described in Figure 5.4.16 was 94% (±1.3) before the PM had been spiked, at PM concentrations of 1 µg.L^{-1}, 1 mg.L^{-1}, and 10 mg.L^{-1}, COD removal efficiencies were changed less significantly to 93% (±2), 88% (±4), and 91% (±3), respectively ($p = 0.14$, ANOVA). The TOC removal efficiencies in MBR2 were similar to those reported for MBR1 at a PM concentration range of 1 µg.L^{-1} to 1 mg.L^{-1} and were at a steady state (Figure 5.4.16), but TOC removal efficiency decreased at PM concentration 10 mg.L^{-1}. TOC removal efficiencies were 94% before PM spiked and significantly decreased to 92% (±1), 89% (±2), and 81% (±9) at PM concentrations of 1 µg.L^{-1}, 1 mg.L^{-1}, and 10 mg.L^{-1}, respectively ($p<0.05$, ANOVA) (Figure 5.4.16). Similar to MBR1 the specific COD utilisation rates of MBR2 experienced insignificant change with the increase in PM concentrations ($p>0.2$, ANOVA) (Figure 5.4.17). The specific TOC utilisation rates of MBR2 were also showing significant change similar to those of MBR1 ($p<0.05$, ANOVA) (Figure 5.4.17).

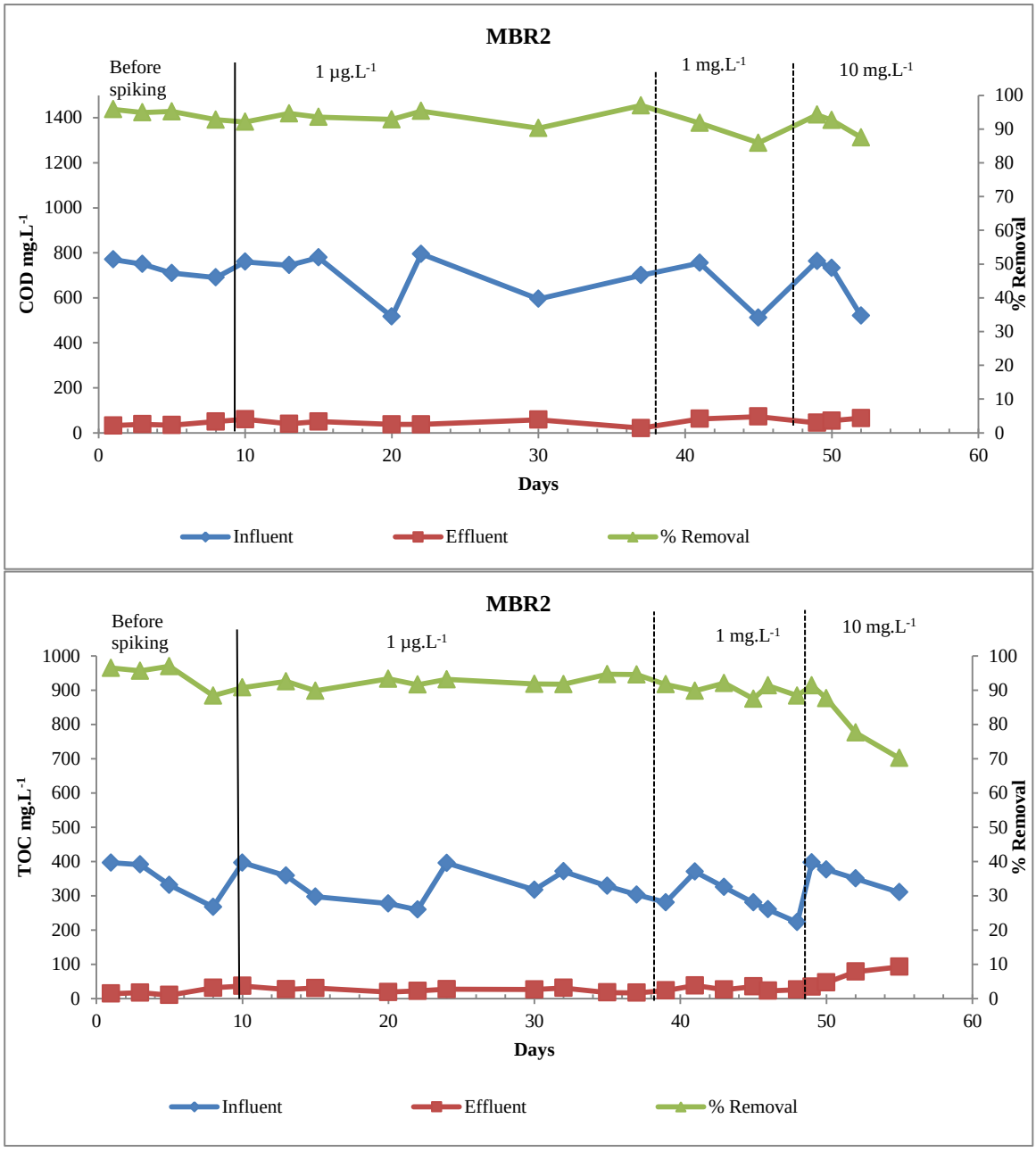


Figure 5.4.16: TOC and COD concentration and removal in MBR2 at different PM concentrations.

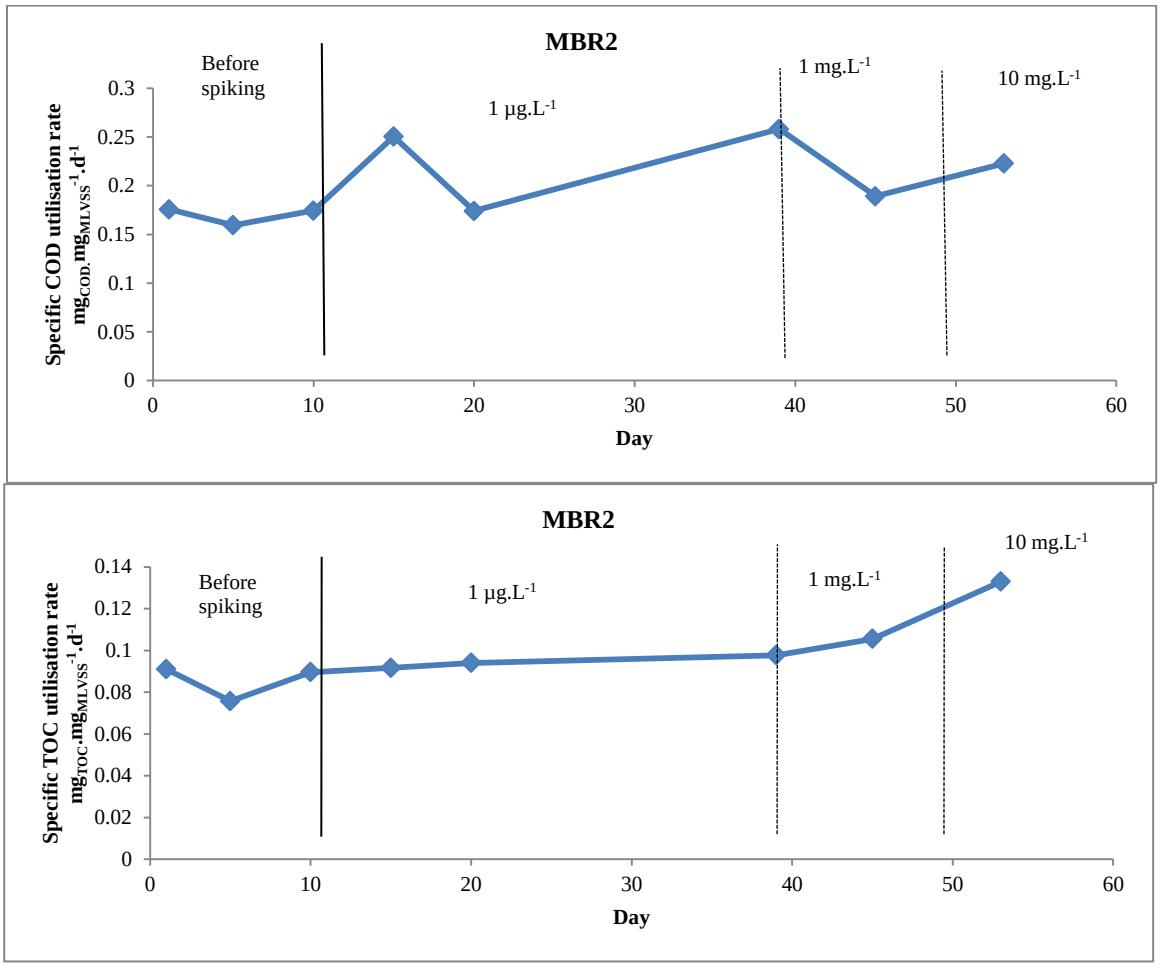


Figure 5.4.17: Specific TOC and COD utilisation rates for MBR2 at different PM concentrations.

5.4.3.5 PM removal efficiency in MBR2 at different concentrations

Unlike the MBR1 the removal of PM spiked into MBR2 was somewhat different. There was less significant removal efficiency of Metronidazole (Figure 5.4.18), namely 80% at concentration 1 µg.L^{-1}, 60% at concentration 1 mg.L^{-1} and 61% at concentration 10 mg.L^{-1} ($p>0.1$, ANOVA). Highly significant removal efficiencies of Trimethoprim in MBR2 were similar to MBR1, where the removal efficiency decreased with increasing Trimethoprim concentration; consequently, 69%, 49, and 26% removal was seen in MBR2 at Trimethoprim concentrations of 1 µg.L^{-1}, 1 mg.L^{-1}, and 10 mg.L^{-1}, respectively ($p<0.05$, ANOVA) (Figure 5.4.18). Sulphamethoxazole was also removed effectively by MBR2, as it was in MBR1, with 91% removal at 1 µg.L^{-1} Sulphamethoxazole, 97% removal at concentration 1 mgL^{-1}, but only 67% removal at concentration 10 mg.L^{-1} ($p>0.1$, ANOVA). The removal of Paracetamol in MBR2 was comparable with MBR1, with 82% removal efficiency at a concentration of 1 µg.L^{-1}, 91% at 1 mg.L^{-1}, and 67% removal at 10 mg.L^{-1} ($p>0.2$, ANOVA). The removal of Ranitidine in the MBR2 showed the highest similarity to MBR1, with 98% removal at a concentration of 1 µg.L^{-1}, increasing to 99% removal at 1 mg.L^{-1} and 10 mg.L^{-1} ($p>0.2$, ANOVA).

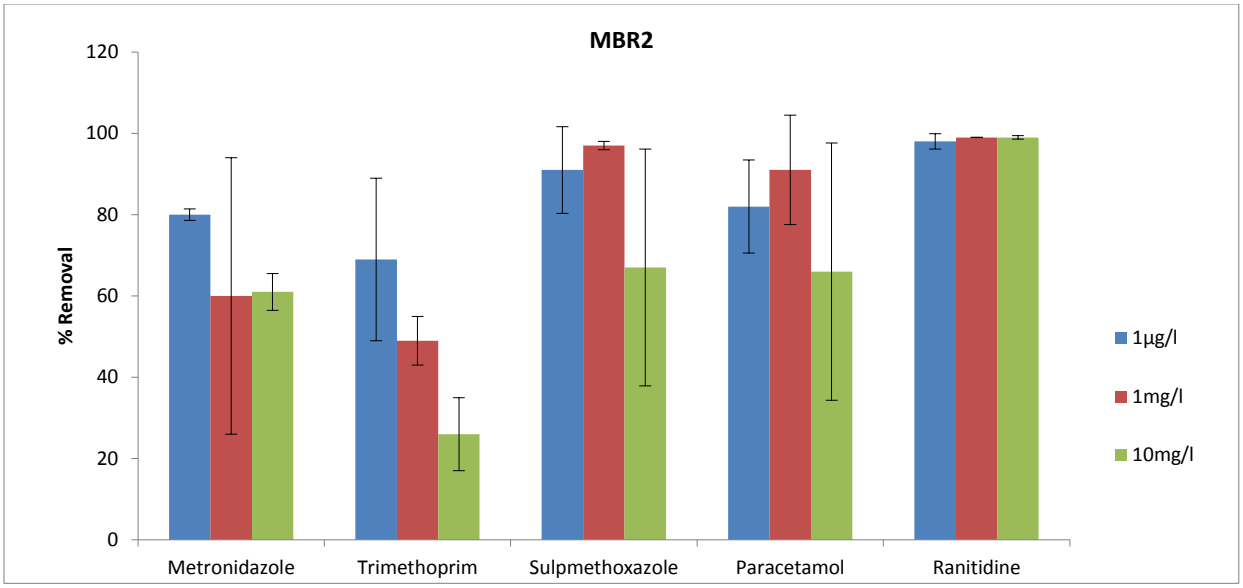


Figure 5.4.18: The removal efficiencies of pharmaceuticals in MBR2 at different PM concentrations.

The specific utilisation rates of Metronidazole, Trimethoprim, Sulphamethoxazole, Paracetamol, and Ranitidine in MBR2 were similar to those observed in MBR1, which generally increased with increasing concentration in the range of 1 $\mu g.L^{-1}$, 1 $mg.L^{-1}$, and 10 $mg.L^{-1}$ (Figure 5.4.19). This suggests a similar effect of the initial PM concentration on the microorganisms in the activated sludge of both MBR1 and MBR2. As shown in Figure 5.4.19, the specific utilisation rates increased with the increase of initial PM concentration, indicating no apparent inhibition of any pharmaceutical on its biodegradation.

Potentially no effects of increases in PM concentration on the specific pharmaceutical utilisation rate were observed. The specific pharmaceutical utilisation rates ($mg_{ph}.mg_{MLVSS}^{-1}.d^{-1}$) increased linearly with an increase in the PM concentration with a correlation coefficient (R^2) of 0.99 for all pharmaceuticals ($p<0.05$, ANOVA) (Figure 5.4.19). The highest specific pharmaceutical utilisation rate for MBR2 was found in Ranitidine followed by Sulphamethoxazole, then Paracetamol and Metronidazole, and the lowest was Trimethoprim.

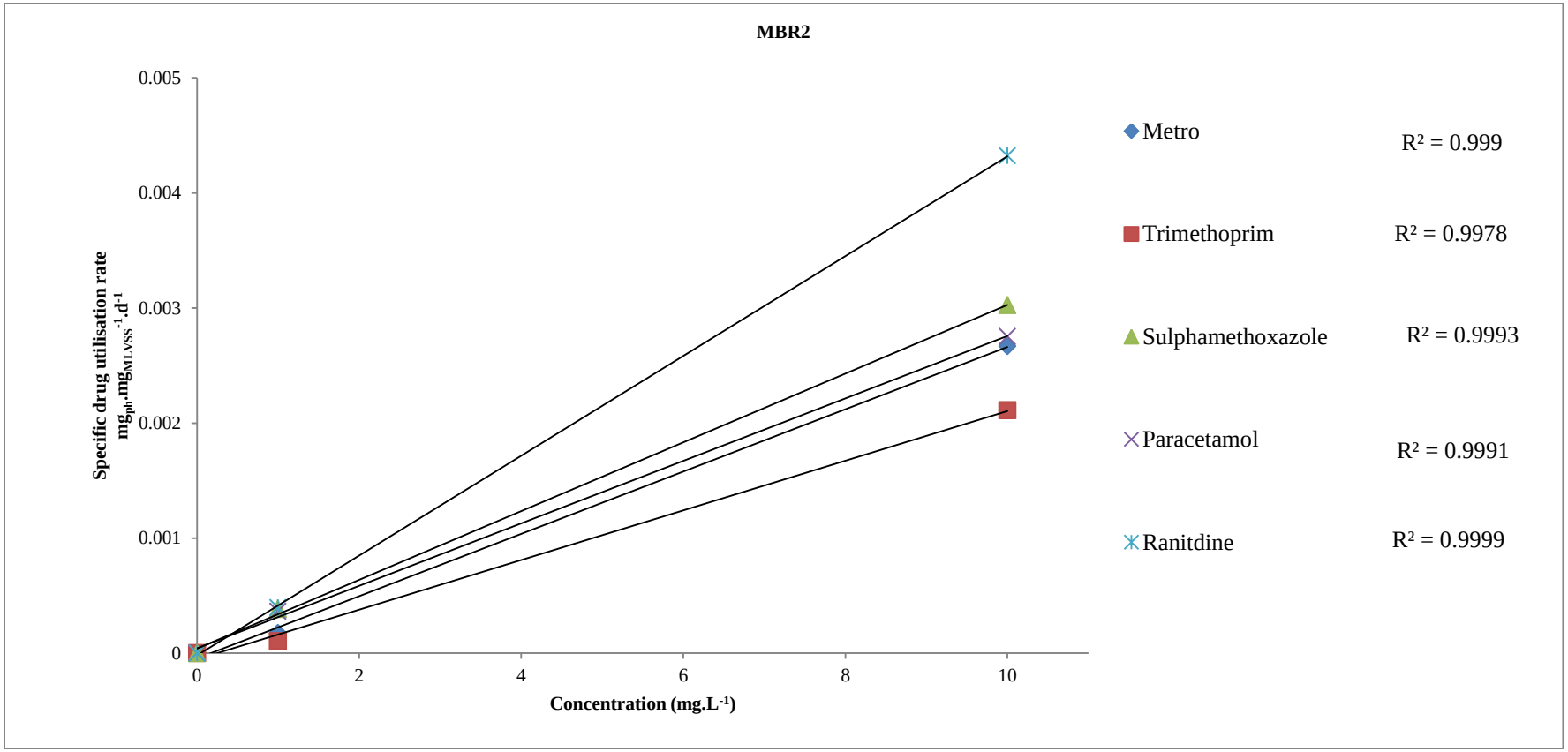


Figure 5.4.19: Specific pharmaceutical utilisation rates at different PM concentrations in MBR2 with regression analysis.

5.4.4 Performance of SBR1 treating PM at three different concentrations under strictly aerobic conditions

The fate of the studied PM was also investigated under strictly aerobic conditions in the sequencing batch reactor (SBR1) at different PM concentrations range (1 µg.L^{-1}, 1 mg.L^{-1}, and 10 mg.L^{-1}) (Table 4.1) (see Section 4.3). The SBR1 was operated at a hydraulic retention time (HRT) of 18 hours every day for 53 d.

5.4.4.1 Variation of dissolved oxygen (DO)

The variation in DO in SBR1 under different PM concentrations was similar to those observed in the SBR control and MBR1 (Figure 5.4.20). The DO decreased from4.05 mg.L^{-1}initially to 3.45 mg.L^{-1}at day 10 before spiking with the PM. After spiking with 1 µg.L^{-1} of PM the DO decreased to 3.05 mg.L^{-1}. The DO remained steady and in the same range and was 3.05 mg.L^{-1} when the PM concentration increased to 1 mg.L^{-1}, and remained stable when the PM concentration was increased to10 mg.L^{-1} at day 53.

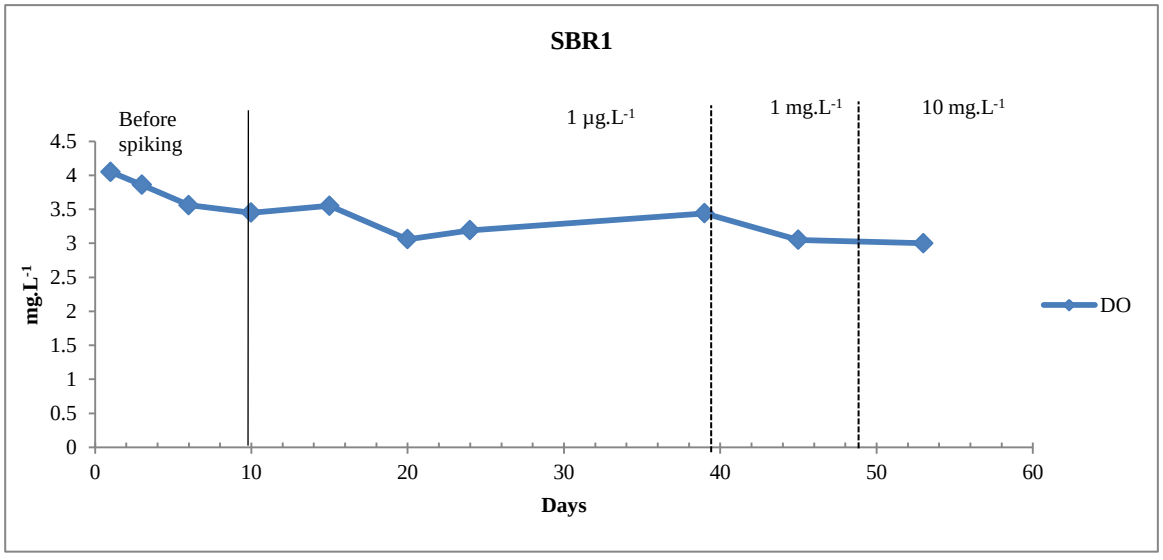


Figure 5.4.20: Changes in DO concentration with different PM concentrations in SBR1.

