Cranfield University

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Impact of Chemical Shock Loads on a Membrane Bioreactor for Urban Water Reuse

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Supervisor: Dr B. Jefferson

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ABSTRACT

The performance of an MBR under chemical shock loading conditions was investigated, to ascertain the robustness of the treatment system for urban water reuse. 32 household products and industrial substances, likely to be found in urban wastewater were assessed for toxicity, using Microtox and respirometry to obtain EC_{50} values. Six of these toxins were dosed into bench scale porous pots to observe any detrimental effects on the treatment system, in terms of effluent quality and potential foulant release. Four toxins were dosed into a pilot scale MBR to observe the effects of scale and enhanced biomass retention on the perturbations seen at bench scale. Mitigation of the foulants observed was investigated by the addition of ancillary chemicals.

10 household products and 6 industrial products were identified as being of risk to a biological treatment system with EC_{50} concentrations of the order that could be present in urban wastewater. 2 of the 6 toxins dosed into the porous pots caused a serious impact on the system reducing COD removal rates to 45%, compared with 92% average for the control pots, and increasing SMP turbidity to 11 NTU. 1 of the 4 toxins dosed into the MBR caused an impact, although less than observed in the porous pots, with the COD removal rate reducing to 77% and SMP turbidity increasing to a maximum of 9 NTU. Jar tests carried out to investigate mitigation potential of SMP turbidity found the cationic polymers MPE50 and high molecular weight polyDADMAC most efficient with reductions of SMP turbidity to <1 NTU possible although the toxins increased the dose necessary to achieve this.

Keywords:
chemical shock loading, MBR for reuse, unsteady state operation, chemical mitigation.

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BOD – biological oxygen demand

BOD_7 – 7 day biological oxygen demand

COD – chemical oxygen demand

CST – capillary suction time

CTMAB – cetyltrimethylammoniumbromate

EC_{50} – effective concentration which results in a 50% reduction in the behaviour being measured

EPS – extracellular polymeric substances

EQS – environmental quality standard

HRT – hydraulic retention time

MBR – membrane bioreactor

MLSS - mixed liquor suspended solids

MLVSS – mixed liquor volatile suspended solids

NTU – nephelometric turbidity units

N_{tot} – total nitrogen

PAC – powdered activated carbon

PACl – poly aluminium chloride

PSD – particle size distribution

P_{tot} – total phosphorus

SDS – sodium dodecyl sulphate

SMP – soluble microbial products

SS – suspended solids

SRT – solids retention time

TOC – total organic carbon

TS – total solids

VS – volatile solids

XOC – xenobiotic organic compounds

1 Introduction

Water demand in the UK has been increasing over the last 50 years, as quality of life increases; all houses now have flushing toilets, washing machines, baths and showers, all of which exert an increased water demand. Management strategies to meet this increasing demand include the provision of more storage or the reduction in demand for fresh water by conservation and/or reuse. The former has limited political support and is of decreasing benefit due the unreliability of replenishing rainfall. For instance, rainfall for 18 out of the 22 months from November 2004 onwards, was below the 1961-1990 long term average (Met Office, 2006). Consequently alternative solutions need to be sought, such as water recycling in urban, industrial and agricultural applications. Research and development have focussed on the last two (Asano, 1998) such that recycling in urban environments is a relatively poorly explored application yet it's potential is significant. For example, the Environment Agency's report on greywater recycling states that less than 3% of water consumed in a household is used for drinking and cooking purposes (EA, 2000), yet 100% of the water delivered to a household is of drinking water quality, wasting valuable resources. If recycled water was used for toilet flushing alone a saving of at least 30% would be achieved (Jefferson *et al.*, 2001, Almeida *et al.*, 1999, Butler, 1996.)

Urban water recycling is defined as small decentralised plants which could provide water for defined reuse at a much lower cost, both economically and environmentally (Eriksson *et al.*, 2003). Grey water recycling is slowly becoming more accepted; with 25 operational or proposed systems as of 2001, however only 6 blackwater or sewage effluent systems have been installed in the same timeframe (Jeffrey *et al.*, 2001).

Urban water recycling would exert a greater demand on the treatment process employed; studies on the characteristics of grey water from all household activities identified the potential for a wide range of xenobiotic organic

compounds (XOCs) to be present (Eriksson *et al.*, 2003, Palmquist and Hanaeus, 2005). Most research to date has focussed on the capability of the technology to remove various constituents of wastewater but have not explored the effects that any of these detrimental constituents may have on the efficiency of the process (Jefferson *et al.*, 2004, Eriksson *et al.*, 2002). The introduction of small decentralised plants would accentuate any fluctuations in influent water quality (Butler, 1996, Butler *et al.*, 1995) in the absence of the dampening effects of a large sewerage network, but would provide a significant cost saving by retaining the water resource in the locality where it is collected and reused (Anderson, 1996). The majority of installed recycling systems are used where human contact with the recycled water is limited (Melin *et al.*, 2006). However, if the recycled water is to be used in domestic households then the reliability and robustness of the process becomes more important as the potential for human contact also increases.

Membrane bioreactors have been identified as one of the more robust technologies for water reuse (Jefferson *et al.*, 2004), however, the effects of chemical shock loads, resulting from industrial and household discharges, on the limits of this treatment technology need to be defined further to ascertain its suitability for decentralised (approximately 2000 population equivalent) urban water reuse.

2 Literature Review

2.1 Water Availability and Demand

There are a finite number of sources of fresh water in the world and this fresh water is in a constant cycle. In northern Europe the quantity of fresh water available has not been a pressing issue, however some droughts of the last decade have changed this view whereas in southern Europe the availability of water is becoming more of an issue (Angelakis and Bontoux, 2001). As water demand increases the availability of fresh water becomes less and less. Predictions for 2025 show that in for example Cyprus, as the worst case scenario, as the population grows and the demand for water increases the available freshwater per person will decline to less than 50% of that available per person in 1955. In the UK this is predicted to be 80% of the availability in 1955 (Table 2-1). It is becoming increasingly understood that the urban model of centralised waterbourne wastewater collection is both resource and economically expensive and decentralised treatment systems that can reuse water and require less water for the transportation of waste will become necessary (Eriksson *et al.*, 2003).

As well as population growth which has increased in the past few decades and is set to rise further in the decades to come, the population is also becoming increasingly urbanised with more concentrated clusters, which further increases the demand on the finite water supplies available in a geographic area (Asano, 2002). Water demand in the developed world, and the United Kingdom specifically, has been increasing over the last 50 years, as quality of life increases; most houses now have flushing toilets, washing machines, baths and showers, all of which exert an increased water demand. Water demand in the UK has been estimated to have as large a range as between 5 and 585 $l.p^{-1}.d^{-1}$ (Edwards *et al.*, 1995) but a use of 145 $l.p^{-1}.d^{-1}$ is an accepted average for Northern Europe for domestic purposes (Butler, 1996).

Table 2-1 – Availability of freshwater per inhabitant in several European countries for 1955, 1990 and 2025 (from Angelakis and Bontoux 2001).

Country	***Fresh water availability in m^3.inhabitant^{-1}***					
	1955		***1990***		***2025***	
	Population (thousands)	***Availability ($m^3.inh^{-1}.yr^{-1}$)***	***Population (thousands)***	***Availability ($m^3.inh^{-1}.yr^{-1}$)***	***Population (thousands)***	***Availability ($m^3.inh^{-1}.yr^{-1}$)***
Belgium	8868	1906	9951	1698	10407	1624
Cyprus	530	1698	702	1282	927	971
France	43,428	4260	56718	3262	61247	3021
Greece	7966	7406	10238	5763	9868	5979
Ireland	2921	17117	3503	14273	3882	12880
Netherlands	10751	8371	14952	6019	16276	5530
Portugal	8610	7666	9868	6688	9685	6815
Spain	29199	3802	39,272	2826	37571	2954
UK	51199	2344	57411	2090	61476	1952

Management strategies to meet this increasing demand include the provision of more storage of freshwater, transportation over large distances or the reduction in demand by conservation and/or reuse. The former have limited political support, with the economic and environmental costs gaining greater recognition (Asano, 2005), and are of decreasing benefit due the unreliability of replenishing rainfall. For instance, rainfall for 18 out of the 22 months from November 2004 onwards, in the UK, was below the 1961-1990 long term average (Met Office, 2006). Consequently, alternative solutions need to be sought, such as water recycling in urban, industrial and agricultural applications. Research and development have focussed on the last two (Asano and Levine, 1998) such that recycling in urban environments is a relatively poorly explored application yet it's potential is significant.

A break down of the usage in domestic households reveals that the water consumed in the bath and shower alone, if collected, would provide two thirds of the water needed to flush the toilet (Chambers *et al.,* 2005) (Figure 2-1). If this could be combined with water from the washing machine and the internal tap to provide the full demand for toilet flushing then 30% of the total water demand from a domestic household could be saved, on average 50 litres of water per day.

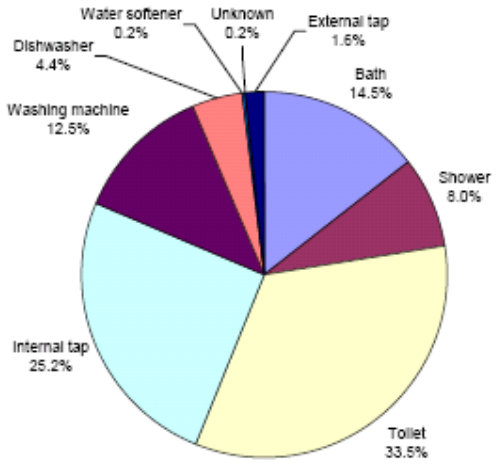


Figure 2-1 – Uses of water in a domestic household broken down by appliance (WRc Report CP 187 Chambers *et al*., 2005).

The Environment Agency's report on greywater recycling (Environment Agency, 2000) states that much of the drinking water quality water used in homes and businesses is often used for activities that could easily use a lower quality of water that needs less intensive treatment, e.g. first rinse of washing programmes, washing the car, irrigation, toilet flushing or even fire fighting. In fact, less than 3% of water consumed in a household is used for drinking and cooking purposes, yet 100% of the water delivered to a household is of drinking water quality, wasting valuable resources (Environment Agency, 2000). Recycled water could be used for these applications with little impact on the outcomes and this would then ease the burden on abstraction and potentially enable the water cycle to recover.

The issue of reuse does not hinge solely on water conservation but on the quality of water being discharged after treatment both in terms of the sensitivity of the receiving environment (nitrates, phosphates) and the quality of water available as a source for drinking water that is not detrimental to health (e.g. pesticide residues) (Angelakis and Bontoux, 2001). Diverting water away from a source, for example a river, concentrates the remaining pollutants as well as adding others that are not legislated for in wastewater treatment consents. Aquarec (2005) describe an example (Figure 2-2) where 75 % of the river flow is diverted to be used in agriculture or urban activities, with the resultant flow in the river being less than half of the original after the treated wastewater has been discharged downstream of the city. The pollutant load downstream of the city, in terms of COD, is almost ten times the original amount.

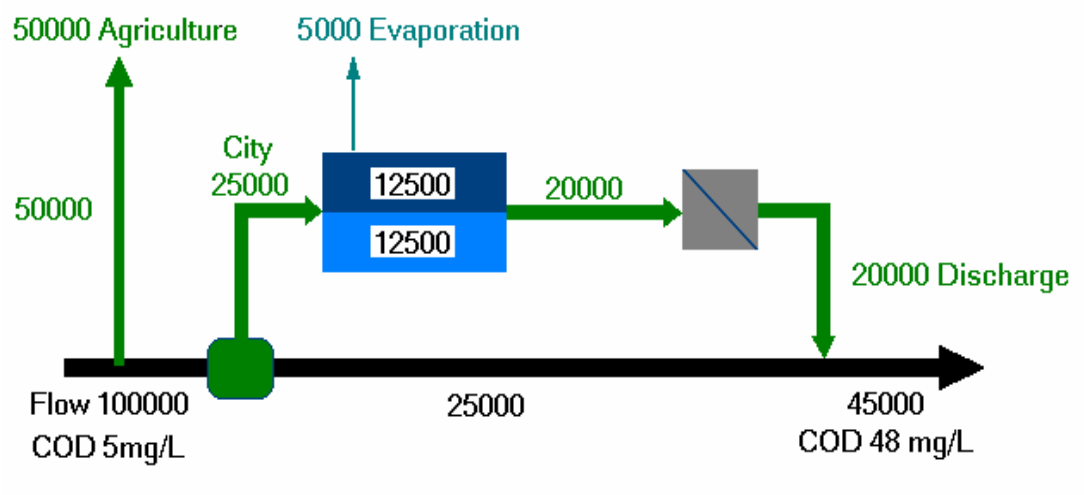


Figure 2-2 – Typical impact on a water source, in an urban scenario, in terms of water demand and pollutant load (Aquarec, 2005).

2.2 Water Reuse.

Water reuse has been employed for a century, with examples from the USA where recycled water has been used in California for irrigation purposes in public spaces since 1912 (Asano, 2002). There are seven categories of water reuse listed in order of greatest use (Asano, 2002):

- Agricultural irrigation,
- Landscape irrigation,
- Industrial activities,
- Groundwater recharge,
- Recreational and environmental uses,
- Non-potable urban uses,
- Potable reuse.

2.2.1 Benefits of water reuse

The potential benefits of water reuse are (Asano, 2005):

- Water reuse conserves water supplies: water recycling increases the total available water supply. High quality water supplies can be conserved by substituting reclaimed water where appropriate.
- Water reuse is environmentally responsible: it can preserve the health of waterways, wetlands, flora and fauna. It can reduce the level of nutrients

and other pollutants entering waterways and sensitive marine environments by reducing effluent and storm water discharge.

- Water reuse makes economic sense: reclaimed water is at the doorstep of the urban development where water supply reliability is most crucial and water is priced highest.
- Water reuse can save resources: recycled water originating from treated effluent contains nutrients if this water is used to irrigate agricultural land less fertiliser needs to be applied to the crops. By reducing pollution and nutrient flows into waterways tourism and fishing industries are also helped.

The scenario depicted previously (Figure 2-2) can now be shown with water reuse and conservation incorporated into the urban setting (Figure 2-3). The river flow is maintained, at more than half of the original, as less needs to be extracted, resulting in a lower pollutant load after the discharge of the residual wastewater after reuse. In this case the COD increases by only 2 $mg.l^{-1}$ whereas previously it increased by 43 $mg.l^{-1}$.

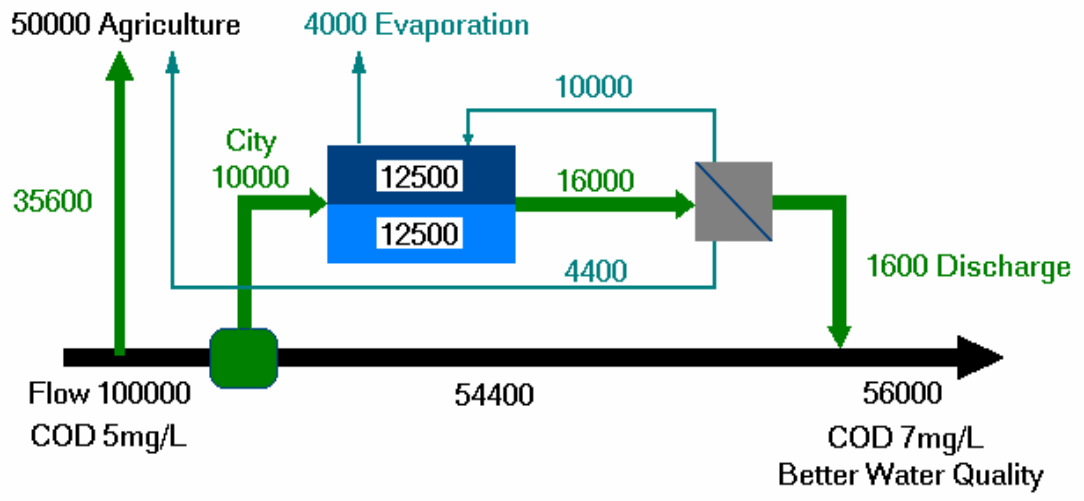


Figure 2-3 – Typical water demand in an urban scenario incorporating water reuse (Aquarec, 2005).

2.3 Urban Water

2.3.1 Definition

For this study urban wastewater has been defined as the sum of all discharges from households, as well as any discharges from any light industry in the wastewater catchment area and any surface runoff. It is likely that, unlike municipal wastewater which is becoming increasingly dominated by controlled industrial discharges (Tchoboglanous *et al.*, 2003), urban water will consist of mainly domestic wastewater. In turn, 75% of domestic wastewater is made up of greywater which is usually understood to be from household sources from washing facilities (bath, shower and wash basin), waste from the kitchen sink, dishwashers and laundry waste (Palmquist *et al.*, 2005, Jefferson *et al.*, 2004, Erikkson *et al.*, 2003). The remainder of wastewater discharged from households is defined as black water i.e. that from the toilet (Palmquist *et al.*, 2005, Ramon *et al.* 2004, Butler, 1996.)

2.3.2 Characterisation

Urban water, as defined for this study, is mainly made up of domestic wastewater which is highly heterogeneous in its character and varies from country to country and even household to household, depending on demographics, cultures, lifestyles and customs (Eriksson *et al.* 2002, Environment Agency, 2000). Palmquist and Hanaeus (2005) highlight these discrepancies, reporting that there is little information on the character of grey water, which accounts for 70 – 75% of domestic wastewater and increasing knowledge in this area is of prime importance, to further understand the impact of potential household pollutants on treatment systems. Attempts have been made to characterise grey water (Eriksson *et al.*, 2002, Palmquist and Hanaeus, 2005, Jefferson *et al.*, 2004) and all the studies agree that it is complex with a multitude of variations in source pollutants, dependant on lifestyles, product choice, water usage and washing habits. Jefferson *et al.*, 2004, go on to observe that “the main characteristic of grey water is its variability”, again underlining the complexity of the wastewater.

Palmquist and Hanaeus (2005) have compared typical greywater, blackwater and domestic wastewater characteristics, in terms of flows, chemical, biological and physical parameters (Table 2-2). Blackwater flows tend to be lower than greywater but the nutrient load is much higher with BOD and COD values being over twice that of greywater. Nitrogen levels are over ten times higher in blackwater than greywater and twice that observed in domestic wastewater with average concentrations of 9.68, 150 and 20 -70 $mg.l^{-1}$, respectively. The total COD of 2260 $mg.l^{-1}$ for blackwater is almost four times that of 588 $mg.l^{-1}$ for greywater and approximately three times the 250 – 800 $mg.l^{-1}$ given for domestic wastewater.

Table 2-2 – Comparison of the characteristics of greywater, blackwater and domestic wastewater (Palmquist and Hanaeus, 2005).

Parameter	***Greywater***		***Blackwater***		***Domestic***
	Average	***Min (Max)***	***Average***	***Min (Max)***	
Q ($m^3.h^{-1}$)	0.54	0.16 (1.02)	0.17	0.16(0.18)	-
P_{tot} ($mg.l^{-1}$)	7.53	4.6(11)	42.7	21 (58)	4-12
N_{tot} ($mg.l^{-1}$)	9.68	8.0 (11)	150	130 (180)	20-70
BOD_7 ($mg.l^{-1}$)	418	350 (500)	1037	410 (1400)	160-300
COD_{Cr} ($mg.l^{-1}$)	588	495 (682)	2260	806 (3138)	250-800
TS ($mg.l^{-1}$)	630	570 (700)	3180	920 (4320)	390-1230
VS ($mg.l^{-1}$)	330	300 (360)	2560	420 (3660)	95-315
pH	7.50	6.06	8.94	8.87 (9.08)	-

Reinforcing the observation that grey and black water are highly heterogeneous another study by Henze and Ledin (2001) observed much lower levels for grey and black water with COD ranging between 200 -700 $mg.l^{-1}$ for grey water and 900 – 1500 $mg.l^{-1}$ for black water (Table 2-3). Total nitrogen of only 20 – 40 $mg.l^{-1}$ for black water was in the range quoted above for domestic wastewater.

Table 2-3 – Comparison of the characteristics of grey and black wastewater (Henze and Ledin, 2001).

Parameter	***Grey Wastewater***		***Black Wastewater***	
	High	***Low***	***High***	***Low***
BOD total ($mg.l^{-1}$)	400	100	600	300
COD total ($mg.l^{-1}$)	700	200	1500	900
Total nitrogen ($mg.l^{-1}$)	30	8	40	20

Almeida *et al*. (1999), further split these characteristics by contribution from various household appliances with relation to volume, COD ammonia nitrogen

and total suspended solids (Table 2-4). The WC is the largest individual contributor in terms of volume and COD, ammonia nitrogen and total suspended solids, however, if the remaining appliances are combined they provide 69.2% volume of the discharged wastewater and are the most likely sources of xenobiotics (Eriksson *et al.*, 2003). In terms of COD and ammonia nitrogen the kitchen sink and washing machine provide the majority of the remaining contribution with 45.5% between them, while the WC provides 97.1% of the ammonia nitrogen.

Table 2-4 - % volume contribution of various household applicances (taken from Almeida *et al.*, 1999).

Appliance	***Volume***	***COD_t***	***NH_3-N***	***TSS***
WC	30.8	43.9	97.1	77.4
Kitchen Sink	13.0	23.2	0.3	10.1
Wash Basin	12.6	1.7	0.1	2.1
Bath	15.7	2.5	0.6	1.3
Shower	11.7	6.4	0.7	5.1
Washing Machine	16.2	22.3	1.2	4.0

In order to investigate the potential compounds from household product usage that could be discharged in greywater, Eriksson *et al.* (2002) conducted a study that identified over 900 xenobiotic compounds in household products, on the basis of their ingredients lists and tonnage of industrial manufacturing. 13 groups were analysed for (Table 2-5) with the most common found to be fragrances and flavours with 197 identified. Detergents made up a significant part of the remainder with a total of 192 identified, including anionic (most common), non-ionic, amphoteric and cationic. Preservatives which by their nature are toxic to micro organisms were also found to be a significant group with 79 identified. In particular, product usage in the bathroom has an impact on the nature of the water, with substances highly toxic to bacteria being discharged on an irregular basis (Jefferson *et al.*, 2004).

Table 2-5 – Groups of compounds found in common household chemicals in Denmark (Eriksson *et al.*, 2002).

Compound Group	***Number of Substances in group***
Fragrances and flavours	197
Preservatives	79
Anionic detergents	73
Solvents	67
Non-ionic detergents	65
Cationic detergents	34
Softeners	29
Emulsifiers	28
Dyes	26
UV filters	23
Amphoteric detergents	20
Bleaches	16
Enzymes	4
Miscellaneous	238

Another study by Eriksson *et al.*, conducted in 2003, obtained the weekly usage of common household products for a housing complex in Copenhagen, Denmark, by inhabitants keeping a daily diary of all usage in the household. This study identified that shampoo was the most consumed product with 353 g per week being used, giving an approximate concentration in wastewater from the complex of 11 $mg.l^{-1}$ (averaged over 7 days, assuming a water usage of 150 $l.p^{-1}.day^{-1}$). This was the most used product with all the other products contributing less than 10 $mg.l^{-1}$ to the final concentration in the wastewater.

Table 2-6 – Weekly comsumption of household products, in order of decreasing amount, from a residential complex in Copenhagen, Denmark (Eriksson *et al.*, 2003).

Type of Product	***Consumed amount (g)***	***Approximate concentration in wastewater[1] ($mg.l^{-1}$)***
Shampoos	353	11
Soaps	245	8
Oral hygiene products	131	4
Hair conditioners	91	3
Skin care products	74	2
Shower gels	62	2
Cleaners	61	2
Lime deposit removers	21	1
Hair styling products	19	-
Deodorants	16	-

Type of Product	*Consumed amount (g)*	*Approximate concentration in wastewater[1] (mg.l^{-1})*
Shaving foam	13	0.4
Powder laundry detergents	8	0.25
Glass and window cleaners	6	0.2

[1] assuming usage of 150 l.p^{-1}.day^{-1} and 30 residents

2.4 Biological Response to Changes in Influent Wastewater.

2.4.1 Types of changes

As discussed in the previous section the characterisation of domestic wastewater is a complex and involved procedure, however, there are four fundamental variations to the biological treatment system environment influenced by the influent wastewater, that will have an effect on the biological community in a wastewater treatment process:

1. pH,
2. temperature,
3. organic loading,
4. presence or absence of toxic compounds.

The presence of toxic compounds is the factor most likely to change abruptly in urban wastewater and which will have the most effect on the microbial community, and which is highly unpredictable. The differing mechanisms of toxicity will be discussed in the following sections as well as the differing effects on various parts of the microbial community in an aerobic biological treatment system.

2.4.2 Mechanisms of toxicity.

Section 2.3.2 illustrated the components of urban wastewater are many and varied and it is difficult to predict the substances or compounds that could be discharged, however, the characteristics of the biomass in a biological wastewater treatment process are influenced by their environment and which have an effect on the filterability of the sludge (Chang and Lee, 1998, Rosenberger and Kraume, 2002) therefore, it is important to have an understanding of the mechanisms involved in the biodegradation of the components of influent wastewater and the effects that these variations could

have on the biomass. Toxicity in biological oxidation systems may be due to one of several causes (Roš, 1993):

1. Organic substances which are toxic in high concentrations, but biodegradeable in low concentrations (such as phenol, formaldehyde etc).
2. Substances which have a toxic threshold dependent on the operating conditions (such as heavy metals).
3. Inorganic salts and ammonia that exhibit retardation at high concentrations.

Toxicity will affect the microbial community in two basic ways; either the osmotic balance of the cell or the enzyme action (Roš, 1993) which can result in inhibition or cell death. The mechanisms can be split further into four broad categories where the oxidant (e.g. chlorine, peroxides) and lytic (e.g. silver, copper, mercury, isothiazolones) mechanisms interfere with the cell membrane and the electrophiles (e.g. phenoxyethanol,) and protonophores (e.g. parabens) interfere with metabolism or enzyme action (Chapman, 2003):

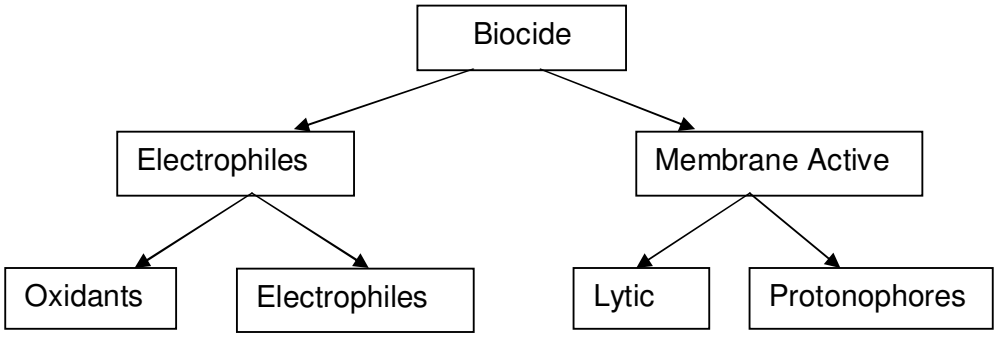


Figure 2-4 – Categories of toxic mechanisms.

The success of any biological treatment system for wastewaters is based on two fundamental activities – substrate utilisation to “clean” the water and growth kinetics to maintain a sustainable microbial community for substrate utilisation. Some substrates are easily degradable and provide a readily available carbon

source for the microbial community whereas others are more difficult to be broken down and these are termed xenobiotics. Xenobiotics can also be toxic but do not have to be. If a xenobiotic causes enzyme inhibition it can have three effects on substrate removal (Volskay and Grady, 1990, Roš, 1993):

1. cause substrate inhibition, preventing the biodegradation of the xenobiotic compound itself (uncompetitive inhibition)
2. influence the rate of substrate utilisation by competition (competitive inhibition) and
3. inhibit substrate utilisation by micro-organisms that are incapable of biodegradation of the xenobiotic (non competitive inhibition) .

Growth kinetics are described by kinetic theory which relates substrate concentration with biomass growth (Daigger and Grady, 1982). In a steady state system these are easily defined and the relationship is a relatively simple equation, first described by Monod, however, in the presence of xenobiotic compounds the kinetics become more complicated, even though ultimately the breakdown of a xenobiotic compound is the same as for any other substrate:

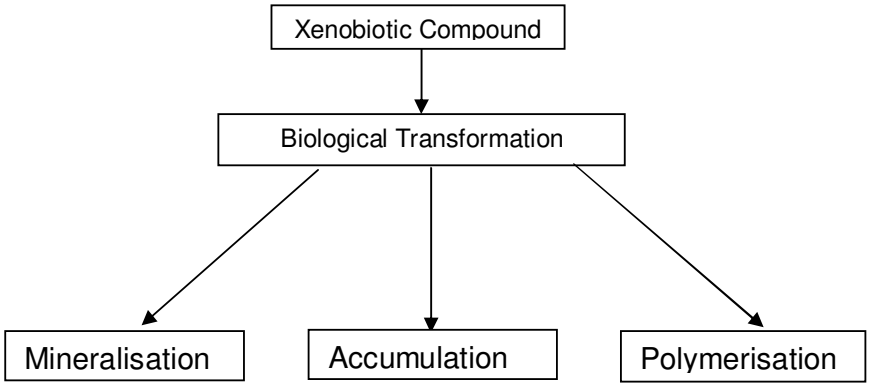


Figure 2-5 – Breakdown of a xenobiotic compound (Bitton, 1997).

Toxicity of any form will interfere with the purpose of the treatment system either by inhibiting substrate utilisation which could then result in inferior effluent quality or by jeopardising the viability of the microbial community by causing cell death.

2.4.3 Effects of toxicity on a biological system

The presence of toxic compounds will affect a biological system in five fundamental ways:

1. impact on substrate removal,
2. impact on flocculation ability of the biomass,
3. release of extracellular polymeric substances,
4. viability of the microbial community,
5. increased oxygen demand.

These five effects will not necessarily happen in isolation, for example, the effects of dosing sodium chloride into an activated sludge process at concentrations of up to 20, 000 $mg.l^{-1}$ induced poor flocculation, reduced efficiency in substrate removal, generated higher effluent solids and an increase in the respiration rate (Kincannon and Gaudy, 1968). Phenol containing waters inhibit the biological degradation of other compounds and inhibit microbial growth resulting in an increase in effluent COD and impacts on the process efficiency (Barrios-Martinez *et al.,*2006).