5.4.4.2 Variation of pH

The influent pH of SBR1 was similar to the influent of MBR1, and the effluent pH dropped in the first 10 d to 6.39 (±0.33) which was similar to MBR1 and the SBR control as it is 6.16 (±0.23) (Figure 5.4.21). The effluent pH remained steady after spiking with PM at concentration 1 µg.L^{-1} and 1 mg.L^{-1} and was recorded as 5.6 (±0.29) and 5.57 (±0.36), respectively. When the PM concentration was increased to 10 mg.L^{-1} the effluent pH increased to 7.7 then decreased to 6.1.

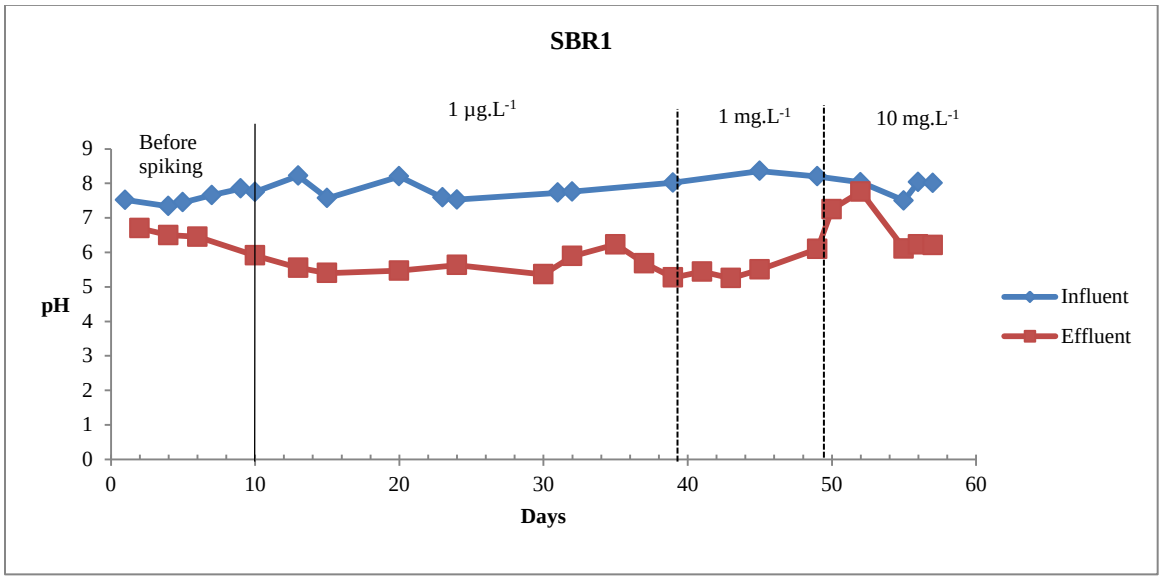


Figure 5.4.21: pH value of the influent and effluent of synthetic wastewater containing different PM concentrations in SBR1.

5.4.4.3 Variation of mixed liquid suspended solids (MLSS) and mixed liquid volatile suspended solids (MLVSS)

MLSS and MLVSS of SBR1 changed relatively differently than in the SBR control and MBR1 during a period of 53 days. As can be seen in Figure 5.4.22, MLSS increased before spiking with PM from 3565 mg.L^{-1} initially to 3699 mg.L^{-1} at day 10. After the PM was spiked at concentration 1 µg.L^{-1} MLSS gradually decreased to 2448 mg.L^{-1} at the end of day 39 with no signs of achieving MLSS relative stability. However, the MLVSS concentration change was similar to MLSS, where MLVSS

increased to 3280 mg.L^{-1} at day 20 at a PM concentration 1 µg.L^{-1} then decreased to 2042 mg.L^{-1} at day 39. The MLVSS decreased with an increase in the PM concentrations to 1800 mg.L^{-1} and 1706 mgL^{-1} at PM concentrations of 1 mg.L^{-1} and 10 mg.L^{-1}, respectively. MBR2 is affected by the concentration of PM similarly to MBR1 from concentration 1 µg.L^{-1}to 10 mg.L^{-1}.

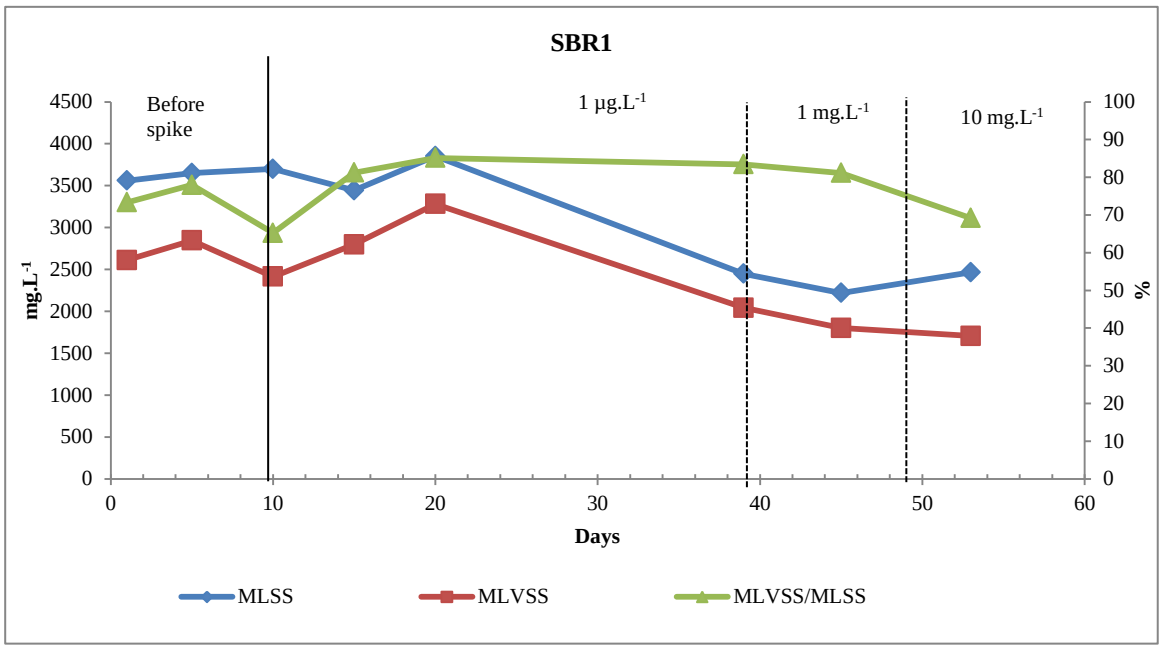


Figure 5.4.22 Variation of MLSS and MLVSS with change of PM concentration in SBR1.

5.4.4.4 Removal of COD and TOC in SBR1 at different PM concentrations

The COD removal efficiencies of SBR1 were similar to MBR1 at different concentrations of PM. The COD removal efficiency was 95% (±0.69) before the PM had been spiked; at PM concentrations 1 µg.L^{-1}, 1 mg.L^{-1}, and 10 mg.L^{-1}, COD removal efficiencies remained stable at concentration 1 µgL^{-1} and decreased to 85% (±6) and 85% (±2) for concentrations 1 mg.L^{-1} and 10 mg.L^{-1},respectively ($p<0.05$, ANOVA) (Figure 5.4.23). The TOC removal efficiencies of SBR1 were similar to MBR1 at PM concentrations 1 µg.L^{-1} and 1 mg.L^{-1} and were at steady state (Figure 5.4.23), but the TOC removal efficiency decreased when the PM concentration increased to 10 mg.L^{-1}.TOC removal efficiencies were at 96% (±0.8) before the PM

spiked and decreased to 92% (±5), 83% (±7), and 78% (±10) at PM concentrations 1 $\mu g.L^{-1}$, 1 $mg.L^{-1}$, and 10 $mg.L^{-1}$, respectively ($p<0.05$, ANOVA).

Specific COD and TOC utilisation rates of SBR1 were similar to those observed in the SBR control and MBR1 during the increased PM concentrations, while insignificant changes of specific COD and TOC utilisation rates were observed with the increased PM concentration, except that the specific TOC utilisation rate increased to 0.142 $mg_{TOC}.mg_{MLVSS}^{-1}.d^{-1}$at PM 1 $mg.L^{-1}$(Figure 5.4.24) ($p>0.2$, ANOVA).

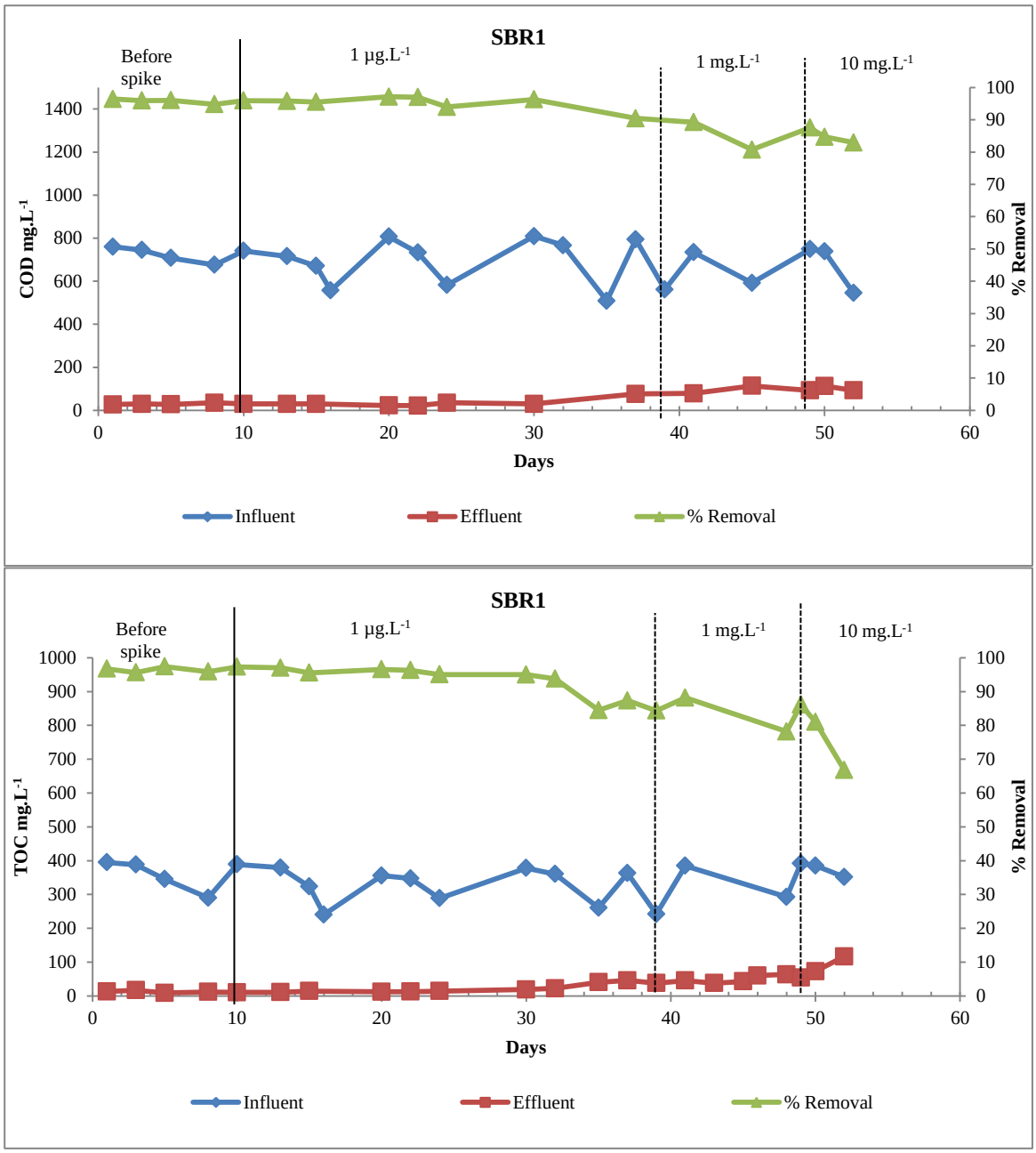


Figure 5.4.23: TOC and COD concentration and removal in SBR1 at different PM concentrations.

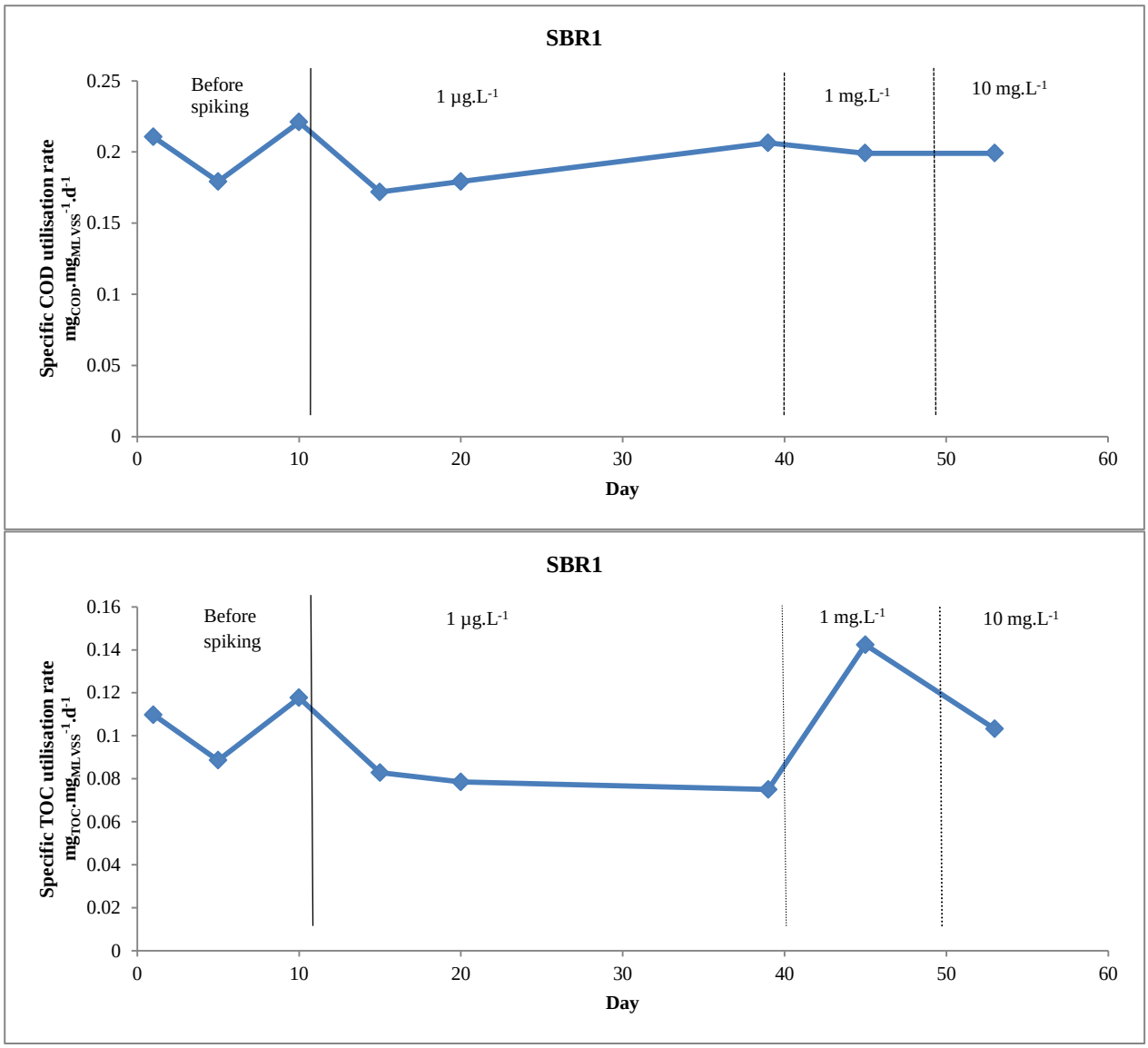


Figure 5.4.24: Specific TOC and COD utilisation rates for SBR1 at different PM concentrations.

5.4.4.4 PM removal efficiency in SBR1 at different concentrations

The removal of the PM spiked into SBR1 was different to that of MBR1. The removal efficiency of Metronidazole was 70% at a concentration of 1 µg.L^{-1}, 57% at a concentration of 1 mg.L^{-1} and further decreased to 45% at a concentration of 10 mg.L^{-1} ($p>0.1$, ANOVA) (Figure 5.4.25). The removal efficiencies of SBR1 for Trimethoprim were similar to MBR1 at a concentration of 1 µg.L^{-1} (67%) but decreased to 17% at a concentration of 1 mg.L^{-1} and 18% at 10 mg.L^{-1} concentration ($p>0.1$, ANOVA). Sulphamethoxazole showed a lower removal efficiency for SBR1 compared to MBR1 with 70% removal at a concentration of 1 µg.L^{-1}, 92% at 1 mg.L^{-1},but decreased sharply to 47% at a concentration of 10 mg.L^{-1} ($p>0.1$, ANOVA). Paracetamol removal in SBR1 was higher than MBR1, with 90% removal efficiency at a concentration of 1 µg.L^{-1} and 1 mgL^{-1}, and 92% at 10 mg.L^{-1} ($p>0.2$, ANOVA). The removal efficiency of Ranitidine in the SBR1 was also the highest, as observed in MBR1, with 95% removal at a concentration of 1 µg.L^{-1} and 99% at concentrations of 1 mg.L^{-1} and 10 mg.L^{-1} ($p<0.05$, ANOVA).

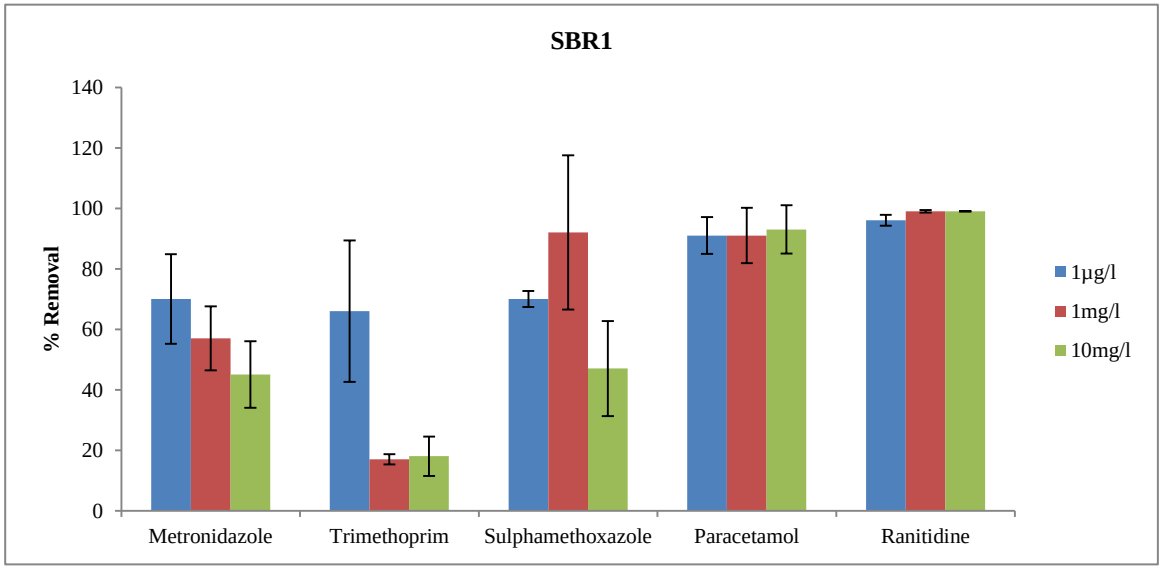


Figure 5.4.25: The removal efficiencies of pharmaceuticals in SBR1 at different PM concentrations.

The specific utilisation rates of Metronidazole, Trimethoprim, Sulphamethoxazole, Paracetamol, and Ranitidine in SBR1 were also similar to those in MBR1, generally increasing with an increase in initial PM concentration in the range of 1 $\mu g.L^{-1}$, 1 $mg.L^{-1}$, and 10 $mg.L^{-1}$ (Figure 5.4.26), indicating no apparent inhibition of the biodegradation of the pharmaceuticals. The specific pharmaceutical utilisation rates ($mg_{ph}.mg_{MLVSS}^{-1}.d^{-1}$) increased linearly with the increase of pharmaceutical concentration with a correlation coefficient (R^2) of 0.99 for all pharmaceuticals. The highest specific pharmaceutical utilisation rate for SBR1 was found for Ranitidine followed by Paracetamol, then Metronidazole and Sulphamethoxazole, and the lowest was Trimethoprim (Figure 5.4.26). The change of specific pharmaceutical utilisation rates with the increase of PM concentrations was highly significant for Metronidazole, Sulphamethoxazole, Paracetamol, and Ranitidine ($p<0.05$, ANOVA), but insignificant in Trimethoprim ($p>0.2$, ANOVA).

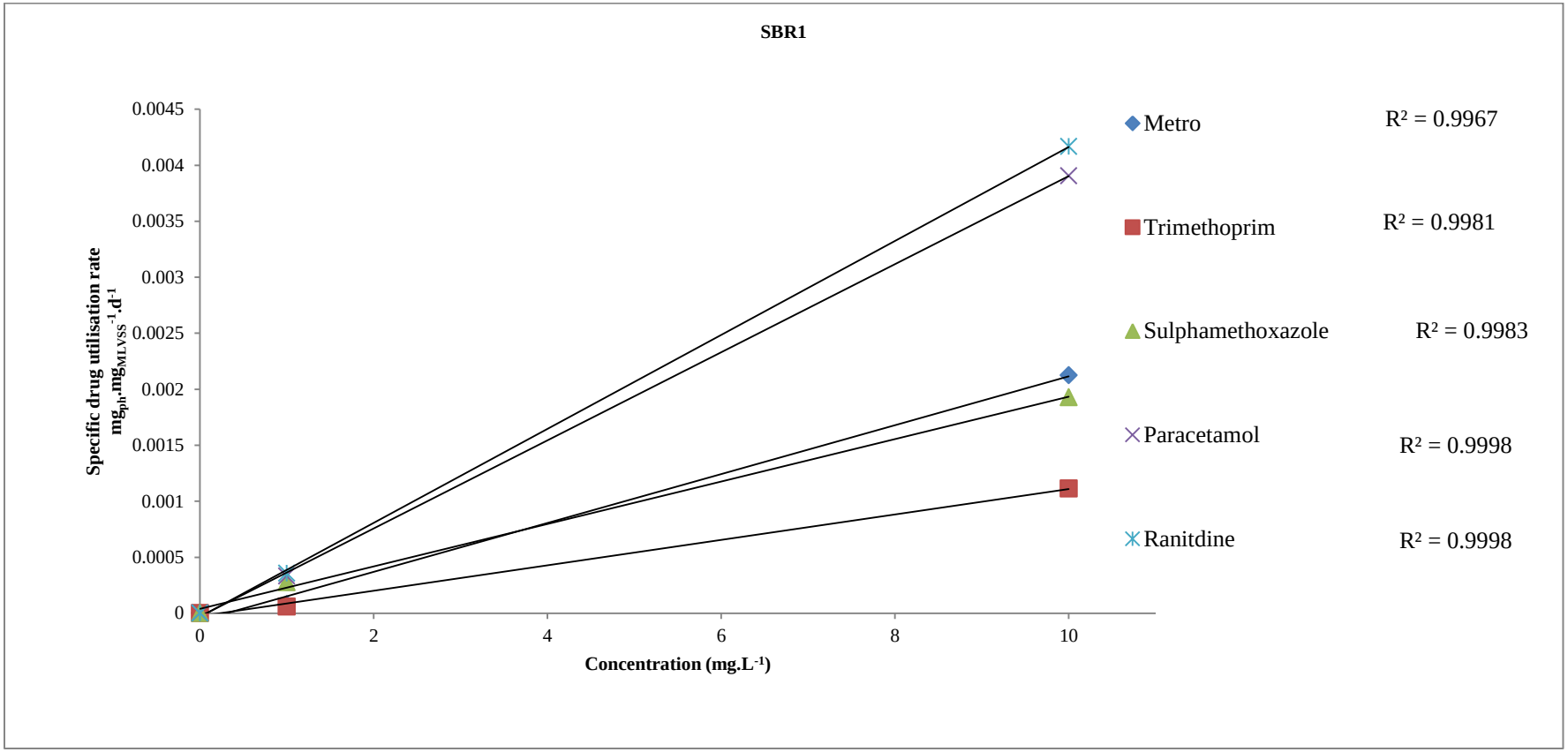


Figure 5.4.26: Specific pharmaceutical utilisation rates at different PM concentrations in SBR1 with regression.

5.4.5 Performance of SBR2 treating PM at three different concentrations under anoxic and aerobic condition

In the SBR2 the fate of PM was studied under a combination of anoxic and aerobic conditions using the sequencing batch reactor (SBR2) at different PM concentrations ranges (1 µg.L^{-1}, 1 mg.L^{-1}, and 10 mg.L^{-1}). The effects of PM concentration were studied by the operational scheduled anoxic/aerobic cycle (anoxic period 2 hours, aerobic period 16 hours), a hydraulic retention time (HRT) of 18 hours, and a solids retention time (SRT) of 53 d (Table 4.1) (see Section 4.3).

5.4.5.1 Variation of dissolved oxygen (DO)

The variation of the DO in SBR2 under different PM concentrations was similar to MBR2 except that the DO increased further at a concentration of 10 mg.L^{-1} (Figure 5.4.27). The DO decreased from 4.15 mg.L^{-1} initially to 3.49 mg.L^{-1} at day 10 before spiking the PM. The DO decreased to 2.67 mg.L^{-1} at the end run of 1 µgL^{-1} concentration, then started to increase to 2.87 mgL^{-1} and 3.24 mgL^{-1} at a concentrations of 1 mgL^{-1}, and 10 mgL^{-1}, respectively.

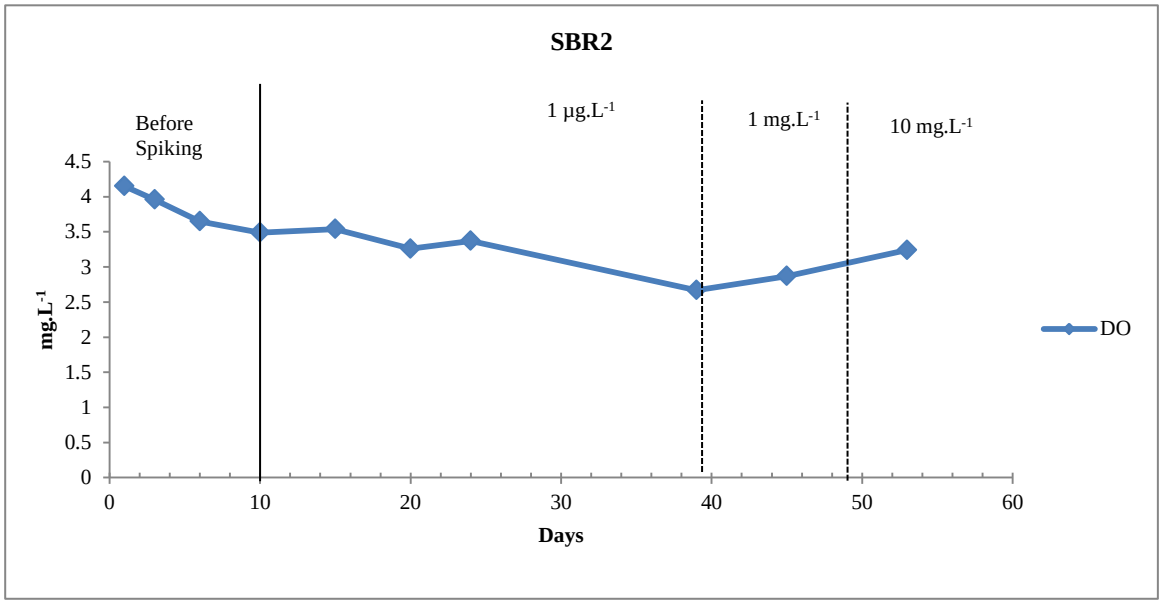


Figure 5.4.27: Changes in DO concentration with different PM concentrations in SBR2.

5.4.5.2 Variation of pH

As shown in Figure 5.4.28, the first 10 d of the experiment the effluent pH dropped to 6.46 (±0.38) which was similar to MBR2 and SBR1 and comparable to the SBR control. The effluent pH, after spiking the PM at a concentration of 1 µg.L^{-1}, decreased to 6.08 (±0.46) and remained steady even when the PM concentration increased to 1 mg.L^{-1}, and increased to 8.17 (±0.32) at a concentration of 10 mg.L^{-1}.

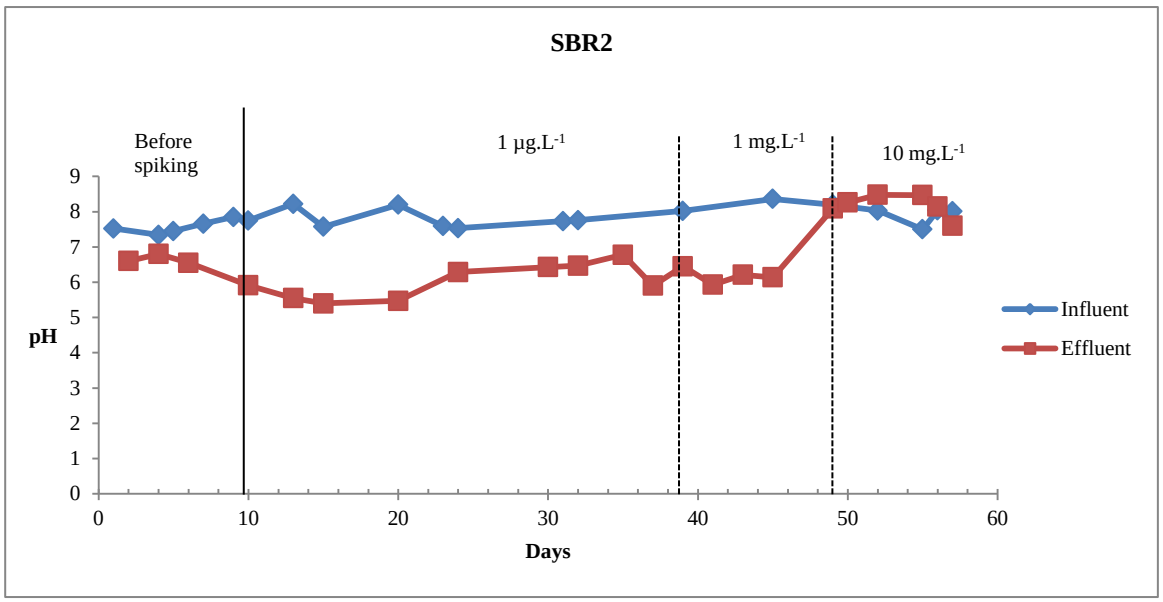


Figure 5.4.28: pH value of the influent and effluent of synthetic wastewater containing different PM concentrations in MBR2.

5.4.5.3 Variation of mixed liquid suspended solids (MLSS) and mixed liquid volatile suspended solids (MLVSS)

The MLSS and MLVSS concentrations in SBR2 were similar to those observed in the SBR1 during a period of 53 days. As can be seen in Figure 5.4.29, the MLSS increased before spiking the PM from 3155 mg.L^{-1} initially to 3311 mg.L^{-1} at day 10. The MLSS firstly increased to 3575 mg.L^{-1} when the PM was spiked at concentration 1 µg.L^{-1} at day 15, then gradually decreased to 2315 mg.L^{-1} at day 39, and also decreased when the PM concentration increased to 1 mg.L^{-1} and 10 mg.L^{-1} (Figure 5.4.29). Furthermore, the MLVSS concentration was changed similar to the MLSS where the MLVSS was the highest concentration at day 15 (2942 mg.L^{-1}) at a

concentration of 1 µg.L^{-1}, then the MLVSS decreased to 2014 mg.L^{-1} at day 39, and it decreased further when the PM concentration increased (Figure 5.4.29). SBR2 is affected by the concentration of PM similarly to MBR1 from concentration 1 µg.L^{-1} to 10 mg.L^{-1}.

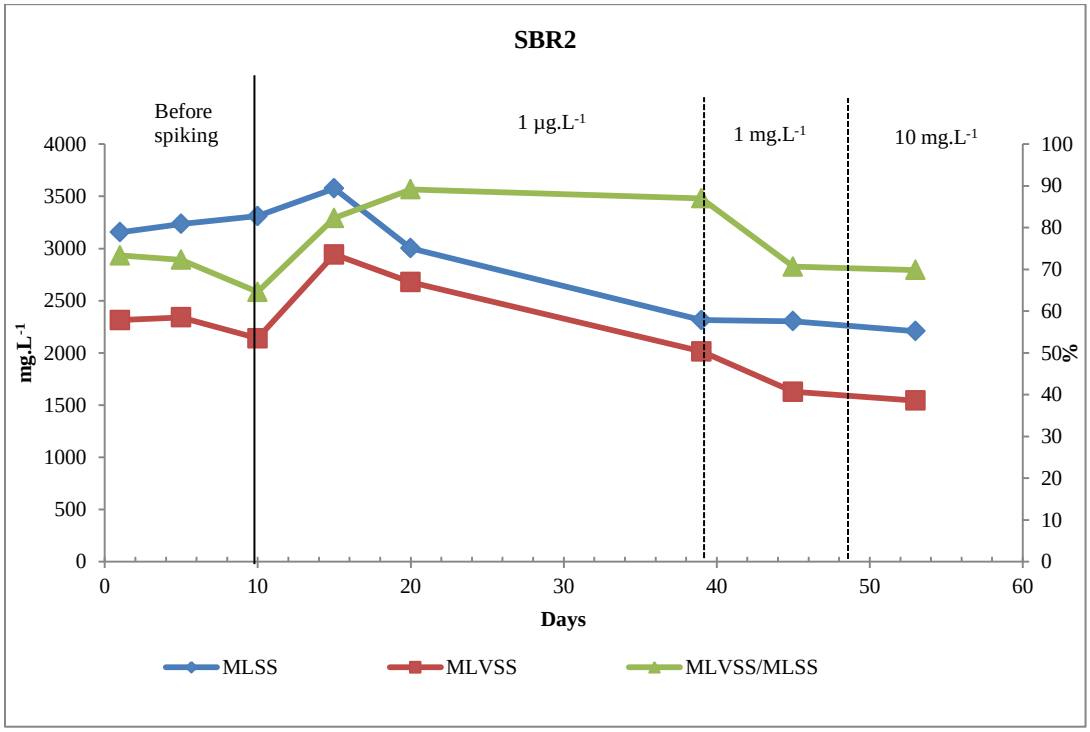


Figure 5.4.29: Variation of MLSS and MLVSS with change of PM concentration in SBR2.

5.4.5.4 Removal of COD and TOC in SBR2 at different PM concentrations

The COD removal efficiencies of SBR2 were similar to those found in the SBR1 at different ranges of PM concentrations. The COD removal efficiency was 96% (±0.26) before the PM had been spiked, when PM spiked at concentrations 1 µg.L^{-1}, 1 mg.L^{-1}, and 10 mg.L^{-1}, COD removal efficiencies was at steady state (95% (±1.23)) at a concentration of 1 µg.L^{-1}, and decreased to 89% (±3.9) and 85%

(±0.51) for a concentration of 1 $mg.L^{-1}$ and 10 $mg.L^{-1}$, respectively (Figure 5.4.30). COD removal efficiency decreased with increasing PM concentration ($p<0.05$, ANOVA). The TOC removal efficiencies of SBR2 were 96% (±0.69) before PM spiked, and were steady similar to SBR1 when fed with PM at concentrations of 1 $\mu g.L^{-1}$l and 1 $mg.L^{-1}$, decreased significantly to 93% (±3.1), 86% (±7.6), respectively, and decreased to 54% (±6.74) at a concentration 10 $mg.L^{-1}$ ($p<0.05$, ANOVA) (Figure 5.4.30).

The specific COD utilisation rates of SBR2 were relatively similar to SBR1, changing significantly with the increasing PM concentrations, except that it increased at a concentration of 1 $mg.L^{-1}$ at day 45 (ANOVA, $p=0.08$) (Figure 5.4.31). Furthermore, the specific TOC utilisation rates of SBR2 were also similar to SBR1, and the specific TOC utilisation rate changed less significantly with the increase of PM concentrations (ANOVA, $p = 0.18$) (Figure 5.4.31).

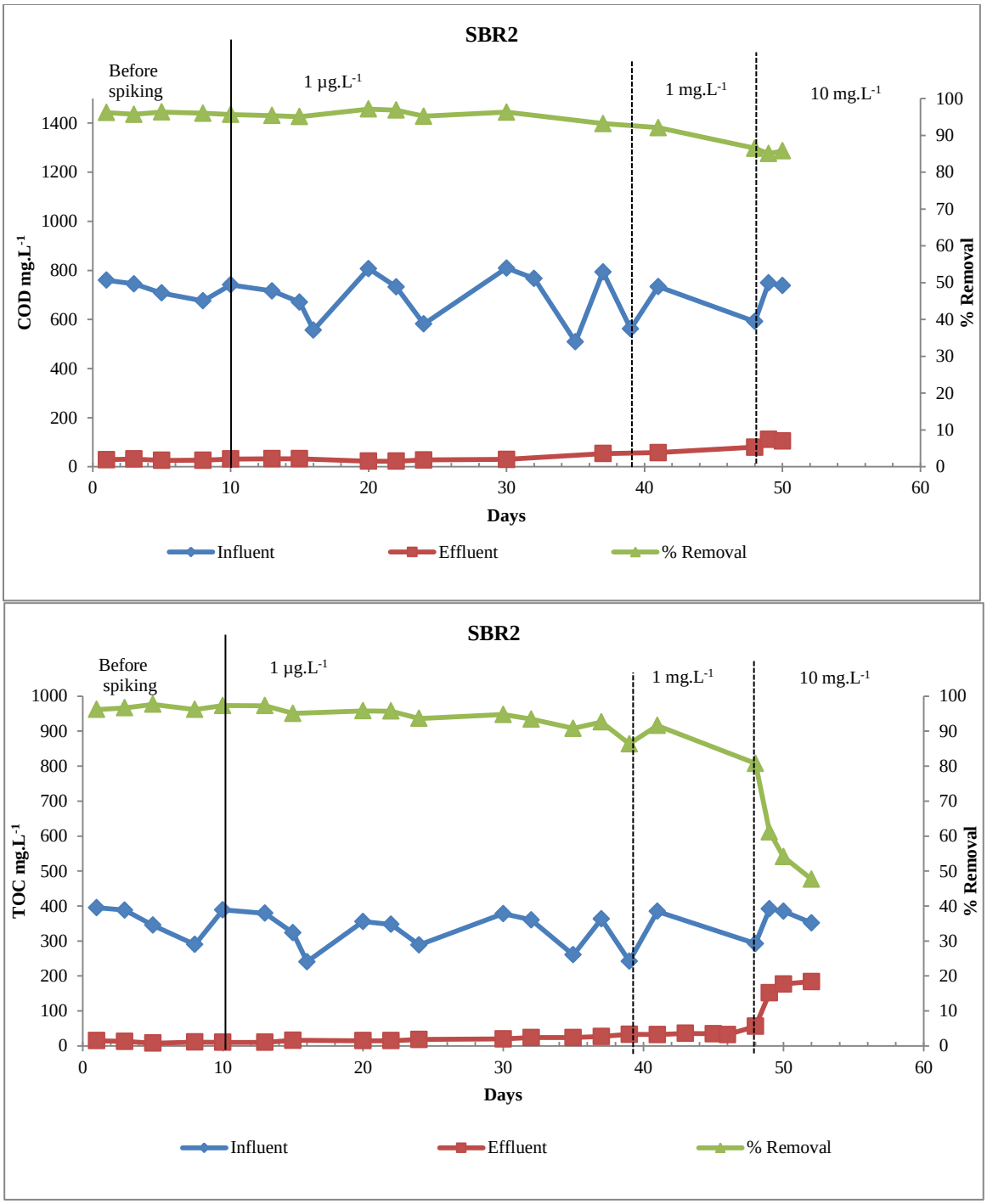


Figure 5.4.30: TOC and COD concentration and removal in SBR2 at different PM concentrations.