Although the previous examples illustrate an apparent general toxicity some parts of the microbial community may be more sensitive to the presence of toxic compounds than others. Pagga *et al.*, (2006) found that the inhibition levels for nitrate formation were consistently lower than for heterotrophic bacterial respiration across a wide range of compounds (Table 2-7). For example, phenol had an EC_{50} for nitrate formation of 0.8 $mg.l^{-1}$ whereas the EC_{50} for heterotrophic respiration was >100 $mg.l^{-1}$. Contrary to these findings, Madoni *et al.*, 1999, reported that neither nitrifiers nor heterotrophs are more sensitive to heavy metals on the whole, with both ammonium uptake rate and oxygen uptake rate being inhibited by 50% for a concentration of soluble zinc of < 1 $mg.l^{-1}$. The EQS for zinc is 0.0075$mg.l^{-1}$.

Table 2-7 – Comparison of inhibition of nitrifiers and hetertrophic bacteria (Pagga *et al.*, 2006).

Chemical	***ISO 9509 Inhibition for nitrate formation, EC_{50} ($mg.l^{-1}$)***	***ISO 8192 Inhibition of heterotrophic respiration EC_{50} ($mg.l^{-1}$)***
3-Chlorophenol	0.9	10-100
2-Chloromandelonitrile	<1	10-50
2,4-Dichlorophenol	0.15	50-100
Imidazole	6.2	>100
N-Methylaniline	1.2	>100
Nitrobenzene	56	>100
Phenol	0.8	>100
Pyrazole	0.16	>10
Thioacetamide	0.2	>5
Thiourea	0.3	>5
Zn^{2+}	200	500-100

Within the context of MBRs the effects of perturbations in influent water quality and/or the presence of toxic compounds is most apparent in the decrease of permeability of the membrane (Reid *et al.*, 2006) caused by the increased production by micro-organisms of soluble microbial products (SMP).

Soluble microbial products can be produced by micro-organisms under various conditions: to establish a concentration equilibrium, during starvation conditions, in the presence of an energy source, when there is a sudden change in substrate availability from starvation conditions, if essential nutrients are only available in low concentrations, to relieve environmental stress and lastly as the process of normal cell growth and metabolism (Barker and Stuckey, 1999). The most notable being the response to environmental stress which could include the presence of toxic substances. Barker and Stuckey (1999) go on to report that SMP produced in a completely stirred mixed reactor fed with phenol produced more SMP than that fed with glucose but the SMP in the phenol fed reactor was more easily degradable, indicating that the type of toxin will have an influence on the type and degradeability of the SMP produced. SMP have been identified as an important factor in membrane fouling that is discussed later in the review (Section 2.7.2).

2.5 Guidelines for water reuse

Standards for water reuse have been introduced mainly in the countries that have experienced prolonged periods of drought or that have large areas of designated desert. At the present time there are no specific European Directives on water reuse quality (Angelakis and Bontoux, 2001) and it is an accepted norm to use bathing water and/or drinking water standards as the accepted water quality standards, however, Aquarec, a research project partially funded by the European Commission and the Australian government has proposed a set of water quality parameters that amalgamates all the available guidelines into one proposal. These are subdivided into seven microbiological and four chemical quality classes to specify water quality parameters depending on the end use of the water (Table 2-8).

The most stringent quality parameters are for the water that is most likely to come into human contact e.g. garden watering and toilet flushing or to protect groundwater recharge while the least stringent are for water used industrial cooling waters where human contact will be minimal. Controversially, the microbial parameters are less stringent for water used for bathing than for that used in car washing.

Table 2-8 – Microbial and chemical water quality categories for different final uses of reclaimed water (Aquarec, 2005).

Microbial Category	***Chemical Category***	***Specific Final Use***
I	1	-Residential uses: private garden irrigation, toilet flushing, home air conditioning systems, car washing. -Aquifer recharge by direct injection into the soil for irrigation purposes.
II	1	-Bathing water.
III	1	-Urban uses and facilities: irrigation of open access landscape areas (parks, golf courses, sport fields....). Street cleaning, fire fighting, ornamental impoundments and decorative fountains. -Greenhouse crop irrigation. -Irrigation of raw consumed food crops. Sprinkler irrigated fruit trees. -Unrestricted irrigation.
IV	1	-Irrigation of pasture for milking or meat animals. -Irrigation of industrial crops for canning industry and crops not raw-consumed. Irrigation of fruit trees except by sprinkler irrigation.

Microbial Category	*Chemical Category*	*Specific Final Use*
		-Irrigation of industrial crops, nurseries, fodder, cereals and oleaginous seeds.
	2	-Impoundments, water bodies and streams for recreational us in which the public's contact with the water is permitted (except bathing)
V	1	-Irrigation of forested areas, landscape areas and restricted access areas. Forestry.
	2	-Aquaculture (plant or animal biomass).
	3	-Aquifer recharge by localised percolation through the soil.
VI	2	-Surface water quality, impoundments, water bodies and streams for recreational use, in which the public's contact with the water is not permitted.
VII	4	-Industrial cooling, except for the food industry.

The microbial parameters for each class are based on both bacterial count and other micro-organisms, dependant on the end use of the water, only the total bacteria and faecal coliform limits have been included for information (Table 2-9):

Table 2-9 – Microbiological parameters for water reuse dependant on class of end use (Adapted from Aquarec, 2005).

Use	Total Bacteria ($cfu.ml^{-1}$)	Faecal Coliforms ($cfu.100ml^{-1}$)
I	<1000- <10,000	abs
II	<1000	<20 - <1000
III	<1000	abs - <1000
IV	<10,000 - <100,000	abs - <10,000
V	<100,000	abs - <10,000
VI	<10,000	<200 - <10,000
VII	<10,000	abs - <10,000

The chemical parameters are wide ranging and are grouped based on frequency of monitoring (daily – weekly, monthly, monthly – once yearly, once per year – once per five years), only the daily and monthly parameters have been included for information (Table 2-10).

Table 2-10 – Frequently analysed chemical water quality parameters dependant on class of reuse (Aquarec, 2005).

Parameter	*1 Private, urban and irrigation*	*2 Environmental and aquaculture*	*3 Indirect aquifer recharge*	*4 Industrial cooling*
Very high analytical frequency (daily – weekly)				
pH	6.0 – 9.5	6.0 – 9.5	7 -9	7.0 – 8.5

Parameter	*1 Private, urban and irrigation*	*2 Environmental and aquaculture*	*3 Indirect aquifer recharge*	*4 Industrial cooling*
BOD ($mg.l^{-1}$)	10 - 20	10 - 20		
COD ($mg.l^{-1}$)	100	70 - 100	70 - 100	70
Dissolved Oxygen ($mg.l^{-1}$)	>0.5	>3	>8	>3
UV 254 absorbance ($cm^{-1}x10^{3}$)	30 - 70	30 - 70	10	
Conductivity ($\mu S.cm^{-1}$)	3000	3000	1400	
TSS ($mg.l^{-1}$)	10 - 20	10 - 20		10 – 20
Ammonium – N ($mg.l^{-1}$)	2 - 20	1.5	0.2	1.5
High analytical frequency (monthly)				
Na ($mg.l^{-1}$)	150	150 -200		
Nitrate ($mg.l^{-1}$)			25	
Chloride ($mg.l^{-1}$)	250	250 - 400	100	400
Sulphate ($mg.l^{-1}$)	500	500	100	
Total P ($mg.l^{-1}$)	2-5	0.2 - 1		0.2

2.6 Technologies used for water reuse.

The technologies employed for urban water reuse are dependant on the end use of the reclaimed water, the volume of water to be treated, the water quality standards to be met and the cost of the treatment (Salgot and Angelakis, 2001). In general, the higher the quality of water required the more technology is involved. A treatment matrix has been developed which details the level of treatment required for a designated end use (van der Graaf *et al.,* 2005) (Figure 2-6):

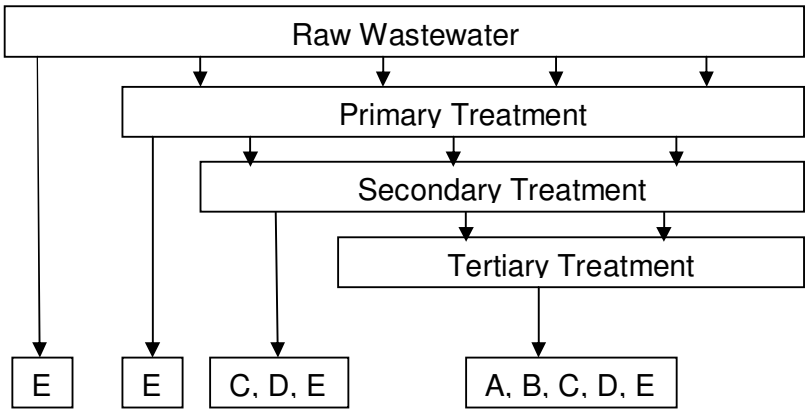


Figure 2-6 – Treatment matrix for different water reuse applications (A = industry, B = Domestic (Household - non potable), C = Domestic (Irrigation), D = Natural and E = Agriculture)

Primary treatment removes particulate matter from the wastewater, typically using sedimentation, filtration or screening, secondary treatment removes organic constituents and nutrients from the wastewater by an aerobic biological process, typically suspended or attached growth. Suspended growth systems include stabilisation ponds, activated sludge or aerated lagoons with some form of biomass/liquid separation mechanism e.g. clarifiers or a membrane, whereas attached growth are trickling filters, rotating biological contactors or other media filters. Tertiary treatment is employed for removal of specific pollutants if required and a final disinfection either by UV or chlorine (Asano and Levine, 1998).

Biological systems vary considerably in their performance. A study carried out by Jefferson *et al*., 2001 compared a MBR with a biological aerated filter and the performance of the MBR was consistently better for grey water, grey black water and black water for both physical and microbiological removal rates, with COD removal rates of ≥ 90% effluent turbidity of < 0.4 NTU and up to an 8 log removal for total coliforms compared with COD removal rates of 30-77%, effluent turbidity of 3 – 12 NTU and a 2-3 log reduction for total coliforms. van der Graaf *et al*., (2005) compared the estimated removal efficiencies of seven secondary treatment systems (activated sludge (AS), trickling filter (TF), rotating biological contactor (RBC), submerged aerated filter (SAF), stabilisation ponds (StabP), constructed wetlands (CW) and an MBR). The differences in removal rates were dependant on the proposed loading rates with greater loading rates producing lower removal rates. The MBR was the only technology that was capable of removing pollutants in all of the categories presented demonstrating that this is the best process for high quality effluent (Table 2-11).

Table 2-11 – Comparison of estimated removal efficiencies for seven secondary treatment processes.

Treatment process	**AS**	**TF**	**RBC**	**SAF**	**StabP**	**CW**	**MBR**
COD/BOD	+++	++/+++	+++	+++	++/+++	++/+++	+++
phosphorus	++/+++	++	++	++	+/+++	+/+++	++/+++
nitrogen	++/+++	++	++	++	+/+++	++/+++	++/+++
SS	+++	+++	+++	+++	+/+++	+++	+++
pathogens	+/+++	+++	+++	+++	+++		+++
viruses/helminths	+++	+/+++			+++		+++
micropollutants							**+/++**

removal efficiency: +: 0 - 35%, ++:35 - 70%, +++: 70 -100%

2.7 Membrane Bioreactors

2.7.1 Membrane Bioreactor Treatment Process

Membrane bioreactors (MBRs) utilise a biological treatment system, either aerobic or anaerobic, with a membrane for biomass separation. There are various different configurations of membrane bioreactors, but for wastewater treatment membrane bioreactors are operated in crossflow operation where the flow is tangential to the membrane, to encourage turbulent flow close to the membrane (Belfort *et al.*, 1994). The membrane can be housed in the same tank as the biological system, known as a submerged membrane bioreactor or it can be housed in an external loop, where the biomass is pumped to the membrane and recirculated back to the tank, known as a sidestream membrane bioreactor (Fane and Chang, 2002). Submerged membrane bioreactors tend to be used for municipal wastewater treatment whereas sidestream MBRs tend to be used for more specialist applications where the wastewater is normally of industrial origin with extremes of pH, temperature, organic loading or other difficult conditions (Yang *et al.*, 2005). Different types of membrane configurations have been developed, including flat plate, hollow fibre, tubular and plate and frame.

Membrane bioreactors were developed in the 1970s and were first used commercially in Japan the 1980s. Since the 1990s MBRs have experienced a rapid growth in Europe and North America, becoming more of a mainstream treatment process (Pearce, 2008). The majority of MBRs have been installed to

treat municipal wastewater. Worldwide, as of 2005, there were 2259 installations from four major MBR providers (Zenon, USFilter, Kubota, Mitsubishi-Rayon) with 1527 treating municipal wastewater and 732 treating industrial wastewater (Yang *et al.*, 2005).

MBRs have many benefits over conventional activated sludge plants, namely a higher quality effluent, as the membrane acts as a physical barrier rather than being reliant on the settleability of the sludge flocs, as in a conventional activated sludge process, intensification of the process resulting in a smaller footprint, (Stephenson *et al.*, 2002), reduced sludge yield and increased process reliability (Visvanathan *et al.*, 2000). Most importantly the hydraulic retention time and solids retention time are decoupled allowing the process to be intensified and run with significantly higher MLSS concentrations, resulting in a smaller footprint with a lower sludge yield (Brindle and Stephenson, 1996). MLSS concentrations of 12 – 15 $g.l^{-1}$ are recommended for submerged MBRs (Melin *et al.*, 2006) however bioreactors have been run at concentrations of 0.3 – 27 $g.l^{-1}$ (Pollice *et al.* 2005). Curtis, Head and Graham (2003) utilised island biogeography theories to demonstrate that the volume of a bioreactor has an influence on the diversity of a microbial community and the diversity of the community has an effect on the robustness of the system, potentially limiting how small a treatment plant can be. The majority of wastewater treatment systems depend on micro-organisms to degrade undesirable contents of effluents and small scale water recycling systems are no different. It is therefore imperative that the microbial population is maintained, and encouraged in its diversity, in order to meet process requirements (van der Gast *et al.*, 2006).

These attributes of the membrane bioreactor lend themselves well to using the technology for small decentralised wastewater treatment and for water reuse. Melin *et al.*, 2006 report that there are around 6 companies that now offer MBR technology for this purpose, however, to date few operational MBRs have effluent that is actively reused, despite the high quality.

The performance of membrane bioreactors is dependant on the ability of the membrane to filter the biomass matrix efficiently. This is influenced by the resistance of the membrane, the resistance of the fluid being filtered and the interaction between the membrane and the fluid. The resultant flow through the membrane per unit area is termed the permeate flux and is given by the resistance in series model (Visvanathan *et al.*, 2000) (1):

$$J = \frac{\Delta P}{\mu R_t} \qquad (1)$$

Where J = permeate flux ($m^3.m^{-2}.h^{-1}$), ΔP = transmembrane pressure (Pa), μ = viscosity (Pa.s) and R_t = total resistance to filtration (m^{-1}). The total resistance to filtration, R_t, has several components; the resistance of the membrane itself, R_m, the resistance of the polarization layer due to the concentration gradient, R_p, reversible or external fouling resistance due to the physicochemical interactions with the membrane, R_{ef}, and irreversible or internal fouling due to absorption into the membrane pores, R_{if}. These components are additive and in reality, there is little to distinguish the resistance due to the concentration gradient, R_p, and the reversible fouling, R_{ef}, and these can be combined to R_e for external fouling. The total resistance is then (Visvanathan *et al.*, 2000) (2):

$$R_t = R_m + R_e + R_{if} \qquad (2)$$

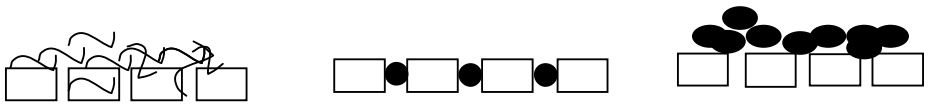
Equation 1 illustrates that flux is proportional to the driving force across the membrane given by the transmembrane pressure, but is inversely proportional to the fouling resistance hence the focus of many research papers on this subject (Yang *et al.*, 2005).

2.7.2 Fouling

Membrane processes are normally run at a constant permeate flow with a varying transmembrane pressure, which increases as resistances to the flow increase (Cho and Fane, 2002). Fouling is the process by which the membrane used for biomass separation becomes less permeable and requires either greater pressure to drive the liquid through the membrane or more regular cleaning to remove the matter that is causing the interference. A flux has been defined, known as the critical flux (Field *et al.*, 1995), specific to each

membrane bioreactor, above which the rate of fouling increases rapidly and below which the rate of fouling is much reduced but still present. Pollice *et al.*, 2005, comment that the source of fouling below the critical flux is due to colloidal elements in the biomass matrix and components that are released due to biological activity, e.g. soluble microbial products or cell detritus from cell lysis, whereas fouling above the critical flux is due to the suspended biomass itself.

There are three main types of fouling that are recognised: adsorption of SMP, pore clogging and particle deposition (Liao *et al.*, 2004) (Figure 2-7):



(a) adsorption of SMP (b) pore clogging (c) particle deposition

Figure 2-7 – Three main types of fouling that occur in membrane bioreactors.

Fouling is the main obstacle to a more widespread uptake for membrane bioreactors as a mainstream treatment process. In recent years much research has been dedicated to understand the causes and mechanisms of fouling, but to date no unifying theory has been proposed. According to Zhang *et al.*, (2006), there are three factors that contribute to fouling:

1. the nature of the biomass matrix,
2. the membrane properties,
3. the hydrodynamic environment experienced by the membrane.

Of these three the last two are controllable by the operator of the MBR as some decision can be taken as to the properties of the membrane to be used and the rate of aeration is normally advised by the manufacturer. The nature of the biomass matrix however, is very dependant on the influent wastewater which is constantly changing and the state of the biomass at the time, which is also constantly changing. It is this cause of fouling that has attracted the most

research and several studies have presented different conclusions as to the reasons (Table 2-12).

It is likely all the relationships found will contribute in some way to the fouling of a membrane. There are many studies (around 30% of recent MBR publications (Drews *et al*., 2006)) on this subject with a number of variables linked with the biomass matrix being associated with fouling (Table 2-12). No clear unifying theory has been proposed and findings are often relevant only to the specific operational and physical set up of the MBR.

Bouhabila *et al*., 2001, linked the colloidal fraction in the biomass with fouling, reporting a contribution of 50% to the overall fouling, however, this was at a very short hydraulic retention time (HRT) of 3.3 hours which Meng *et al*., (2006a) discovered to have a strong influence on fouling with a short HRT resulting in a high extracellular polymeric substance (EPS) concentration, high mixed liquor volatile suspended solids (MLVSS) and a high sludge viscosity. The MBR run at an HRT of 10 -12 hours maintained a flux twice that of the MBRs with HRTs <8 hours after 35 days filtration. Itonaga *et al.,* (2004) also linked the colloidal fraction to a decline in flux for dead end filtration with the colloidal element contributing only 18% to the overall fouling resistance, using biomass from an MBR with an HRT of <6 hours, resulting in a high loading rate. Nagaoka *et al*., (1998), observed that the loading rate had an influence on the fouling when an MBR running at a high loading rate of 1.5 $gTOC.L^{-1}.day^{-1}$ fouled irreversibly after 40 days whereas a low loaded MBR (0.5 $gTOC.L^{-1}.day^{-1}$) did not foul until 120 days of filtration. On the other hand Rosenberger *et al*., (2006) observed colloids to have a significant influence on fouling at a longer HRT of 11 hours, but a short solids retention time (SRT) of 8 -15 days, whereas Bouhabila *et al*, (2001) had observed the longer the SRT, the less the effect of fouling from colloids.

It can be concluded from these studies that SRT and HRT have a strong influence on colloidal fouling and an increase in both parameters will have a positive effect on mitigation of fouling by colloids.

A more robust relationship is that of SMP carbohydrates with fouling which has been shown for a variety of operating parameters. Drews *et al*., (2006) observed that flux decline is proportional to polysaccharide content for an HRT of 11 hours and a 30 day SRT. The MBR was run with intermittent sludge removal and although the relationship was generally linear there were deviations of up to 100%, thought to be caused by a variation in polysaccharides that had a varying fouling effect. Lesjean *et al.,* (2005) reported that SMP carbohydrates and fouling rate formed a linear relationship at a short SRT of 8 days but this could not be replicated at a longer SRT of 15 days. Again it can be concluded that the relationship of SMP carbohydrates is dependant on the specific operating environment of the MBR.

Table 2-12 – Fouling relationships from literature.

Fouling parameter	***Relationship to fouling***	***Operating parameters***	***Reference***
Colloidal fraction from biomass SRT	Flux decline was proportional to the amount of colloids present. Flux decline was inversely proportional to SRT.	Flux = 12 $l.m^{-2}.h^{-1}$ SRT = 10,20, 30 days HRT = 3.3 h Reactor volume = 20 l Membrane type = Zenon HF Feed = synthetic dairy	Bouhabila *et al.*, 2001.
Particle size	Flux decline accelerated with a decrease in particle size.	Flux = various SRT = nd HRT = nd Reactor volume = nd Membrane type = HF Feed = synthetic	Chang and Kim, 2005.
Polysaccharide content	Flux decline is proportional to polysaccharide content.	Flux = 10 $l.m^{-2}.h^{-1}$ SRT = $30d^{1}$ HRT = 10.7 h Reactor volume = 140 l Membrane type = plate and frame. Feed = domestic.	Drews *et al.*, 2006.
Colloidal fraction	Flux decline in dead end filtration tests was attributed to the colloidal fraction.	Flux = 17 $l.m^{-2}.h^{-1}$ SRT = nd HRT = 4.5 h Reactor volume = 180 l Membrane type = HF Feed = municipal	Itonaga *et al.*, 2004.

Fouling parameter	*Relationship to fouling*	*Operating parameters*	*Reference*
Dynamic membrane	Flux decline was more rapid for an attached growth system where the biomass was grown out of suspension than for a suspended growth MBR. 80% of filtration resistance was observed for the cake layer in the suspended growth MBR.	Flux = 25 $l.m^{-2}.h^{-1}$ SRT = nd HRT = 8 h Reactor volume = 5 l Membrane type = HF Feed = synthetic	Lee, J. *et al.*, 2001.
Microbial floc (Amount of EPS protein, ratio of protein:carbohydrate, hydrophobicity, surface charge and microbial activity).	Increase in all parameters was significant indication of fouling.	Flux = 9 $l.m^{-2}.h^{-1}$ SRT = 20, 40, 60 days. HRT = 7.8 h Reactor volume = 7 l Membrane type = HF Feed = synthetic	Lee, W. *et al.*, 2003.
SMP polysaccharide content	A linear relationship was observed for fouling vs. polysaccharide content at an 8 day SRT but this was not observed at a longer 15 day SRT.	Flux = 21.5 – 23.5 $l.m^{-2}.h^{-1}$ SRT = 26, 20-12, 15, 8 days HRT = 11 h Reactor volume = 2000 l Membrane type = HF Feed = municipal	Lesjean *et al.*, 2005.
Sludge morphology	Fractal dimension had a strong positive correlation with fouling rate.	Flux = 10 $l.m^{-2}.h^{-1}$ SRT = 30,90,120 days HRT = 12 h	Li *et al.*, 2008.
Proteins	Fouling rate is proportional to the amount of proteins present.	Reactor volume =12 l Membrane type = flat sheet Feed = synthetic	

Fouling parameter	*Relationship to fouling*	*Operating parameters*	*Reference*
Effect of HRT	A low HRT resulted in high EPS concentration, high MLSS concentration and high sludge viscosity. These were all attributed to the excessive growth of filamentous bacteria and caused increased fouling in the MBR.	Flux = variable (fixed water head drop) SRT = 30 days HRT = 12-5 h Reactor volume = 12 l Membrane type = HF Feed = synthetic	Meng *et al.*, 2006a.
Sludge viscosity	A decrease in HRT resulted in an increase in sludge viscosity decreasing the cross flow velocity of the sludge which in turn decreased the fouling effect on the membrane and increased fouling.	Flux = variable (fixed water head drop) SRT = 30 days HRT = 12-5 h Reactor volume = 12 l Membrane type = HF Feed = synthetic	Meng *et al.*, 2006a
MLSS	Fouling rate and MLSS followed an exponential relationship.	Flux = variable (fixed water head drop)	Meng *et al.*, 2006b.
PSD	The smaller the particle size the faster the fouling rate.	SRT = nd HRT = nd	
Microbial products	SMP and colloidal products induced from EPS had a strong influence on the fouling rate.	Reactor volume = 12 l Membrane type = HF Feed = synthetic	

Fouling parameter	***Relationship to fouling***	***Operating parameters***	***Reference***
Filamentous bacteria	An increased concentration of filamentous bacteria resulted in a more rapid fouling of the membrane.	Flux = variable (fixed water head drop) SRT = nd HRT = nd Reactor volume = 12 l Membrane type = HF Feed = synthetic	Meng *et al.*, 2007.
Organic loading	The membrane in the high organic loading MBR fouled quicker than that with the low organic load.	Flux = 6.25 $l.m^{-2}.h^{-1}$ SRT = nd HRT = nd Reactor volume = 28 l Membrane type = flat sheet Feed = synthetic	Nagaoka *et al.*, 1998.
Decomposition of EPS on membrane surface	The EPS accumulated on the surface of the flat sheet membranes was sampled over a 40 day period and was found to decay to lower MW EPS over this time resulting in a lower fouling rate.	Flux = 6.25 $l.m^{-2}.h^{-1}$ SRT = nd HRT = nd Reactor volume = 82 l Membrane type = flat sheet Feed =synthetic	Nagaoka and Akoh, 2008.
Suspended EPS (SMP)	The higher the concentration of suspended EPS (SMP) the higher the fouling rate.	Various pilot and full scale MBRs. Feed = municipal, domestic, industrial.	Rosenberger and Kraume, 2002.

Fouling parameter	*Relationship to fouling*	*Operating parameters*	*Reference*
SMP polysaccharide content	Fouling rate is proportional to polysaccharides concentration.	Flux = 19 – 21 $l.m^{-2}.h^{-1}$ SRT = 8 – 15 days HRT = 11 h	Rosenberger *et al.*, 2006.
Supernatant colloidal and soluble fraction	Fouling rate is proportional to the colloidal and soluble fraction of supernatant.	Reactor volume = 1900 and 2100 l Membrane type = HF Feed = municipal	

[1]intermittent sludge removal, nd = not defined, HF = hollow fibre.

2.8 Mitigation of the effects of fouling in MBRs

2.8.1 Physical mitigation

There are several strategies for mitigation of fouling in membranes by manipulation of operating parameters that will have a limited effect (Howell *et al.*, 2004). These are increased aeration, backwashing/relaxation, chemical cleaning and operating at a reduced flux (Judd, 2005). Each will have a limited effect on reversible and/or irreversible fouling.

Increasing the aeration to a membrane will enhance the crossflow velocity in the vicinity of the membrane and increase the turbulence next to the membrane, thereby reducing fouling, by discouraging particle deposition, but becomes ineffective above a certain threshold and increases operational costs (Visvanathan *et al.*, 2000, Le-Clech *et al.*, 2003, Chang and Fane, 2000).

Backwashing for hollow fibre submerged membrane entails reversing the permeate flow back through the membrane to dislodge reversible fouling by dislodging the cake layer and build up within the pores of the membrane. Longer less frequent backwashes have been proven to be more effective than shorter frequent ones (Le-Clech *et al.*, 2006). Backwashing is not possible for flat plate membranes and these are relaxed at frequent intervals where permeate is no longer withdrawn and the continued aeration is effective at removing solids build on the membrane (Le-Clech *et al.*, 2006). Again both methods must take into consideration the impact on the process efficiency and additional operational costs.

Chemical cleaning of membranes has been proven to be very effective at removing organic and inorganic build up on membranes. Solutions of either sodium hypochlorite (organic foulant) or citric acid (inorganic foulant) are generally used but cleaning agents and regimes are dependant on the foulant present and are often developed specifically for individual sites (Liao *et al.*, 2004, Le-Clech *et al.*, 2006).

Operating at a reduced flux will certainly reduce or delay the fouling experienced but will reduce the throughput of the system adding to operational costs (Chua *et al.*, 2002).

2.8.2 Chemical mitigation

The premise behind chemical mitigation of fouling is to stop the fouling fraction from coming into contact with the membrane. This is achieved through coagulation or adsorption of the fouling fraction (in this instance believed to be colloidal particles or soluble microbial products as discussed in the fouling section above). Coagulation will enable larger flocs to form that will trap colloidal particles and soluble microbial products, whereas adsorption acts by adsorbing the particles or SMP onto the additive. The most commonly used chemicals for flux enhancement by coagulation or adsorption in MBRs are polymers and metal salts for coagulation and activated carbon for adsorption.

Coagulation, by the addition of cationic polymers or inorganic salts, results in the neutralisation of the zeta potential of the negatively charged particles in the wastewater. This destabilisation of colloidal particles allows the particles to overcome their natural repulsive forces and agglomerate, through macrokinetic flocculation, within the turbulent environment in the aerated MBR (Tchobanoglous *et al.*, 2003). Typical dose response curves for metal salts, for zeta potential and residual turbidity, show a minimum turbidity at, or close to, a neutral zeta potential. This is followed by a subsequent increase in turbidity with an increase in concentration of the metal salt, as the zeta potential becomes increasingly positive and colloidal particles restabilise (Adin and Asano, 1998). This action removes the colloidal element within the biomass matrix, as evidenced by the reduction in residual turbidity, and increases the particle size within the matrix, significantly reducing fouling (Baek and Chang, 2009). The main disadvantage of using metal salts is the increase in sludge production (Yoon *et al.*, 2005). Dosing with a cationic polymer has a similar effect on as the metal salt causing destabilisation of colloids through charge neutralisation

(Koseoglu *et al.*, 2008), however, they have the added advantage of polymer bridging if they are of a very high molecular weight. Polymer bridging is the action of numerous particles being adsorbed to the polymer at varying points along the chain thus providing a bridge between particles (Tchobanoglous *et al.*, 2003). Dosing with cationic polymer does not increase sludge production significantly.

Cationic polymers, inorganic salts, activated carbon and other coagulants have been used in many studies for flux enhancement in MBRs (Table 2-13). The majority have been carried out on a case by case basis with varying degrees of success. In general, although it is hard to compare the studies the cationic polymers seem to perform better than the activated carbon and inorganic salts. Collins *et al.,* (2006) and Yoon *et al.,* (2005) found that a dose of 400 $mg.l^{-1}$ of MPE50 provided close to a 70% increase in flux, whereas a 2000 $mg.l^{-1}$ dose of powdered activated carbon (PAC) only produced a 22% reduction in filtration resistance for Fang *et al.*, (2006). The most comprehensive study carried out was by Koseoglu *et al.*, (2008) which compared 3 cationic polymers (MPL30, MPE50, KD452), 2 metal salts ($FeCl_3$, PACl), a biopolymer (chitosan) and starch under the same experimental conditions. This found that the inorganic salt poly aluminium chloride (PACl) performed the worst, with only a 33% reduction in fouling rate whereas the cationic polymers produced between a 74% (KD452) and a 96% (MPE50) reduction in fouling rate. Although KD452 resulted in a smaller reduction in the fouling rate this was at a dose of 70 $mg.l^{-1}$ compared with 500 $mg.l^{-1}$ for MPE50. Interestingly, the starch and biopolymer produced reductions of 90% at doses of 1500 $mg.l^{-1}$ and 250 $mg.l^{-1}$ respectively.