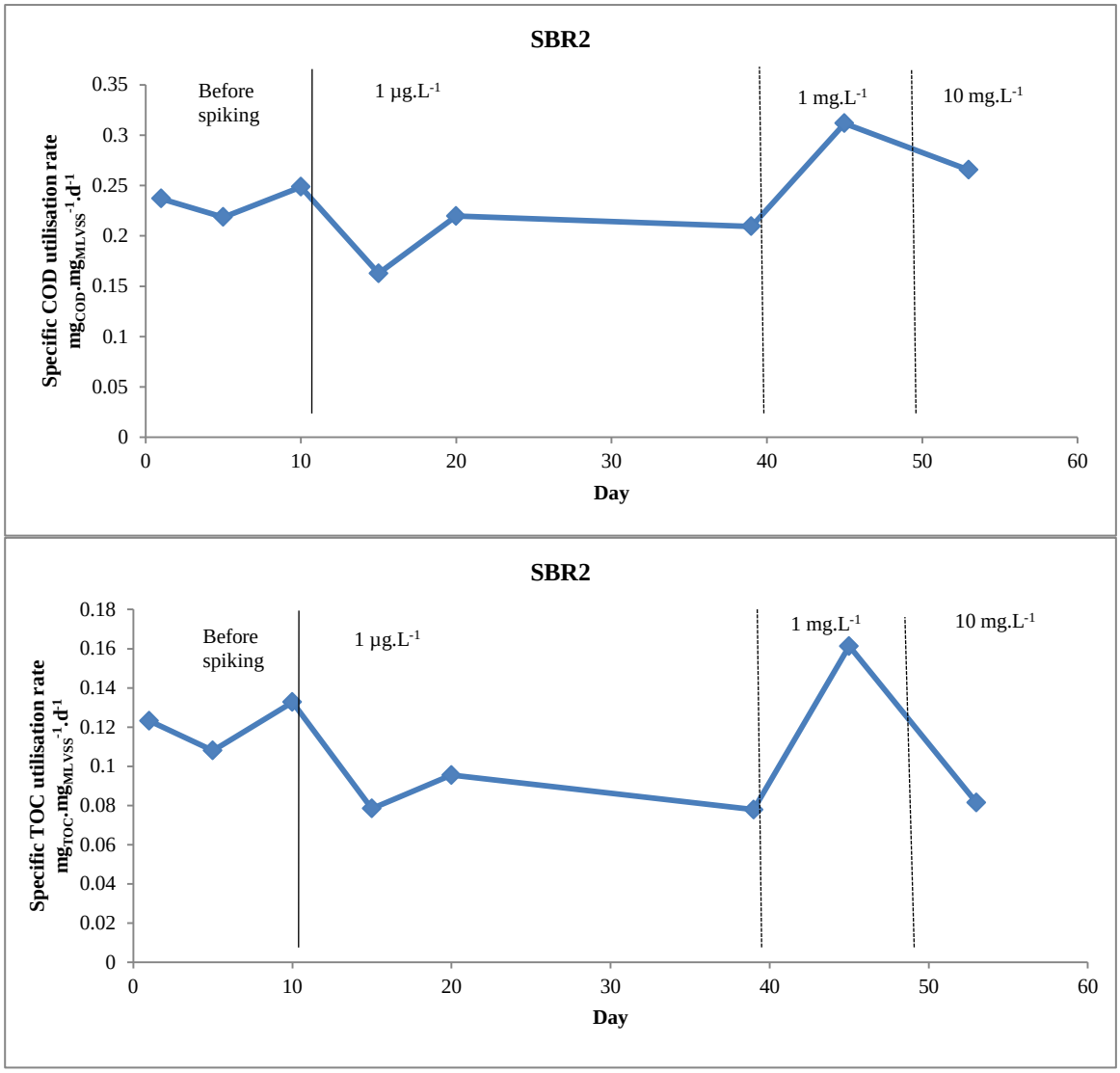


Figure 5.4.31: Specific TOC and COD utilisation rates for SBR2 at different PM concentrations.

5.4.5.4 PM removal efficiency in SBR2 at different concentrations

The removal of the PM spiked into synthetic wastewater was studied using SBR2. The removal efficiency of Metronidazole fluctuated insignificantly with the increasing PM concentrations ($p>0.2$, ANOVA), and the removal was 64%, 69% and 38% at concentrations 1 µg.L^{-1}, 1 mg.L^{-1} and 10 mg.L^{-1}, respectively (Figure 5.4.32). The removal efficiencies of SBR2 for Trimethoprim was the highest (89%) at

concentration 1 µg.L^{-1}compared with other reactors (MBR1, MBR2 and SBR1), and decreased significantly to 42% and 27% at a concentration of 1 mg.L^{-1} and 10 mg.L^{-1}, respectively ($p<0.05$, ANOVA) (Figure 5.4.32). Sulphamethoxazole removal efficiency in SBR2 was 81% at concentration 1 µg.L^{-1}, then increased to 95% at a concentration of 1 mg.L^{-1} and decreased significantly to 32% at a concentration of 10 mg.L^{-1}($p<0.05$, ANOVA) (Figure 5.4.32). Insignificant changes were found in the removal efficiency of Paracetamol with the increase of PM concentrations in the SBR2, 87% removal at concentration 1 µg.L^{-1}, which then increased to 93% and 92% at concentrations of 1 mg.L^{-1} and 10 mg.L^{-1}, respectively ($p>0.2$, ANOVA) (Figure 5.4.32). The removal efficiency of Ranitidine in the SBR2 was also high, 97% at concentrations 1 µg.L^{-1} and 1 mg.L^{-1} and increased to 98% removal at a concentration of 10 mg.L^{-1}, while the changes of removal efficiencies with the increase of PM concentrations was insignificant ($p>0.2$, ANOVA) (Figure 5.4.32).

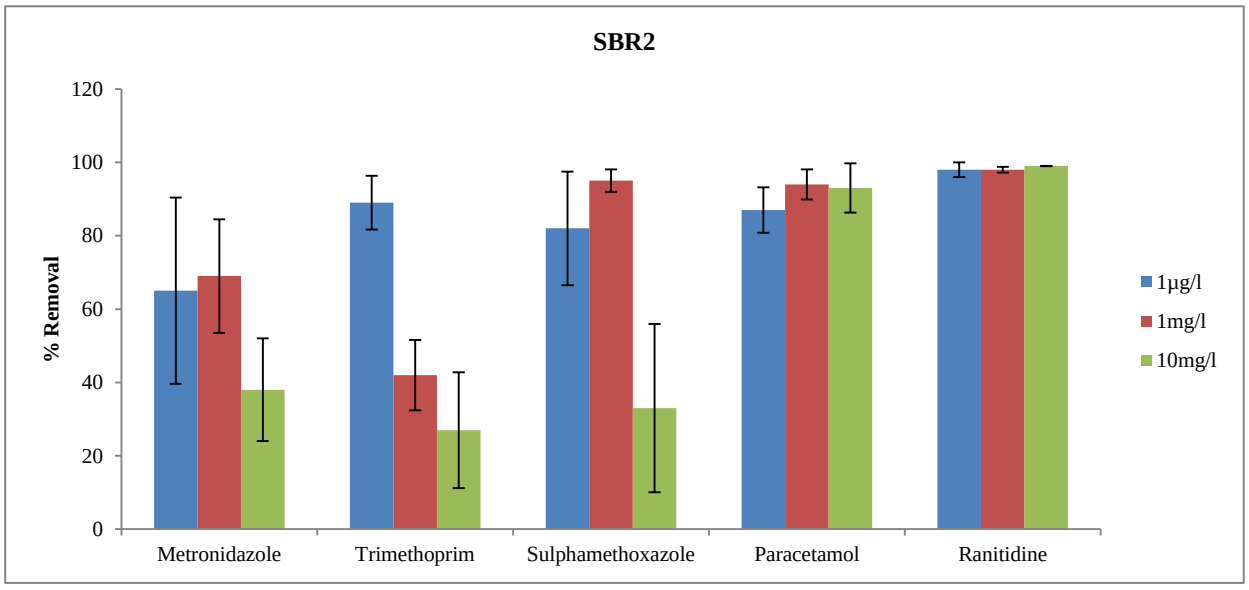


Figure 5.4.32: The removal efficiencies of pharmaceuticals in SBR2 at different PM concentrations.

The specific utilisation rates of Metronidazole, Trimethoprim, Sulphamethoxazole, Paracetamol, and Ranitidine in SBR2 were similar to those of SBR1, which increased with increasing concentration in the range of 1 $\mu g.L^{-1}$, 1 $mg.L^{-1}$, and 10 $mg.L^{-1}$ (Figure 5.4.33). The specific utilisation rates increased with the increase of initial PM concentration, demonstrating that no apparent inhibition in the biodegradation of the PM studied. The specific pharmaceutical utilisation rates ($mg_{ph}.mg_{MLVSS}^{-1}.d^{-1}$) increased linearly with the increase of the PM concentration with a correlation coefficient (R^2) of 0.99 for all the pharmaceuticals studied. The effect of microorganisms in the activated sludge on each pharmaceutical was the same as in SBR1 at all concentrations. The highest specific pharmaceutical utilisation rate for SBR2 was found for Ranitidine followed by Paracetamol, then Metronidazole and Sulphamethoxazole, and the lowest was Trimethoprim. The increase in specific pharmaceutical utilisation rate changes were highly significant with increasing concentrations for Trimethoprim, Paracetamol, and Ranitidine ($p<0.05$, ANOVA), while it was significant in Sulphamethoxazole ($p=0.07$, ANOVA) and insignificant in Metronidazole ($p>0.2$, ANOVA).

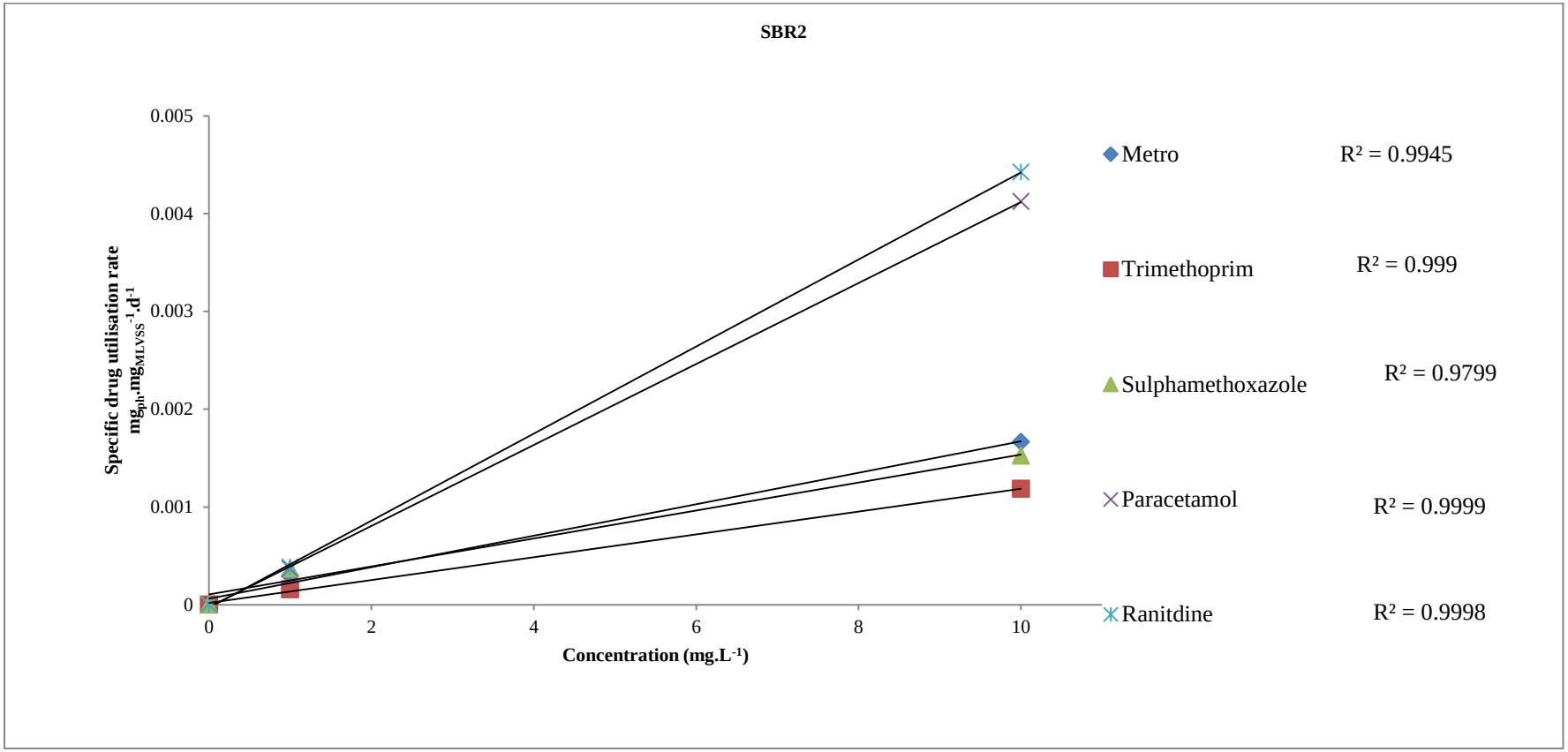


Figure 5.4.33: Specific pharmaceutical utilisation rates at different PM concentrations in SBR2 with regression.

5.4.6 Performance of bioreactors with real municipal wastewater

Performance of the four reactors (MBR1, MBR2, SBR1, and SBR2) was tested with a real municipal wastewater feed spiked with PM at a concentration of 1 $mg.L^{-1}$ to evaluate the removal efficiencies. Real municipal wastewater was used towards the end of the experimental study, and these treatability studies were conducted for comparison with the results of the synthetic wastewater studies. Comparisons of overall treatability of the four reactors on the actual municipal wastewater was based on effluent quality and pharmaceutical degradation patterns.

The initial results had been obtained with synthetic wastewater as the sole organic carbon source, and this was highly removed in all the control reactors (without PM). The synthetic wastewater COD was also removed efficiently at PM concentrations of 1 $\mu g.L^{-1}$ (Figure 5.4.34). Analysis always detected remaining soluble COD at the end of each run, which mostly revealed the presence of PM, or residuals of low biodegradable products. The results suggest that the PM was probably adsorbed by the sludge, based on the assumption that they are practically non-biodegradable with short-term exposure to non-acclimated biomass.

During the test of real municipal wastewater that contained 1 $mg.L^{-1}$ of PM, the effluent pH increased to 6.8 (±0.56), 7.32 (±0.25), 7.26 (±0.28), and 7.41 (±0.13) for MBR1, MBR2, SBR1, and SBR2, respectively (Table 5.4.1). All the reactors using real municipal wastewater showed good COD removal of 79, 88, 86, and 82 % for MBR1, MBR2, SBR1, and SBR2, respectively, when compared with the COD removal for synthetic wastewater (Figure 5.4.34). On the other hand, the TOC removal efficiency with real municipal wastewater was less than that for synthetic wastewater at all PM concentrations, with TOC removal of 66, 62, 73, and 59 % for reactors MBR1, MBR2, SBR1, and SBR2, respectively. This can be explained by the relatively low concentration of easily biodegradable organic compounds present in

real municipal wastewater, which is only partially soluble and contains greater component of hard COD and complex molecules, unlike synthetic wastewater which contains highly soluble simple molecules as the organic matter. Consequently, the removal rates of COD for municipal wastewater were low in comparison with those found for the synthetic wastewater. A similar trend was observed for removal rates of TOC (Table 5.4.1). Furthermore, the DO concentration was relatively high in all reactors treating real municipal wastewater, being in the range 4.75 to 5.07 $mg.L^{-1}$, indicating lower levels of microbial activity. Interestingly, the MLVSS was relatively high in the real municipal wastewater, where it decreased with increasing PM concentrations in synthetic wastewater. Furthermore, the specific COD and TOC utilisation rates were lower in municipal wastewater compared with synthetic wastewater at most pharmaceutical concentrations, with 0.059 to 0.074 $mg_{COD}.mg_{MLVSS}^{-1}.d^{-1}$ for COD, and 0.024 to 0.031 $mg_{TOC}.mg_{MLVSS}^{-1}.d^{-1}$ for TOC (Table 5.4.1).

The observations from the current study are similar to results obtained from other studies conducted under aerobic conditions. Due to the acute inhibititory effect of PM observed in aerobic systems, the consumption of dissolved oxygen was significantly reduced upon first exposure to the PM. The study by Orhon et al. (2010), observed significant reductions in the amount of DO consumption due to the acute effect of 2,6-dihydrobenzoic acid on the biodegradation of a peptone mixture. Similarly, the acute effect of the same pharmaceutical on the biodegradation of organics in wastewater under aerobic conditions presented the same results in the reduction in the amount of oxygen consumed with the corresponding portion of the organic available in the experiments remaining unused (Cetecioglu, 2011; Ozkok et al., 2011).

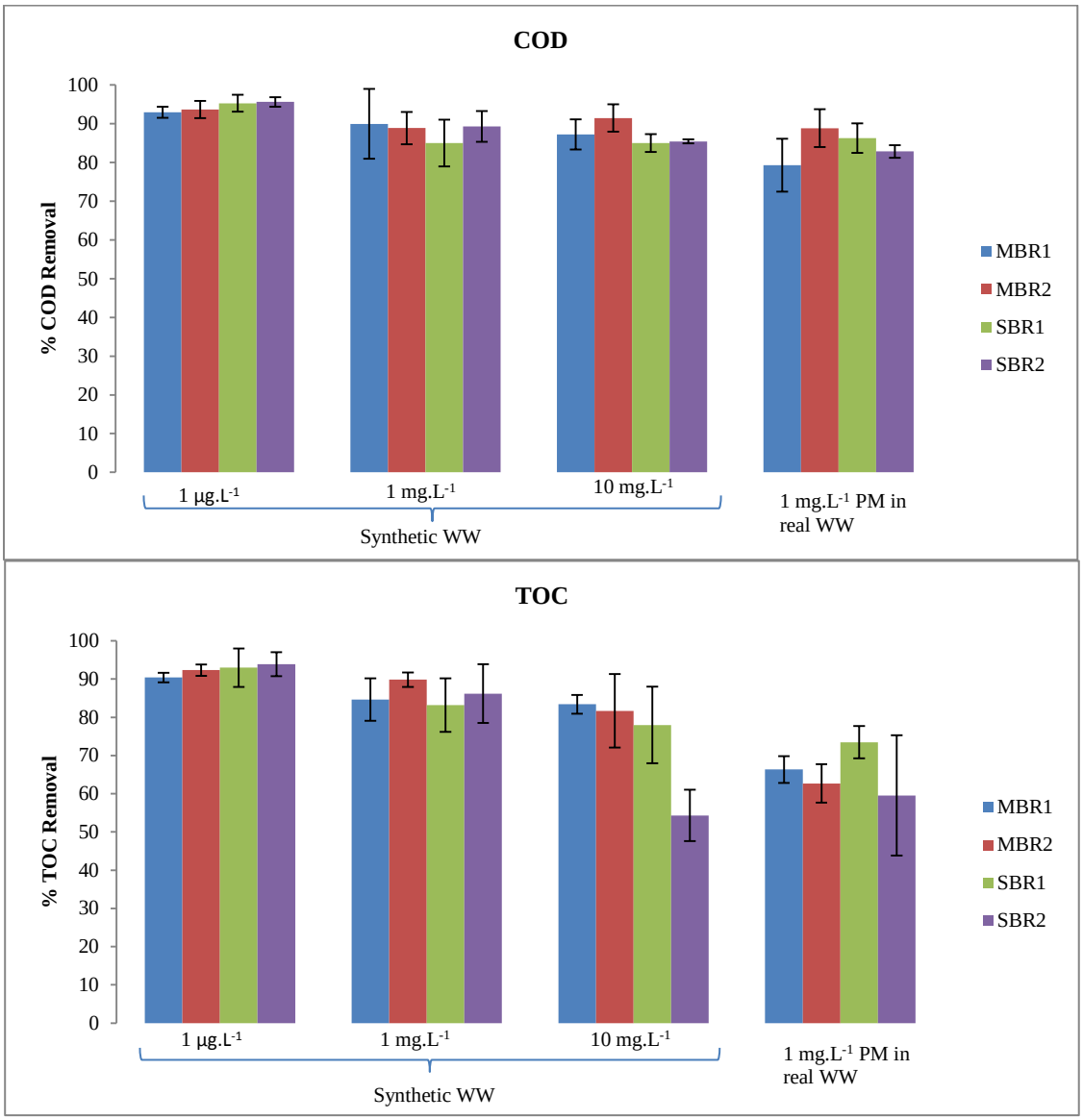


Figure 5.4.34: The TOC and COD removal at all reactors at different PM concentrations in synthetic wastewater and real municipal wastewater.

Table 5.4.1: Summary of performance data from the four bioreactors at different PM concentrations and types of wastewaters.

Reactor	MBR1					MBR2					SBR1					SBR2				
PM concentration	0	1 μg.L^{-1}	1 mg.L^{-1}	10 mg.L^{-1}	municipal WW + 1mg.L^{-1}	0	1 μg.L^{-1}	1 mg.L^{-1}	10 mg.L^{-1}	municipal WW + 1mg.L^{-1}	0	1 μg.L^{-1}	1 mg.L^{-1}	10 mg.L^{-1}	municipal WW + 1mg.L^{-1}	0	1 μg.L^{-1}	1 mg.L^{-1}	10 mg.L^{-1}	municipal WW + 1mg.L^{-1}
Influent pH	7.56 ±0.22	7.80 ±0.31	8.27 ±0.03	7.97 ±0.28	7.61 ±0.32	7.56 ±0.22	7.80 ±0.31	8.27 ±0.03	7.97 ±0.28	7.61 ±0.32	7.56 ±0.19	7.81 ±0.26	8.28 ±0.11	7.95 ±0.26	7.61 ±0.32	7.56 ±0.19	7.81 ±0.26	8.28 ±0.11	7.95 ±0.26	7.61 ±0.32
Effluent pH	6.19 ±0.22	5.99 ±0.22	6.03 ±0.44	6.37 ±0.11	6.80 ±0.56	6.39 ±0.35	5.63 ±0.24	6.12 ±0.66	7.38 ±0.32	7.32 ±0.25	6.39 ±0.33	5.56 ±0.29	6.28 ±0.88	6.71 ±0.74	7.26 ±0.28	6.46 ±0.38	6.08 ±0.46	7.49 ±1.17	8.19 ±0.35	7.41 ±0.13
DO	3.77	3.27	2.94	2.93	4.78	3.83	3.18	3.61	3	4.75	3.73	3.31	3.05	3	4.98	3.81	3.21	2.87	3.24	5.07
MLSS	2758	2369	2396	2331	3195	3645	3241	2662	2388	2978	3636	3247	2218	2466	3031	3234	2964	2303	2209	2859
MLVSS	2476	2198	1906	1437	2647	3113	2073	1741	1532	2442	2623	2706	1800	1706	2197	2264	2544	1627	1543	2290
MLVSS/MLSS	89	92	79	61	82	85	64	65	64	82	72	83	81	69	72	70	86	70	69	80
TOC_{inf}	346.3 (±60)	325 (±48)	308 (±57)	358 (±37)	138 (±12)	346 (±60)	325 (±48)	291 (±57)	358 (±37)	138 (±12)	354 (±48)	327 (±55)	338 (±65)	376 (±21)	138 (±12)	354 (±48)	327 (±55)	338 (±65)	376 (±21)	138 (±12)
TOC_{Eff}	15 (±7)	31 (±4)	42 (±12)	58 (±5)	46 (±6)	18 (±9)	25 (±6)	29 (±6)	62 (±27)	51 (±9)	12 (±3)	21 (±13)	50 (±11)	77 (±27)	36 (±7)	11 (±2)	19 (±7)	38 (±10)	173 (±14)	56 (±24)
% TOC Removal	95 (±3)	90 (±1)	84 (±5)	83 (±2)	66 (±3)	94 (±4)	92 (±1)	89 (±1)	81 (±9)	62 (±5)	96 (±0.8)	92 (±5)	83 (±7)	77 (±10)	73 (±4)	96 (±0.6)	93 (±3)	86 (±7)	54 (±6)	59 (±15)
COD_{Inf}	730 ±36	698 ±104	633 ±172	671 ±132	259 ±16	730 ±36	698 ±104	633 ±172	671 ±132	259 ±16	722 ±37	687 ±108	663 ±100	677 ±114	259 ±16	722 ±37	687 ±108	663 ±100	677 ±114	259 ±16
COD_{Eff}	31 (±7)	48 (±3)	56 (±39)	82 (±8)	54 (±21)	38 (±8)	43 (±13)	67 (±7)	54 (±10)	29 (±15)	30 (±3)	34 (±17)	96 (±24)	99 (±11)	36 (±12)	28 (±2)	32 (±9)	69 (±15)	108 (±4)	44 (±4)
% COD Removal	95 (±1)	92 (±1)	89 (±9)	87 (±3)	79 (±6)	94 (±1)	93 (±2)	88 (±4)	91 (±3)	88 (±4)	95 (±0.6)	95 (±2)	84 (±6)	85 (±2)	86 (±3)	96 (±0.2)	95 (±1)	89 (±3)	85 (±0.5)	82 (±1)
Spec. COD utilistion rate (mg.mg$_{MLVSS}^{-1}$.d^{-1})	0.216	0.208	0.168	0.224	0.059	0.169	0.214	0.189	0.222	0.07	0.203	0.194	0.199	0.199	0.074	0.234	0.21	0.338	0.265	0.067
Spec. TOC utilistion rate (mg.mg$_{MLVSS}^{-1}$.d^{-1})	0.108	0.092	0.087	0.149	0.024	0.085	0.093	0.105	0.132	0.024	0.105	0.088	0.142	0.103	0.031	0.121	0.096	0.161	0.081	0.027

Results of the PM removal in the studied bioreactor configurations showed that the different treatment designs (MBR versus SBR) could also affect the removal of the PM since it fluctuated between bioreactors. In addition, correlations between removal efficiencies of the pharmaceuticals, and the removal efficiencies of general parameters, were observed. However, some similar trends were observed between the aerobic bioreactors and the anoxic-aerobic bioreactors. These results imply that the aerobic microorganisms are mostly responsible for the degradation of the pharmaceuticals.

The overall removal of the pharmaceuticals by all the bioreactors under continuous PM loading was relatively steady over the experimental period of 63 days. It was observed that higher removal rates for Ranitidine occurred during the first day, suggesting that equilibrium was reached rapidly. Paracetamol was also seen to be removed more effectively with average elimination efficiencies of 88-93% by all bioreactors, whereas the average removal for Ranitidine ranged between 96-99%, indicating that Ranitidine is more biodegradable. On the other hand, Sulphamethoxazole also showed relatively high removal rates in all bioreactors, average elimination efficiencies being 70-97%, whereas the removal of Trimethoprim ranged between 18-69%, indicating that Trimethoprim is more resistant to biological treatment than the Sulphamethoxazole. However Metronidazole showed only moderate removal in all bioreactors, with average removal ranging between 29-83%.

Figure 5.4.35 shows the removal of Metronidazole in the four different reactors. The removal of Metronidazole spiked in real municipal wastewater was very low compared with its removal in synthetic wastewater at similar conditions, real wastewater showing only 28, 53, 33, and 47 % Metronidazole removal in MBR1, MBR2, SBR1, and SBR2, respectively. The low and highly variable removal of the Metronidazole is in good agreement with the report of Beier et al. (2010), and may

be attributed to the presence of a strong electron withdrawing group EWG nitro group in its structure. Metronidazole was not biodegradable in the laboratory-based batch experiment and is relatively hydrophilic according to Alexy et al. (2004). Because of these factors Metronidazole was not expected to be effectively removed during conventional wastewater treatment. On the other hand, an excellent removal of Metronidazole (95%) was observed with an MBR by Dolar et al. (2012).

Similarly, the removal of Trimethoprim was also low in real municipal wastewater (Figure 5.4.36), but was higher than at PM concentration of 1 $mg.L^{-1}$ in synthetic wastewater for all reactors (Figure 5.4.36). The removal of Trimethoprim was highest in MBR2 and SBR2 at 58%, followed by MBR1 at 57%, and lowest in SBR1 (33%). A previous study by Batt el al. (2006) reported that enhanced biodegradation of Trimethoprim does occur in nitrifying activated sludge where the removals were approximately 20% in both nitrifying and inhibited nitrifying activated sludge reactors (Batt et al., 2006). On the other hand, research by Göbel et al. (2007) reported comparable elimination rates for SRTs of 16 and 33 days (30%), while 87% removal of Trimethoprim was obtained for SRTs in the range of 60–80 days in an MBR. In contrast, Tambosi et al. (2010) observed the highest removal efficiencies for Trimethoprim at 86% and 94% at 15 and 30 SRT respectively.

In contrast, the removal of Sulphamethoxazole was relatively high compared with the previous pharmaceuticals but was degraded less efficiency in real municipal wastewater than in synthetic wastewater (Figure 5.4.37). The removal of Sulphamethoxazole in municipal wastewater was the highest in MBR2 at 87%, followed by MBR1 at 79%, and lowest in SBR2 and SBR1, at 52% and 28%, respectively. Göbel et al. (2007), who studied the elimination of Sulphamethoxazole, reported an elimination efficiency of around 80%. On the other hand, Tambosi et al. (2010) reported that Sulphamethoxazole was eliminated by 55% to 64% in the MBR treatment processes.

The removal of Paracetamol in real municipal wastewater was relatively high for all reactors but still lower than that found in the synthetic wastewater (Figure 5.4.38). The highest removal of Paracetamol was in MBR2 at 85%, followed by SBR1 and

MBR1 at 82% and 81%, respectively. The lowest removal of Paracetamol was in SBR2 at 68%. The structure of Paracetamol allows the bacteria and enzymes to readily attack the molecule. This removal of Paracetamol could be attributed to biological conversion, resulting in quite stable transformation products which are conjugates of Paracetamol. These results are in agreement with other reported studies which obtained 99% Paracetamol removal during the treatment of municipal sewage in an MBR (Kim et al., 2007; Tambosi et al., 2010).

The removal of Ranitidine from real municipal wastewater was the highest at 97% compared with other spiked pharmaceuticals and had the same level of removal in all reactors (Figure 5.4.39). Therefore, the effect of an increase in pharmaceutical concentration on the Ranitidine removal was negligible. High and steady Ranitidine removal of more than 80% in the MBR was observed by Radjenovic et al. (2007). Similarly, Dolar et al. (2012) observed a removal efficiency of Ranitidine in wastewater equal to 89%, whereas Radjenovic et al. (2009) observed a lower removal of Ranitidine of 44.2%.

The mechanism of pharmaceutical biodegradation is well studied in the literature, mostly by enzyme analogy, and conveniently associating into two enzymatic mechanisms; firstly, competitive drugs which compete on the substrate for the same reactive site on the enzyme and secondly, non-competitive pharmaceuticals which can also bind with the enzyme and deactivate the bound enzyme sites (Campell and Farrell, 2007). In competitive or non-competitive experiment approaches, as in studies by Fountoulakis et al., 2008, the important point to consider in the results of this study is that the pharmaceutical impact is kinetic, slowing down the rate of different reactions for substrate utilisation. Thus, it would take more time for the completion of microbial activity ending with complete utilisation of available organic substrate, but the same stoichiometry would be observed at anytime along the pathway of biochemical reactions, (Campell and Farrell, 2007). The same would be true for a possible toxic effect of a pharmaceutical inactivating a part of the microbial community. However, remained substrate observed in the current study gives indication of a stoichiometric disturbance, as contrasted with the kinetic impact

reported for pharmaceutical effect studies. It is interesting to note that the results of Fountoulakis et al., 2008 related to the effect of ofloxacine on an organic substrate providing supporting experimental evidence as they showed the same disagreement between model simulation and experimental values.

In this current study, the substrate was removed by microorganisms and the partially used pharmaceutical which could be interpreted from the substrate binding effect. The corresponding enzyme analogy probably has an uncompetitive effect, where the pharmaceutical could bind the enzyme substrate complex but not the free enzyme; this would correspond to the blocking of the substrate after enzymatic uptake, within the biomass. As previously mentioned, a similar binding effect of organic substrate by the same pharmaceutical at lower doses was also observed under aerobic conditions (Ozkok et al., 2011).

Sorption onto sludge is one of the mechanisms which can be described as absorption and adsorption. According to Carballa et al. (2005), absorption is a hydrophobic interaction of the aromatic and aliphatic groups of a compound with the lipophilic cell membrane of the microorganisms, or with the sludge (depending on their K_{ow} value), while adsorption is an electrostatic interaction of positively charged groups of dissolved chemicals with the negatively charged surfaces of the microorganisms (characterized by the dissociation constant pK_a). Göbel et al. (2007) concluded that the removal of pharmaceuticals in an MBR and conventional activated sludge treatment by activated sludge adsorption was less than 6%. Therefore, the adsorption in the system was negligible, because this is within the analytical variance of the method.

Physical retention of the membranes is another mechanism responsible for the removal of pharmaceutical compounds in the MBR. However, due to the molecular weight cut off of ultrafiltration MBR membranes are around 100–200 kDa which is much greater than the molecular weights of these pharmaceuticals (200-300 Daltons) and highly soluble at neutral pH (logD values < 2), the mechanism would not have

led to retention of the pharmaceuticals. The rejections were expected due to electrostatic interactions (attractive or repulsive) rather than size exclusion and hydrophobic interaction. The membranes are often negatively charged at neutral pH (Nghiem et al., 2005; Schafer et al., 2003; Simon et al., 2009), and thus negatively charged pharmaceuticals are rejected mainly through electrostatic repulsion; while the positively charged pharmaceuticals are removed by a combination of attractive electrostatic interaction with the membrane surface and Donnan equilibrium (Schaep et al., 2001; Verliefde et al., 2008).

Metronidazole is a significantly hydrophilic compound and its removal was low to moderate in this study. This can be explained by the presence of one or more strong EWG (amide group and nitro group) or absence of strong electron donating group EDG in their structures. Our results regarding the removal efficiency of Metronidazole are in agreement with previous reports (Clara et al., 2005; Joss et al., 2005; Radjenovic et al., 2007; Tadkaew et al., 2011).

Sorption onto the membranes is also limited because of the available membrane surface area. Nhat LE (2011) found the amounts of Trimethoprim, which were partially positively charged at pH near 7, adsorbed to the membrane surface was 2.93% while the adsorption of Sulphamethoxazole, which is mostly negatively charged at neutral pH, was much lower (less than 0.53%) and these figures reduced over the 4 hour experiment. This decrease in rejection rates may be due to the charge equilibrium occurring on the membrane surface.

Sulphamethoxazole has a hydrophilic nature with two ionizable amine groups. As a result, in an aqueous solution, it can be present in positive, neutral and negative forms. At pH values between 1.4 - 5.8, the pKa value of Sulphamethoxazole is present predominantly as a neutral, while above pH 5.8 the pKa value becomes negatively charged. These properties indicate that in all reactors at pH 7.2 the sludge adsorption mechanism played a negligible role, due to electrostatic repulsion between the negatively charged Sulphamethoxazole and the negatively charged surfaces of the sludge. Therefore, biodegradation processes can be considered the main mechanism responsible for the removal.

One anomalous result obtained was the high removal of Ranitidine, despite containing a strong EWG (amide and nitro group) (Tadkaew et al., 2011). A possible explanation is that the presence of methyl groups (weak EDG) led to conversion of the methyl group to alcohol (Shaw and Harayama, 1992), bypassing the problematic amide conversion. In contrast, Paracetamol is a less hydrophobic compound (logD < 3.2) containing a strong EDG hydroxyl group, and was consistently removed to a high degree, which agrees well with reports in other literature (Visvanathan et al., 2005).

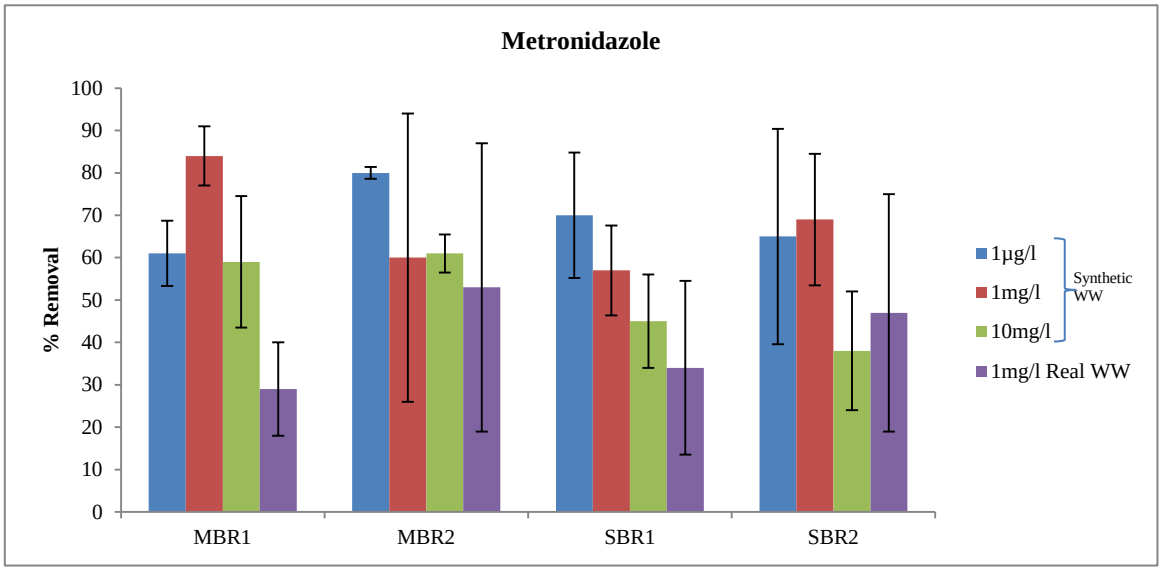


Figure 5.4.35: The removal efficiencies of Metronidazole in four reactors at different PM concentrations with synthetic and real wastewaters.

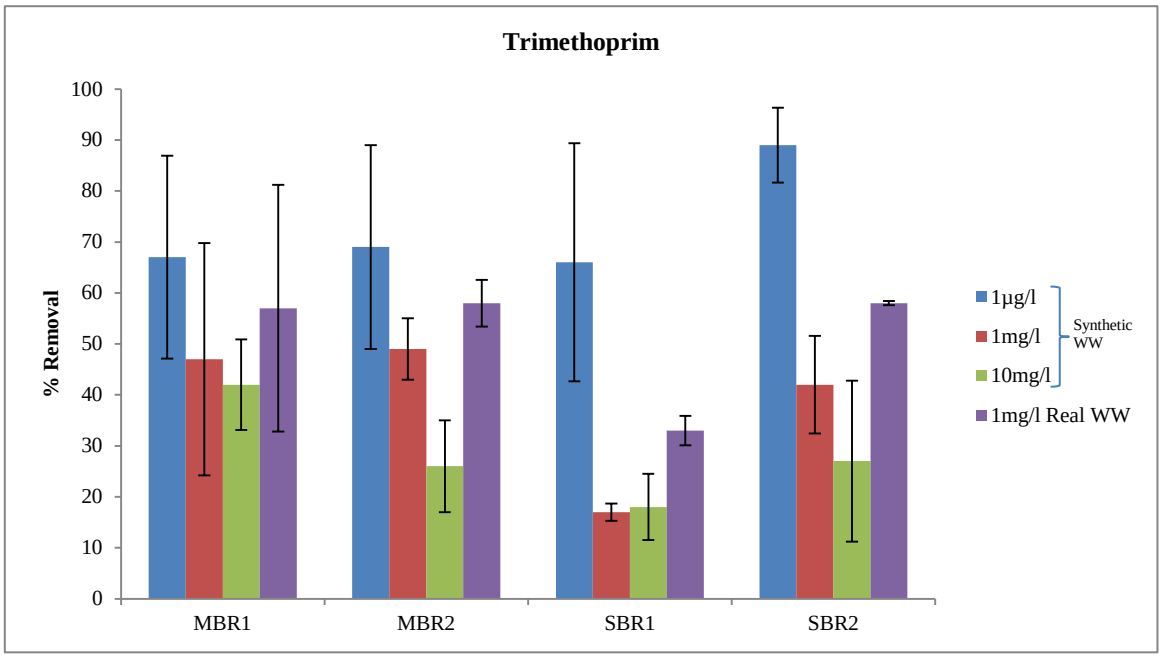


Figure 5.4.36: The removal efficiencies of Trimethoprim in four reactors at different PM concentrations with synthetic and real wastewaters.

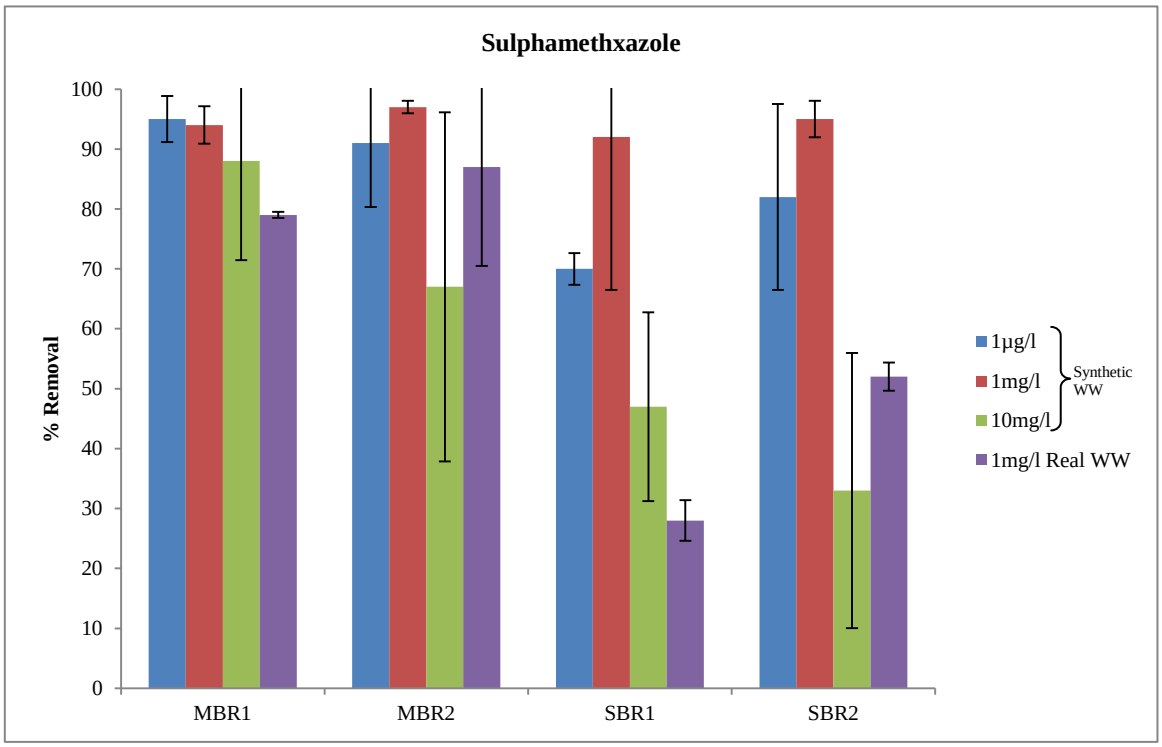


Figure 5.4.37: The removal efficiencies of Sulphamethoxazole in four reactors at different PM concentrations with synthetic and real wastewaters.