Table 2-13 – Flux enhancers for MBRs and their effectiveness

Chemical	***Type***	***Flux ($l.m^{-2}.h^{-1}$) (min and max)***	***Enhancement***	***Dose ($mg.l^{-1}$)***	***MLSS ($g.l^{-1}$)***	***Scale***	***Ref***
MPE50	Cationic polymer	25 – 50	65% lower TMP	400	10-11	Pilot/Full	Collins *et al.*, 2006.
MPE50	Cationic polymer	10.2 – 17.5	72% higher flux	400 + 10 daily	8 – 12	Full	Collins *et al.*, 2006.
MPE50	Cationic polymer	17	Increased the critical flux from 17 to 33 $l.m^{-2}.h^{-1}$.	50 - 1000	12 – 30	Pilot	Yoon *et al.*, 2005.
MPE 50	Cationic polymer	Ave 47.25	50% increase in flux (short term) 68% increase in flux (long term)	400	12	Full	Yoon *et al.*, 2005.
MPE50	Cationic polymer	27	96% reduction in fouling rate	500	8-9	Bench	Koseoglu *et al.*, 2008.
MPL30	Cationic polymer	27	80% reduction in fouling rate	600	8-9	Bench	Koseoglu *et al.*, 2008.
KD452	Cationic polymer	27	74% reduction in fouling rate	70	8-9	Bench	Koseoglu *et al.*, 2008.
Alum ($Al_2(SO_4)_3$	Inorganic salt	15	Maintained flux at low TMP (<0.5 bar) for 14 days compared with fouling of the control after ~150 hours	Ratio of Al:P of 1.5	5.2 – 9.2	Lab	Lee *et al.*, 2001.
PACl	Inorganic salt	27	33% reduction in fouling rate.	85	8-9	Bench	Koseoglu *et al.*, 2008.

Chemical	***Type***	***Flux ($l.m^{-2}.h^{-1}$) (min and max)***	***Enhancement***	***Dose ($mg.l^{-1}$)***	***MLSS ($g.l^{-1}$)***	***Scale***	***Ref***
PAC	Organic	Stirred cell	22% reduction in filtration resistance	2000	4.76	Bench	Fang *et al.*, 2006.
PAC	Organic	42	10 $g.l^{-1}$ PAC ~doubled filtration time to set TMP, 40 $g.l^{-1}$ PAC trebled filtration time to set TMP compared to control.	10000 and 40000	NR	Bench	Kim *et al.*, 2005.
Zeolite	Inorganic	15	Maintained flux at low TMP (<0.5 bar) for 14 days compared with fouling of the control after ~150 hours	1000	7.1 – 7.4	Lab	Lee *et al.*, 2001.
Chitosan	Biopolymer	27	90% reduction in fouling rate	250	8-9	Bench	Koseoglu *et al.*, 2008.
Starch	Starch	27	90% reduction in fouling rate	1500	8-9	Bench	Koseoglu *et al.*, 2008.

NR = not reported.

The review of the literature has highlighted the need for water reuse to provide a valuable alternative water source for increasing water demand. Of the biological treatment systems used for water reuse the MBR has been identified as the most efficient and robust. Characterisation studies of domestic wastewater have shown the potential for a large variation in chemical constituents in urban wastewater. Some constituents have been identified as xenobiotic which could have a detrimental effect on the biomass of the biological treatment system in a small scale MBR. MBRs are susceptible to membrane fouling and this has been shown to be due to the production of SMP carbohydrates and the colloidal fraction within the biomass matrix, both of which can be exacerbated with the introduction of unsteady state operation. To date the effects of the common household products or xenobiotic constituents of industrial wastewater have not been investigated on the performance and operation of a MBR.

3 Aim and Objectives

3.1 Aim

To understand the nature of intermittent chemical events that could be present with respect to urban blackwater, to understand the effect these events may have on the performance and robustness of a membrane bioreactor (MBR) and to investigate the impact of chemical control solutions at alleviating these effects.

3.2 Scope

Urban wastewater, or blackwater, can consist of any combination of chemicals that are present in products used in the domestic household and those discharged from industrial activities. These chemicals may have an adverse effect on biological treatment systems, in this case an MBR.

The scope of this project is to investigate and understand, firstly, the risk that is presented by these household or industrial chemicals and, secondly, the likely effects that they may have on an MBR. Monitoring of the effects will focus on increased fouling of the membrane, failure of compliance of the effluent of the system with current reuse guidelines, any increased maintenance needs (e.g. more frequent chemical cleaning) and increases in sludge production.

When an understanding of these risks and effects has been gained then a control strategy can be formulated to limit the impact on the system. This control will be in the form of chemical addition to the system, either as planned addition of chemicals at defined times or in the form of a continuous addition.

3.3 Objectives

- To identify the risks presented by household products or industrial chemicals in wastewater to biological systems.
- To investigate the effects of these products on a biological system with reference to system performance and the potential of foulant release.

- Using one or more of the toxicants identified in the previous stage to further study the effect on an MBR with reference to foulant potential, system performance and maintenance needs.
- To identify which chemicals could be added to the system to alleviate the impacts observed in the previous stage.
- To trial at pilot scale the addition of toxins followed by the addition of the control chemical to ensure the intended effects are reproducible at scale.

4 Materials and Methods

4.1 Choice of toxins

The toxins to be assessed were chosen on the basis of those that were most likely to be present in urban wastewater with either a domestic or industrial origin (Eriksson *et al.*, 2002, Almeida *et al.*, 1999). 23 household products and 9 industrial toxins were chosen (Table 4-1).

The household products were chosen to represent those that would be used on a regular basis in a normal domestic household. These were further categorised by cost and ecological credentials. Two were chosen on the basis of cost, with one being a more expensive leading brand and one being a low cost supermarket own brand and two were chosen from ecological brands, one being a widely available ecologically marketed brand and one being a more niche brand with stricter definitions of its impact on the environment. The household products that were used throughout the project were purchased at one of the main supermarket chains in the UK or from websites in the case of the more niche environmental brands.

The industrial toxins were chosen to represent as wide a spectrum as possible to investigate different actions of toxicity on the biomass or bacteria. These were agreed on in discussion with the industrial sponsor of the project, KeppelSeghers, and represented some toxins that had been indentified as being of particular interest to the sponsors from case studies or those that gave a broad representation of toxins found in industrial wastewaters (KeppelSeghers, 2006). Some of the individual industrial toxins form part of the ingredients list of the household products.

Table 4-1 – Toxins tested in scoping work split into origin and category.

Origin	*Category*	*Substance*	*Supplier*
Domestic	All purpose cleaner	Nest Anti-bacterial with essential oils (lavender and bergamot)	NEST (www.eco-nest.co.uk)
		Ecover natural lemon	Morrisons supermarket
		Morrisons Orange	Morrisons supermarket
		Mr Muscle Orange	Morrisons supermarket
	Shampoo	Henna Plus Natural	Spirit of nature (www.spiritofnature.co.uk)
		Naked Volumising	Boots the chemist
		Morrisons bettabuy	Morrisons supermarket.
		Pantene Pro-V (Sleek and Smooth)	Morrisons supermarket
	Shower Gel	Lavera Orange and Seabuckthorn	Spirit of nature (www.spiritofnature.co.uk)
		Ecover Aloe and Lavender	Morrisons supermarket
		Original Source Mint and Tea Tree	Morrisons supermarket
		Morrisons peach shower creme	Morrisons supermarket
	Bleach	Nest	NEST (www.eco-nest.co.uk)
		Morrisons Thick	Morrisons supermarket
		Domestos	Morrisons supermarket
	Washing powder	Nest	NEST (www.eco-nest.co.uk)
		Ecover	Morrisons supermarket
		Morrisons cyclon for colours	Morrisons supermarket
		Persil Aloe Vera tablets	Morrisons supermarket
	Washing up liquid	Nest (with essential oil lavender)	NEST (www.eco-nest.co.uk)
		Ecover Chamomile	Morrisons supermarket
		Morrisons concentrated lemon	Morrisons supermarket
		Persil Fresh	Morrisons supermarket
Industrial	Surfactants	Anionic (sodium dodecyl sulphate)	Fisher Scientific, UK.
		Cationic (cetyl trimethyl ammonium bromate)	Fisher Scientific, UK.
	Metals	Zinc (as zinc sulphate)	Fisher Scientific, UK.
		Copper (as copper sulphate)	Fisher Scientific, UK.
	Oxidants	Sodium hypochlorite	Fisher Scientific, UK.
		Hydrogen peroxide	Fisher Scientific, UK.
	Salts	Sodium chloride	Fisher Scientific, UK.
		Magnesium (as magnesium sulphate)	Fisher Scientific, UK.
	Organic	Phenol	Fisher Scientific, UK.

4.2 Why use the whole household product?

Most studies have focussed on testing single toxins or well known chemicals. However, in the case of domestic wastewater it is the whole household product that is discharged to sewer and consequently it is important to test the whole product as there may be antagonistic or synergistic effects in the combination of any ingredient with another. The individual toxicities of the ingredients do not

necessarily have an additive effect, in fact some combinations of toxins will be more toxic than the additive effect of the individual toxins whereas some will be less toxic (Kahru *et al.*, 1996). Some of the ingredients that are included in household products were tested as part of the industrial portion of the toxins tested and illustrate the point that individual toxicities do not necessarily have a bearing on the overall toxicity. For example, sodium dodecyl sulphate (also known as the much used sodium lauryl sulphate) showed low toxicity to the biomass using respirometry, yet most of the shower gels and shampoos, which include this as a main ingredient, showed a high toxicity. Although all products give an ingredients list this is not exhaustive and some only contain an indication of the contents of the bottle e.g the ingredients listed for Morrisons thick bleach are >5% sodium hypochlorite, >5% anionic surfactants and >5% limescale deterrent. All ingredients are given in descending order of magnitude, however, the absolute quantities are not known. As the Microtox® and respirometry demonstrated all substances have an EC_{50}, and this can vary dramatically from substance to substance, illustrating that although an ingredients is listed low on the list this does not reduce its potential toxicity. The surfactants used in household products are not of analytical grade as this would be prohibitively expensive but can vary in chain length which can have an effect on their toxicity (Madsen *et al.*, 2000). Household products are a complex mixture of ingredients that are formulated to produce an end result which depends on each individual ingredient in the mix, moreover, these ingredients will interact with each other to produce this desired effect which would not be gained if each ingredient was used in isolation. Combinations of compounds can increase or decrease the toxic effect of each in isolation, for example, the presence of phenol with copper decreased the inhibition rate of nitrifying bacteria (Kim *et al.*, 2006).

Whole effluent toxicity testing is a potential tool for aquatic toxicity and has been proposed for analysing industrial effluent being discharged to wastewater treatment works (Dalzell *et al.*, 2002, Koh and Ellis, 2005). One study conducted to assess the inhibitory effect of municipal wastewaters on

nitrification, identified that 60% of wastewater treatment plants contained substances that were inhibitory but no one element could be isolated that could cause this (Jönsson *et al.*, 2000). Without testing the complete influent wastewater these effects would not have been identified.

4.3 Toxicity assessment

Acute toxicity is typically measured in two different ways. The effective concentration, which is the concentration where a specific effect is observed in a specific time frame (e.g. EC_{50} at 15 minutes) and the lethal concentration, where a specific percentage of the population is killed in a specific time frame (e.g. LC_{50}) (Tchobanoglous *et al.*, 2003). It was decided to use the EC_{50} measurement in this assessment as even if the observed behaviour was inhibited rather than the bacteria actually being killed it would mean a reduction in the system efficiency and potential discharge consents violations (Gutiérrez *et al.*, 2002).

Microtox and respirometry were chosen for the toxicity assessment. Microtox uses a standard cultured luminescent marine bacteria that allows multidisciplinary comparison of results, even if at first inspection it would appear to bear little or no relation to a biological wastewater treatment system. Respirometry on the other hand uses a microbial community taken from the biological treatment process under assessment but produces results that are often difficult to compare and are dependant on many outside variables (feed matrix of the treatment plant from where the biomass is taken, pH of the biomass, prior acclimatisation to the toxin under test, f:m ratio, colloidal matter present). If the ranking of the individual toxicities are the same for the two methods then the Microtox will provide a quick, repeatable and reproducible method for attaining an indication of a wastewater's toxicity.

4.4 Microtox

4.4.1 Materials

The Microtox experiments were carried out using a Microtox Model 500 Analyser (*SDI Europe, Hampshire, UK.*) The serial dilutions necessary were made using a diluent, which is 2% NaCl in ultra pure water. Osmotic adjusting solution, 22% NaCl, was added as directed and the bacteria used was *Vibrio fischeri* (formerly known as *Photobacterium phosphoreum,* NRRL number B-11177) a marine species. The household products used were diluted in deionised water.

Figure 4-1 – Microtox 500 Analyser.

The light readings taken from the bioluminescing bacteria were read using the Microtox analyser and transferred to a PC running Microtox Omni software Version 1.18.

4.4.2 Methods

Each of the Microtox tests were carried out following the 89% standard procedure detailed in the Microtox manual, which gave EC_{50} values at 5 and 15 minutes for nine serial dilutions. Tests were carried out on a trial and error basis until a concentration was found where the EC_{50} level was at

approximately half way through the dilutions. The test was then repeated three times to ensure accuracy.

4.5 Respirometry

4.5.1 Materials

The respirometry was carried out using a manometric respirometer (*CES, Cornwall*, *UK*). The biomass used was from a pilot scale activated sludge plant that had been running for over one year on the Cranfield University campus, fed with sewage from the primary basin (settled sewage). The biomass used was washed three times with deionised water that had 0.0125% m/V ferric sulphate, 2.75% w/v calcium chloride, 2.5% w/v magnesium sulphate and phosphorus buffer solution (dilution water used in biological oxygen demand analysis) added, to ensure that the subsequent substrate added was the only food source available to the biomass. Copper sulphate provided the electrolytic solution for the oxygen generation.

Figure 4-2 – Manometric respirometer

4.5.2 Methods

The solutions to be tested were contained in 500ml glass bottles. Each bottle contained 150ml of substrate (settled sewage), 100ml biomass and 50ml of the potential toxin. 11 channels were used with one as a control channel with 50ml

of deionised water instead of any toxin and five concentrations of toxin in the remaining 10 channels. The oxygen was measured for the first 20 hours, the maximum time observed before endogenous respiration. The graph of oxygen uptake was drawn and the gradient obtained to give the oxygen uptake rate. This was normalised to the MLVSS of the biomass to give the specific oxygen uptake rate (SOUR). The SOUR of each concentration was then compared to the control SOUR and the EC_{50} determined when the SOUR of a concentration was 50% of the control SOUR.

4.6 Porous Pots

4.6.1 Materials

The porous pots are purpose built 3.8 litre capacity pots for performing biodegradeability tests. Each pot is made of a PVC outer shell with a membrane inner shell, with a pore size of 60 – 90 µm, that slots into the pot to capture the biomass and stop it escaping from the system. Effluent overflows through a spout at the front of the pot to a communal drain to waste (Figure 4-3 and Figure 4-4). Each pot was aerated using a stone diffuser and the air flow regulated by an adjustable valve. The air flow was set to 0.9 $l.s^{-1}$, after five months of monitoring the dissolved oxygen (D.O.), as being the lowest flow to ensure the D.O. was >2$mg.l^{-1}$. Five spare liners were kept soaked in 0.1 % sodium hypochlorite. When a liner became blocked and the contents of the pot was close to overflowing, the biomass was removed and a new liner that had been thoroughly rinsed with tap water, was inserted into the pot and the biomass replaced. The dirty liner was then washed with tap water to remove as much of the biomass as possible and soaked in sodium hypochlorite solution, as described above, until needed.

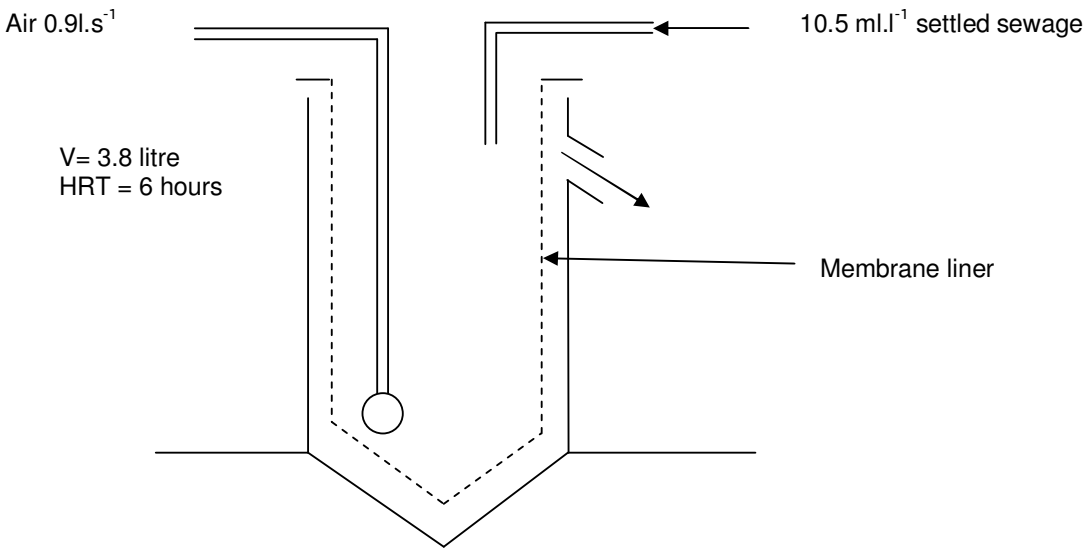


Figure 4-3 – Diagrammatic representation of a porous pot.

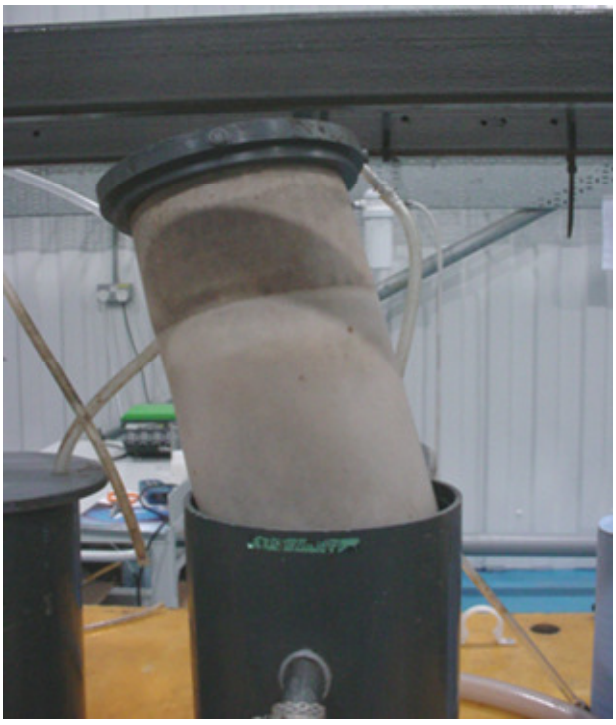


Figure 4-4 – Photograph of a porous pot showing the membrane liner.

Each porous pot was fed using a multi channel peristaltic pump (*Watson Marlow,* UK) with settled sewage from a small reservoir of approximately 50 litres capacity, positioned close to the porous pots. The reservoir maintained a

constantly fresh supply of settled sewage by draining from the bottom of the tank to drain, and being periodically cleaned thoroughly. The supply lines to feed the pots were also positioned to draw sewage from the middle of the reservoir to avoid scum floating on the top of the reservoir and any solids that had settled to the bottom.

4.6.1.1 Operating parameters

The porous pots were operated with the same parameters throughout the baseline monitoring period and the trial periods (Table 4-2). These parameters were chosen to maximise the MLVSS concentration in order for it to be as close as possible to the concentration in the pilot MBR. The pots were run on a no wastage policy apart from sampling 100 ml from the biomass three times per week. This resulted in an approximately 90 day solids retention time (SRT).

Table 4-2 – Operating parameters for the porous pots.

Parameter	Porous Pots
Aeration $l.s^{-1}$	0.9
HRT(hours)	8
SRT (days)	~90

4.6.2 Methods

4.6.2.1 Baseline Analysis for Porous Pots

The following analysis was carried out for the baseline analysis of the pots over an eighteen month period to build up a steady state picture of the variation of the parameters (Table 4-3). All samples were analysed within 24 hours of collection.

Table 4-3 – Baseline analysis for porous pots.

Parameter	Influent	Effluent	Biomass
COD	3 x weekly	3 x weekly	
BOD	1 x weekly	1 x weekly	
Ammonia	1 x weekly	1 x weekly	
Turbidity	3 x weekly	3 x weekly	
CST			1 x weekly
pH			3 x weekly
Conductivity			3 x weekly
MLSS/MLVSS			3 x weekly
PSD			1 x weekly

4.6.2.2 Chemical Oxygen Demand (COD)

The chemical oxygen demand was determined using Merck cell tests at the lowest concentration range to ensure accuracy. The standard procedure was followed as set out in the manufacturer's instructions and the results were read using a Spectroquant Nova 60 spectrophotometer.

4.6.2.3 5 Day Biological Oxygen Demand (BOD)

The 5 day biological oxygen demand was determined using the standard APHA methods.

4.6.2.4 Ammonia

The ammonia concentration in the effluent and influent was measured using Merck cell tests at the lowest concentration range possible to ensure accuracy. The standard procedure set out in the manufacturer's instructions was followed and the results were read using a Spectroquant Nova 60 spectrophotometer.

4.6.2.5 Turbidity

Influent and effluent turbidity were measured using a Hach 2100N turbidimeter (*Camlab, Cambridge, UK*). A 30 ml sample was decanted into the specific glass vials for the turbidimeter. The sample was then sonicated for 2-3 minutes and the turbidity read from the turbidimeter. Each sample was read three times to provide an average reading. The turbidimeter was calibrated each time it was used against a standard set of solutions.

4.6.2.6 Capillary Suction Time (CST)

The capillary suction time was measured using apparatus supplied by Triton Electronics, UK (*Triton Electronics, Essex, UK*). This consisted of a control box attached to a Perspex frame, containing two concentric metallic rings each with an electronic contact, that was laid over a piece of thick filter paper (Figure 4-5). A small stainless steel reservoir with a volume of 5.5ml was placed inside this frame and in contact with the filter paper. The sludge was then injected into the reservoir and the time for the water in the sludge to move by capillary action

from the first electronic contact to the second was timed in seconds. Capillary suction time gives an indication of the dewaterability of the sludge.

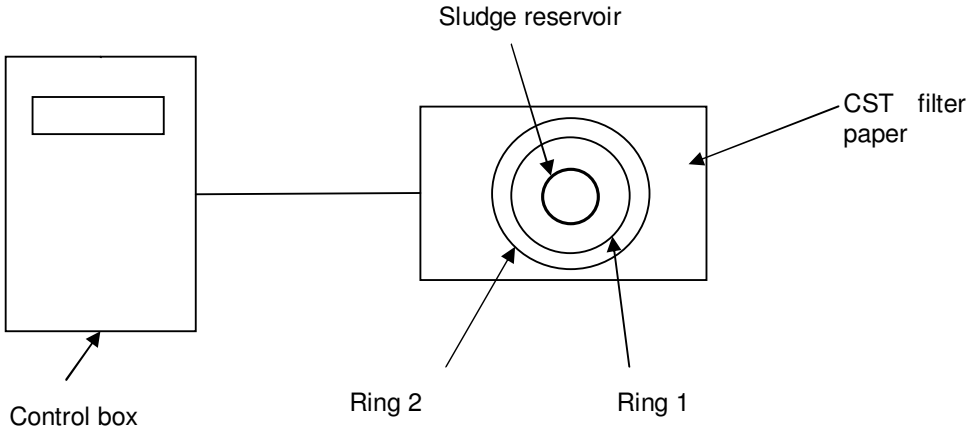


Figure 4-5 – Capillary suction time apparatus.

4.6.2.7 pH and Conductivity

The pH was measured using a Jenway 3540 combined pH and conductivity meter (*Camlab, Cambridge, UK)*. This was calibrated with standard solutions each time it was used.

4.6.2.8 Mixed Liquor Suspended Solids/Mixed Liquor Volatile Suspended Solids

The mixed liquor suspended solids and mixed liquor volatile suspended solids were measured using APHA standard methods 2540D and 2540E.

4.6.2.9 Particle Size Distribution (PSD)

The particle size distribution was measured using a Malvern Mastersizer 2000 (*Malvern Instruments, Malvern, UK).* This utilises light dispersion from the particles in suspension and reports the results as a %volume of the total against the particle size. The measurements were taken over 5 20 second cycles and an average used. The samples measured were dispersed in deionised water.

4.6.2.10 Toxin dosing

4.6.2.11 Sampling regime

The pots were sampled over a 24 hour period, as it was projected that most effects would be seen within 1 to 2 HRTs. More sampling was carried out closer to the dosing time to ensure that any immediate effects were monitored (Table 4-4). The parameters monitored were split into groups: effluent quality analysis (COD, ammonia and turbidity), biomass analysis (pH, conductivity, MLSS, MLVSS, CST and PSD) and foulant potential analysis (SMP tubidity, SMP proteins and SMP carbohydrates).

Table 4-4 – Sampling regime for two control and two test pots during dosing trials.

Parameter	*Before dosing*	*t=0 mins (0 HRT)*	*t = 30 mins (0.08HRT)*	*t = 1 hr (0.2 HRT)*	*t = 6 hrs (1 HRT)*	*t = 12 hrs (2 HRT)*	*t = 24 hrs (4 HRT)*
COD	1,2	2	2	2	2	2	2
Ammonia	1,2	2	2	2	2	2	2
Turbidity	1,2	2	2	2	2	2	2
pH	3	3	3	3	3	3	3
Conductivity	3	3	3	3	3	3	3
MLSS/MLVSS	3	3	3	3	3	3	3
CST	3	3		3	3		3
PSD	3	3		3	3		3
SMP turbidity	3	3	3	3	3	3	3
SMP proteins	3	3	3	3			3
SMP carbs	3	3	3	3			3

1 = influent, 2 = effluent, 3 = biomass.

4.6.2.12 Analysis

COD, ammonia, turbidity, pH, conductivity, MLSS/MLVSS, CST and particle size distribution were carried out as described in 4.6.2.2 to 4.6.2.9.

4.6.2.13 Soluble Microbial Product (SMP) Turbidity

SMP turbidity was determined by centrifuging 250ml of sample at 10,000g for 20 minutes (Sorvall Legend RT+). The supernatant was then carefully decanted into the glass vessels used for reading turbidity and the turbidity determined as for turbidity (Section 4.6.2.5).

4.6.2.14 SMP proteins

Proteins were determined following the Lowry *et al.,* method (1951). Three reagents were used: 143mM NaOH mixed with 270mM Na_2CO_3, 57 mM $CuSO_4$ and 124mM Na_2-tartat. The three reagents were mixed at a ratio of 100:1:1 respectively. The indicator was Folin Cioucalteau reagent diluted 1:2 with deionised water. The absorption was measured at 750nm against a blank using a Jenway Aquanova spectrophotometer and a calibration curve was produced using the protein standard bovine serum albumin (BSA) (Figure 4-6).

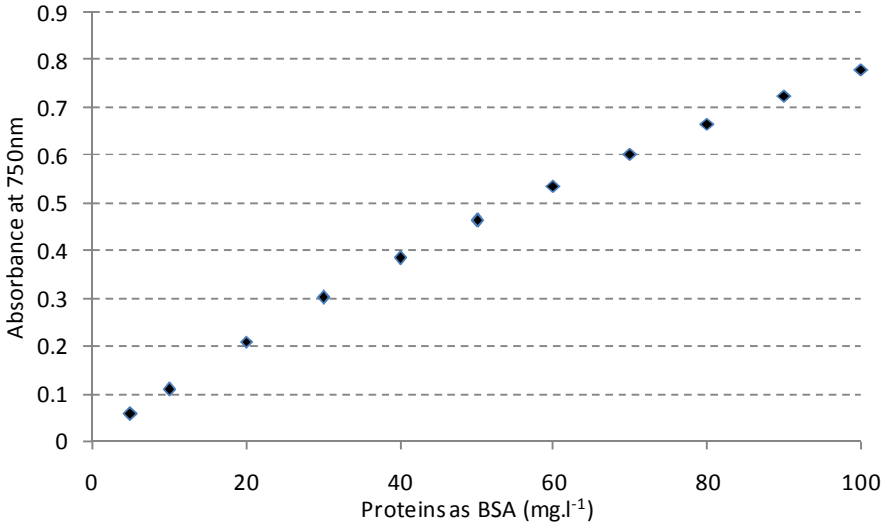


Figure 4-6 – Calibration curve for SMP proteins.

4.6.2.15 SMP carbohydrates

SMP carbohydrates were determined using a method developed by Dubois *et al.* (1956). The sample was mixed with phenol at 5% w/v and then sulphuric acid. The absorbance was measured at 490nm against a blank using a Jenway Aquanova spectrophotometer and a calibration curve produced using monohydrate glucose (Figure 4-7).