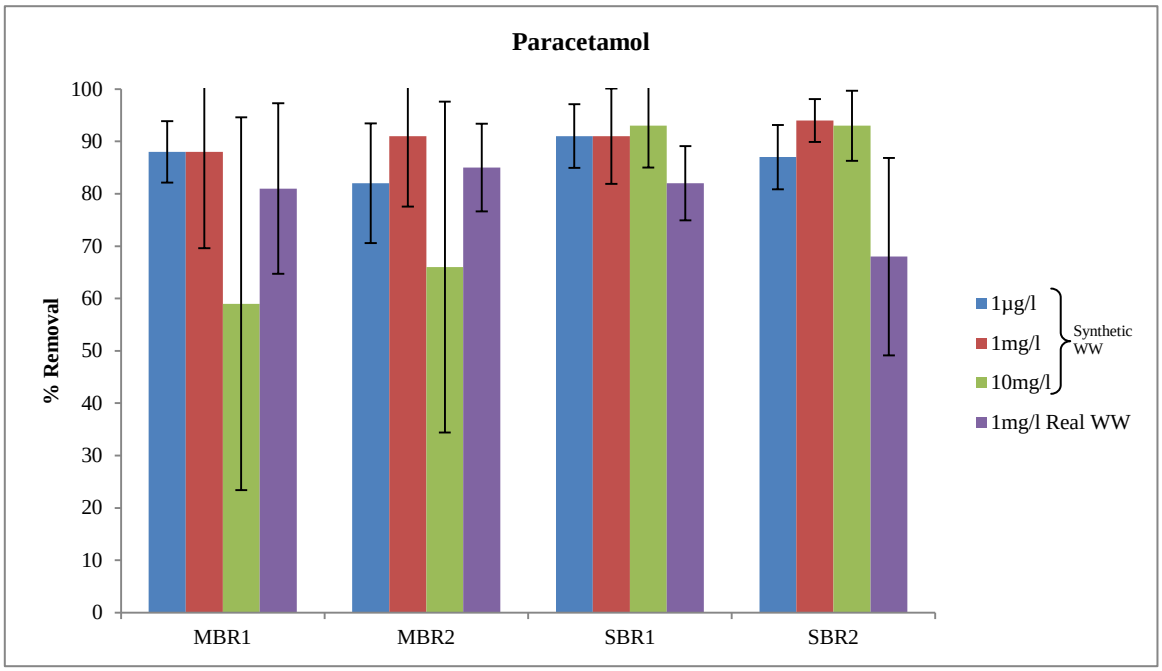


Figure 5.4.38: The removal efficiencies of Paracetamol in four reactors at different PM concentrations with synthetic and real wastewaters.

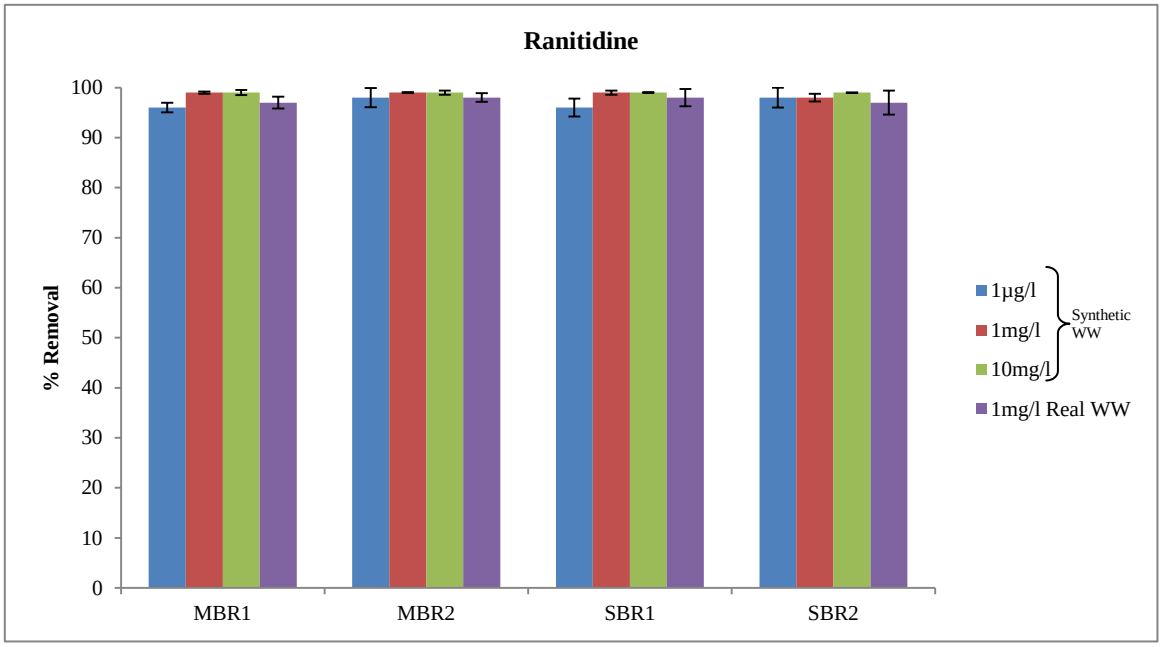


Figure 5.4.39: The removal efficiencies of Ranitidine in four reactors at different PM concentrations with synthetic and real wastewaters.

5.4.7 COD/TOC ratio

The removal of TOC followed a similar trend to COD removal. The COD test consists of measuring all the organic materials that can be oxidized by a strong oxidizing agent, but the COD test has some restrictions and cannot oxidize some substances present in the wastewater such as sulfides, sulfites and ferrous iron. In addition, the COD test cannot completely oxidize some aromatic compounds. While the TOC test does not measure other organically bound elements such as nitrogen, hydrogen and inorganics that can be measured by COD, it also is independent of the oxidation state of the organic matter. Furthermore, the TOC test is not affected by the presence of organics that are difficult to oxidize completely.

The most important observation made in the current study is that COD removal was high, but the amount of COD removal decreased depending on the dose of PM, which indicated that the pharmaceutical could be utilized in metabolic reactions (Figures 5.4.34). For this purpose COD with its soluble and particulate fractions, was measured for the pre-spiked PM, and measured for the additions of PM at different concentration runs. The decrease of the COD/TOC ratio in both the influent and the effluent with increasing PM in all reactors (Table 5.4.2) indicates the increase of the oxidative state of carbon in the organic solution and lower reactivity (Hsu et al., 2004). In contrast, the increase in the COD/TOC ratio in the experiments carried out indicates a degradation pathway driven primarily by radical oxidation leading to different reaction intermediates. These results may be justified as COD utilization by biomass could be slightly changed during the different phases of the metabolic reactions. The ratio was the lowest with the municipal wastewater due to its complexity. However, the COD/TOC ratio of the effluent decreased as the PM concentration increased, and was the lowest in the case of real municipal wastewater except in the reactor SBR2.

Table 5.4.2: COD/TOC ratio in four reactors at different PM concentrations spiked in synthetic and real wastewater.

	Influent					Effluent				
	0	1 $\mu g.L^{-1}$ synthetic	1 $mg.L^{-1}$ synthetic	10 $mg.L^{-1}$ synthetic	1 $mg.L^{-1}$ real municipal WW	0	1 $\mu g.L^{-1}$ synthetic	1 $mg.L^{-1}$ synthetic	10 $mg.L^{-1}$ synthetic	1 $mg.L^{-1}$ real municipal WW
MBR1	2.14 (±0.3)	2.24 (±0.45)	1.93 (±0.15)	1.78 (±0.25)	1.80 (±0.2)	2.13 (±0.46)	1.64 (±0.08)	1.32 (±0.2)	1.38 (±0.05)	1.13 (±0.29)
MBR2	2.14 (±0.3)	2.24 (±0.45)	1.93 (±0.15)	1.78 (±0.25)	1.80 (±0.2)	2.38 (±0.74)	1.71 (±0.31)	1.84 (±0.29)	1.09 (±0.23)	0.52 (±0.16)
SBR1	2.05 (±0.19)	2.1 (±0.21)	1.96 (±0.08)	1.79 (±0.2)	1.8 (±0.2)	2.46 (±0.64)	2.1 (±0.63)	1.76 (±0.03)	1.35 (±0.48)	0.96 (±0.28)
SBR2	2.05 (±0.19)	2.1 (±0.21)	1.96 (±0.08)	1.79 (±0.2)	1.8 (±0.2)	2.52 (±0.54)	2.06 (±0.81)	1.61 (±0.26)	0.66 (±0.1)	0.87 (±0.35)

5.5 MICROBIAL DIVERSITY ANALYSIS

The analysis of microbial diversity present in each reactor during the experiment was carried out using the PCR-DGGE technique. This technique was used to characterise and understand how the stability and diversity of the microbial communities was influenced by the different concentrations of doses of the PM in the reactors, i.e. to investigate the effects of pharmaceutical concentration on microbial community structure.

5.5.1 Analysis of DGGE profile

DGGE profiles of bacterial 16S rRNA gene fragments from MBR1, MBR2, SBR1, and SBR2, and control reactors represented by MBR3 and SBR3, are shown in Figure 5.5.1. DGGE profiles of bacterial gene fragments reveal that different populations were present in the same reactor at different doses of PM, and there was a difference between the populations in different reactors even though differences in the overall patterns of bacterial populations in all reactors were clear, as evidenced by the loss and the appearance of some bands in the different phases of each reactor run. Moreover, there were some particular bands that obviously showed an increase and decrease in band intensity, indicating changes in the number of the predominant population at certain doses of a PM. These changes of band intensity were observed in MBR1, MBR2, SBR1 and SBR2.

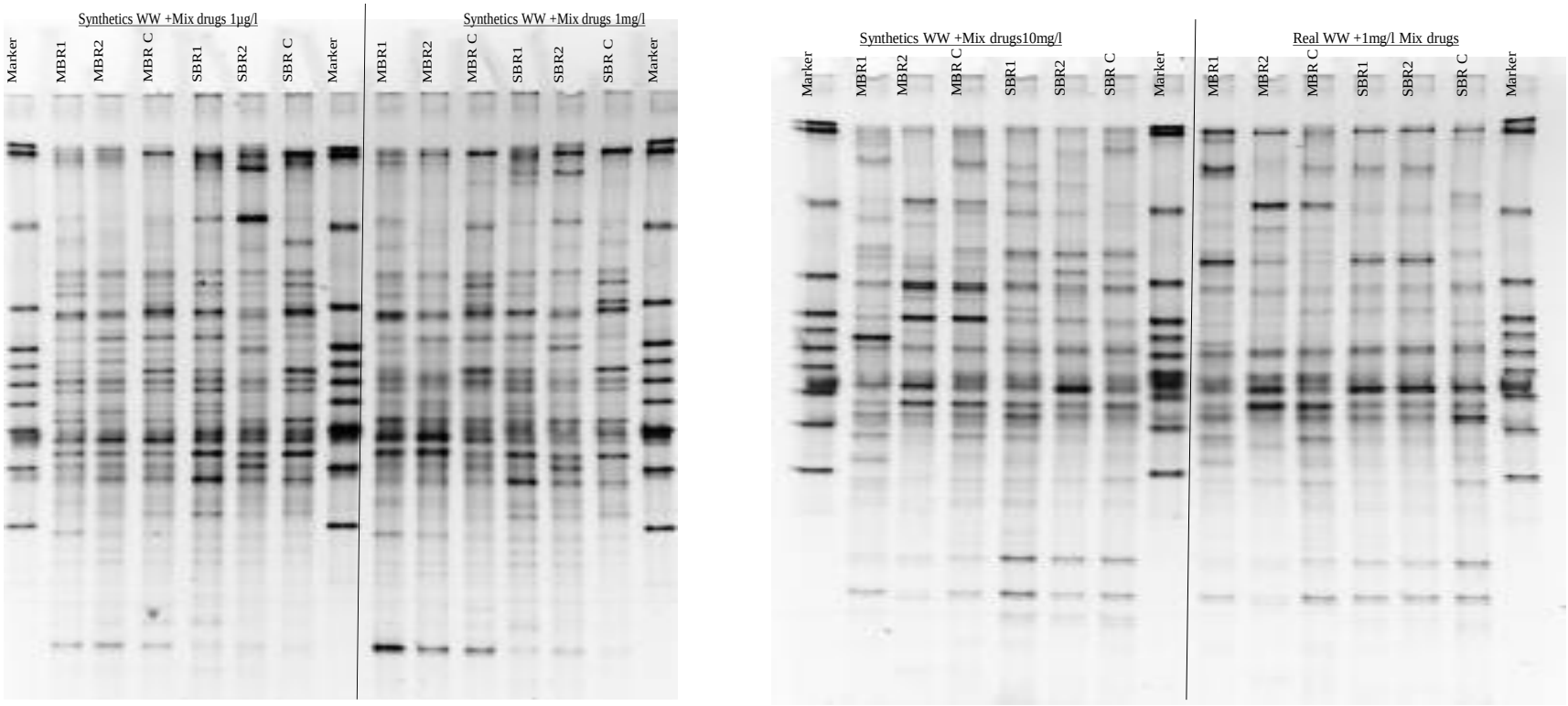


Figure 5.5.1: DGGE profile of bacterial communities from MBR1, MBR2, SBR1, SBR2 and Control (MBR C and SBR C) (M = the marker band, run to correct for variation across different gels).

5.5.2 Effect of PM concentration on microbial diversity

The observations on the DGGE gel showed that for each dose of PM or different experiment diversity produced a different band pattern. The bands count and its change throughout the experiment showed that the change in PM concentration appears to affect the diversity of the microbial population.

DGGE is a largely qualitative method, thus analysis was done on bands which clearly appeared, disappeared or changed in intensity relative to the control treatment. The DGGE analysis suggests that changes in microbial diversity may have occurred in the presence of PM as compared to the control. There was a significant change in microbial community for MBR1, MBR2, SBR1 and SBR2 (Figure 5.5.2) with an increase of the PM concentration ($p< 0.05$; ANOVA), where the number of bands decreased. The result was expected since the MLVSS decreased due to reduced microbial growth, and a reduction in COD removal was seen. Therefore, it is clear that the microbial diversity was affected by high doses of pharmaceuticals.

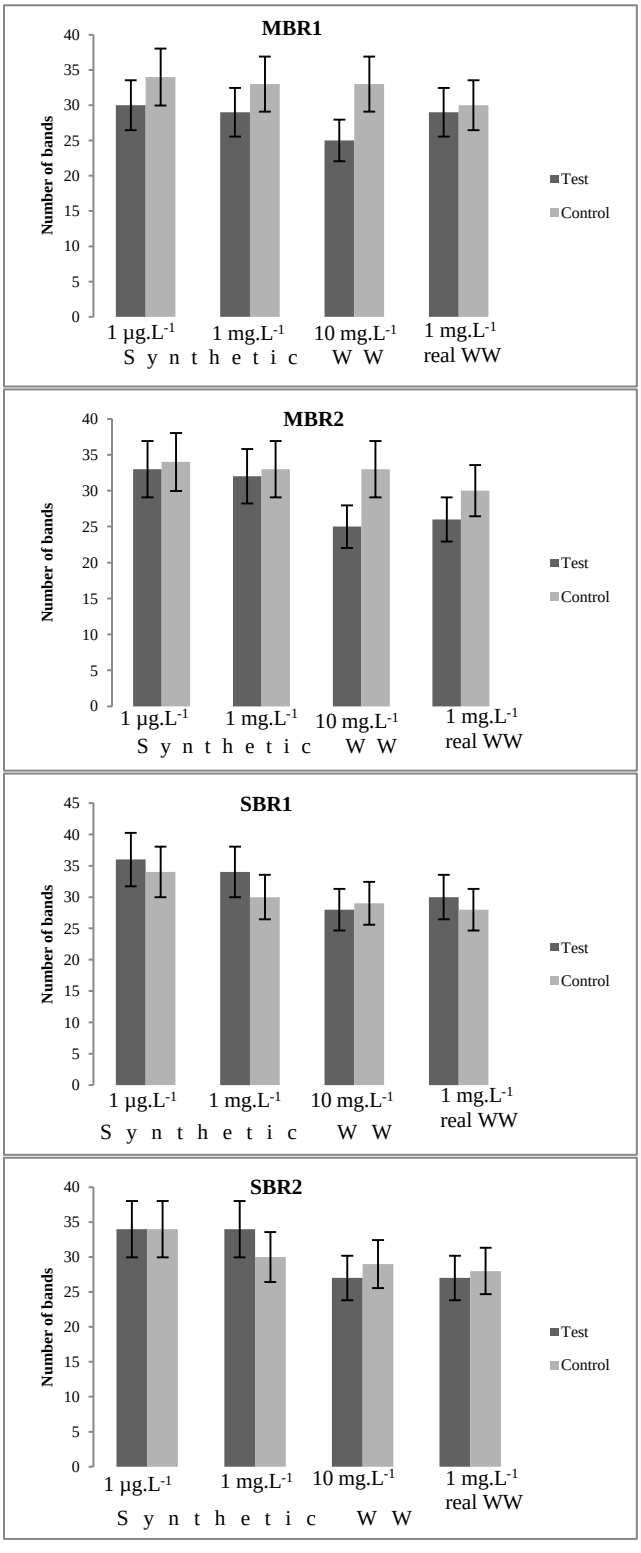


Figure 5.5.2: Microbial diversity changes shown by number of bands present in DGGE profile of different bioreactors, at different doses of PM concentration in synthetic and real municipal wastewater.

5.5.3 Similarities in microbial diversity

Cluster analysis permitted a comparison of the levels of similarities between bacterial communities according to PM concentration and bioreactors operational design. This showed a small similarity between the samples according to the method of operational design and PM concentration. This similarity decreased with increasing PM dose (Figure 5.5.3). The similarity between MBR1 and MBR2 at a concentration of 1 $\mu g.L^{-1}$ of PM was 86.5%, whereas the similarity between SBR1 and SBR2 was 82.5%. All reactors at a concentration of 1$\mu g.L^{-1}$ showed 73.5% similarity. The similarity decreased to 69.8% for all reactors when the PM concentration was increased to 1$mg.L^{-1}$, where the similarity of sludge communities in MBR1 and MBR2 was 84%, and SBR1 and SBR2 was 83% at concentration 1$mg.L^{-1}$. The similarity decreased to 57.5% in all reactors when fed by real municipal wastewater containing 1 $mg.L^{-1}$ of PM. All these results showed that the concentration of PM and different conditions changed the diversity of bacterial communities according to the similarity profile.

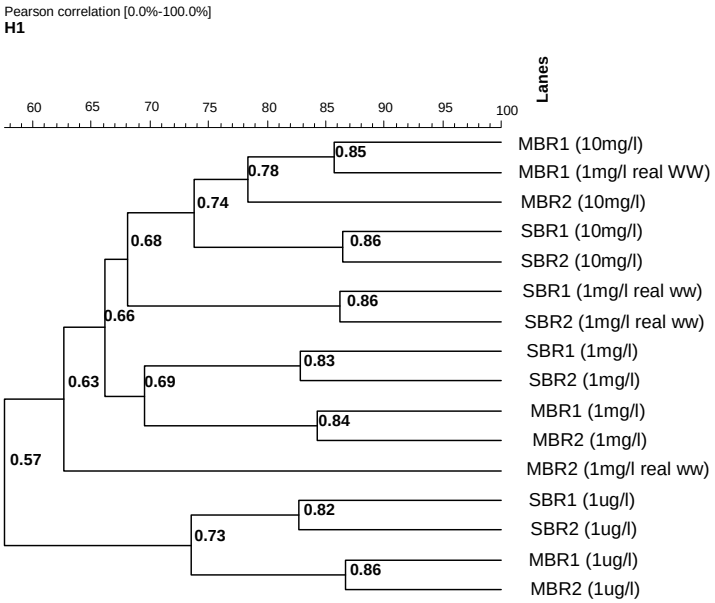


Figure 5.5.3: Dendogram for similarity of bacterial communities in different bioreactors at different PM concentrations in synthetic and real municipal wastewater.

5.5.4 Similarity of diversity of continuous batch bioreactors inoculated with isolated bacteria

The microorganisms present in the activated sludge from the batch reactors experiment in Section 5.3 were isolated by both spread plate and streak plate method on selective media containing single a pharmaceutical such as Metronidazole, Trimethoprim, Sulphamethoxazole, Paracetamol and Ranitidine. Different bacterial strains were isolated based on each pharmaceutical being the only carbon source in order to find the similarity of these isolated bacteria with non-inoculated bioreactors in Section 5.4. Figure 5.5.4 shows the visual comparison of the DGGE profiles of bacterial 16S rRNA gene fragments and reveals that isolated bacteria were present in the bioreactor during the experiments and there was a difference between different reactors (Figure 5.5.4). The loss and the appearance of some bands were still observed in the different phases of each reactor. Moreover, there were some particular bands that obviously showed an increase and decrease in band intensity, indicating changes in the number of the predominant population at different operating conditions. These changes in band intensity were also observed for some reactors with the isolated bacteria. The differences between the isolated bacteria were expected, as they grown in different pharmaceuticals. However, similarities were found between these isolated bacteria and bacterial communities of different reactors.