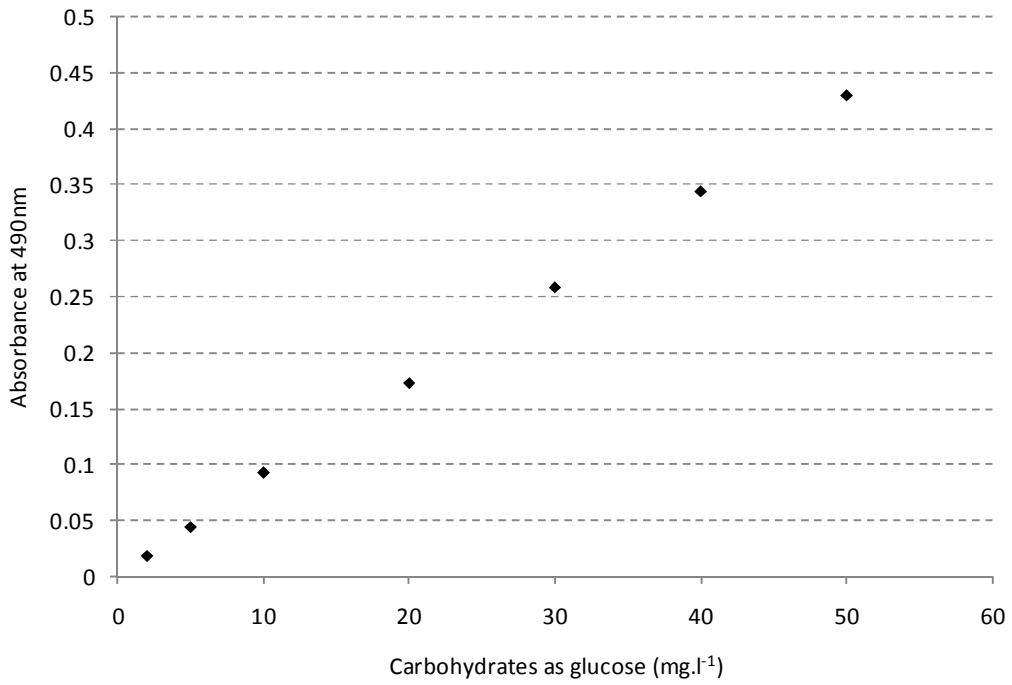


Figure 4-7 – Calibration curve for SMP carbohydrates.

4.7 Membrane Bioreactor

4.7.1 Materials

4.7.1.1 Pilot Rig Mark One

A ready built pilot rig was supplied by KeppelSeghers in a sea container. This was supplied in the "plug and play" concept; in theory all that was required was to attach electricity and sewage and the MBR would be operational.

The plant consisted of a 12m^3 aeration tank, a denitrification tank of 3m^3, a membrane tank of 1m^3 and influent and effluent tanks of 1m^3 (Figure 4-8, Figure 4-9 and Figure 4-10). Feed was pumped from the feed tank to the denitrification tank, then pumped to the aeration tank, overflowed by gravity to the membrane tank and pumped back from the bottom of the membrane tank to the aeration tank.

Figure 4-8 – The containerised pilot plant showing the tops of the membrane and aeration tanks.

Figure 4-9 – The containerised pilot plant delivered from Belgium.

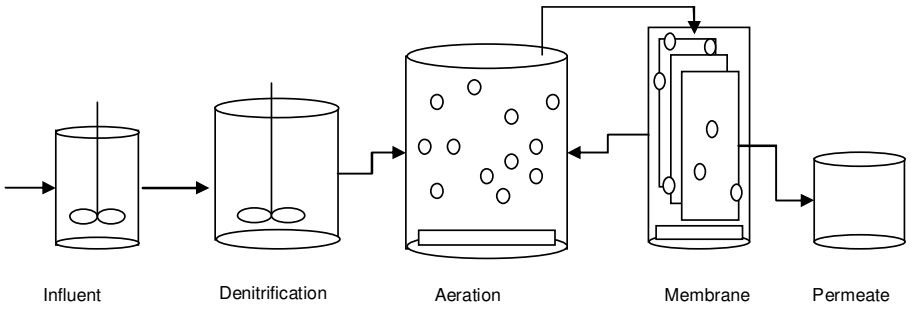


Figure 4-10 – flow diagram for the containerised plant.

Two permeate pumps acted on duty and standby and were automatically swapped every week. All levels and pumps were controlled via a central controller which had been programmed by the technicians at KeppelSeghers. The membranes supplied were flat sheet, nominal pore size 0.1 µm with a total area of 25m^2. The membranes were continuously aerated and permeate withdrawn for 9 minutes of a 10 minute cycle. The remaining 1 minute was for relaxation of the membranes to aid fouling attenuation. A steady state operation was not reached for the pilot plant although it was attempted to run the plant with an initial flux of 14 $l.m^{-2}.h^{-1}$ giving an HRT of 46 hours.

Due to various technical problems with operation the containerised pilot plant was decommissioned in November 2006 and returned to Belgium.

4.7.1.2 Pilot Rig Mark Two

The second pilot rig was assembled within the pilot hall facilities on campus. It consisted of a large aeration tank and a smaller membrane tank giving a total volume of 1950 litres (Figure 4-11).

Feed was fed by gravity from the ring main in the pilot hall via a ball and float valve to ensure that the tank would not overfill. The biomass was recirculated from the aeration tank to the membrane tank with a variable speed positive displacement pump (*Seepex Ltd, Belgium*) at 2 $m^3.h^{-1}$ and then overflowed back to the aeration tank. Permeate was withdrawn from the membrane using

stainless steel piping, via a variable speed positive displacement pump (*Seepex Ltd, Belgium*). Permeate flow was measured using a digital flow meter. Automatic pneumatic valves, programmed via the controller, reversed the flow for 1 minute in every 10 to backwash the membrane. The permeate tank provided a reservoir for the membrane backwashing. The transmembrane pressure was measured using a pressure transducer and recorded on a digital display (*Wika Electronics, Germany*). Air was applied to the bottom of the membrane module via a customised aeration fitting, in the membrane tank and this was adjustable via a mechanical flow meter. Air for the aeration tank was supplied from the same line to a disc aerator on the bottom of the aeration tank, this flow was not controllable.

The MBR was seeded with return activated sludge (~6 $g.l^{-1}$ MLSS) from the local municipal sewage works. In order to acclimatise the biomass to Cranfield sewage the MBR was run at a relatively low flux of 10 $l.m^2.h^{-1}$ for two months, after which the flow was steadily increased until an instantaneous flux of 21 $l.m.h^{-1}$ was reached. This gave the shortest HRT of 8.8 hours, that the rig was run at.

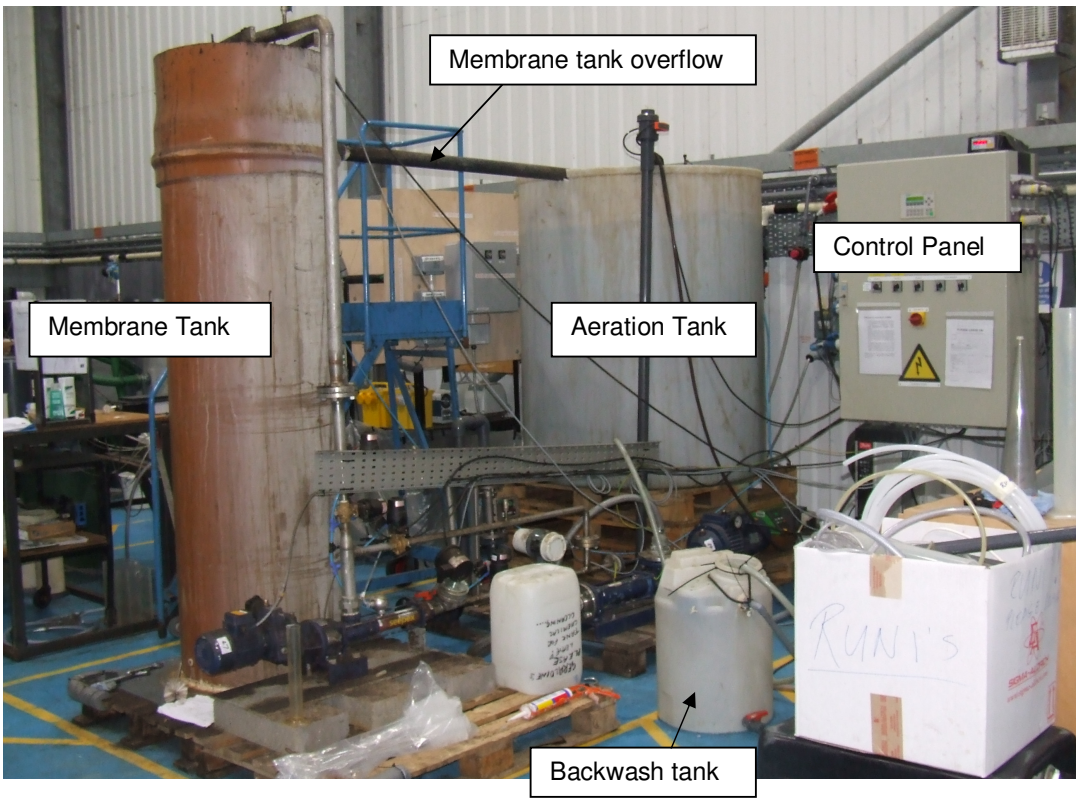


Figure 4-11 – Replacement pilot plant.

4.7.1.3 Operating parameters

The operating parameters for the MBR pilot plant were more variable than the porous pots. To ensure the acclimatisation of the biomass, the HRT was reduced steadily from 21 hours to 8 hours over the first two months of operation. For the monitoring and trial period the HRT was maintained at 11 hours. The membranes for the small pilot plant were hollow fibre, the membrane material was PVDF, with an area of 12.5m^2 and the nominal pore size was 0.1 µm.

Table 4-5 – Operating parameters for the pilot MBR.

Parameter	MBR	Porous Pots
Aeration (l.s^{-1})	1.5 – 1.6	0.9
HRT (hours)	8 – 11	6
SRT (days)	∞	~90
Membrane	PVDF	N/A
Instantaneous Flux	17.6 - 21	N/A

4.7.2 Methods

4.7.2.1 Membrane Cleaning

Both in situ and off line cleans were performed on the membrane when needed, normally if the TMP was greater than 200 - 250 mbar. An in situ clean consisted of pumping 0.5% NaOCl into the membrane over a 2 hour period and leaving it to soak for several hours. The permeate was run to drain and the membrane operated for three to four cycles with no backwash until the sodium hypochlorite solution had been removed from the system. An offline clean was performed when the in situ clean no longer proved effective. The membrane was removed from the membrane tank, as much biomass as possible removed using a hose, and placed in a temporary tank with 0.5 % NaOCl solution. Aeration was applied at 1.5 $l.s^{-1}$ to maximise the cleaning process and the membrane left for 24 hours. Before reinstating the membrane, it was thoroughly rinsed in clean water and, once reinstalled, run for three to four cycles with no backwash to remove any residual hypochlorite.

4.7.2.2 Baseline monitoring

Baseline monitoring was carried out in the MBR for a period of fourteen months to establish the steady state range of the parameters analysed. Analysis was carried out three times per week and all samples were analysed within 24 hours of collection (Table 4-6).

Table 4-6 – Baseline monitoring for MBR.

Parameter	*Influent*	*Effluent*	*Biomass*
COD	3 x weekly	3 x weekly	
Ammonia	3 x weekly	3 x weekly	
Turbidity	3 x weekly	3 x weekly	
CST			1 x weekly
pH			3 x weekly
Conductivity			3 x weekly
MLSS/MLVSS			3 x weekly

4.7.2.3 Toxin dosing

4.7.2.3.1 Sampling regime

The sampling period was kept to 4 HRTs with the final sample being taken at 48 hours, slightly over 4 HRTs (Table 4-7). Sampling was still concentrated to the initial period after dosing to ensure that any immediate effects were detected but a sample was also taken at 0.5 HRTs to give a more even spread. The analysis was split into groups: effluent quality analysis (COD, ammonia and turbidity), biomass analysis (pH, conductivity, MLSS, MLVSS, CST and PSD) and foulant potential analysis (SMP turbidity, SMP proteins and SMP carbohydrates).

Table 4-7 – Sampling regime for the MBR during dosing trials.

Parameter	***t***							
	Before dosing	***0 mins (0 HRT)***	***30 mins (0.05HRT)***	***1 hr (0.1 HRT)***	***5.5hrs (0.5HRT)***	***11 hrs (1 HRT)***	***22 hrs (2 HRT)***	***48 hrs (>4 HRT)***
COD	1,2	1,2	2	2	1,2	1,2	1,2	1,2
Ammonia	1,2	1,2	2	2	1,2	1,2	1,2	1,2
Turbidity	1,2	1,2	2	2	1,2	1,2	1,2	1,2
pH	3	3	3	3		3	3	3
Conductivity	3	3	3	3		3	3	3
MLSS/ MLVSS	3	3	3	3		3	3	3
CST	3	3		3		3		3
PSD	3	3	3	3	3	3	3	3
SMP turbidity	3	3	3	3		3	3	3
SMP proteins	3	3	3	3				3
SMP carbs	3	3	3	3				3

1 = influent, 2 = effluent, 3 = biomass.

4.7.2.4 Analysis

COD, ammonia, turbidity, pH, conductivity, MLSS/MLVSS, CST and particle size distribution were carried out as described in 4.6.2.2 to 4.6.2.9 above.

SMP turbidity, SMP proteins and SMP carbohydrates were determined using the methods described in Sections 4.6.2.13 to 4.6.2.15.

4.8 Jar testing

4.8.1 Materials

The jar testing was carried out using a Phipps and Bird programmable jar tester (*Camlab, Cambridge, UK*) with six stirred cells of 1 litre each (Figure 4-12). The biomass used in the jar testing was taken from a 2 m^3 MBR pilot plant using hollow fibre membranes with an HRT of 11 hours and an SRT of 20 days, a different, but similar, MBR to that used for the MBR dosing trials described above (Section 4.7.2.3). Repeat tests, comparing the two MBR biomasses, were carried out as described in the methods section, for high molecular weight polyDADMAC, MPE50 and Ferripol XL, to ensure that the biomass was not significantly different (see Section 4.8.2.1).

4.8.1.1 Toxins

The four toxins that had been tested at the MBR pilot stage, bleach, washing powder, sodium dodecyl sulphate and zinc sulphate, were carried forward to this part of the research to observe in more detail the release of potential foulants and to investigate the interaction with the ancillary chemicals tested.

Figure 4-12 – Jar tester used for the ancillary chemical investigation.

4.8.1.2 Coagulants

The ancillary chemicals that were chosen were a polymer polydialyldimethylammoniumchloride (polyDADMAC) at varying molecular weights (Table 4-8), a metal salt (ferric sulphate), a commercially available membrane bioreactor performance enhancer (MPE50), manufactured by Nalco, and powdered activated carbon (Table 4-9). The polyDADMAC, MPE 50 and ferric sulphate were in the form of aqueous solution, whereas the activated carbon was in powdered form.

Table 4-8 – range of molecular weights for polyDADMAC.

polyDADMAC	***Molecular weight range (Daltons)***
very low MW	<100,000
low MW	100,000 - 200,000
medium MW	200,000 – 350,000
high MW	400,000 - 500,000

Table 4-9 – Coagulants used in jar testing.

Category	***Ancillary chemical***	***Form***	***Supplier***
Polymer	polyDADMAC (very low MW)	aqueous solution	Sigma Aldrich
	polyDADMAC (low MW)	aqueous solution	Sigma Aldrich
	polyDADMAC (medium MW)	aqueous solution	Sigma Aldrich
	polyDADMAC (high MW)	aqueous solution	Sigma Aldrich
	MPE50[1]	aqueous solution	Nalco
Metal salt	ferric sulphate (Ferripol XL)	aqueous solution	EA West
Carbon	Powdered activated carbon (Plusorb 207AP)	powder	Chemviron

[1]MPE50 (Membrane Performance Enhancer 50) is a commercially available product formulated specifically to enhance flux in MBRs.

4.8.2 Methods

4.8.2.1 Comparison of biomass from MBRs.

The MBR that was used for the toxin dosing trials was decommissioned during the jar tests therefore biomass from a similar MBR that was running for the duration of the tests was used. To ensure that the new biomass was comparable to the biomass from the original MBR some concurrent tests were carried out (Figure 4-13).

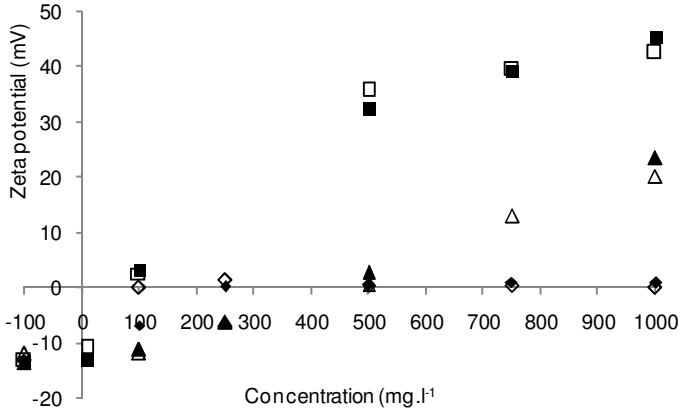


Figure 4-13 – Comparison of MBR biomass for MBR 1 (solid shapes) and MBR 2 (outlined shapes) for high molecular weight polyDADMAC (■,□) MPE50 (▲,Δ) and Ferripol XL (♦,◊)

4.8.2.2 Toxin only testing

1 jar was used for the toxin only testing and the samples taken sequentially in time. 800 ml of biomass was added to the jar and stirred for 1 minute. A 50ml sample of the biomass was taken at this stage as the baseline. The toxin was added and samples taken at 10, 20, 30, 40, 50 and 60 minutes after dosing, as the maximum perturbation of parameters monitored for the porous pots and the MBR trials had occurred in the first hour after dosing. Each sample was analysed for CST, SMP turbidity, SMP proteins and SMP carbohydrates as described previously.

4.8.2.3 Toxin and ancillary chemical

6 jars were used in each run of the jar test; 1 as a control with only the toxin present and the other five at varying concentration of ancillary chemical. Each beaker or jar contained 1 litre of biomass. The biomass was stirred for 1 minute after which the toxin was added and the mixture stirred for another minute. The ancillary chemical was added and the resulting mixture stirred for 20 minutes, as this was when the maximum SMP turbidity had been observed in the toxin only jar tests.

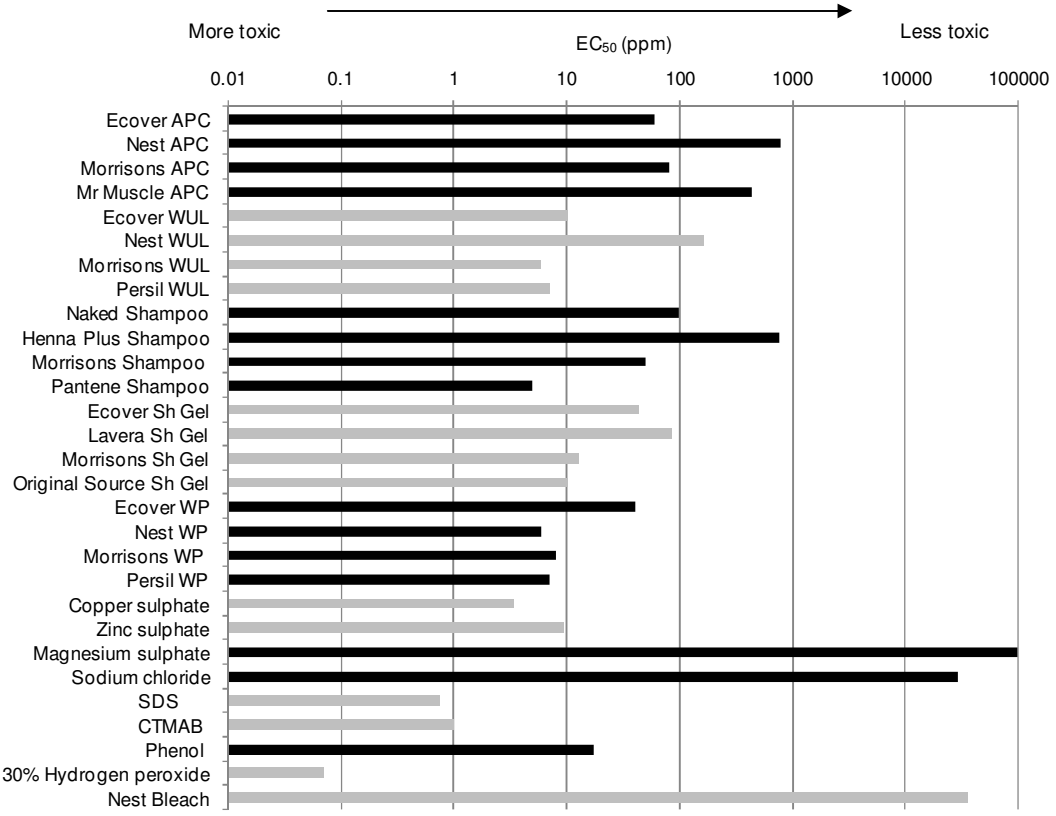
250 ml of the biomass was centrifuged at 10,000g for 20 minutes and the resultant supernatant was analysed for SMP turbidity (as described previously)

and zeta potential. The zeta potential was obtained by using a Malvern Zetasizer 2000 (*Malvern Instruments, Malvern, UK*). Three repeat readings were taken and an average used.

5 Microtox® and Respirometry.

5.1 Microtox®

EC_{50} values were obtained for a total of 29 toxins tested (Figure 5-1). The three hypochlorite based products are excluded (Morrisons bleach, Domestos bleach and sodium hypochlorite) as dechlorination of samples is required as a preparation stage in the Microtox® method. EC_{50} values are given in ppm for ease of comparison; those in liquid form (all purpose cleaners, washing up liquid, shampoo, shower gel, hydrogen peroxide and bleach) were used in $\mu l.l^{-1}$ concentrations and those in solid form (washing powder, metals, salts surfactants and phenol) in $mg.l^{-1}$ concentrations. The equivalence in ppm is approximate for the washing up liquid, shampoo, shower gel and bleach as they are likely to have specific weights of greater than 1.



SDS = sodium dodecyl sulphate CTMAB = cetyltrimethyammoniumbromate.
APC = all purpose cleaner, WUL = washing up liquid, WP = washing powder

Figure 5-1 – Microtox® EC_{50} values for all toxins tested.

The EC_{50} values ranged from the most toxic, 0.07±0.03 $\mu l.l^{-1}$ for 30% H_2O_2, to the least toxic, 98000±16000 $mg.l^{-1}$ for magnesium sulphate, both from the industrial compounds. Both sodium chloride and magnesium sulphate were very much less toxic than the other industrial compounds with EC_{50} values of >10,000 ppm whereas the rest of the group had EC_{50} values of <15 ppm. The range of EC_{50} values for the household products was 5±1 $\mu l.l^{-1}$ for Pantene Pro V shampoo (the most toxic) to 35440±6720 $\mu l.l^{-1}$ for the Nest bleach. Although initially surprising that a bleach would be least toxic of the household products, on inspection of the ingredients the Nest bleach was 6% hydrogen peroxide in water, a very dilute oxidant. In general, shower gels, washing powders and washing up liquids were more toxic than the other household products, while the all purpose cleaners and shampoos had two that were more toxic and two that were less toxic.

In four out of the five household product categories (all purpose cleaner, washing up liquid, shampoo and shower gel) the niche environmental product was the least toxic. This is most evident in the washing up liquid category, the environmental product is much less toxic than the other products in the category (Nest EC_{50} = 163±57 $\mu l.l^{-1}$ compared with Ecover = 10±1 $\mu l.l^{-1}$, Morrisons Ultra = 6±1 $\mu l.l^{-1}$ and Persil = 7±2 $\mu l.l^{-1}$). A comparison of the ingredients of all the products (Appendix A) reveals that the three more toxic products are made up of many more ingredients than the Nest product and all have surfactants as their main ingredients after water, whereas the Nest is based on vegetable soap which clearly has a less toxic effect on the bioluminescent bacteria. In fact, the Morrisons Ultra washing up liquid has either surfactants or biocides as its top six ingredients. As a marine bacteria, *Vibrio fischeri,* is sensitive to changes in osmotic pressure, which disrupts cell metabolism (Fernández-Alba *et al*., 2002), indeed one of the preparation steps for Microtox® is to add an osmotic adjusting solution (Microtox® manual, 2000) and the surfactants present in the products interfere with this mechanism. The two surfactants tested in isolation were the next most toxic industrial compounds after hydrogen peroxide (Figure 5-1).

In contrast to this, in the washing powder category, the Nest product (niche environmental product) was not significantly less toxic than the Morrisons or Persil washing powders but all were more toxic than the Ecover washing powder, when analysed using ANOVA analysis with a 95 % confidence interval, resulting in an F-ratio of 14.7, and a p value 0.001, indicating that there was a significant difference between the EC_{50} values. Inspection of the ingredients for these four products does not, however, provide a clear picture of why there is such a difference between them. All four products have sodium carbonate as an ingredient but in varying proportions (Nest second ingredient, Ecover first or main ingredient, Persil as the sixth ingredient and Morrisons Cyclon it appears seventh). Sodium carbonate is strongly alkaline and Microtox® is sensitive to changes in pH operating at an optimum of pH 6 (Fulladosa *et al.*, 2005). The Nest product has only two ingredients of sodium carbonate (soda crystals) and vegetable flakes (assumed to be vegetable soap), which could account for the increased toxicity. Sodium carbonate also appears as the top product for the Ecover washing powder but the alkalinity is clearly counteracted by other ingredients to give a lower toxicity. The other two washing powders, Persil and Morrisons Cyclon, have many similarities in terms of their ingredients, however, only sodium carbonate in common with the Nest washing powder. Morrisons Cyclon and Persil additionally contain bleaching agents, perfumes, complexing agents and surfactants but there is no prominent group that can explain the toxicity. Synergistic or antagonistic toxicities have been demonstrated in simple mixtures of toxicants (Fernández-Alba *et al.*, 2002, Farré *et al.*, 2008) and it is likely that the complex set of ingredients will have a mixed effect on the bacteria with no dominant effect being evident.

Ranking of the household products by toxicity revealed no clear overall pattern in relation to the consumer groupings. Two out of the five categories (shampoo and shower gel) had toxicity rankings of leading brand>own brand>widely available environmental product>niche environmental product. The other three categories had rankings of: All purpose cleaner - widely available environmental product>own brand>leading brand>niche environmental, washing up liquid – own brand>leading brand>widely available environmental>niche environmental

and washing powder – niche environmental>leading brand>own brand>widely available brand. On the whole, the washing up liquids and washing powders were of a similar toxicity (EC_{50} <10 $\mu l.l^{-1}$ and <10 $mg.l^{-1}$ respectively) and were more toxic than the shower gels and shampoos (EC_{50} <100 $\mu l.l^{-1}$ for both categories). The all purpose cleaners had two products that were much less toxic than the other categories (EC_{50} >500 $\mu l.l^{-1}$) and two that were as toxic as the shampoos and shower gels (EC_{50} <100 $\mu l.l^{-1}$).

In the industrial toxin category a general pattern was much more evident with the hydrogen peroxide being most toxic (0.07±0.03 $\mu l.l^{-1}$), followed by the two surfactants (sodium dodecyl sulphate (0.75±0.04 $mg.l^{-1}$) and cetyltrimethylammoniumbromate (1.0±0.2 $mg.l^{-1}$)). The increase in hypochlorite concentration from 6% in the Nest bleach to a solution of 30% has had a profound effect on the EC_{50}, reducing it from over 35000 ppm to >1 ppm. Copper and zinc sulphate were toxic in the <10 $mg.l^{-1}$ range and phenol at <20 $mg.l^{-1}$, whereas sodium chloride and magnesium sulphate were toxic only at very high concentrations. This gives a ranking of oxidant>surfactant>heavy metals>organics>salts for the compounds tested.

The method used for the Microtox® gave an EC_{50} value at 5 and 15 minutes. Using ANOVA analysis with a 95% confidence interval to analyse the difference between the mean EC_{50} values for 5 minutes and 15 minutes it was found ten of the tested substances had a significant difference (Table 5-1). Nine toxins had EC_{50} 5 minute values greater than the 15 minute value (i.e. the 15 minute value was more toxic) with a range of between 1:0.8 for Ecover all purpose cleaner to 1:0.2 for zinc sulphate and copper sulphate. The remainder had ratios of 1:0.6 (Naked and Pantene shampoo), 1:0.5 (Ecover and Morrisons Ultra washing up liquid) and 1:0.4 (Nest washing powder and sodium dodecyl sulphate). Only Henna Plus shampoo showed an EC_{50} 15 minute value greater (i.e. less toxic) than the 5 minute value (1100 $\mu l.l^{-1}$ compared with 750 $\mu l.l^{-1}$ respectively or a ratio of 1:1.5). Values in bold in the table denote the value used as the EC_{50} for that toxin.

The largest effect was seen with the heavy metals with an increase of 80 % over the 15 minute period (Copper sulphate EC_{50} 5 minute was 19.7 $mg.l^{-1}$ and EC_{50} 15 minute was 3.4 mg.l-1, zinc sulphate EC50 5 minute was 38.1 mg.l-1 and EC50 15 minute was 9.5 mg.l-1).

Table 5-1 – EC_{50} values at 5 and 15 minutes, for household products tested using Microtox™ (+ standard error of the mean, n=3).

Category	***Brand/ Compound***	***EC_{50} 5 mins (ppm)***	***EC_{50} 15 mins (ppm)***	***EC_{50}5m:EC_{50}15m***
All purpose cleaner	Ecover	80 (±3)	**60 (±3)**	1:0.8
	Nest	**780 (±250)**	840 (±250)	1:1
	Morrisons	**80 (±6)**	110 (±12)	1:1
	Mr Muscle	**430 (±90)**	440 (±126)	1:1
Washing up liquid	Ecover	21(±3)	**10(±1)**	1:0.5
	Nest	**163(±57)**	168(±56)	1:1
	Morrisons Ultra	11(±1)	**6(±1)**	1:0.5
	Persil	13(±2)	**7(±2)**	1:1
Shampoo	Naked	160(±20)	**100(±6)**	1:0.6
	Henna Plus	**750(±40)**	1100 (±110)	1:1.5
	Morrisons	98(±19)	**50(±10)**	1:1
	Pantene	9(±1)	**5(±1)**	1:0.6
Shower Gel	Ecover	58(±17)	**43(±8)**	1:1
	Lavera	**85(±6)**	93(±2)	1:1
	Morrisons	21(±2)	**13(±2)**	1:1
	Original Source	15(±3)	**10(±2)**	1:1
Bleach	Nest	42250 (±1280)	**35440 (±6720)**	1:1
	Morrisons	-	-	-
	bettabuy			
	Domestos	-	-	-
Washing powder	Ecover	**41 (±8)**	54 (±18)	1:1
	Nest	14 (±1)	**6 (±1)**	1:0.4
	Morrisons	14 (±2)	**8 (±2)**	1:1
	Persil	8 (±1)	**7 (±1)**	1:1
Heavy metal	$CuSO_4$	19.7(±0.2)	**3.4(±0.1)**	1:0.2
	(as Cu^{2+})	12.4(±0.1)	**2.2(±0.07)**	
	$ZnSO_4$	38.1(±0.9)	**9.5(±0.3)**	1:0.2
	(as Zn^{2+})	15.5(±0.3)	**3.8(±0.1)**	
Salt	NaCl	**29000(±6000)**	29000(±6000)	1:1
	$MgSO_4$	125000(±4000)	**98000(±16000)**	1:1
Surfactant	SDS[1]	1.72(±0.15)	**0.75(±0.04)**	1:0.4
	CTMAB[2]	1.7(±0.4)	**1.0(±0.2)**	1:1
Organic	Phenol	**17.4(±0.8)**	17.5(±0.3)	1:1
Oxidant	30% H_2O_2	0.100(±0.040)	**0.07(±0.03)**	1:1
	NaOCl	-	-	-

[1] SDS = sodium dodecyl sulphate [2]CTMAB = cetyltrimethylammoniumbromate

Dutka *et al.*, (1983) observed a similar effect with the heavy metals tested, zinc (in the form Zn^{2+}) and copper (in the form Cu^{2+}) with EC_{50} values at 5 minutes of 13.8 and 19.5 ppm, respectively, compared with EC50 values of 3.8 and 3.45 ppm at 15 minutes, a greater than 70 % increase in toxicity. In contrast, the

EC_{50} at 5 minutes for sodium lauryl sulphate was 3.19 ppm and the 15 minute value 1.8 ppm, a 44 % increase and the phenol gave an increase in EC_{50} from 28 ppm at 5 minutes to 34.3 ppm at 15 minutes. These findings were echoed in research by Petala *et al.* (2005) where the five organics tested showed little change over a 15 minute timeframe (e.g. pentachlorophenol 5 min EC_{50} = 1.81 $mg.l^{-1}$ and 30 min EC_{50} = 1.20 $mg.l^{-1}$) whereas the five heavy metals showed an increase in toxicity (e.g. nickel 5 min EC_{50} = 74.5 $mg.l^{-1}$ and 30 minute EC_{50} = 7.6 $mg.l^{-1}$).