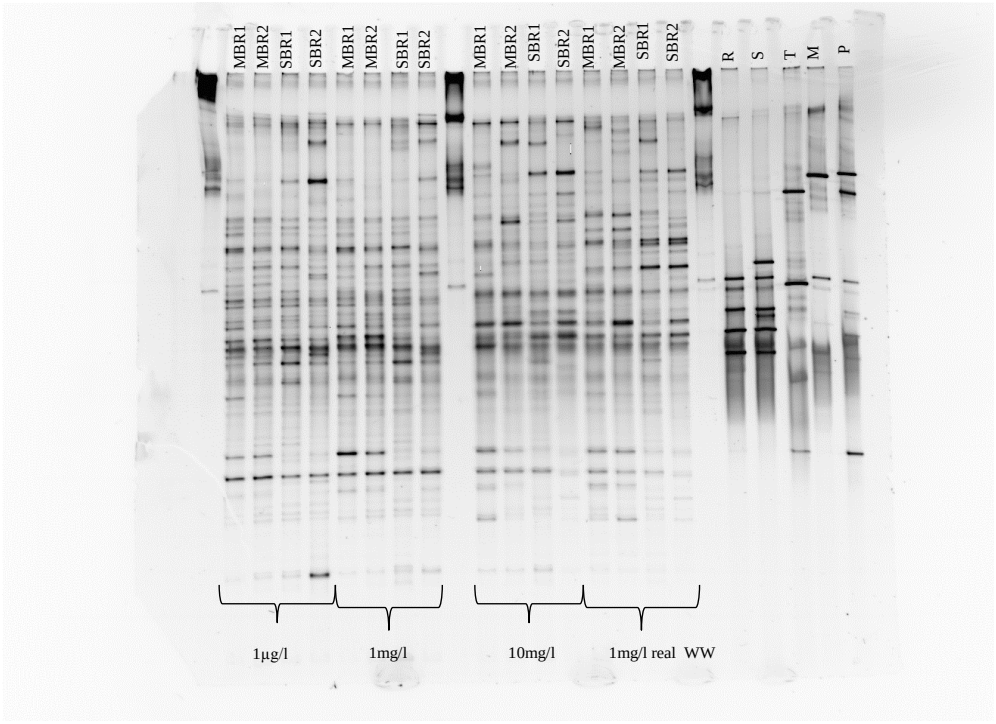


Figure 5.5.4: DGGE profile of bacterial communities from MBR1, MBR2, SBR1, and SBR2 compared with the profile of specific isolated bacterial from Metronidazole (M), Trimethoprim (T), Sulphamethoxazole (S), Paracetamol (P) and Ranitidine (R).

All bands were detected which clearly either appeared or disappeared when comparing treatments with pharmaceuticals at a specific concentration. However, all bands that were identified in the Metronidazole, Trimethoprim, Sulphamethoxazole, Paracetamol and Ranitidine showed increasing intensity in reactor communities with increasing concentration of PM (Figure 5.5.4). Furthermore, since other bands remained at similar intensities when comparing between different bioreactors and different concentration of PM, an increase in a specific band's intensity could be explained by an increase in that species' population and thus enrichment.

6. CONCLUSIONS

1. Development of an analytical method

An analytical method for the determination of Metronidazole, Trimethoprim, Sulphamethoxazole, Paracetamol and Ranitidine in wastewater at nano concentrations was successfully developed using LC-MS-MS after SPE extraction of samples. The method detection limits for the detection of Metronidazole, Trimethoprim, Sulphamethoxazole, Paracetamol and Ranitidine in wastewater were 5, 5, 1, 5 and 5 $ng.L^{-1}$, respectively.

2. Removal of pharmaceuticals in Sulaibiya WWTP

The results of raw wastewater analysis at Sulaibiya WWTP showed that the five target pharmaceuticals were always present, however, their concentrations varied throughout the year of study, depending on seasonal temperature variations, the prevalence of certain diseases intend to these use, and seasonal precipitation values. Generally, the concentration of pharmaceuticals found in the raw wastewater at Sulaibiya WWTP was lower than reported elsewhere around the world, which might indicate a lower level of their consumption in Kuwait compared to other countries.

The effluent of the secondary treatment stage of Sulaibiya had a high quality removal regarding the organic and nitrogen contents due to the efficiency of the new technologies implemented, such as activated sludge processes in the wastewater treatment plant. Despite the high removal of organic compounds by the secondary wastewater treatment process, the removal efficiencies of pharmaceuticals fluctuated during the year, and that was considered to be mainly due to seasonal changes in temperature.

The removal efficiency for trace organic compounds (TOC) was high as a result of implementing dual MF/ RO membrane systems at the Sulaibiya WWTP.

The concentration of pharmaceuticals in RO permeate were very low levels, the maximum concentrations detected for Ranitidine and Trimethoprim being 15 and 19 $ng.L^{-1}$, respectively. The RO membrane served as a large reservoir for organic matter as well as trace organic compounds, such as pharmaceuticals, due to the adsorption of contaminants on the membranes and their likely release in the brine. The concentrations

of pharmaceuticals in the brine were at ng.L^{-1} levels, and the concentration factor ranged between one and six. Consequently, the disposal of this brine would cause a real pollution concern.

The removal efficiency for conventional wastewater parameters was excellent, so that the product water could be used for various reuse applications. Chlorine was added to the product water as disinfectant to allow safe water reuse for irrigation of raw vegetables. Levels of pharmaceuticals remaining in the product water were either greatly reduced to very low concentrations or to undetectable levels or transform to by-product after chlorine treatment, which could cause environmental health problems.

3. Chemical removal of pharmaceuticals by the oxidation processes indicated that chlorination removed pharmaceuticals more effectively than the ozonation process. Chlorination, at a concentration dose of 10 mg.L^{-1}, removed pharmaceuticals by more than 92%, except for Metronidazole (58% removal).

4. Studies on the effect of pharmaceutical concentrations on their removal in the batch reactors showed the TOC removal was fast and high during the first 5 hours at all concentration doses of every pharmaceutical. High removal of pharmaceuticals was observed at a concentration of 0.1 mg.L^{-1}, then this decreased with increasing pharmaceutical concentration, except for Paracetamol which gave high removal for all concentrations doses.

5. The removal efficiency of Metronidazole, Trimethoprim, Sulphamethoxazole, Paracetamol and Ranitidine was assessed in laboratory-scale MBR1, MBR2, SBR1 and SBR2 installed in parallel. Ranitidine showed the highest removal rate for all concentrations tested. Metronidazole was removed moderately in all reactors and the effect of increased pharmaceuticals mixture (PM) concentration on the removal efficiencies of Metronidazole was negligible in MBR1 and MBR2, and was significant in SBR1 and SBR2. The removal efficiency of Trimethoprim was similar to Metronidazole except that the effect of increased PM concentration on the removal efficiencies of

Trimethoprim was significant in all reactors. The removal efficiency of Paracetamol inSBR1 and SBR2 was much better than in MBR1 and MBR2, and the effect of increased PM concentration was negligible in SBR1 and SBR2, and was significant in MBR1 and MBR2.

6. The effect of the PM concentration on the removal efficiency of the COD and the TOC in MBR1, MBR2, SBR1 and SBR2 was negligible at concentration 1 $\mu g.L^{-1}$, compared with the control reactors. But the removal efficiency of the COD and the TOC started to decline with increased PM concentration at 1 and 10 $mg.L^{-1}$ for all reactors. This was in agreement with the PCR-DGGE results, which showed the microbial diversities in MBR1, MBR2, SBR1 and SBR2 were lower at higher concentrations of the PM. The results of the PCR-DGGE analysis indicate the importance of microbial diversity on PM removal efficiency, higher removal efficiency of PM being observed in reactors containing higher microbial diversity and higher concentration of these microbial as in the increase in the intensity of DNA bands.

Further Research

There is further research needed to develop an analytical method in order to determine pharmaceuticals concentration lower than the $ng.L^{-1}$ level. This would allow the removal of low level pharmaceuticals which were detected in the recycled water to be assessed.

This research has focused on the analysis of the pharmaceutical parent compounds. Some of the pharmaceutical metabolites are also biologically active compounds which may also be excreted at high concentrations. Degradation products of pharmaceutical are also a potential source of persistent biologically active contaminants and it is important to identify metabolites and degradation products if the full input on the environment is to be known.

There is still uncertainty about the fate of pharmaceuticals once they have become adsorbed to sludge, as this may be applied to farmland, incinerated, or occasionally disposed to landfill. Consequently, there is the potential for uptake by crops if used on agricultural land, which could be returned to the human food chain. Further research is

therefore required to establish the fate of pharmaceuticals when sludge is disposed to land.

During the WWTP pharmaceutical could not be completely degraded though the treatment. Chlorine and ozone treatment may produce by-product compounds that cause environmental and health problem. Further research should be done in the persistence and toxicity of these by-products.

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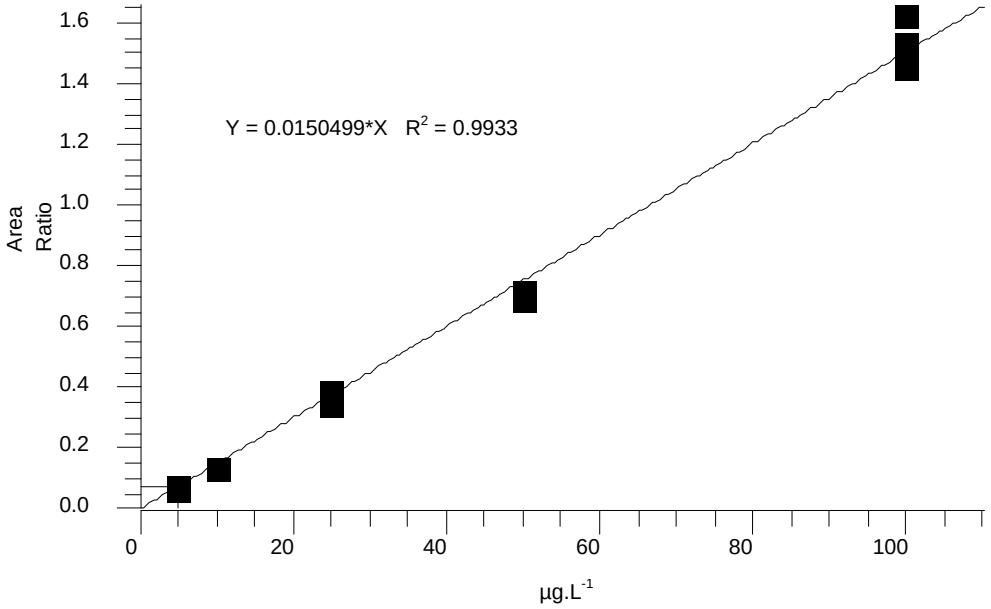
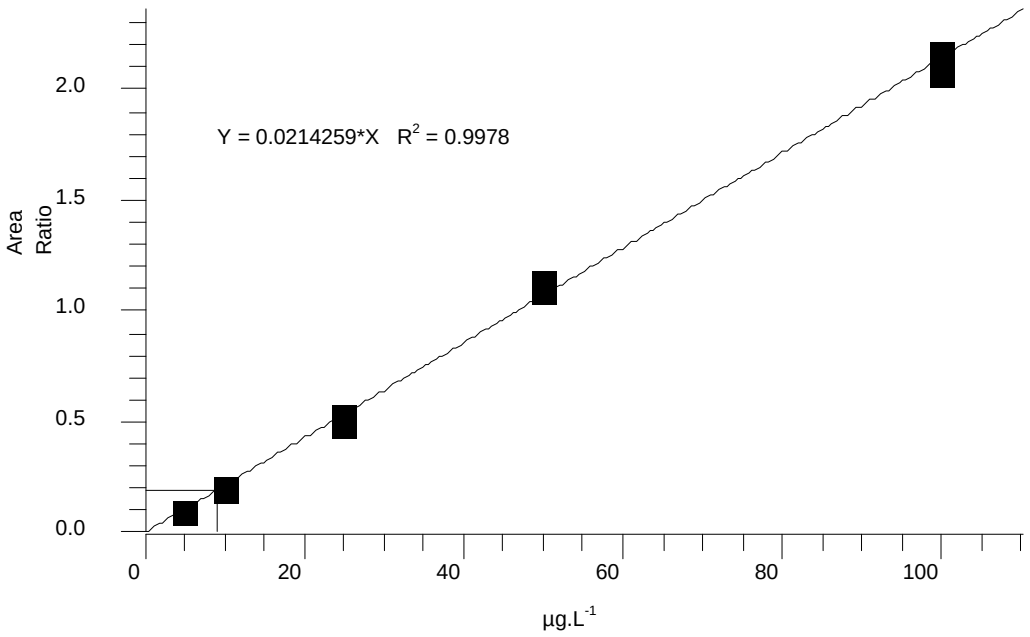
Appendix: A

Calibration curve of authentic pharmaceutical in liquid chromatography Mass spectrometry (LC-MS-MS)

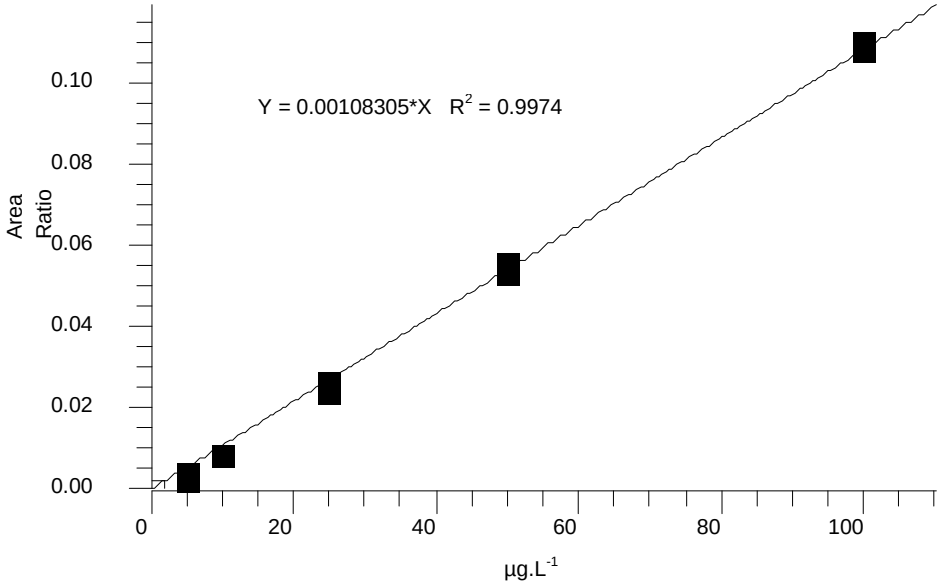
Metronidazole



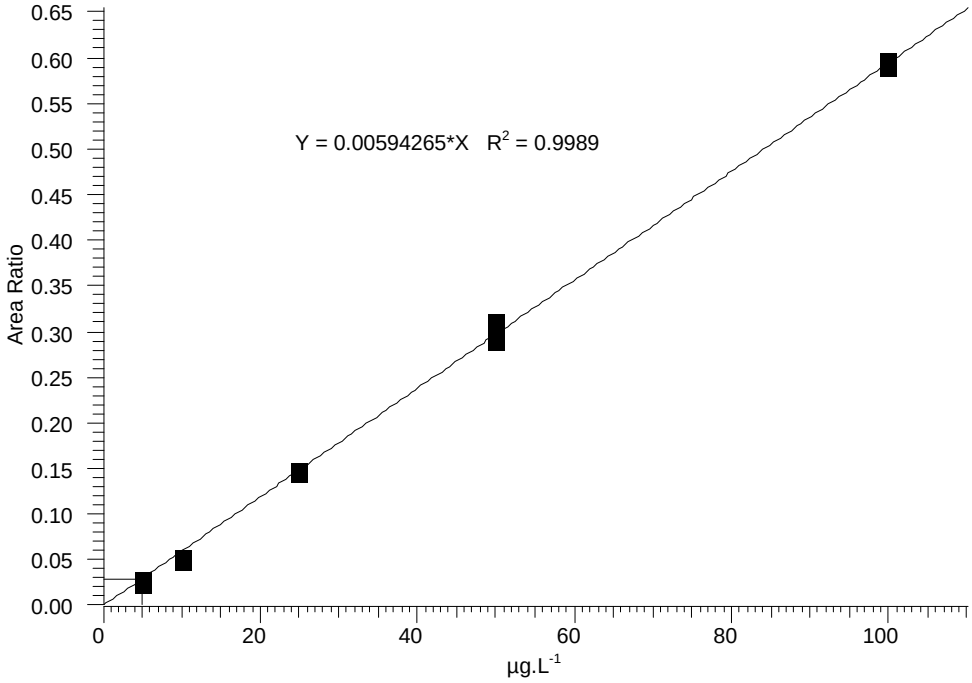
Trimethoprim



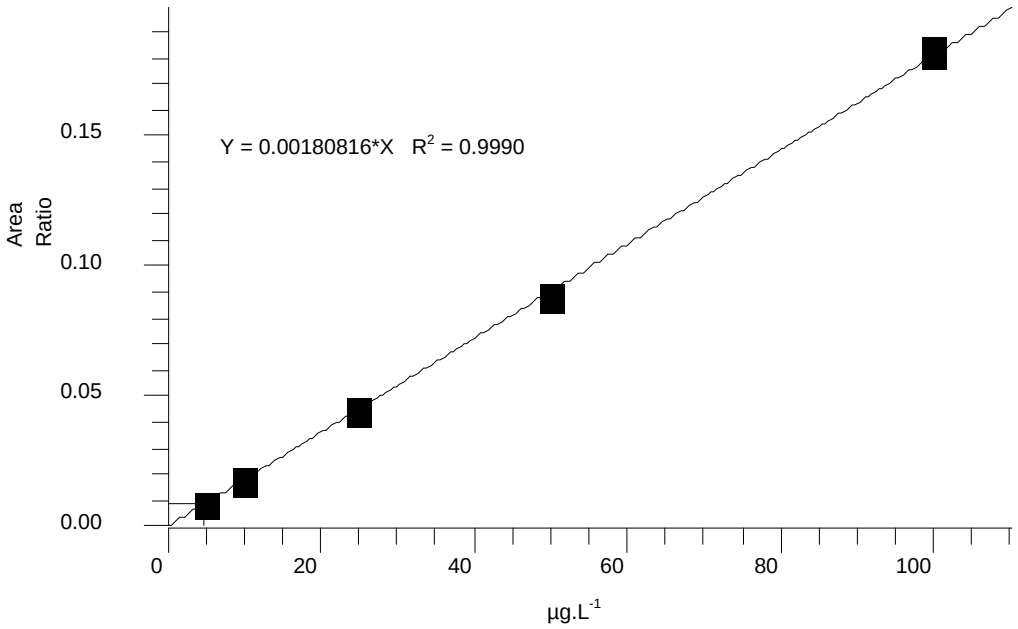
Sulphamethoxazole



Paracetamole



Ranitidine


Y = 0.00180816*X R2 = 0.9990
Area Ratio
0.00
0.05
0.10
0.15
0
20
40
60
80
100
µg.L-1

Appendix: B

Isolating Genomic DNA from Gram Positive and Gram Negative Bacteria

Materials to Be Supplied by the User

• 1.5ml microcentrifuge tubes

• water bath, 80°C

• water bath, 37°C

• isopropanol, room temperature

• 70% ethanol, room temperature

• water bath, 65°C (optional; for rapid DNA rehydration)

• 50mM EDTA (pH 8.0) (for gram positive bacteria)

• 10mg/ml lysozyme (Sigma Cat.# L7651) (for gram positive bacteria)

• 10mg/ml lysostaphin (Sigma Cat.# L7386) (for gram positive bacteria)

1. Add 1ml of an overnight culture to a 1.5ml microcentrifuge tube.

2. Centrifuge at 13,000–16,000 × *g* for 2 minutes to pellet the cells. Remove the supernatant. For Gram Positive Bacteria, proceed to Step 3. **For GramNegative Bacteria go directly to Step 6.**

3. Resuspend the cells thoroughly in 480µl of 50mM EDTA.

4. Add the appropriate lytic enzyme(s) to the resuspended cell pellet in a total volume of 120µl, and gently pipet to mix. The purpose of this pretreatment is to weaken the cell wall so that efficient cell lysis can take place.

Note: For certain *Staphylococcus* species, a mixture of 60µl of 10mg/ml lysozyme and 60µl of 10mg/ml lysostaphin is required for efficient lysis.

However, many Gram Positive Bacterial Strains (e.g., *Bacillus subtilis*, *Micrococcus luteus*, *Nocardiaotitidiscaviarum*, *Rhodococcusrhodochrous*, and*Brevibacteriumalbidium*) lyse efficiently using lysozyme alone.

5. Incubate the sample at 37°C for 30–60 minutes. Centrifuge for 2 minutes at 13,000–16,000 × *g* and remove the supernatant.