Those toxins tested with an equal 5 and 15 minute EC_{50} value, exhibit behaviours of oxidant or lytic biocides, that produce a rapid kill response either by oxidation or destabilisation of the cell membrane leading to rapid cell lysis e.g. hydrogen peroxide, Original Source shower gel containing tea tree oil. Those toxins with a greater 5 minute than 15 minute EC_{50} value (i.e. more toxic after 15 minutes) exhibit behaviours of electrophiles or protonophores which interrupt enzymes and metabolism resulting in a slower impact on the cells e.g. copper sulphate and zinc sulphate (Chapman, 2003).

No data could be found in the literature on complete household products, however, the values of industrial toxins found in this project were within the range of those found in the literature (Table 5-2). For example, Dutka *et al.*, (1983) found the EC_{50} for Zn^{2+} to be 3.45 ppm compared to 3.8±0.1 ppm for this study. The study carried out by Farré *et al.*, in 2006 illustrated the variation in values obtained by different laboratories analysing the same toxins using the same methods. E.g. a range of 8.28 – 16.8 ppm was reported for phenol.

Table 5-2 – Comparison of values found in literature with those obtained in this study for tested toxins.

Category	***Brand/ Compound***	***EC_{50} from this study (ppm)***	***EC_{50} (ppm)***	***Reference***
Heavy metal	$CuSO_4$ (as Cu^{2+})	3.4(±0.1)	3.8 (as Cu^{2+})	Dutka *et al*., 1983
		2.2(±0.1)	<0.3 (as Cu^{2+})*	Dalzell *et al*., 2002.
			0.35 (as Cu^{2+})	Fulladosa *et al*., 2005.
	$ZnSO_4$ (as Zn^{2+})	9.5(±0.3)	3.45 (as Zn^{2+})	Dutka *et al*., 1983.
		3.8(±0.1)	18.8 – 31.9	Farré *et al*., 2006.
			0.76 (as Zn^{2+})*	Gutiérrez *et al*., 2002.
			<1 (as Zn^{2+})*	Dalzell *et al*., 2002.
			0.86 (as Zn^{2+})	Fulladosa *et al*., 2005.

Category	*Brand/ Compound*	*EC_{50} from this study (ppm)*	*EC_{50} (ppm)*	*Reference*
Surfactant	SDS[1]	0.75	1.8	Dutka *et al*., 1983.
	LAS[2]	-	14.29*	Gutiérrez *et al*., 2002.
			<20*	Dalzell *et al*., 2002.
	CTMAB[3]	1.0(±0.2)	0.86 (CTMAC[4])	Dutka *et al*., 1983.
Organic	Phenol	17.4(±0.8)	25.9	Petala *et al*., 2005.
			28	Dutka *et al*., 1983.
			8.28 – 16.8	Farré *et al*., 2006.
			18	Ren *et al*., 2003.

* EC_{50} determined at 30 minutes.
[1]SDS = sodium dodecyl sulphate or sodium lauryl sulphate.
[2]LAS = linear alkyl benzene sulphonate.
[3]CTMAB = cetyltrimethylammoniumbromide. [4]CTMAC = cetyltrimethylammoniumchloride.

Examination of the full dose response curves gives a further insight into the effects of the individual products and compounds over and above the EC_{50} values (Appendix B). Three different types of response were observed; a natural logarithmic shape (Figure 5-2) shown by the majority of the toxins tested (20 from 29 tested), a linear response (Figure 5-3) (6 from 29 tested) and a negative effect or stimulatory response at low concentrations with a linear inhibitory effect being apparent at higher concentrations (Figure 5-4) (3 from 29 tested).

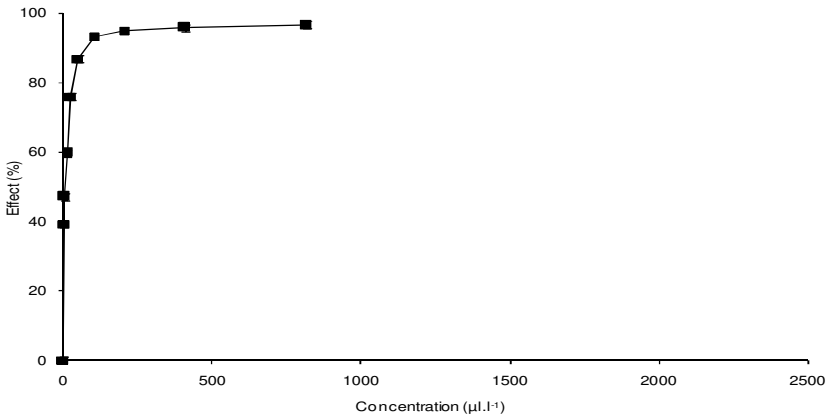


Figure 5-2 - Pantene Pro V shampoo showing a natural logarithmic dose response (■ = 15 minute data).

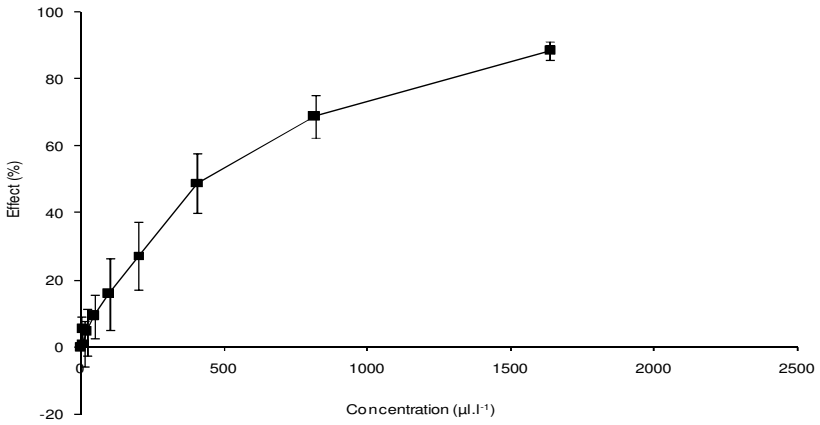


Figure 5-3 - Mr Muscle all purpose cleaner showing a linear response (■ = 15 minute data).

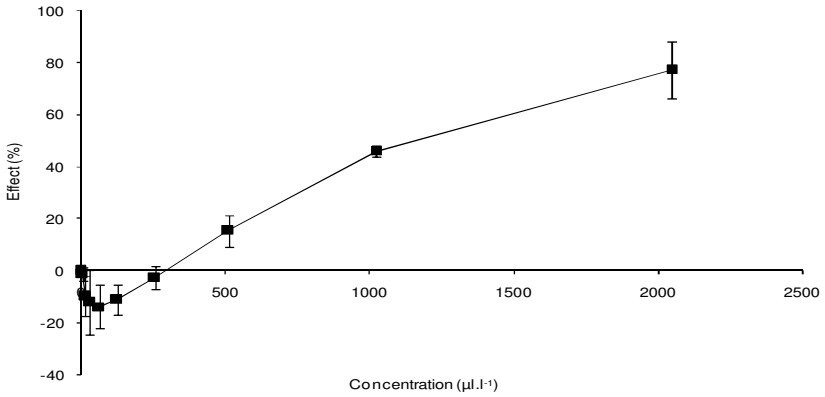


Figure 5-4 – Henna Plus shampoo showing a stimulatory followed by a linear response (■ = 15 minute data).

The gradient of the linear portion of each curve was calculated and if the response plateaued the %effect and concentration that this occurred at were also noted to assess the sensitivity of the system to each product. A steeper gradient gives a more sensitive system while the concentration at the plateau indicates the maximum concentration above which no increased effect will be observed on the system (Table 5-3).

Table 5-3 – Microtox® dose response types, gradients and plateau values.

Category	***Brand/ Compound***	***Response Type***	***Max gradient (%effect.ppm^{-1})***	***Plateau height (% effect)***	***Concentration at plateau (ppm)***	***Plateau gradient (%effect.ppm^{-1})***	***EC_{50} (ppm)***
All purpose cleaner	Ecover	ln	0.31	77	200	0.01	60(±3)
	Nest	sl	0.04	-	-	-	780(±250)
	Morrisons	ln	0.35	72	200	0.02	80(±6)
	Mr Muscle	l	0.05	-	-	-	430(±91)
Washing up liquid	Ecover	ln	1.2	77	64	0.002	10(±1)
	Nest	sl	0.04	-	-	-	163(±57)
	Morrisons Ultra	ln	3.5	95	25	0.01	6(±1)
	Persil	ln	0.9	90	102	-0.03	7(±2)
Shampoo	Naked	ln	0.2	80	409	0.002	100(±6)
	Henna Plus	sl	0.04	-	-	-	750(±40)
	Morrisons	ln	0.7	72	102	0.01	50(±10)
	Pantene	ln	0.9	93	102	0.003	5(±1)
Shower Gel	Ecover	ln	0.4	87	205	0.003	43(±8)
	Lavera	ln	0.3	80	256	0.003	85(±6)
	Morrisons	ln	0.4	82	205	0	13(±2)
	Original Source	ln	0.8	85	102	0.003	10(±2)
Bleach	Nest	ln	0.0008	89	102400	0	35440(±6720)
Washing powder	Ecover	l	0.5	-	-	-	41(±8)
	Nest	l	0.3	-	-	-	6(±1)
	Morrisons	ln	1.9	77	41	0.1	8(±2)
	Persil	ln	3.4	69	20.5	0.1	7(±1)
Heavy metal	$CuSO_4$	ln	7.7	99	13	0.002	3.4(±0.1)
	$ZnSO_4$	l	1.1	-	-	-	9.5(±0.3)
Salt	$MgSO_4$	l	0.3	-	-	-	98000(±16000)
	NaCl	ln	1.5	77	51.2	0.01	29000(±6000)
Surfactant	SDS[1]	ln	33	85	2.61	0.05	0.75(±0.04)
	CTMAB[2]	ln	54	70	1.29	0.02	1.0(±0.2)
Organic	Phenol	l	1.5	-	-	-	17.4(±0.8)
Oxidant	30% H_2O_2	ln	0.4	90	205	0.004	0.07(±0.03)

A comparison of these responses shows that the two surfactants had the most pronounced affect with gradients of 33 %effect.mg.l^{-1} and 54 %effect.mg.l^{-1} for SDS and CTMAB respectively, further reinforcing the case for the *V. fischeri* bacteria being sensitive to surfactants. This is most likely to be caused by the disruption of the osmotic pressure for the *V. fischeri*, that is adjusted at the beginning of the method (Microtox operating manual, 2000). The CTMAB plateaued quickly after the EC_{50} of 1 mg.l^{-1} was reached at 1.29 mg.l^{-1}, at a 70 %effect.

Within the household product category there was a broad range of responses. Morrisons Ultra washing up liquid and Persil washing powder had the steepest gradients of 3.5 %effect.µl.l^{-1} and 3.4 %effect.mg.l^{-1} respectively. In the case of the washing up liquid this translates to a 3500 % increase in effect for every 1 ml.l^{-1} increase in concentration. Most products were in the 0.2 – 1.0 %effect.ppm^{-1} range (Ecover and Morrisons all purpose cleaner, Persil washing up liquid, Naked, Morrisons and Pantene shampoo, Ecover, Lavera, Morrisons and Original Source shower gel and Ecover and Nest washing powder). At the other end of the range the Nest bleach would result in a 0.8 % increase in effect for every 1 ml.l^{-1} increase in concentration; a considerable difference (Table 5-3).

Interestingly, comparison of the response type, maximum gradient, plateau height and concentration at plateau did not necessarily provide a good indicator of the EC_{50} value. For example, in the case of the shower gels the maximum gradients were broadly similar (Ecover 0.4 %effect.µl.l^{-1}, Lavera 0.3 %effect.µl.l^{-1}, Morrisons 0.4 %effect.µl.l^{-1} and Original Source 0.8 %effect.µl.l^{-1}), each started to plateau at a similar %effect (Ecover 87 %effect, Lavera 80 %effect, Morrisons 82 % effect and Original Source 85 %effect) with a similar concentration, apart from the Original Source (Ecover 205 µl.l^{-1}, Lavera 256 µl.l^{-1}, Morrisons 205 µl.l^{-1} and Original Source 102 µl.l^{-1}) and each had a true plateau with a gradient very close to zero. However, each had different EC_{50} values (Ecover 43±8 µl.l^{-1}, Lavera 85±6 µl.l^{-1}, Morrisons 13±2 µl.l^{-1} and Original Source 10±2 µl.l^{-1}).

5.2 Respirometry

The respirometry analysis provided EC_{50} values with respect to oxygen uptake rate for 32 toxins tested. 25 produced EC_{50} values while the others were not toxic at the maximum concentrations tested. The EC_{50} values gained ranged from 0.04 $ml.l^{-1}$ for 30 % sodium hypochlorite (NaOCl) to 110 $ml.l^{-1}$ for Ecover shower gel. The household products ranged from 0.48 $ml.l^{-1}$ for Domestos bleach to 110 $ml.l^{-1}$ for Ecover shower gel and the industrial toxins ranged from 0.04 $ml.l^{-1}$ for NaOCl to 55000 $mg.l^{-1}$ for magnesium sulphate (Figure 5-5). This is a wide range of concentrations of EC_{50} values illustrating the differing effects of different toxins. Those products or compounds that did not reach an EC_{50} value at the maximum concentration tested (Ecover and Mr Muscle all purpose cleaner, Henna Plus and Morrisons shampoo, NaCl and sodium dodecyl sulphate) have been omitted from the graph but included in Table 5-4.

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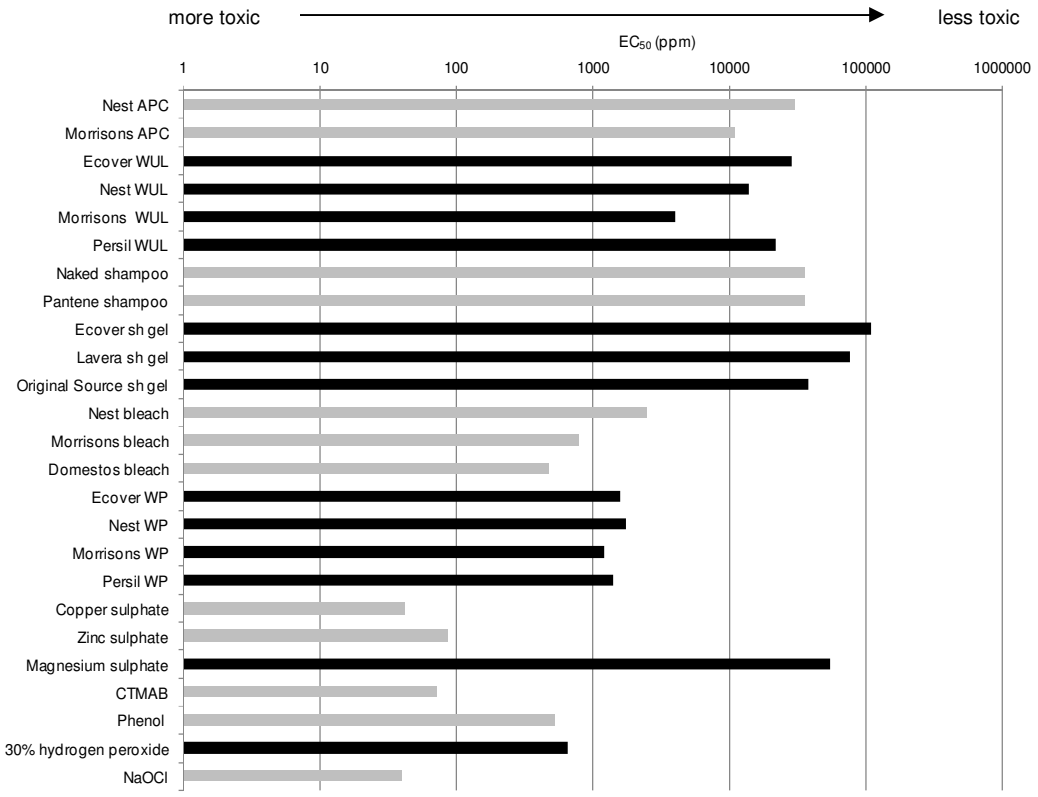


Figure 5-5 – Respirometry EC_{50} values for toxins tested.

Within the household product category the hypochlorite based bleaches were the most toxic (0.48 and 0.8 $ml.l^{-1}$ for Domestos and Morrisons respectively), showing that the microbial community is sensitive to strong oxidants which are a broad spectrum biocide (Cloete, 2003). The washing powders were the next most toxic with EC_{50} values of 1600, 1750, 1200 and 1400 $mg.l^{-1}$ for Ecover, Nest, Morrisons and Persil washing powders, with no one powder being clearly more toxic than the others. It is likely that there are a combination of factors that are affecting the community including pH change (sodium carbonate) and surfactant action (Nest only has sodium carbonate and vegetable soap as ingredients but was not much less toxic). The increase in toxicity for the other washing powders was due to the more complex ingredients and the bleaching agents present (for example sodium carbonate peroxide in the Morrisons Cyclon and Persil powders).

The all purpose cleaners and washing up liquids had broadly similar toxicities of around 10 $ml.l^{-1}$ however there were some exceptions, for example, Morrisons washing up liquid 4 $ml.l^{-1}$ (which includes the specific anti-microbial ingredients (alkyldimethyl amine oxide and 5-Chloro-2-methyl-2H-isothiazol-3-one/2-methyl-2H-isothiazol-3-one)), Morrisons all purpose cleaner 11 $ml.l^{-1}$ (mostly alcohols), Nest washing up liquid 14 $ml.l^{-1}$ (containing essential oils as a biocide). Ecover all purpose cleaner showed no toxicity at the maximum concentration tested of 167 $ml.l^{-1}$ (containing mostly ethanol). The shampoos and shower gels showed similar toxicities and were less toxic than the other categories (Naked and Pantene shampoo 36 $ml.l^{-1}$ and Ecover shower gel 110 $ml.l^{-1}$). The shampoos contained a complex mixture of ingredients from which it was unable to discern which could be contributing to the toxicity (Appendix A).

Within the industrial compounds tested the strong oxidants hydrogen peroxide and sodium hypochlorite were the most toxic with EC_{50} values in the sub $1ml.l^{-1}$ range. CTMAB was significantly more toxic than SDS with an EC_{50} of $72mg.l^{-1}$ compared with $>200mg.l^{-1}$. The metals were the next most toxic with copper sulphate being twice as toxic as zinc sulphate with EC_{50} values of 42 and 86

mg.l^{-1} respectively. Neither of the salts tested, sodium chloride or magnesium chloride showed significant toxicity with EC_{50} values of >10 g.l^{-1} and 55 g.l^{-1}.

Comparison within the categories of household products did not produce any conclusive evidence that the environmental products are less toxic to the activated sludge community. For example in the all purpose cleaner category the cleaners were ranked Morrisons>Nest>Mr Muscle>Ecover however in the shampoo category they were ranked Naked=Pantene>Morrisons>Henna Plus. In the washing powder category there was no distinct difference between the categories with a range of 1200 to 1750 mg.l^{-1}.

Table 5-4 – EC_{50} values obtained using respirometry.

Product/ Compound	***Brand***	***EC_{50} (ppm)***
All purpose cleaner	Ecover	>167000 (40% stimulation)
	Nest	30000
	Morrisons	11000
	Mr Muscle	>100000 (20% inhibition)
Washing up liquid	Ecover	29000
	Nest	14000
	Morrisons Ultra	4000
	Persil	22000
Shampoo	Naked	36000
	Henna Plus	>167000 (40% inhibition)
	Morrisons bettabuy	>100000 (12% inhibition)
	Pantene	36000
Shower Gel	Ecover	110000
	Lavera	77000
	Morrisons	>167000 (14% inhibition)
	Original Source	38000
Bleach	Nest	3000
	Morrisons bettabuy	800
	Domestos	480
Washing powder	Ecover	1600
	Nest	1750
	Morrisons cyclon	1200
	Persil tablets	1400
Heavy metal	$CuSO_4$	42
	$ZnSO_4$	86
Salt	NaCl	>10000 (20% inhibition)
	$MgSO_4$	55000
Surfactant	SDS[1]	>200 (24% inhibition)
	CTMAB[2]	72
Organic Oxidant	Phenol	525
	30% H_2O_2	650
	NaOCl	40

Note: 167ml.l^{-1} was maximum concentration able to be tested with method.
[1]SDS = sodium dodecyl sulphate.
[2]CTMAB = cetyl trimethyl ammonium bromate.

The respirometry method produced oxygen uptake curves for each of the toxins tested. Two interesting examples of these are given in Figure 5-6 and Figure 5-7.

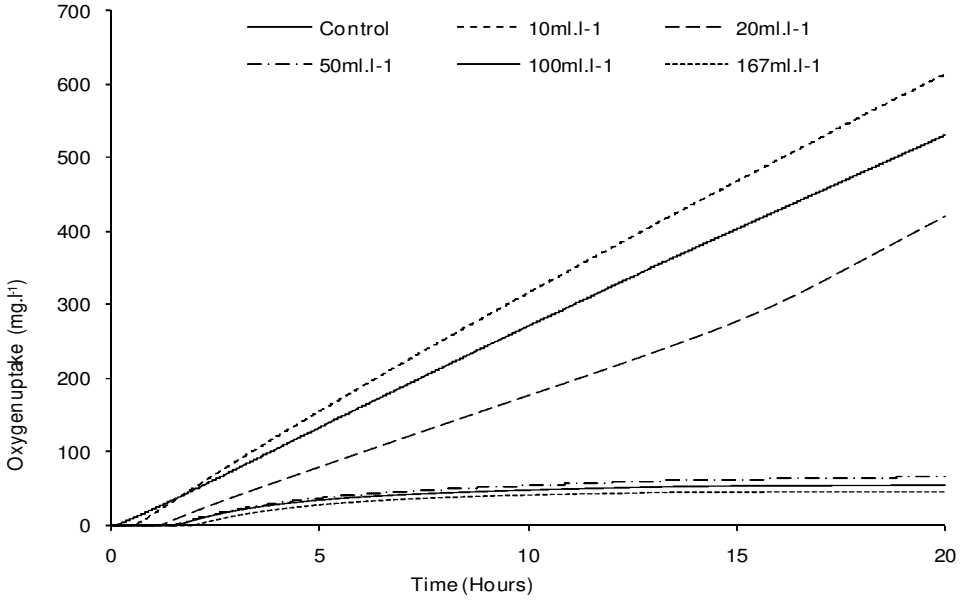


Figure 5-6 – Oxygen Uptake rates for Nest Antibacterial all purpose cleaner showing inhibition increasing with increasing concentration.

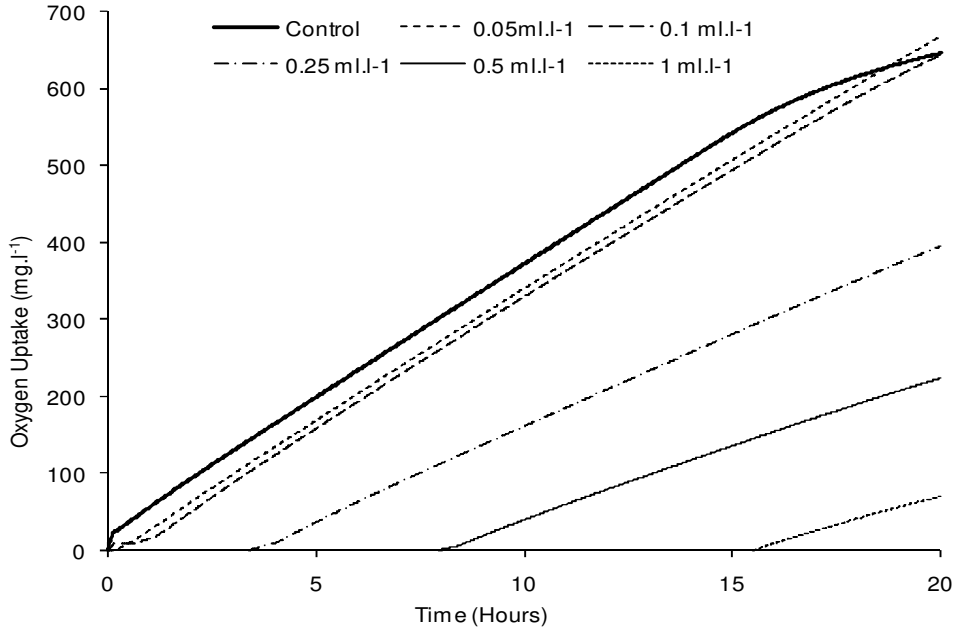


Figure 5-7 – Oxygen uptake for hydrogen peroxide showing initial inhibition followed by return to close to control uptake rates.

There are two distinct differences: the first shows an inhibition of the biomass for the duration of the experiment, proportional to the concentration of the toxin, whereas the second shows a complete inhibition of the biomass for a specific length of time (directly proportional to the concentration of the toxin in the sample) followed by an oxygen uptake at the same rate as the control.

The Nest antibacterial all purpose cleaner contained essential oils in its ingredients and these inhibit the biomass at higher concentrations however at lower concentrations it is stimulatory to the microbial community. At 10 $ml.l^{-1}$ the effect is stimulatory, whereas for 20 $ml.l^{-1}$ some inhibition is evident and by 50 $ml.l^{-1}$ it is close to 100% inhibition. A subsequent increase in concentration of the toxin brings about little change in oxygen uptake. This is caused by the essential oils in the cleaner which result in disturbance of the cell membrane and contents causing leakage of key components and, in some instances, death (Burt, 2004).

In contrast, Figure 5-7 illustrates the response for an oxidant, hydrogen peroxide. Here inhibition is total for a specific number of hours, directly proportional to the concentration of toxin. E.g., at a concentration of 250$\mu l.l^{-1}$ the inhibition is for four hours before the oxygen uptake resumes at a slightly lower rate than the control (24 $mgO_2.l^{-1}.h^{-1}$ compared with 33.5 $mgO_2.l^{-1}.h^{-1}$).

Table 5-5 – oxygen produced by hydrogen peroxide.

Concentration of H_2O_2 ($\mu l.l^{-1}$)	***H_2O_2 present (μl)***	***Theoretical oxygen available from H_2O_2 (mg O_2)***	***Length of inhibition (hours)***	***Oxygen needed (mg O_2)[1]***
50	300	2.88	0.5	1.67
100	600	5.76	1	3.35
250	1500	14.4	4	13.4
500	3000	28.8	8	26.8
1000	6000	57.6	16	53.6

[1] with 100ml of biomass and an oxygen uptake rate of 33.5 $mgO_2.l^{-1}.h^{-1}$ (Control OUR).

Hydrogen peroxide will oxidise any organic material in the solution producing oxygen, hence the lack of oxygen consumption in the first portion of the curve. This acts akin to the chlorine demand in clean water with the majority of the cells themselves remaining unharmed. If the theoretical oxygen produced from

the hydrogen peroxide is calculated it relates well to the required oxygen of the biomass for the length of the inhibition observed (Table 5-5). Oxygen uptake is registered again once the oxygen produced by the oxidising action of the hydrogen peroxide is consumed (Chapman, 2003).

The dose response curves for respirometry provide a more in-depth analysis of the toxin action (Appendix C). Four responses were observed for the respirometry; a linear response (Nest washing up liquid, Pantene shampoo, Persil washing powder, copper sulphate, zinc sulphate, magnesium sulphate, sodium chloride, CTMAB, phenol and hydrogen peroxide) (Figure 5-8 (a)), a natural logarithmic response (Nest and Morrisons all purpose cleaner, Ecover, Morrisons and Persil washing up liquid, Original Source shower gel, Morrisons and Domestos bleach, Ecover washing powder and sodium hypochlorite) (Figure 5-8 (b)), a stimulatory followed by a linear inhibitory response at higher concentrations (Ecover and Mr Muscle all purpose cleaner, Henna Plus and Morrisons shampoo, Ecover, Lavera and Morrisons shower gel and sodium dodecyl sulphate) (Figure 5-8 (c)) and an s shaped curve with little inhibition at lower concentrations, increasing rapidly over a small concentration increase with a plateau effect at higher concentrations (Naked shampoo, Nest bleach and Nest and Morrisons washing powder) (Figure 5-8 (d)).

From the gradients, plateau values and EC_{50} values the differences and similarities of the toxins are further revealed. In general, the household products had shallow gradients with the Morrisons bleach and three of the washing powders (Ecover, Nest and Morrisons) having the steepest gradients. The microbial community is most sensitive to the hypochlorite based bleaches and the washing powders, whereas the shampoos and shower gels have a much lesser effect.

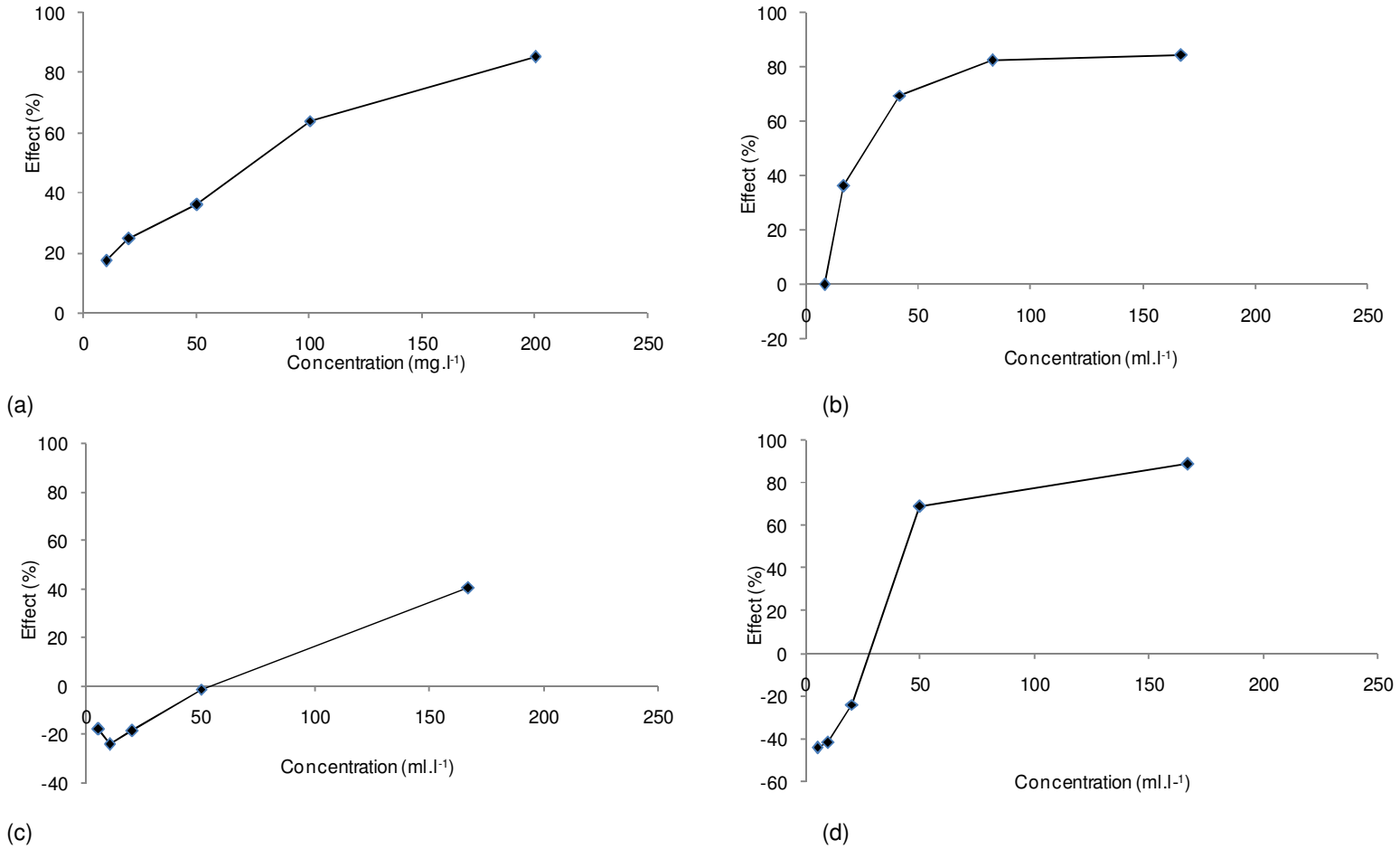


Figure 5-8 – Respirometry dose response curve for (a) CTMAB (linear response) (b) Persil WUL (natural logarithmic) (c) Henna Plus shampoo (stimulatory linear) (d) Naked shampoo (stimulatory s-shaped curve).

Shampoos and shower gels are likely to have up to 50% surfactant content compared to 25% in washing powders indicating the microbial community is more sensitive to other ingredients than the surfactants (Madsen *et al.*, 2001). Pantene and Naked shampoos have the same EC_{50} values but the maximum gradients are very different: Naked produced a maximum of 3 %effect.ml.l^{-1} whereas Pantene had a maximum of 0.8 %effect.ml.l^{-1}. The response curves themselves are remarkably similar at higher concentrations, with both shampoos causing an abrupt step change in toxicity to 70 % effect at 50 ml.l^{-1}, however, Naked shampoo produced an initial stimulatory response of -20 % effect at concentrations of 5 – 20 ml.l^{-1} whereas Pantene produced 10 – 20 % effect at the same concentrations. Both shampoos have mainly surfactants as ingredients which clearly have a threshold value at which they become more toxic, however, the advertised 97% natural ingredients in the Naked shampoo has a much less toxic effect at the lower concentrations.

Within the industrial toxins category the hypochlorite had the biggest effect with the steepest gradient of all the toxins of 0.93 %effect.µl.l^{-1} followed by copper sulphate and the cationic surfactant CTMAB, with a maximum gradient of 0.4 %effect.mg.l^{-1}. The sodium dodecyl sulphate also had an impact with a maximum gradient of 0.2 %effect.mg.l^{-1} although this was not found to be toxic at 200 mg.l^{-1}. These results reinforce the observations that the system is most sensitive to hypochlorite, the same as the Microtox system, but although SDS (an anionic surfactant) produced one of the steeper gradients it had a much lesser toxic effect, in contrast to the cationic surfactant, CTMAB. Anionic surfactants are used much more in household products than cationic surfactants (European estimated consumption in 1998 of 780,000 tons of anionic surfactant compared to 98,000 cationic surfactant (Madsen *et al.*, 2001)) and tend to be more biodegradeable (Eriksson *et al.*, 2002).

Table 5-6 - Dose response types, gradients and plateau values for respirometry.

Category	*Brand/ Compound*	*Response Type*	*Max gradient (%effect.ppm^{-1})*	*Plateau height (% effect)*	*Concentration at plateau (ppm)*	*Plateau gradient (%effect.ppm^{-1})*	*EC_{50} (ppm)*
All purpose cleaner	Ecover	sl	0.0007	-	-	-	>167000
	Nest	ln	0.003	86	50000	0.00003	30000
	Morrisons	ln	0.004	70	42000	0.0002	11000
	Mr Muscle	sl	0.0005	-	-	-	>100000
Washing up liquid	Ecover	ln	0.002	78	50000	-0.00002	29000
	Nest	l	0.002	-	-	-	14000
	Morrisons Ultra	ln	0.009	84	10000	0.0004	4000
	Persil	ln	0.002	82	83000	0	22000
Shampoo	Naked	s	0.003	-	-	-	36000
	Henna Plus	sl	0.0004	-	-	-	>167000
	Morrisons	sl	0.0003	-	-	-	>167000
	Pantene	l	0.0008	-	-	-	36000
Shower Gel	Ecover	sl	0.0009	-	-	-	110000
	Lavera	sl	0.0008	-	-	-	77000
	Morrisons	sl	0.00005	-	-	-	>167000
	Original Srce	ln	0.001	86	100000	0.00001	38000
Bleach	Nest	s	0.024	98	5000	0.0004	3000
	Morrisons	ln	0.06	94	2000	0.0004	800
	Domestos	ln	0.025	92	2000	0.0002	480
Washing powder	Ecover	ln	0.06	86	3330	0.002	1600
	Nest	s	0.06	82	2500	0.0016	1750
	Morrisons	s	0.06	98	3330	0.0004	1200
	Persil	l	0.03	-	-	-	1400
Heavy metal	$CuSO_4$	l	0.4	-	-	-	42
	$ZnSO_4$	l	0.3	-	-	-	86
Salt	$MgSO_4$	l	0.0007	-	-	-	55000
	NaCl	l	0.001	-	-	-	>10000
Surfactant	SDS[1]	sl	0.2	-	-	-	>200
	CTMAB[2]	l	0.4	-	-	-	72
Organic	Phenol	l	0.07	-	-	-	525
Oxidant	30% H_2O_2	l	0.07	-	-	-	650
	NaOCl	ln	0.93	86	100	0.007	40

No comparison data was found in the literature for household products. All comparative values from literature are similar to those found in this study (Table 5-7).

Table 5-7 – Comparison of EC_{50} values from this study with those found in literature.

Category	*Brand/ Compound*	*EC_{50} from this study (ppm)*	*EC_{50} (ppm)*	*Reference*
Heavy metal	$CuSO_4$	42	32.07 (as Cu^{2+})	Gutiérriez *et al.*, 2002.
	(as Cu^{2+})	16.7	~30 (as Cu^{2+})	Dalzell *et al*., 2002.
			17 (as Cu^{2+})	Dutka *et al*., 1983.
	$ZnSO_4$	86	55.79 (as Zn^{2+})	Gutiérriez *et al.*, 2002.
	(as Zn^{2+})	34	~80 (as Zn^{2+})	Dalzell *et al*., 2002.
			5.2 (as Zn^{2+})	Dutka *et al*., 1983.
Surfactant	SDS[1]	>200	135	Dutka *et al*., 1983.
	LAS[2]	-	non toxic	Gutiérriez *et al.*, 2002.
			non toxic	Dalzell *et al*., 2002.
Organic	Phenol	525	520	Volskay *et al*., 1990.

[1] SDS = sodium dodecyl sulphate.
[2]LAS = linear alkylbenzene sulphate.

5.3 Comparison of Microtox® and Respirometry.

On the whole the Microtox® system was more sensitive to the toxins tested and produced lower EC_{50} values than the respirometry in this study and others found in literature (Figure 5-9). There were, however, two exceptions in this study: Nest bleach and magnesium sulphate which produced EC_{50} values for the respirometry that were lower than for the Microtox® (35 $ml.l^{-1}$ for Microtox® and 3 $ml.l^{-1}$ for respirometry and 98 $g.l^{-1}$ for Microtox® and 55 $g.l^{-1}$ for respirometry, respectively). Toxins represented by 0.01 ppm values for Microtox® were too toxic for the *V.fischeri* and those represented by 1,000,000 ppm values for respirometry were not found to be toxic at the concentrations tested.

The differing results observed between the two methods are due to the differences between the pure marine microbial culture used in the Microtox test and the mixed community present in the biomass from a biological wastewater treatment works. The *V. Fischeri* do not exist in a habitat that is similar to the biomass and thus will react in different ways to the different toxins present (Ricco *et al*., 2004). The transition from salt water to fresh water for salt tolerant species is more detrimental than for freshwater to saltwater shocks (Kincannon and Gaudy, 1968). There are a number of factors that influence the microbial

community's response to acute toxicity including floc size, which protects the biomass (Henriques *et al.*, 2005), and the diversity of the community being tested (Curtis *et al.*, 2003).

Several studies have compared Microtox® and respirometry for a range of single organic or inorganic toxins and all have found that the Microtox® method produces a more sensitive result compared to respirometry (Figure 5-9) (Ricco *et al.*, 2004, Dutka *et al.*, 1983, Dalzell *et al.*, 2002, and Gutiérrez *et al.*, 2002). All draw the conclusion that as the Microtox method uses only a single pure culture of bacteria it will not react in the same way as the mixed community present in the activated sludge used for respirometry tests. The respirometry results will therefore present the most meaningful results for toxicity assessment.

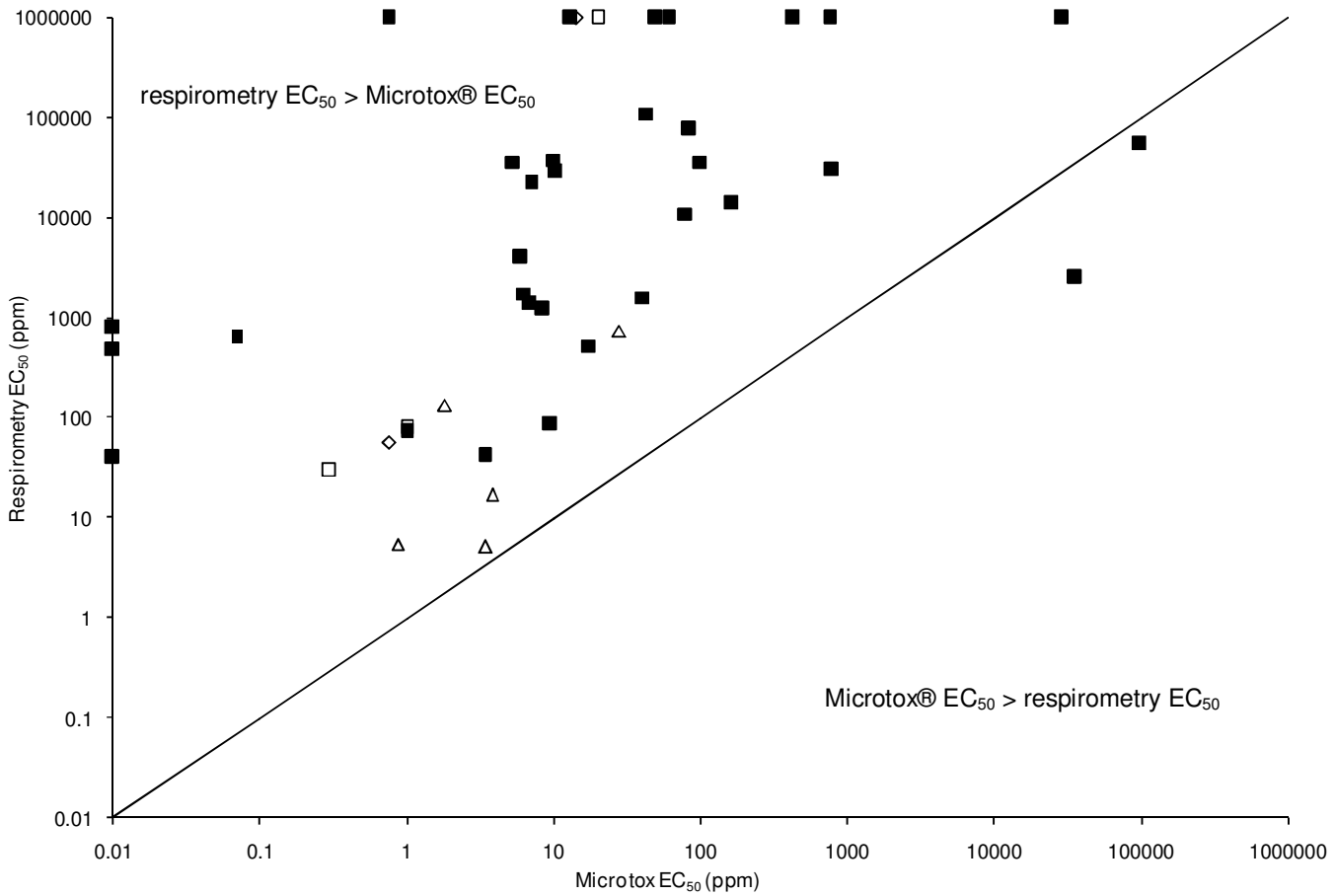


Figure 5-9 – Comparison of EC_{50} values by Microtox® and respirometry [■ values from this study, □ Dalzell *et al.*, 2002, Δ Dutka *et al.*, 1983, ◊ Gutiérrez *et al.*, 2002 (solid line represents Microtox® EC_{50} = respirometry EC_{50})].

Three of the categories tested produced the same ranking of toxicities irrespective of method used for analysis: bleach, oxidants and the heavy metals. These have the same action on any type of bacteria and the presence of a community of micro-organisms does not affect the toxicity mechanisms of these compounds. The remainder of the categories (all purpose cleaner, washing up liquid, shampoo, shower gel, washing powder, salts and surfactants) showed differences between the ranking of the toxins depending on which method was used.

The respirometry biomass was more sensitive to the environmental household products containing essential oils than the *Vibrio fischeri* bacteria used in Microtox®. For example, in the all purpose cleaner category (Nest cleaner contains essential oils) the ranking was Ecover>Morrisons>Mr Muscle>Nest for Microtox® and Morrisons>Nest>Mr Muscle>Ecover for respirometry. In the washing up liquid category (Nest washing up liquid containing essential oils) a similar effect was seen; Microtox® gave a Morrisons>Persil>Ecover>Nest ranking compared with Morrisons>Nest>Mr Muscle>Ecover for respirometry. Both methods were sensitive to the Morrisons washing up liquid which contained anti microbials (Appendix B).

There was little discrimination between the washing powders for respirometry (Ecover 1600 $mg.l^{-1}$, Nest 1750 $mg.l^{-1}$, Morrisons 1200 $mg.l^{-1}$, Persil 1400 $mg.l^{-1}$) although for Microtox® the Ecover powder was less toxic (Ecover 41 $mg.l^{-1}$, Nest 6 $mg.l^{-1}$, Morrisons 8 $mg.l^{-1}$, Persil 7 $mg.l^{-1}$). The washing up liquids, all purpose cleaners, shampoos and shower gels had a broadly similar effect. Interestingly, the Microtox® was equally sensitive to both the surfactants tested (sodium dodecyl sulphate 0.75 $mg.l^{-1}$, CTMAB 1 $mg.l^{-1}$) whereas the respirometry was only sensitive to the cationic surfactant (SDS >200 $mg.l^{-1}$, CTMAB 72 $mg.l^{-1}$).

Table 5-8 – ranking of toxins using both Microtox® and respirometry.

Category	*Method*	*EC_{50} Most toxic* →			*EC_{50} Least toxic*
All purpose cleaner	Micro	Ecover (60±3)	Morrisons (80±6)	Mr Muscle (430±90)	Nest (780±250)
	Resp	Morrisons (11000)	Nest (30000)	Mr Muscle (>167000)	Ecover (>167000)
Washing up liquid	Micro	Morrisons (6±1)	Persil (7±2)	Ecover (10±1)	Nest (163±60)
	Resp	Morrisons (4000)	Nest (14000)	Persil (22000)	Ecover (29000)
Shampoo	Micro	Pantene (5±1)	Morrisons (50±10)	Naked (100±6)	Henna Plus (750±40)
	Resp	Pantene (36000)	Naked (36000)	Henna Plus (>167000)	Morrisons (>167000)
Shower Gel	Micro	Original Source (10±2)	Morrisons (13±2)	Ecover (43±8)	Lavera (85±6)
	Resp	Original Source (38000)	Lavera (77000)	Ecover (110000)	Morrisons (>1670000)
Bleach	Micro	Domestos (nv)	Morrisons (nv)	Nest (35440±6720)	
	Resp	Domestos (480)	Morrisons (800)	Nest (2500)	
Washing powder	Micro	Nest (6±1)	Persil (7±1)	Morrisons (8±2)	Ecover (41±8)
	Resp	Morrisons (1200)	Persil (1400)	Ecover (1600)	Nest (1750)
Heavy metals	Micro	$CuSO_4$ (3.4±0.1)	$ZnSO_4$ (9.5±0.3)		
	Resp	$CuSO_4$ (42)	$ZnSO_4$ (86)		
Salt	Micro	NaCl (29000±6000)	$MgSO_4$ (98000±16000)		
	Resp	$MgSO_4$ (55000)	NaCl (>10000)		
Surfactants	Micro	SDS (0.75±0.04)	CTMAB (1.0±0.2)		
	Resp	CTMAB (72)	SDS (>200)		
Organic	Micro	Phenol (17.4±0.8)			
	Resp	Phenol (525)			
Oxidants	Micro	NaOCl (nv)	30% H_2O_2 (0.07±0.03)		
	Resp	NaOCl (40)	30% H_2O_2 (650)		

nv = no valid results obtained.

5.4 Risk assessment of toxins

In order to be able to assess the risk that the household products pose it is necessary to link the EC_{50} values to the system that will treat the urban water. Most systems include some kind of buffering tank to equalise flows to the system and it is assumed that the water is stored for 24 hours for this purpose; the optimal time for grey water storage before treatment (Dixon *et al.*, 1999). Using the EC_{50} values from Microtox® and respirometry the volume of any given product needed to be used per person per 24 hours, based on a water use of 150 l.p.day^{-1}, was calculated (Table 5-9).

Table 5-9 – Volumes needed to be discharged to reach EC_{50} levels per 24 hours.

Category	***Brand/ compound***	***Product number***	***Water usage of 150 l.p^{-1}.day^{-1}.***	
			Volume to reach Microtox® EC_{50}	***Volume to reach respirometry EC_{50}***
All purpose cleaner (ml.p^{-1})	Ecover	1	9	-
	Nest	2	117	4500
	Morrisons	3	12	1650
	Mr Muscle	4	64.5	-
Washing up liquid (ml.p^{-1})	Ecover	5	1.5	4350
	Nest	6	24	2100
	Morrisons Ultra	7	0.9	600
	Persil	8	1.05	3300
Shampoo (ml.p^{-1})	Naked	9	15	5400
	Henna Plus	10	112.5	-
	Morrisons	11	7.5	-
	Pantene Pro V	12	0.75	5400
Shower Gel (ml.p^{-1})	Ecover	13	6	165000
	Lavera	14	13.5	11550
	Morrisons	15	1.5	-
	Original Source	16	1.5	5700
Bleach (ml.p^{-1})	Nest	17	5316	375
	Morrisons	18	-	120
	Domestos	19	-	72
Washing powder (g.p^{-1})	Ecover	20	6	240
	Nest	21	0.9	262.5
	Morrisons	22	1.2	180
	Persil tablets	23	1.05	210

Using the respirometry values as a guide, the bleaches and washing powder pose the greatest threats, as these could reach the EC_{50} values in everyday use (doses of 200 – 300 ml are recommended for cleaning with bleach and washing powder uses around 90 -100 g per wash). Of the remaining categories (all purpose cleaner, washing up liquid, shampoo and shower gel) only the Morrisons washing up liquid is at levels (600ml.p^{-1}) that might be discharged during normal

household activities over one day. The rest of the products would only have an effect if more than 2 litres were discharged on one day. In the worst case scenario a whole bottle of product could be dispensed, however, even in this case products tend to come in 1 litre packages making it very unlikely that more than 1 litre of a product would be discharged per day. In the case of Ecover shower gel 165 litres would need to be discharged in any 24 hours which is extremely unlikely.

A risk matrix can be drawn up to show the likelihood of discharging a specified volume of household product and the potential impact this could have on a treatment works, taking into account whether the respirometry EC_{50} values determined would be exceeded or not. Five categories are normally given for the frequency, or likelihood, and the impact, or severity, of the event (Pollard, 2008). These are numbered 1 to 5, with 1 being the lowest impact and the least likely to 5 being the highest impact and the most likely (Table 5-10).

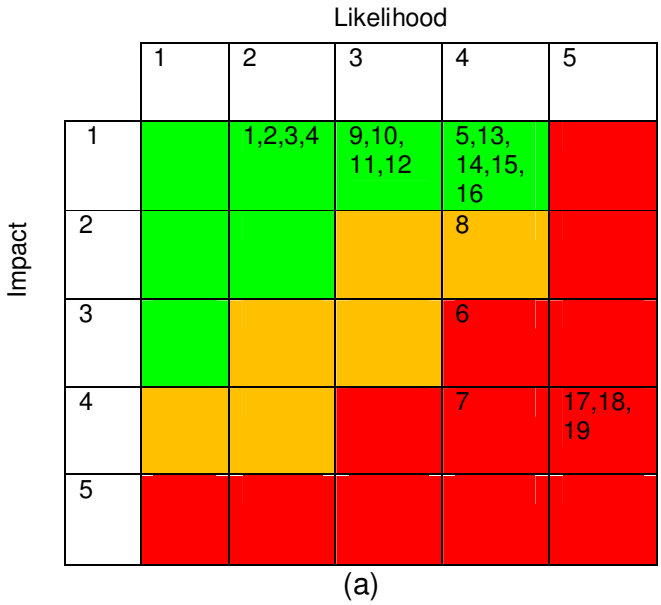
Table 5-10 – Definition of the impact and likelihood categories for the risk matrix.

Category	Impact	Likelihood
1	no impact on treatment process	yearly
2	limited impact	monthly
3	moderate impact	weekly
4	severe impact	daily
5	catastrophic process failure	hourly

This has been translated into a risk matrix and the areas that pose an unacceptable risk to the treatment system have been coloured red, those with a moderate risk have been coloured amber and those with an acceptable risk coloured green. These have been populated for a 0.5 litre and 1 litre discharge of each liquid. Each product has been numbered (Table 5-9) and placed within the matrix, apart from the washing powder.

As the amount of toxin discharged changes the risk to the treatment system also changes. The bleaches present the biggest risk and this does not change from 0.5 to 1 litre discharge. In fact if 0.25 litres of bleach were discharged per person in a 24 hour period this could have an impact on the treatment system as Domestos and Morrisons bleach have critical levels of under 0.15 litres per

person per day. In the case of the other household products only the washing up liquids present an increasing risk to the system, as the amount discharged is increased.



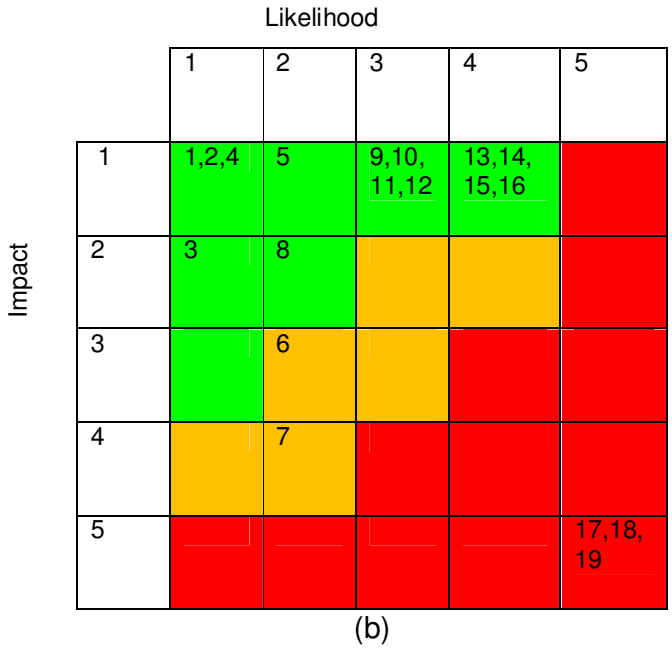


Figure 5-10 – Risk matrix for discharge of toxic products (a) 0.5 litre and (b) 1 litre per day using respirometry EC_{50} values.

6 Porous Pots Trials

6.1 Toxins used

The previous work of the Microtox® and respirometry provided a ranking of the toxins tested. In the household product category, on the whole the leading brand household products were the most toxic and contained the most complex mix of ingredients and these would be used for subsequent experiments. In the industrial product category it was decided to use compounds that were most likely to be discharged in urban wastewater or that were of particular interest to the industrial sponsor KeppelSeghers. The toxins chosen were a mix of products to ensure that as broad a spectrum as possible was covered to investigate the effects on a bench scale biological treatment system. From the 23 household products and 9 industrial toxins it was decided to carry forward 4 household products and 3 industrial products.

From the household products it was sensible to choose the shampoo, shower gel, washing powder and the hypochlorite based bleach as these are the most likely to be discharged from households (Almeida *et al.*, 1999). Although the all purpose cleaner was the next most toxic after bleach it is highly unlikely to be discharged to sewer in any great quantities and even if it was it would have less of an effect than the bleach. It was anticipated that bleach would have the most profound effect on the system and cause the most damage, proving the systems robustness (Bodík *et al.*, 2008). Washing powder is used in large quantities in the home and has been identified as containing many ingredients that could be harmful to wastewater treatment works (Pettersson *et al.*, 2000). Shampoo and shower gel are the next most regularly used in the home along with washing up liquid. Examination of ingredients lists (Appendix A) showed that the shampoo and shower gel were similar to washing up liquid but had more complex mixtures of ingredients which could interact with the biomass in the treatment system (Eriksson *et al.*, 2002).

From the industrial toxins the most likely to be discharged to sewer were zinc sulphate (Palmquist and Hanaeus, 2005), sodium dodecyl sulphate (Eriksson *et al.*, 2002) and phenol (requested by the industrial sponsor as of interest).

6.2 Baseline Monitoring

The hydraulic retention time (HRT) of the porous pots was set at 6 hours and the solids retention time (SRT) of approximately 90 days (wastage due to sampling only), to maintain a biomass concentration as close to that in an MBR as possible. The dissolved oxygen (DO) was monitored for the first 5 months of operation to ascertain the lowest rate needed to ensure that the DO remained >2 $mg.l^{-1}$. This was found to be at an aeration rate of 54 $l.h^{-1}$, which was kept constant for the remainder of the experiments. The porous pots were run for 18 months before any trials took place to ensure full acclimatisation to the Cranfield sewage and to provide a comprehensive baseline data set at steady state.

A complete set of baseline monitoring data is given in Table 6-1 to Table 6-3. The average COD removal rate was >80 % and the average ammonia removal rate was >90 %. Mixed liquor volatile suspended solids (MLVSS) peaked at >10 $g.l^{-1}$ but was on average between 4 and 5 $g.l^{-1}$. This performance is in accordance with pilot MBRs that have been operated in previous studies. Brindle *et al.*, (1996) reviewed a number of pilot MBR systems all of which provided a >80% COD removal rate and operated at between 2.5 – 6 $g.l^{-1}$ MLSS. Rosenberger and Kraume (2002) ran an MBR for 535d and reported a 95% reduction in COD and an ammonia removal rate of 82% with an MLSS that reached a maximum of 20 $g.l^{-1}$. Gander *et al.*, (2000) also reviewed a number of MBRs that achieved a >85% COD removal and >90% ammonia removal.

The feed characteristics of a mean of 344 $mg.l^{-1}$ COD and 24.5 $mg.l^{-1}$ ammonia place the Cranfield sewage in the diluted category of domestic wastewater according to Henze and Ledin (2001) who defined diluted domestic wastewater as having 320 $mg.l^{-1}$ COD and 18 $mg.l^{-1}$ ammonia.

Table 6-1 – COD, Ammonia and Turbidity values for background monitoring of the porous pots.

Parameter	COD[a](mg.l^{-1})				Ammonia[b](mg.l^{-1})				Turbidity[c](NTU)			
	Mean	Range	Standard deviation	n	Mean	Range	Standard deviation	n	Mean	Range	Standard deviation	n
Pot 1	60.57	14 - 260	38.9	86	1.7	0.07 – 22.4	3.7	52	5.17	0.56 – 69.4	11.4	44
Pot 2	57.91	6 - 318	41.0	87	1.5	0.1 – 27.2	4.0	52	5.73	0.7 – 80.4	13.1	44
Pot 3	60.71	14 – 176	33.1	86	2.3	0.08 – 35.8	6.1	52	5.68	0.58 - 97.4	14.9	43
Pot 4	62.76	14 – 174	30.0	87	1.7	0.14 – 24.9	3.9	52	5.10	0.7 - 79.4	12.0	44
Pot 5	59.93	8 – 266	37.6	87	1.5	0.2 – 24.3	3.6	52	5.65	0.49 - 111	16.7	44
Pot 6	59.72	11 – 242	37.4	87	1.5	0.15 – 22.8	3.5	52	5.77	0.66 - 43.1	9.7	44
Feed	344.1	132 - 1172	189.2	85	24.5	12.7 – 36.8	5.7	49	129.0	22.1 - 409	67.2	44

[a] Effluent COD except for feed. [b] Effluent ammonia except for feed [c] Effluent turbidity except for feed

Table 6-2 – MLSS, MLVSS and particle size values for background monitoring of the porous pots.