6. Add 600µl of Nuclei Lysis Solution. Gently pipet until the cells areresuspended.

7. Incubate at 80°C for 5 minutes to lyse the cells; then cool to room temperature.

8. Add 3µl of RNase Solution to the cell lysate. Invert the tube 2–5 times to mix.

9. Incubate at 37°C for 15–60 minutes. Cool the sample to room temperature.

10. Add 200µl of Protein Precipitation Solution to the RNase-treated cell lysate.

Vortex vigorously at high speed for 20 seconds to mix the Protein Precipitation Solution with the cell lysate.

11. Incubate the sample on ice for 5 minutes.

12. Centrifuge at 13,000–16,000 × *g* for 3 minutes.

13. Transfer the supernatant containing the DNA to a clean 1.5ml microcentrifuge tube containing 600µl of room temperature isopropanol.

Note: Some supernatant may remain in the original tube containing the protein pellet. Leave this residual liquid in the tube to avoid contaminating the DNA solution with the precipitated protein.

14. Gently mix by inversion until the thread-like strands of DNA form a visible mass.

15. Centrifuge at 13,000–16,000 × *g* for 2 minutes.

16. Carefully pour off the supernatant and drain the tube on clean absorbent paper. Add 600µl of room temperature 70% ethanol and gently invert the tube several times to wash the DNA pellet.

17. Centrifuge at 13,000–16,000 × *g* for 2 minutes. Carefully aspirate theethanol.

18. Drain the tube on clean absorbent paper and allow the pellet to air-dry for
10–15 minutes.

19. Add 100µl of DNA Rehydration Solution to the tube and rehydrate the DNA by incubating at 65°C for 1 hour. Periodically mix the solution by gently tapping the tube. Alternatively, rehydrate the DNA by incubating the solution overnight at room temperature or at 4°C.

20. Store the DNA at 2–8°C.

Appendix: C

DNA Extraction

The FastDNA® SPIN Kit for Soil (Q.BIOgene, USA.) was used and the DNA extraction technique carried out in this study was based on that detailed in the kit application manual, and summarised below.

The procedure started by adding 978 µl of sodium phosphate buffer and 122 µl of MT buffer to the Lysing Matrix E Tube before 250 µl of completely mixed fixed sample was added to the same tube. The tube was then secured in the Ribolyser and processed at speed 6.5 for 30 seconds before being centrifuged at 14,000×g for 10 minutes. Then, supernatant was transferred to a clean 2 ml Eppendorf tube (Eppendorf AG, Hamburg, Germany) and 250 µl of PPS reagent was added and mixed by inverting 10 times before centrifuging the tube at 14,000×g for 5 minutes. The supernatant was again transferred to a new clean 2 ml Eppendorf tube and 1 ml of shaken DNA Binding Matrix solution was added. The tube was then inverted repeatedly by hand for 2 minutes before being left in a rack for 3 minutes to allow settling of silica matrix. 700-750 µl of supernatant was removed and discarded and the remaining Binding Matrix was resuspended again before approximately 600 µl of the mixture was transferred to a SPIN Filter and centrifuged at 14,000×g for 1 minute. The catch tube was emptied and the remaining mixture was transferred to the same SPIN Filter and centrifuged at 14,000×g for 1 minute, this step was repeated until all of the mixture was transferred. The SPIN Filter was then filled with 500 µl of SEWS-M and centrifuged at 14,000×g for 1 minute. The flow-through was decanted and the SPIN Filter was replaced in a Catch tube before being centrifuged at 14,000×g for 2 minute to dry the residual SEWS-M wash solution. The SPIN Filter was removed and placed in a fresh kit-supplied Catch Tube with the cap opened to air dry the SPIN Filter for 5 minutes at room temperature. The DNA was eluted by adding 50 µl of DES and the matrix gently stirred using a finger flip before centrifuging at 14,000×g for 1 minute to transfer eluted DNA to a Catch Tube. This eluted DNA was ready to be used in the next step.

Polymerase Chain Reaction

The preparation of the polymerase chain reaction (PCR) mix and the general technique used for the different PCR reactions were based on the method described by Devereux and

Willis(1995) with minor modifications. The sample preparation was carried out in the Bio2+ Class II Microbiological Safety Cabinet (Envair, Lancashire, England) in order to minimise contamination of the samples.

The following reaction mix was prepared (volumes per sample).

28 µl forward primer

28 µl of reverse primer

1316 µl Mega Mix Blue

For each sample, 49 µl of reaction mix was transferred to the 1 ml Eppendorf tube followed by the addition of 1 µl of extracted DNA. A blank (same reagents, but no sample) was included with the PCR run, so as to check for contamination.

The P×2 Thermal Cycler (Thermo Electron Corporation, USA) was used in the PCR amplification reactions. In this study the PCR was carried out targeting the whole eubacterial population). Sequences of the different primers utilised are shown in Table 4.4.

Table : List of all the primers and respective sequences used for the PCR reactions, classified according to the oligonucleotide probe database (Alm et al., 1996).

Primer	Sequence (from 5'end to 3'end)	Reference
Vr	ATTACCGCGGCTGCTGG	Muyzer et al. (1993)
Vf	(CGCCCGCCGCGCGCGGCGGGCGGGGCGGGGGCACGGGGGG)[a] CCTACGGGAGGCAGCAG	Muyzer et al. (1993)

[a]Added to the forward primer Vf was a high melting temperature GC-clamp

Eubacterial Specific PCR

The primers used for revealing the whole bacterial population were the universal (eubacterial specific) primers Vr (reverse) and Vf (forward)(Muyzer et al., 1993).

The program used in the PCR machine comprised the following steps.

Initial Denaturation Step	95ºC	3 minutes	1 cycle
Denaturation Step	95ºC	1 minute	
Annealing Step	65ºC	1 minute (to be reduced 1ºC every second cycle)	
Extension Step	72ºC	1 minute	(24 cycles)
Denaturation Step	95ºC	1 minute	
Annealing Step	53ºC	1 minute	(15 cycles)
Extension Step	72ºC	1 minute	
Final Extension Step	72ºC	10 minutes	1 cycle

Agarose Gel

The products of DNA extraction and of PCR reactions were examinedby agarose gel in order to check whether DNA was present in the samples and whether the correct gene fragments had been amplified. 1% agarose gels were prepared, where 1 g of agarose was added to 100 ml of 1×TAE buffer (2 M Tris-Acetate, 0.05 M EDTA, pH 8.3, Eppendorf Scientific Inc., New York, USA). The agarose was, then, melted by heating the mixture on a hot plate. After the agarose was melted, the mixture was allowed to cool to about 60ºC and 2 µl of ethidium bromide were added. The gel was then poured into a plate and allowed to set for 30 minutes. The wells of the gel were loaded with 5 µl of sample and 2 µl of loading buffer. A reference DNA ladder was loaded in one of the wells of the gel to check the size of the DNA in the samples. For the samples from DNA extraction, Lambda DNA Hind III Digest (Sigma, Poole, UK) was used as the marker and 2 µl of this compound was added to 2 µl of loading buffer and to 3 µl of autoclaved deionised water. In the case of PCR products, the marker used was PCR 100 base pair ladder (Sigma, Poole, UK) (5 µl of marker and 2 µl of loading buffer). The gel was run for about 45 minutes using a Power Pac 300 power supply (Biorad,

Hemel Hempstead, UK) in a wide mini sub cell (Biorad, Hemel Hempstead, UK). The gel was then visualised using a dual-intensity transilluminator (UVP, San Gabriel, California).

Denaturing Gradient Gel Electrophoresis (DGGE)

The population fingerprinting method, denaturing gradient gel electrophoresis (Muyzer et al., 1993) was used to analyse the bacterial diversity in the lab-scale bioreactors. The electric field of the gels used in this study is parallel to the denaturing chemical gradients which were formed with 10% (w/v) acrylamide stock solution (acrylamide-N, N'-methylenebisacrylamide, 37:1), containing formamide and urea. PCR samples were directly applied onto a 10% polyacrilamide gel in 0.5×TE (20 mMTris acetate at pH 7.4, 10 mM sodium acetate, 0.5 mM Na_2-EDTA) with a range of denaturants of 30-55% for the Vr/Vf and 25-55% for the CTOs. The D-Gene system (Biorad, Hemel Hempstead, UK) was used to perform the DGGE analysis. Gels were run for 4.5 h at 200V constant voltage and at 60ºC and subsequently stained for 30 minutes in SYBR green I (Sigma, Poole, UK). Stained gels were viewed using an ultraviolet transilluminator (UVP, San Gabriel, California) and photographed with a Polaroid camera (CU-5, GRI, Great Dunmoor, Essex).

Appendix: D

	Temperature [°C]	pH		Conductivity [µs/cm]		Total Suspended Solids [mg/l]			Volatile Suspended Solids [mg/l]		Total Dissolved Solids [mg/l]		
DATE	SULAIBIYA Raw wastewater	ARDIYA Raw wastewater	SECONDARY EFFLUENT	ARDIYA Raw wastewater	SECONDARY EFFLUENT	ARDIYA Raw wastewater	SULAIBIYA Raw wastewater	SECONDARY EFFLUENT	ARDIYA Raw wastewater	SULAIBIYA Raw wastewater	ARDIYA Raw wastewater	SULAIBIYA Raw wastewater	SECONDARY EFFLUENT
Sept	36.4	7.0	7.2	885.6	735.2	188.4	176.3	13.8	152.9	144.9	518.1	511.1	433.1
Oct	34.7	7.0	7.2	867.7	730.7	258.5	133.1	14.1	215.2	111.0	509.2	510.6	430.3
Nov	31.6	7.1	7.1	905.6	780.4	308.7	183.6	22.2	256.4	146.4	531.6	525.9	459.7
Dec	29.5	7.1	7.1	901.5	737.0	262.4	188.4	27.4	210.8	155.9	529.2	512.3	431.6
Jan	27.3	7.1	7.1	911.0	778.0	239.9	209.2	12.9	194.5	174.1	541.2	552.5	458.3
Feb	27.3	7.2	7.2	901.7	736.3	322.2	210.9	7.8	273.9	175.8	529.3	516.2	433.7
Mar	28.4	7.2	7.1	910.5	749.9	366.4	164.7	12.2	267.2	134.1	531.1	518.7	441.6
April	30.7	7.2	7.1	908.0	747.4	351.3	227.1	15.9	279.4	177.4	533.1	521.2	440.3
May	33.3	7.1	7.4	886.9	743.0	284.1	135.0	10.0	191.3	104.2	519.4	510.9	437.6
Jun	35.2	7.0	7.4	798.7	656.5	192.1	150.3	12.3	153.3	123.3	469.2	478.1	386.6
July	36.1	7.1	7.3	813.2	643.5	485.7	216.4	17.4	411.2	167.7	476.6	450.8	378.9
Aug	37.0	7.0	7.2	762.9	592.3	208.8	167.5	7.7	172.4	136.1	447.8	412.1	348.7

Appendix: D

	COD [mg/l]			BOD [mg/l]			Total Phosphate [mg/l]			Total Phosphorus [mg/l]			Total Coliform-Presumptive CFU/100ml
DATE	ARDIYA Raw wastewater	SULAIBIYA Raw wastewater	SECONDARY EFFLUENT	ARDIYA Raw wastewater	SULAIBIYA Raw wastewater	SECONDARY EFFLUENT	ARDIYA Raw wastewater	SULAIBIYA Raw wastewater	SECONDARY EFFLUENT	ARDIYA Raw wastewater	SULAIBIYA Raw wastewater	SECONDARY EFFLUENT	ARDIYA Raw wastewater
Sept	387.6	336.8	34.7	197.4	149.9	9.5	16.1	17.3	5.8	5.3	5.6	1.9	4.2E+07
Oct	520.8	303.5	34.7	245.7	140.2	8.9	16.1	16.4	3.6	5.3	5.4	1.2	1.55E+08
Nov	592.0	375.6	52.0	295.2	189.2	11.8	19.3	19.1	5.4	6.3	6.2	1.8	1.39E+08
Dec	543.9	406.8	57.7	314.7	225.5	14.8	20.6	20.3	9.0	6.7	6.6	2.9	1.25E+09
Jan	495.6	417.5	36.4	281.1	211.6	9.2	18.5	19.2	6.6	6.0	6.3	2.1	3.59E+08
Feb	555.8	406.3	27.5	280.7	199.6	8.0	21.0	17.4	5.7	6.8	5.7	1.8	5.07E+08
Mar	623.1	340.3	32.9	337.5	175.3	9.1	19.2	16.2	4.4	7.3	5.9	1.4	5.89E+08
April	550.8	385.5	34.1	285.5	186.8	11.4	19.1	18.4	3.3	6.2	6.0	1.1	5.9E+08
May	495.1	263.9	23.5	259.8	139.9	7.0	19.0	15.7	1.6	6.2	5.1	0.5	4.92E+08
Jun	424.5	301.1	26.6	239.7	154.7	6.7	19.1	17.8	5.3	6.2	5.8	1.7	4.68E+08
July	687.7	403.7	37.0	353.7	238.9	11.2	19.7	18.6	4.5	7.1	6.1	1.6	5.58E+08
Aug	470.3	395.5	21.8	281.0	241.8	5.8	18.1	17.6	1.8	6.4	6.2	0.7	4.59E+08

Appendix:D

	Organic Nitrogen [mg/l]			Nitrate Nitrogen [mg/l]			Nitrite Nitrogen [mg/l]			Total Kjeldahl Nitrogen [mg/l]			Ammonia Nitrogen [mg/l]		
DATE	ARDIYA Raw wastewater	SULAIBIYA Raw wastewater	SECONDARY EFFLUENT	ARDIYA Raw wastewater	SULAIBIYA Raw wastewater	SECONDARY EFFLUENT	ARDIYA Raw wastewater	SULAIBIYA Raw wastewater	SECONDARY EFFLUENT	ARDIYA Raw wastewater	SULAIBIYA Raw wastewater	SECONDARY EFFLUENT	ARDIYA Raw wastewater	SULAIBIYA Raw wastewater	SECONDARY EFFLUENT
Sept	12.4	12.0	2.3	0.3	0.3	4.4	0.0	0.0	0.2	41.3	33.0	2.8	28.6	19.9	0.3
Oct	16.0	10.5	2.2	0.4	0.3	5.7	0.0	0.0	0.2	43.1	32.2	2.6	26.1	20.9	0.2
Nov	16.6	11.6	2.5	0.5	0.3	6.0	0.0	0.0	0.1	42.4	34.6	3.0	25.3	21.6	0.2
Dec	16.8	13.1	3.4	0.4	0.4	6.9	0.0	0.1	0.2	43.5	35.9	3.9	26.5	22.0	0.2
Jan	14.1	12.2	2.0	0.5	0.4	6.2	0.0	0.0	0.3	42.4	35.7	2.4	27.6	23.5	0.7
Feb	16.4	11.2	1.3	0.5	0.4	6.2	0.1	0.0	0.3	46.7	35.7	1.6	29.0	23.6	0.7
Mar	17.0	10.9	2.1	0.5	0.4	4.6	0.0	0.0	0.0	46.3	34.8	2.5	27.9	23.2	0.4
April	16.2	11.9	2.2	0.4	0.3	5.5	0.0	0.0	0.1	47.2	36.5	2.4	28.7	22.6	0.2
May	13.2	8.8	1.7	0.4	0.4	5.3	0.0	0.1	0.0	43.7	32.3	2.0	29.1	22.3	0.1
Jun	12.4	10.3	2.0	0.4	0.3	5.5	0.0	0.1	0.1	41.3	34.5	2.2	28.1	23.1	0.2
July	13.5	12.9	2.7	0.4	0.3	5.3	0.0	0.0	0.1	42.2	38.1	3.0	28.9	24.0	0.2
Aug	12.9	10.6	1.3	0.4	0.3	4.1	0.0	0.0	0.0	41.5	32.3	1.4	28.2	20.8	0.1

Appendix: D

	Alkalinity CaCO3 [mg/l]			Chlorides [mg/l]		Sulphates [mg/l]		Sulphides [mg/l]		Grease & Oil [mg/l]			Settleable Solids [cm³/l/2hr]	EFF Turbidity NTU	Total Inflow S55 [m³/d]
DATE	ARDIYA Raw wastewater	SULAIBIYA Raw wastewater	SECONDARY EFFLUENT	ARDIYA Raw wastewater	SECONDARY EFFLUENT	ARDIYA Raw wastewater	SECONDARY EFFLUENT	ARDIYA Raw wastewater	SECONDARY EFFLUENT	ARDIYA Raw wastewater	SULAIBIYA Raw wastewater	SECONDARY EFFLUENT	SULAIBIYA Raw wastewater	SECONDARY EFFLUENT	SULAIBIYA Raw wastewater
Sept	219.8	219.4	73.9	83.1	100.6	91.7	107.7	12.0	0.006	23.4	10.3	0.0	3.1	5.6	286897.3
Oct	200.7	219.0	66.5	69.9	102.7	88.6	107.2	5.1	0.008	22.3	10.4	0.0	5.2	6.1	287264.2
Nov	198.2	223.2	59.0	79.5	112.6	115.1	118.0	6.2	0.015	18.1	7.9	0.0	6.2	12.0	281961.8
Dec	192.5	218.7	53.1	88.1	105.0	112.5	114.1	4.7	0.017	18.1	13.9	0.0	4.9	17.8	278559.9
Jan	190.8	206.7	54.8	107.2	114.7	109.3	112.3	3.2	0.010	15.0	11.7	0.0	4.1	6.4	271157.5
Feb	195.2	203.7	53.8	100.7	108.3	117.8	113.4	3.6	0.006	16.5	12.1	0.0	6.6	3.5	275703.7
Mar	222.8	205.2	61.3	82.7	101.4	114.9	118.0	3.5	0.004	13.7	8.9	0.0	6.0	5.0	285955.1
April	204.9	215.6	66.5	66.6	108.1	97.2	109.1	5.8	0.006	28.0	18.7	0.0	6.3	7.0	287160.7
May	196.1	202.2	65.3	88.2	104.0	95.4	111.4	6.9	0.006	35.7	21.4	0.0	3.6	3.6	290870.0
Jun	186.7	188.6	62.4	90.2	86.2	68.2	86.4	4.1	0.008	27.7	17.8	0.0	2.8	4.8	271785.7
July	191.0	185.7	62.0	96.0	86.0	65.7	86.6	5.3	0.012	291.3	21.5	0.1	6.6	7.7	274087.3
Aug	170.1	158.9	59.7	86.9	77.2	68.5	83.5	4.7	0.006	81.2	32.5	0.1	3.1	3.7	298406.4

Appendix: D

	pH			TSS (mg/l)			TDS (mg/l)			Total Coliforms (CFU/100 ml)			BOD5 (mgO2/l)			COD (mgO2/l)		T otal Iron (mg/l as Fe)	
Date	UF inlet	Outlet UF and inlet RO	Outlet	UF inlet	Outlet UF and inlet RO	Outlet	UF inlet	Outlet UF and inlet RO	Outlet	UF inlet	Outlet UF and inlet RO	Outlet	UF inlet	Outlet UF and inlet RO	Outlet	UF inlet	Outlet UF and inlet RO	UF inlet	Outlet UF and inlet RO
Sept	7.06	6.62	7.50	9.9	0.136	0.036	438	453	19.8	216153	211	1	4.99	1.21	0.13	27.7	15.37	1.45	0.017
Oct	7.02	6.62	7.45	9.13	0.283	0.048	442	449	20.2	600160	243	1	4.99	1.16	0.16	27.2	16.12	1.33	0.025
Nov	6.98	6.62	7.38	12.7	0.170	0.076	464	508	17.8	772000	295	1	6.69	0.67	0.08	31.4	15.71	1.39	0.024
Dec	6.91	6.63	7.44	13.3	0.15	0.093	445	451	16.6	1022105	334	1	6.05	1.11	0.05	36.0	13.99	1.58	0.034
Jan	6.90	6.62	7.29	9.36	0.0961	0.058	464	473	16.0	340090	117	1	5.04	1.08	0.04	28.6	15.51	1.39	0.036
Feb	6.97	6.67	7.36	6.18	0.240	0.0285	451	459	14.5	429411	278	1	2.01	0.57	0.06	17.9	12.14	1.17	0.166
Mar	7.04	6.72	7.40	8.1	0.107	0.0225	455	461	16	304285	227	1	2.91	0.70	0.09	22.9	14.38	1.50	0.232
April	7.04	6.73	7.36	8.30	0.08	0.0233	453	459	18.3	386666	235	1	3.33	0.65	0.09	21.7	12.69	1.25	0.017
May	7.16	6.74	7.33	5.11	0.114	0.0354	456	465	20.5	306956	142	1	1.81	0.72	0.08	16.4	11.47	1.30	0.022
Jun	7.11	6.78	7.35	8.22	0.126	0.03	397	402	18.1	229523	148	1	2.62	0.84	0.08	18.6	11.70	1.53	0.044
Jul	7.17	6.74	7.43	9.67	0.088	0.0483	389	396	19.4	285971	167	1	4.32	1.0	0.11	24.5	11.57	1.49	0.048
Aug	7.14	6.83	7.43	5.05	0.042	0	357	362	16.9	55500	37	1	3.45	2.14	0.05	16.6	11.10	1.29	0.053