Parameter	MLSS(g.l^{-1})				MLVSS(g.l^{-1})				Particle Size (d_{50})			
	Mean	Range	Standard deviation	n	Mean	Range	Standard deviation	n	Mean	Range	Standard deviation	n
Pot 1	4.91	0.76 – 11.48	2.4	85	4.20	0.07 – 10.17	2.0	81	169	99.1 – 230.4	49.6	11
Pot 2	5.72	0.69 – 15.23	3.4	85	4.91	0.05 – 13.3	2.9	80	171	89.9 – 220.0	41.7	11
Pot 3	5.64	0.57 – 13.61	2.8	85	4.90	0.49 – 11.74	2.4	79	169	99.1 – 230.4	49.6	11
Pot 4	5.27	0.37 – 10.73	2.5	85	4.58	0.01 – 9.41	2.2	80	188.4	96 – 268.2	59.7	11
Pot 5	5.78	0.94 – 15.82	3.2	85	4.95	0.02 – 13.63	2.8	80	179.8	117.6 – 243.7	49.3	11
Pot 6	5.93	0.63 – 13.01	2.8	84	5.06	0.05 – 10.85	2.4	80	153.21	117.87 – 177.33	20.48	11

Table 6-3 – Conductivity and pH values for background monitoring of the porous pots.

Parameter	Conductivity(µS.cm^{-1})				pH			
	Mean	Range	Standard deviation	n	Mean	Range	Standard deviation	n
Pot 1	733.6	584 – 1060	117.4	43	6.3	4.61 – 7.54	0.7	48
Pot 2	715.5	581 – 997	107.1	43	6.3	4.62 – 7.11	0.6	48
Pot 3	740	592 – 1156	132.6	43	6.2	4.57 – 7.08	0.7	48
Pot 4	730.7	563 – 1035	113.0	43	6.3	4.75 – 7.09	0.6	48
Pot 5	722.5	575 – 1015	111.7	43	6.3	5.01 – 7.23	0.6	48
Pot 6	751.2	589 - 1113	156.4	43	6.3	5.14 – 7.42	0.5	48

The variation of the control pots was fairly large over the 18 months that they were monitored at steady state. This is a reflection of the variation in the sewage feed that was used for the pots and shows the variation of the parameters as a result of this. The outlying values can be accounted for by system failures (i.e. air or sewage failure) so on the whole the pots were consistent and any changes that were seen in the dosing trials can be attributed to the addition of toxins rather than the "steady state" variation of the pots themselves. As neither air nor sewage failures occurred during the dosing trials these can be ruled out as having caused the changes seen in the parameters monitored.

A plot of the cumulative frequency curves for the effluent parameters illustrates the robustness of the system and the spread of the data. For the effluent COD over 80% of the values are ≤100 mg.l^{-1} the limit for the Aquarec guidelines. One of the pots managed to attain this for 100% of measurements taken (Figure 6-1).

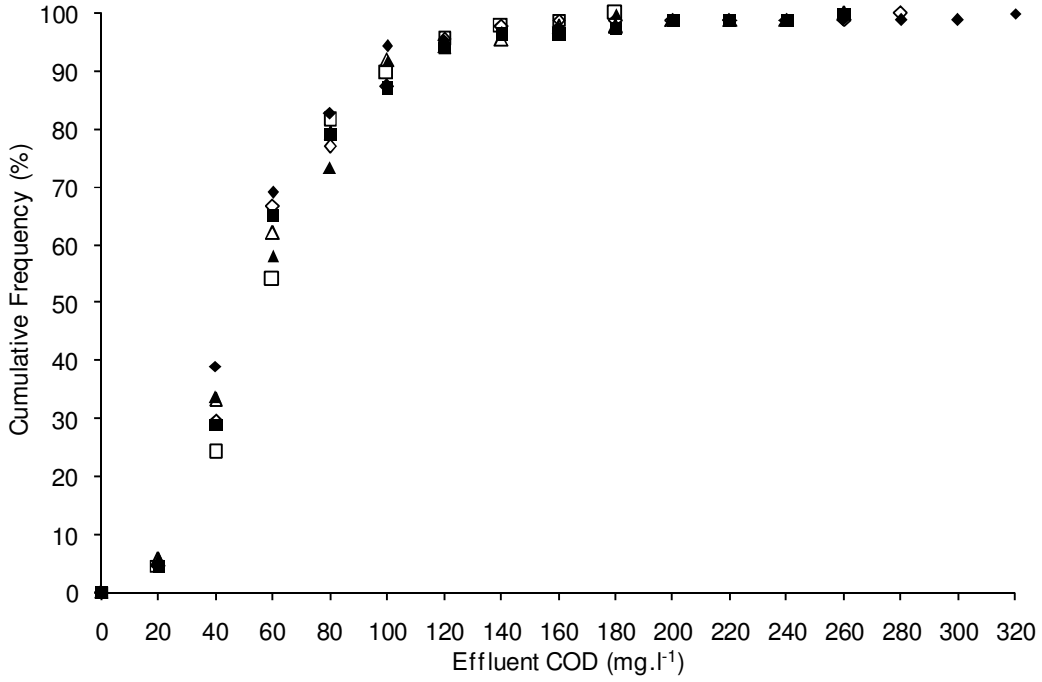


Figure 6-1 – Cumulative frequency curves for the six porous pots for effluent COD (dashed line represents Aquarec reuse guidelines).

Both the ammonia and turbidity curves show the effect of a few outliers where >90% of the data is less than 10 $mg.l^{-1}$ for effluent ammonia and 90% of the effluent turbidity samples are <8 NTU but there are a couple of points that disrupt this hence the large standard deviations for these parameters (Figure 6-2 and Figure 6-3).

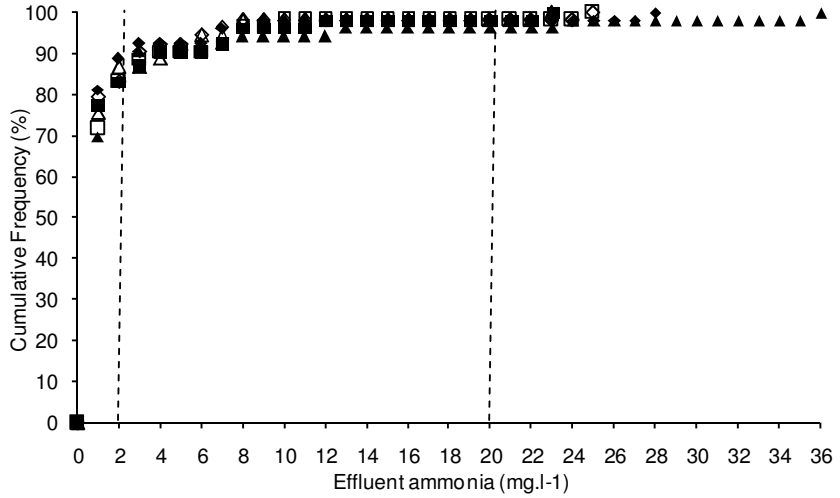


Figure 6-2 – Cumulative frequency curves for all six pots for effluent ammonia (dashed line represents Aquarec guidelines for Class 1 Private, urban and irrigation use).

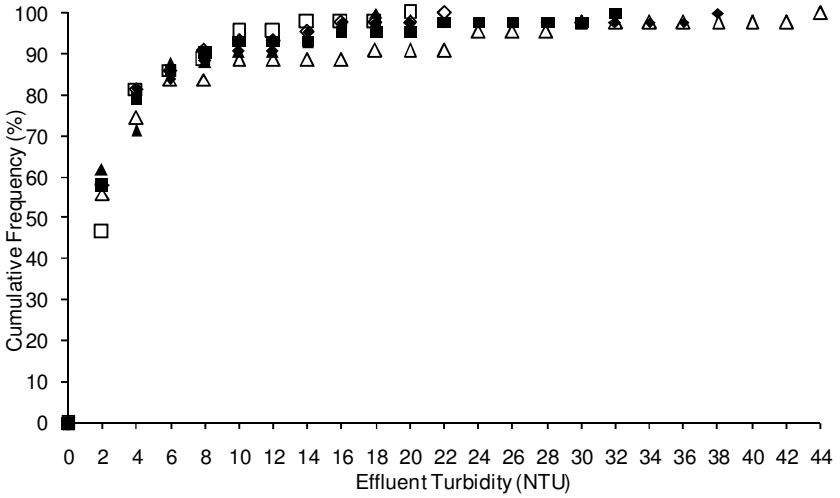


Figure 6-3 – Cumulative frequency curves for all six pots for effluent turbidity.

It was important to ascertain that the pots were behaving similarly so that when dosing with toxins any variation observed could be attributed to the toxin itself rather than the specific pot it had been dosed into. ANOVA analysis of the data (95 % confidence interval) proves that each of the pots is functioning similarly

and that each has reached a steady state that is the same as the others. In this instance the p-value is >0.05, the α value, making the F-ratio small, for all parameters, and acceptance of the hypothesis that all the means are equal, regardless of the pot sampled from (Table 6-4).

Table 6-4 - ANOVA results for all parameters.

Parameter	*F ratio*	*p value*
COD	0.16	0.976
Ammonia	0.29	0.92
Turbidity	0.64	0.669
MLSS	1.51	0.185
MLVSS	1.37	0.234
pH	0.15	0.979
Conductivity	0.45	0.816

6.3 Results for acute toxicity experiments

6.3.1 Shampoo

The shampoo was dosed at the EC_{50} value from respirometry of 36 $ml.l^{-1}$. It immediately became apparent that the limiting factor for the biological process was severe foaming rather than toxicity. At this dose the foaming was not containable and even though the pots are small with a capacity of only 3.8 litres the foaming was substantial (Figure 6-4 to Figure 6-6).

The severe foaming made it impossible to obtain uncontaminated samples, due to the set up of the pots, as effluent samples were taken from a point at the front of the pot where the effluent overflowed to drain. Biomass was carried by the foam and forced out of the pots severely limiting the population. The foaming had greatly subsided after 12 hours but a lot of biomass had been lost in this time. The initial MLVSS was an average of 4.9 $g.l^{-1}$ for the two test pots, after 12 hours this had dropped to an average of 1.66 $g.l^{-1}$ due to the overflow action of the foaming.

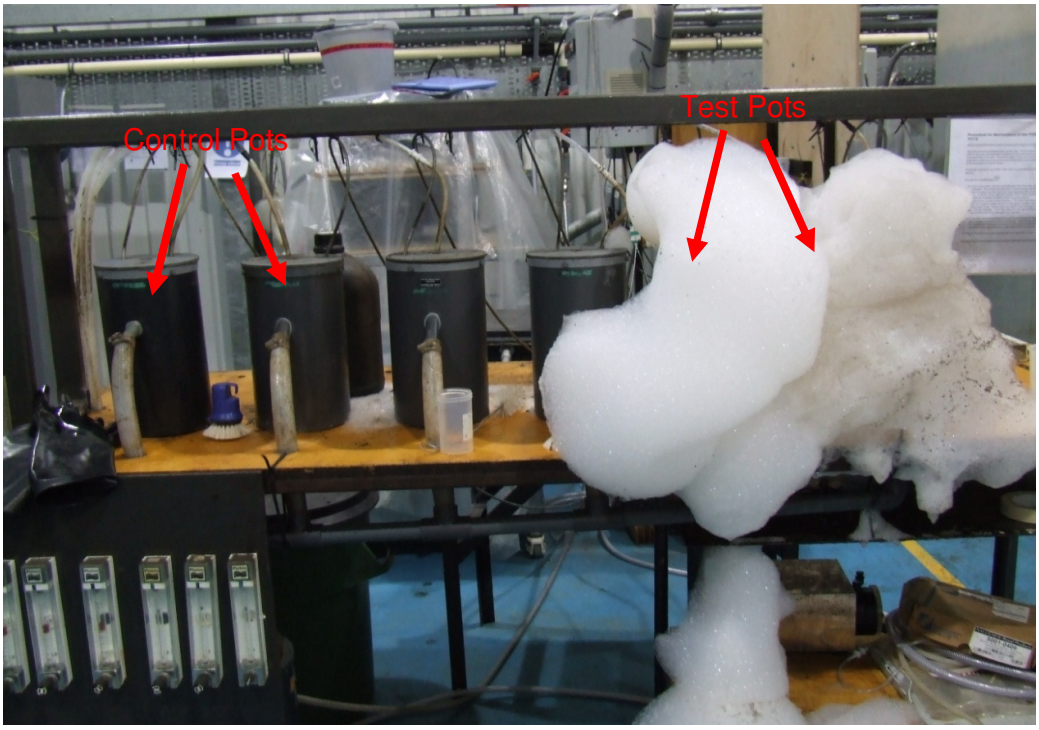


Figure 6-4 – Control and test pots for shampoo dosing.

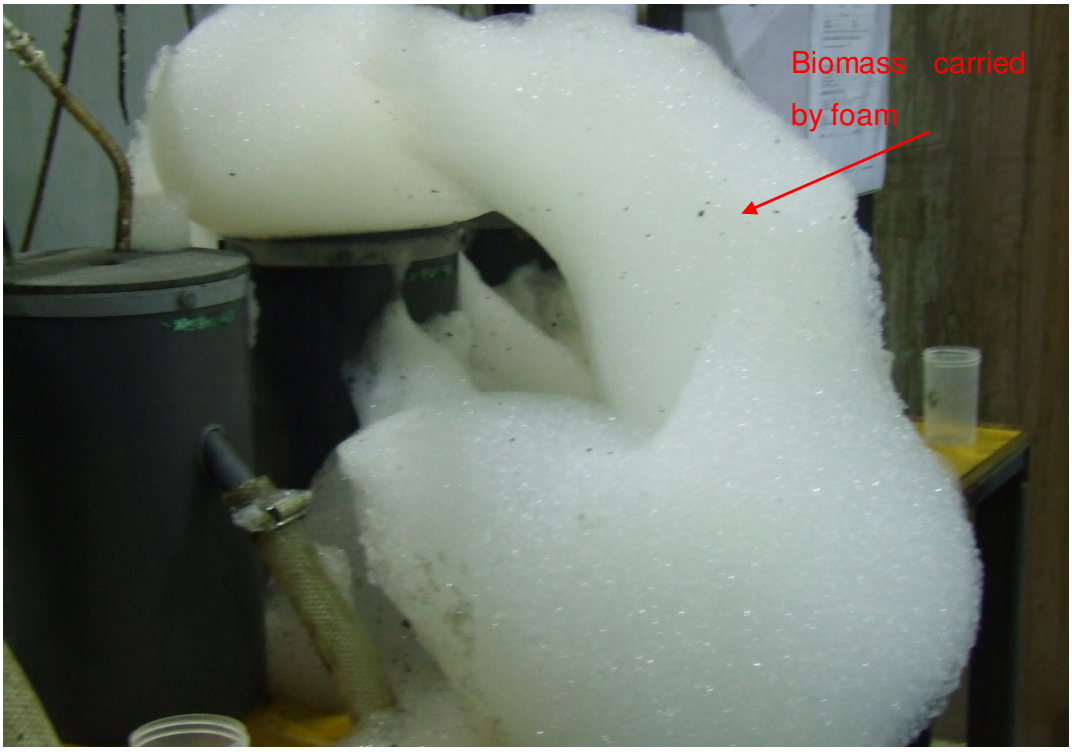


Figure 6-5 – Loss of biomass during foaming with shampoo.

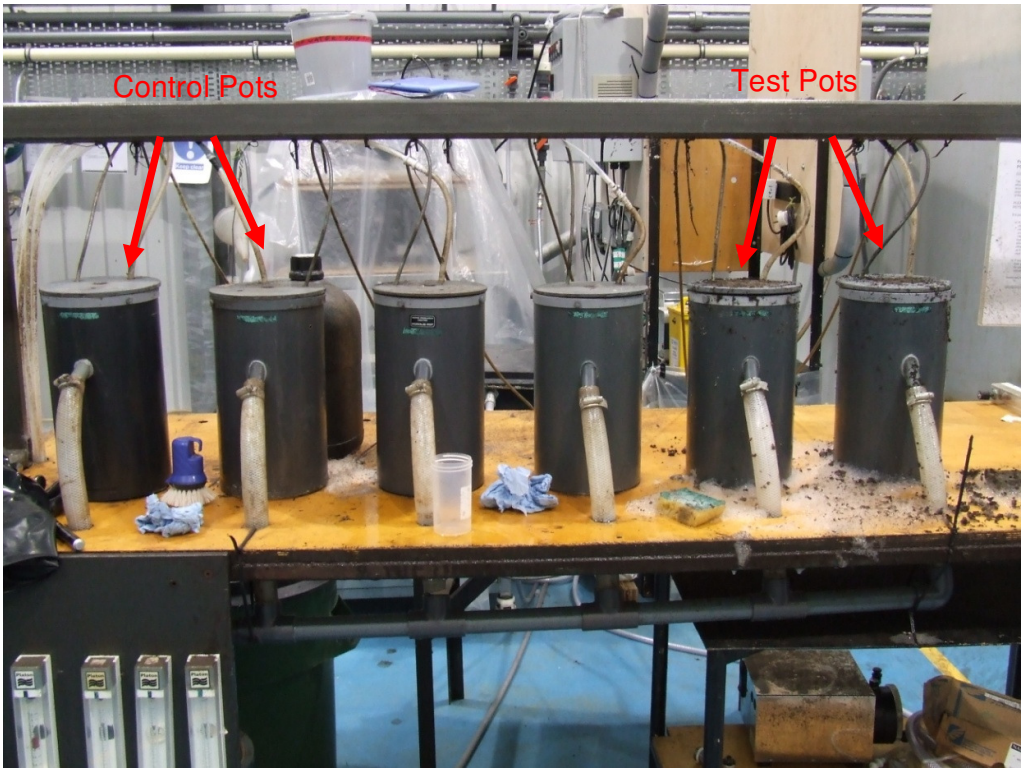


Figure 6-6 – Effects of foaming 12 hours after dosing.

As a result of this all other foaming toxins were subjected to a foaming trial which consisted of adding a solution of each toxin to a beaker of biomass aerated at the same rate as the pots until foaming occurred. The concentration that produced foam was noted and each toxin was dosed below this level. This was done for shower gel, washing powder and sodium dodecyl sulphate (SDS) considerably reducing the dose compared to the EC_{50} obtained by respirometry. Before dosing into the pots it was already apparent that the limiting factor with these toxins was not the acute toxicity but the foaming potential.

6.3.2 Doses of toxins used

Table 6-5 lists the doses used for the porous pot acute toxicity experiments. After the preliminary dosing with shampoo (results given separately in Section 6.3.1) and the effects of the foaming that were observed, all foaming products were reduced to a dose below their foaming point. The other three toxins were all dosed at or close to the EC_{50} levels obtained by respirometry.

Table 6-5 – volume of toxins dosed for acute toxicity experiment using the porous pots.

Toxin	*EC_{50} by respirometry*	*Actual amount dosed*
Shampoo	36ml.l^{-1}	36ml.l^{-1}
Shower gel	38.5ml.l^{-1}	1ml.l^{-1}
Washing powder	1.2g.l^{-1}	0.5g.l^{-1}
Bleach[1]	0.48ml.l^{-1}	0.4ml.l^{-1}
SDS	NT	28mg.l^{-1}
Zinc sulphate	85mg.l^{-1}	85mg.l^{-1}
Phenol	525mg.l^{-1}	525mg.l^{-1}

[1]Hypochlorite based household bleach

6.3.3 Effluent Analysis

6.3.3.1 Hydraulic characteristics of the pots

It was assumed that the porous pot were ideal completely mixed reactors due to the vigorous aeration. For an ideal completely mixed reactor a non reactive tracer, that completely and instantaneously disperses, injected as a spike dose, would affect the effluent of the system in a negative exponential curve (Figure 6-7). τ is the HRT of the system, in this case 6 hours and the concentration of the tracer is given as 1 for the initial concentration, C_0 (Tchobanoglous *et al.*, 2003).

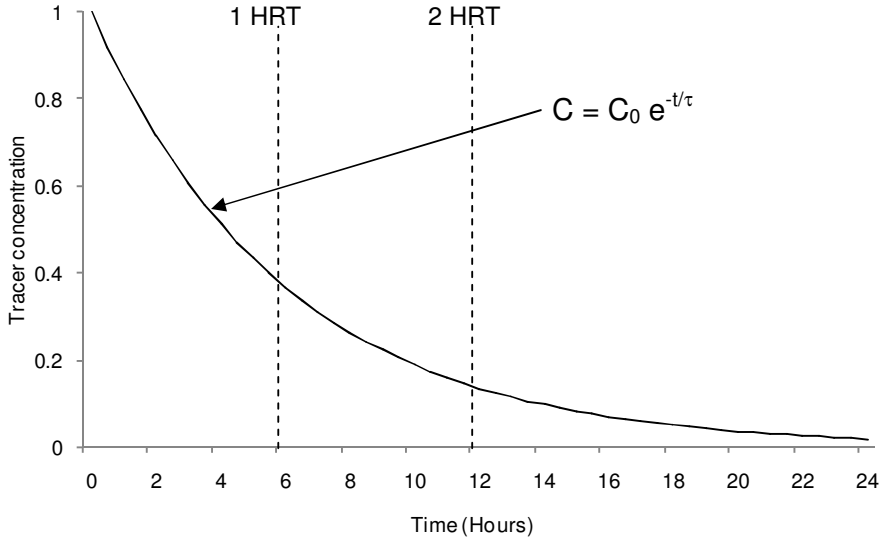


Figure 6-7 – Effluent tracer concentration for an ideal completely mixed reactor

For example, the phenol dosing had an initial COD concentration of 1247 $mg.l^{-1}$, if there was no interaction with the biomass and no contribution of COD from any other source then due to dilution at 1 HRT the effluent COD concentration would be 499 $mg.l^{-1}$. For any of the parameters tested if the effluent profile is different from that shown above it can be assumed that there has been some interaction with the contents of the bioreactor.

6.3.3.2 Effluent Chemical Oxygen Demand

The effluent chemical oxygen demand (COD) measurements were normalised to the control pots to ensure that the response seen was only for the shock loading of COD from the toxins dosed. As all the pots were fed from the same feed reservoir it was assumed that the normalised response seen was due only to the shock loading of the toxin and any other effects were also seen in the control pots which would be accounted for in the normalisation. The shock dose of COD varied for each of the toxins (Table 6-6).

Table 6-6 – COD shock dose from each toxin

Toxin	*COD (mg)*
Bleach	167
Shower gel	1835
Washing powder	566
Phenol	4739
Sodium Dodecyl Sulphate	452
Zinc sulphate	170

Two distinct responses to the spot doses were observed for effluent COD. Bleach, SDS and zinc sulphate had no discernable effect on the effluent COD with no change greater than 20 $mg.l^{-1}$ compared to the control pots (Figure 6-8 and Figure 6-9). However, washing powder, shower gel and phenol showed a rapid increase to a peak at 1 HRT (6 hours) followed by a gradual decline over the 4 HRTs (24 hours) monitored, without returning to control pot levels.

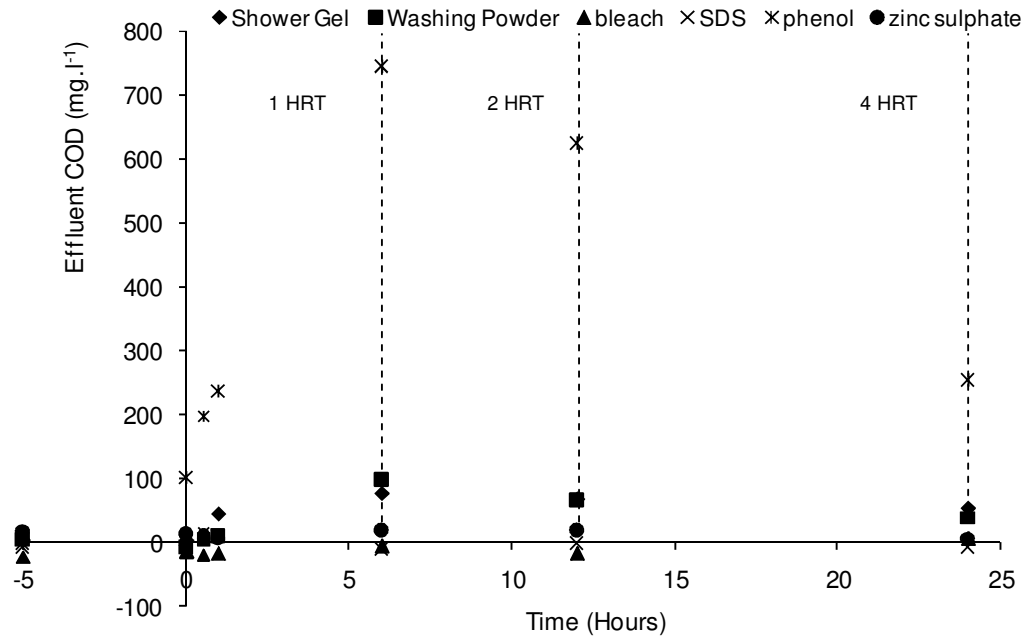


Figure 6-8 –Effluent COD vs time for all six toxins tested normalised to their respective controls

In the case of the phenol, a shock load of 4739 mg of COD (Table 6-6) was applied at time t = 0 (Figure 6-8). The total normalised effluent COD over the 24 hours monitored was 7530 mg COD meaning that 2791 mg COD was released as a result of the action of the phenol on the biomass. The origin of the COD could be a mixture of soluble and insoluble COD. This is clearly different from the effluent concentration expected from an ideal reactor with a non reactive tracer (Section 6.3.3.1) and it can be assumed that the phenol interacts with the biomass. The phenol dose has had an extreme effect on the COD removal of the system and would result in system failure.

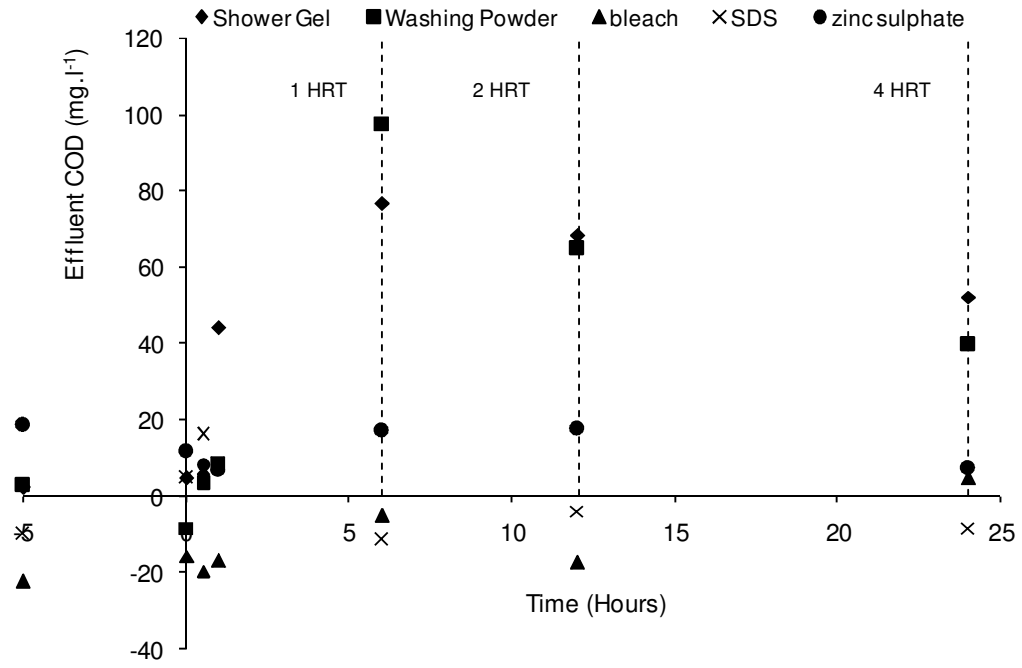


Figure 6-9 – Effluent COD excluding phenol normalised to their respective controls

The washing powder dose resulted in a shock load of 566 mg COD with a resulting 759 mg COD in the effluent, a positive difference of 193 mg COD. The effluent turbidity showed an increase indicating degreasing and separating dirt through surfactants is the most likely action here, with an increase in colloidal particles in the effluent. The evidence does not suggest any toxic action as the other parameters monitored do not show that the biomass was harmed in any way.

Similarly the shower gel produced a shock load of 1832 mg COD, however, the effluent COD over the 24 hours monitored was 880 mg with a removal rate of 52%. Although there is evidence that the biomass has been disturbed there is clearly a part of the community that is still functioning and removing substrate. This indicates true inhibition or kill of a part of the biomass, which is unexpected as the dose for shower gel was 2.5% of the EC_{50} value (1 $ml.l^{-1}$ dosed compared with an EC_{50} by respirometry of 38.8 $ml.l^{-1}$).

6.3.3.3 Effluent Ammonia

None of the products contributed to the ammonia loading. Neither the sodium dodecyl sulphate nor the zinc sulphate had any adverse effects on the effluent ammonia with normalised readings for SDS being a maximum of 0.52 $mg.l^{-1}$ difference and zinc sulphate being a maximum of 1.33 $mg.l^{-1}$ different from the control.

Most notable amongst the effluent ammonia results are those of the shower gel and phenol. Both were constant for the first hour of sampling with levels of 1.3$mg.l^{-1}$ above the control for shower gel and a gradual increase for phenol to just 0.34$mg.l^{-1}$ above the control. At the six hour mark, however, both had increased to over 6 $mg.l^{-1}$ above their respective controls. This gap widened at the 12 hour sample to phenol being at 11.24 $mg.l^{-1}$ above and shower gel to being 8.6 $mg.l^{-1}$. The effluent ammonia for the phenol dosing peaked at this point whereas the shower gel increased again at 24 hours to 11.05 $mg.l^{-1}$ above the control. This gave an absolute effluent ammonia of 13.96 $mg.l^{-1}$. Influent ammonia levels were around 30 $mg.l^{-1}$ for the shower gel giving a minimum removal rate of 53 %. Influent levels were 16.4 $mg.l^{-1}$ for phenol, resulting in the lowest removal rate being just 31%.

It is well known that nitrifiers are more sensitive than other parts of the microbial community (Pagga *et al.*, 2006) and these two dosing experiments demonstrate inhibition of ammonia removal and both show that it takes longer than 4 HRTs for the system to return to pre dosing levels. The lag in reaction time for the shower gel indicates that parent compounds present were degrading into more toxic byproducts (Petersson *et al.*, 2000).

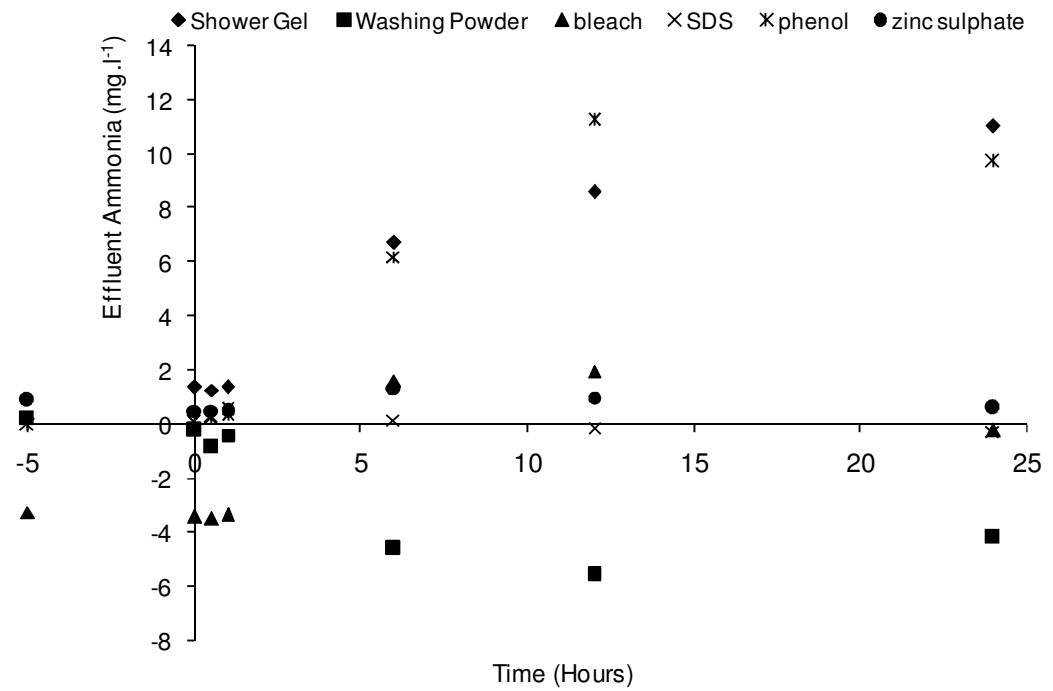


Figure 6-10 – effluent ammonia for all six toxins normalised to their respective controls

In contrast the washing powder dose reduced the effluent ammonia level compared to the control. Again this effect is most prominent 6 hours after the spot dosing, peaks at 12 hours and is still evident after 24 hours. In addition to this, the influent ammonia at the beginning of the trial was above the average recorded during baseline monitoring at 41.5mg.l^{-1}. The washing powder has increased the pH of the system from 6.1 to 7 immediately after dosing (Section 6.3.4.4) which is the optimum operating pH for nitrifying bacteria (Tchobanoglous *et al.*, 2003) and can be seen in the increase in ammonia removal in the test pots to 94 % compared to the control pots removal rate of 80 %.

The bleach dosing is the only trial where the toxin transits the system within the 24 hours and returns to close to steady state. However, as there is not a marked contrast between control and test pots for the bleach trial, this is most likely to be due to environmental factors rather than true inhibition of the

nitrifying bacteria. The test pots went from an effluent level of around 4mg.l^{-1} until an hour after dosing to 8mg.l^{-1} after 6 hours so a doubling of effluent ammonia when the pH was between 5.7 and 6.2. The control pots were also showing a higher than usual effluent ammonia of around 7mg.l^{-1} during most of the trial with a low pH of 6.5 but had decreased to 0.73mg.l^{-1} by the end of the 24 hour period when the pH was at 7, the optimum for the nitrifying bacteria as mentioned above.

6.3.3.4 Effluent Turbidity

The effluent turbidity was affected differently by all six of the toxins (Figure 6-11) and there was no apparent pattern to the effects, in contrast to the effluent ammonia. The sodium dodecyl sulphate seems to have had no significant effect on the effluent turbidity at all and the zinc sulphate dose, although the effluent turbidity for the test pots was high to begin with compared to the controls, steadily decreases over the 24 hour period until it is, to all intents and purposes, the same as the control. These effects are most likely to be the influence of external environmental factors rather than the toxin dosing.

The bleach also had little effect on this parameter for the first 12 hours of sampling but then surprisingly there is an increase after 24 hours. It is, however, difficult to determine whether this can be attributed to the bleach dose or some other environmental factor in the pots.

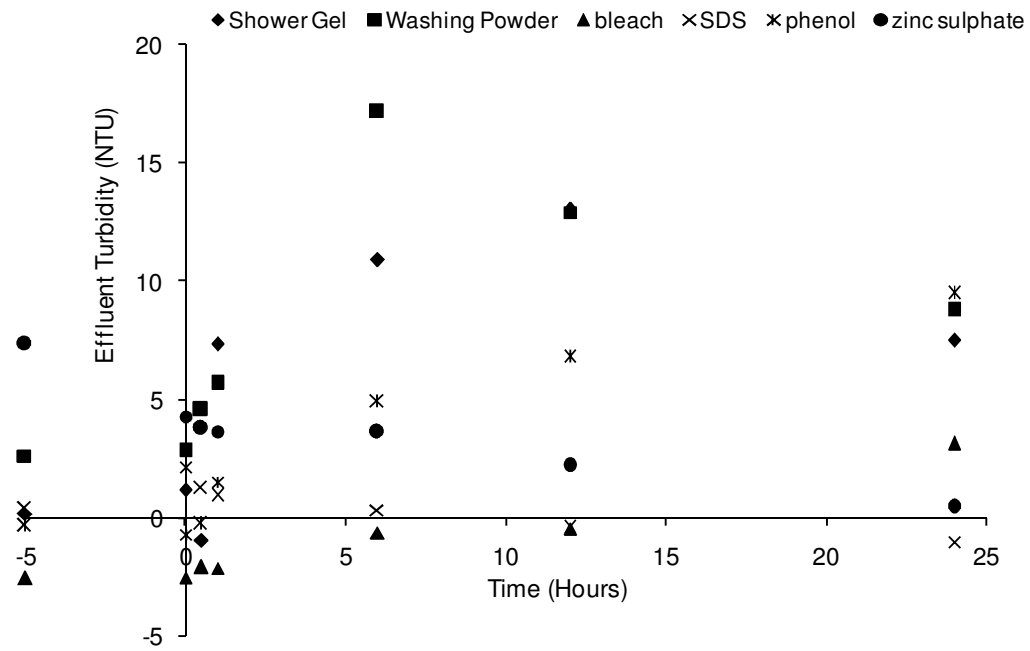


Figure 6-11 – Graph of effluent turbidity for all six toxins tested normalised to their respective controls

The washing powder and shower gel showed an immediate increase in the effluent turbidity after dosing; from 2.82 NTU immediately after dosing to 4.53 at 0.5 hours for washing powder and from -1 NTU at 0.5 hours to 7 NTU after 1 hour for the shower gel. Both these products are predominantly surfactants (Appendix A) and are designed to separate dirt and hold it in aqueous solution to be rinsed either from the washing machine or in the process of showering (Madsen *et al.*, 2001). The increase in effluent turbidity was due to colloidal particles being held in solution and being washed from the system also reflected in the increase in effluent COD. In the case of shower gel this is exacerbated by the action of the essential oils.

The effluent turbidity as a result of the phenol dosing was still increasing after 24 hours. This appears to be true toxicity of the biomass present but whether this is due to the action of the phenol or a lack of oxygen present in the system due to the organic overload produced by the phenol dose is impossible to deduce (Kim *et al.*, 2006).

6.3.4 Biomass Analysis

6.3.4.1 Capillary Suction Time.

Three different responses were observed for the capillary suction time (CST). Sodium dodecyl sulphate, shower gel and bleach had little impact on the CST readings, phenol and zinc sulphate resulted in an initial increase in the time to filter which returned to pre dose levels after 24 hours and washing powder improved the response compared to the control.

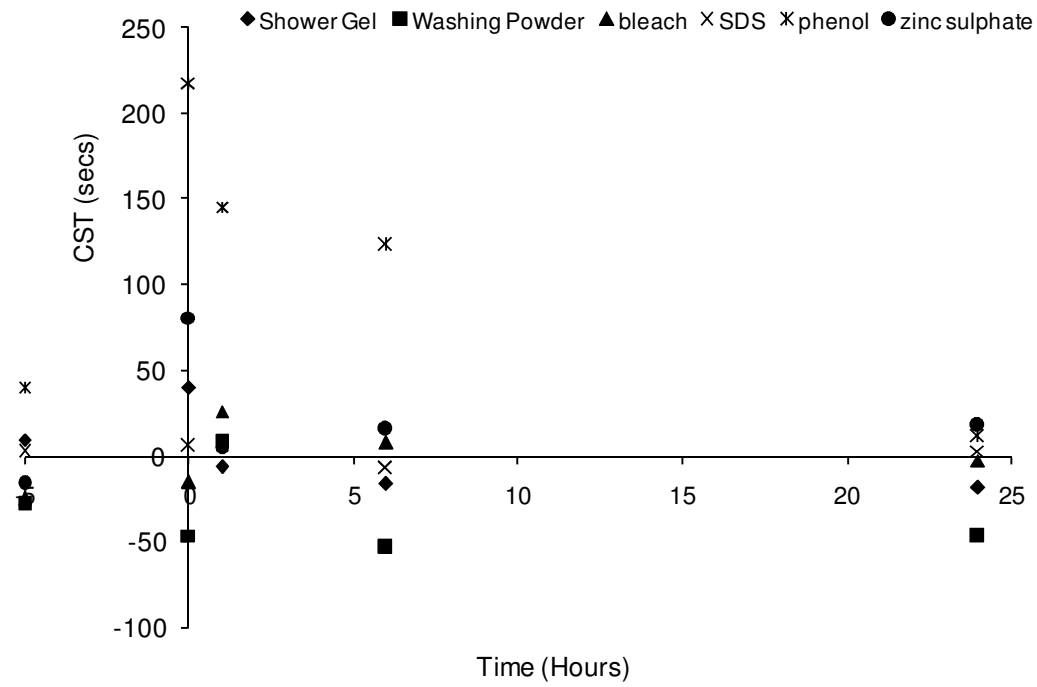


Figure 6-12 – Capillary suction time for six toxins, normalised to their respective controls

The peak CST time measured for the phenol dosing was immediately after dosing which indicates that the source of this is not from colloidal material as it is not registered as effluent turbidity or effluent COD at this time. Both effluent turbidity and COD have a peak at 12 and 6 hours respectively. It is more likely that the microbial products excreted by the bacterial community have affected the dewaterability as there is also a peak in SMP turbidity and carbohydrates immediately after dosing (Section 6.3.5.1 and 6.3.5.2).

The surfactants present in the washing powder are likely to interact with the capillary action on the filter paper used for the test, resulting in lower readings. Interestingly, the shower gel did not produce any difference in readings even though the MLVSS of the pots decreased markedly in the first hour (Section 6.3.4.1) and CST is normally linked to MLVSS concentration; the higher the MLVSS concentration the longer the CST (Houghton *et al.*, 2001).

6.3.4.2 Mixed Liquor Suspended Solids (MLSS) and Mixed Liquor Volatile Suspended Solids (MLVSS).

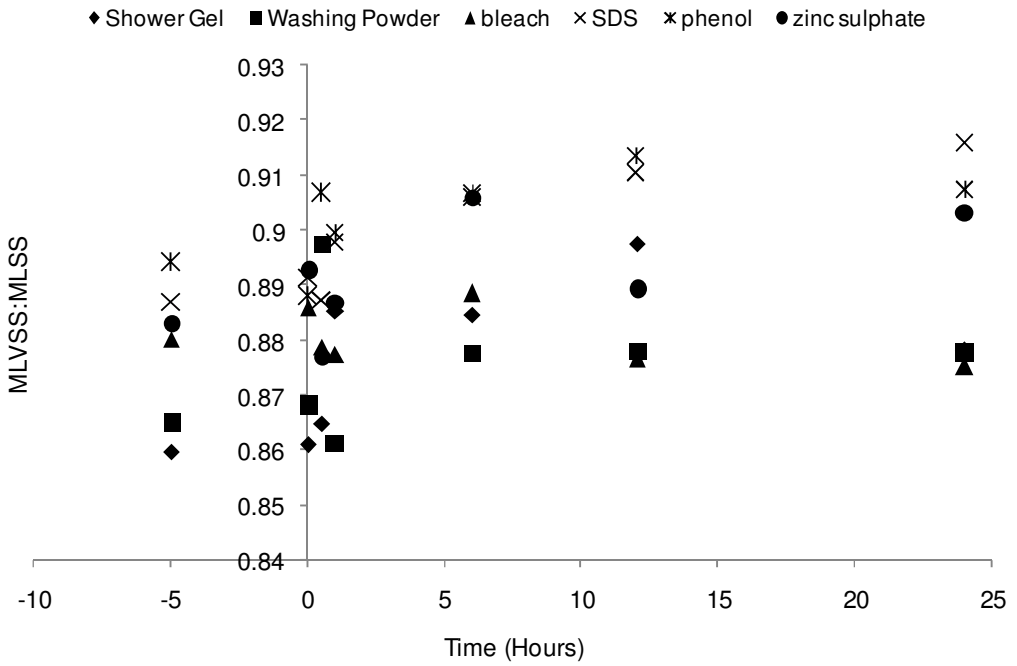


Figure 6-13 – Ratio of MLVSS to MLSS six toxins

The ratio of MLVSS to MLSS stayed broadly the same for washing powder, bleach, phenol and zinc sulphate. The shower gel had an increasing ratio as the MLVSS decreased, as expected.

The sodium dodecyl sulphate, however, had an increasing ratio from 0.89 to 0.92, which indicates that the readily degradeable surfactant was helping to break down other recalcitrant compounds present in the reactor. As there was no wastage from the pots any recalcitrant compounds would eventually be broken down but it would seem that the surfactant either in providing more substrate or changing the nature of these compounds has helped to reduce them over the period monitored.

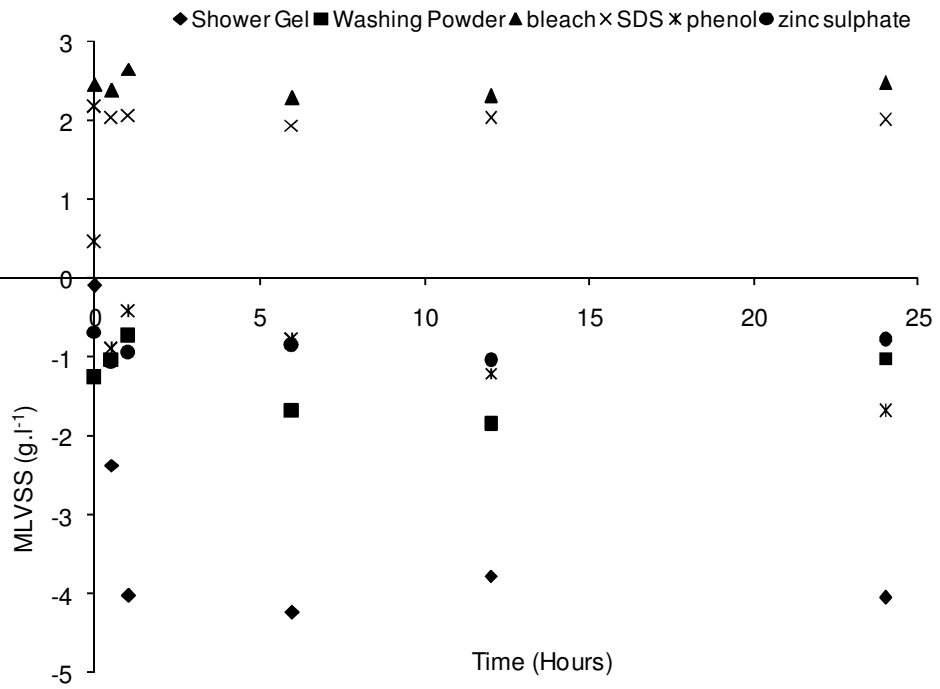


Figure 6-14 – MLVSS for six toxins tested normalised to their respective controls

Zinc sulphate, SDS and bleach had no effect on the MLVSS and all remained at a constant difference from the control pots (Figure 6-14). The most notable toxin to affect the MLVSS was the shower gel (Figure 6-14). There is an immediate reduction on the MLVSS in comparison to the control pots and this continues for the first 6 hours where it stabilises but does not increase again. The initial loss is to 4 $g.l^{-1}$ less than the control pots (Figure 6-15). This is an 82% loss of biomass over an hour and represents a catastrophic event which would impact on the performance of the system as the biomass has been

washed from the system and recovery is impossible (coincident with a large increase in effluent COD and effluent turbidity). This is for a dose that is only 2.5% of the EC_{50} obtained in the respirometry scoping work. The most likely cause of this toxicity is the essential oils present in the particular shower gel used, mint oil (*mentha arvensis*) and tea tree oil (*melaleuca alternifolia*), both of which have been shown to be fungicidal and bactericidal (Mayaud *et al.*, 2008, Kumar *et al.*, 2007).

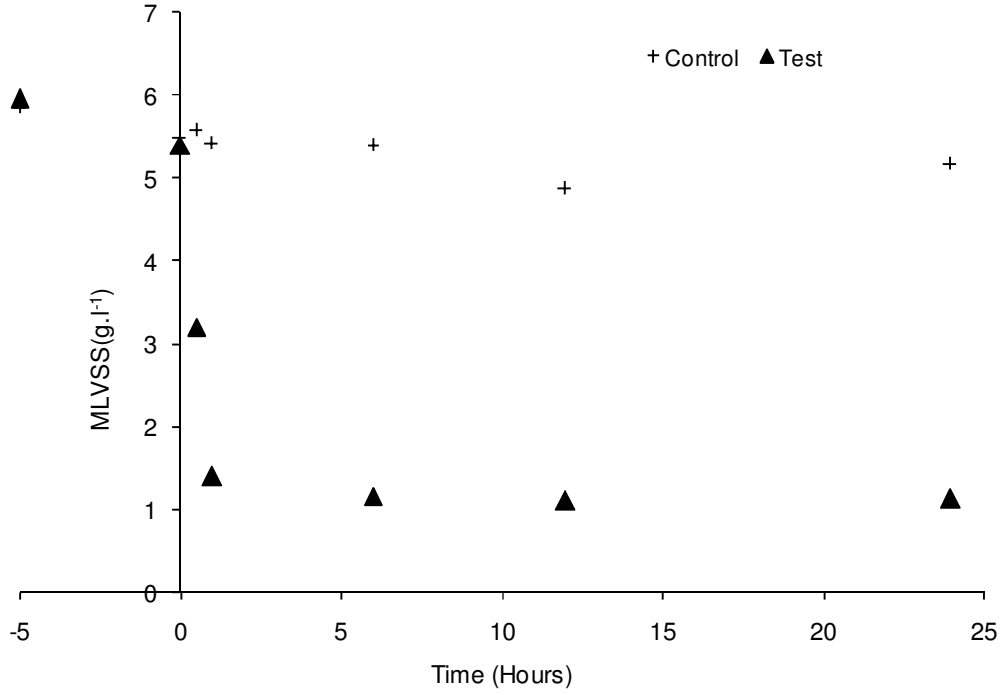


Figure 6-15 – MLVSS, control and test pots, for shower gel 24 hours.

6.3.4.3 Particle Size Distribution

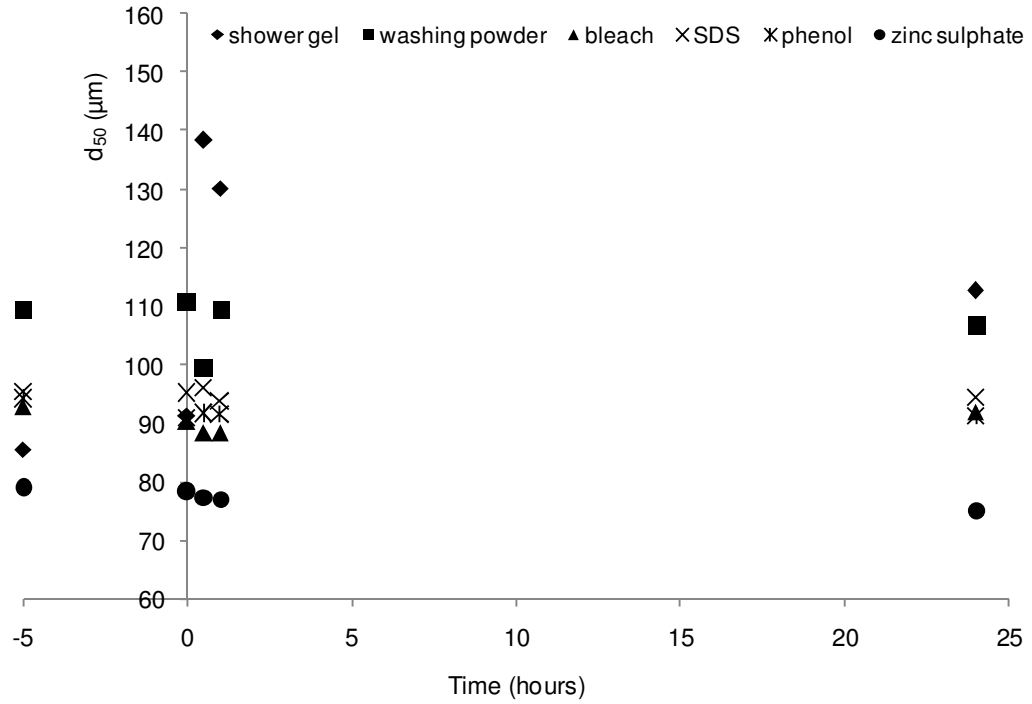


Figure 6-16 – d_{50} values for all six toxins

Only shower gel and washing powder showed any difference for the particle size distribution with the washing powder showing a slight decrease in floc size after 30 minutes followed by a return to pre dosing levels. On the other hand the shower gel caused an increase in the particle size without a return to pre dosing values after the full 24 hours that was monitored (Figure 6-17). It is likely that the shower gel has affected those bacteria that were not in large flocs and these have been washed from the system whereas those in larger flocs have been protected and remain viable (Henriques *et al.*, 2005).

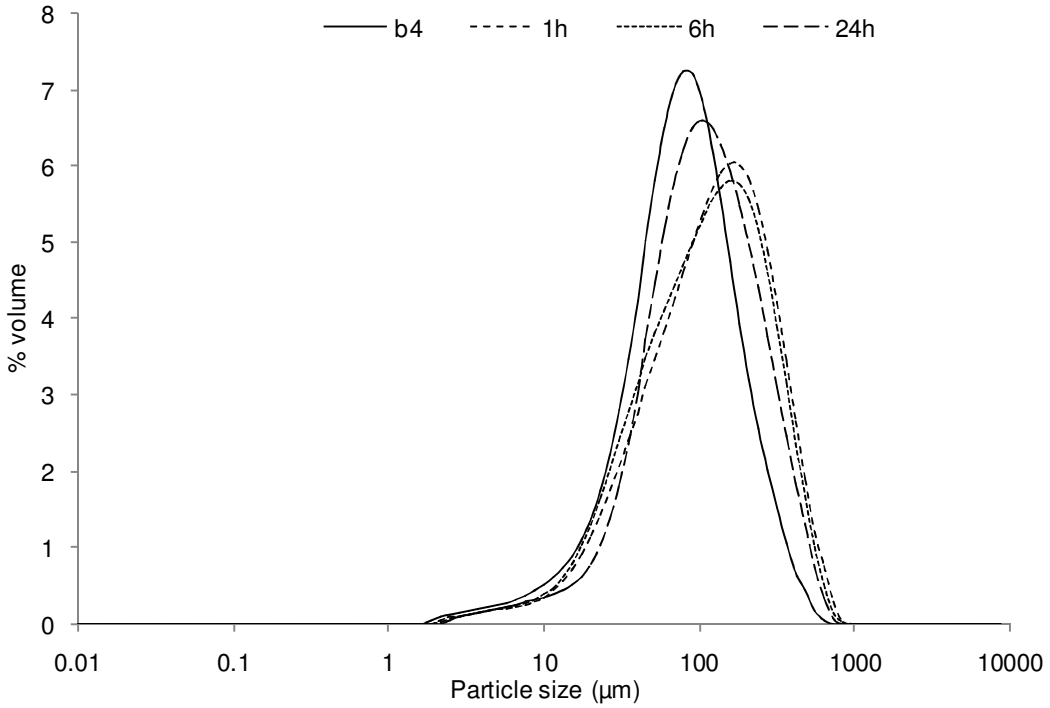


Figure 6-17 – Detailed particle size distribution for shower gel.

6.3.4.4 pH

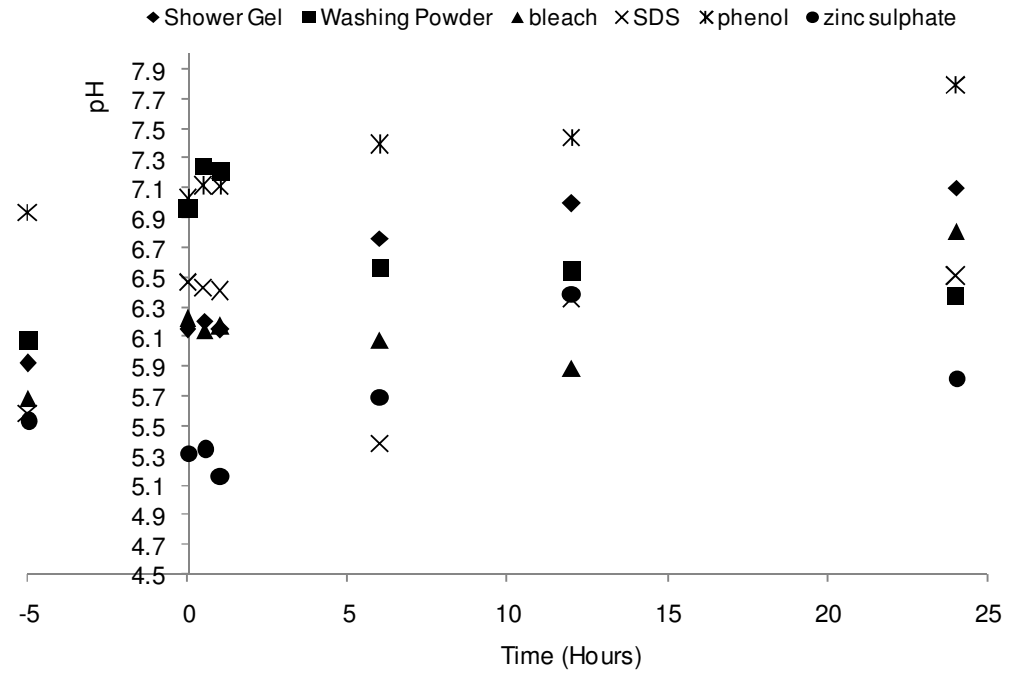


Figure 6-18 – pH of biomass for six toxins.

These results were not normalised against the control pots. Again washing powder, shower gel and phenol had the most noticeable effects on the pH but neither raised the pH above levels that would have a detrimental effect on the biomass (Figure 6-18).

6.3.4.5 Conductivity

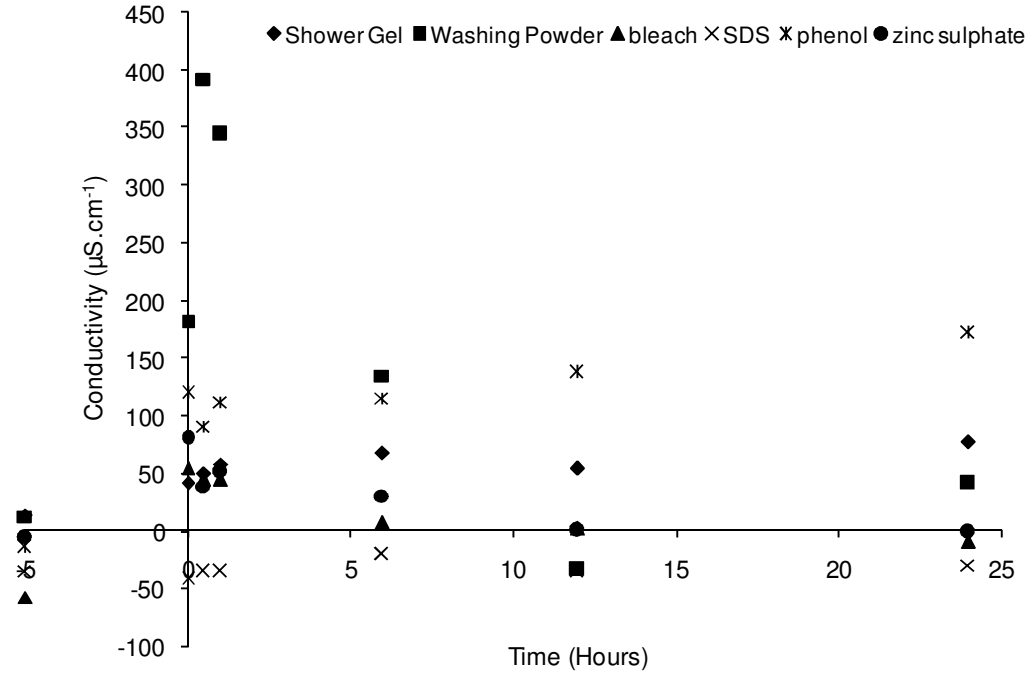


Figure 6-19 – Conductivity of biomass for six toxins normalised to their respective controls.

The sodium dodecyl sulphate had no discernable effect on the conductivity compared to the control pots. The washing powder dose had an immediate and pronounced effect on the conductivity raising the level to over 350 $\mu S.cm^{-1}$ above the control readings within the first hour after dosing. This rapidly diminished again to be around the same as the control by the 12 hour sampling run. On the other hand the phenol dose raised the conductivity to over 100 $\mu S.cm^{-1}$ above the control immediately after dosing and did not reduce this level by the end of the 24 hour trial. In fact the conductivity increased slightly over this period to a final reading of 172 $\mu S.cm^{-1}$ above the control pots.

The shower gel dose produced a steady increase in the conductivity over the 24 hour period to a maximum of 77 $\mu S.cm^{-1}$ over the control from an initial difference of 14 $\mu S.cm^{-1}$ before dosing. The bleach affected the conductivity, in contrast, only over the first 6 hours of the trial giving an increase to 54 $\mu S.cm^{-1}$ immediately after dosing from being 57 $\mu S.cm^{-1}$ below the control before dosing. This increase diminished slightly at the 0.5 and 1 hour samples to 44 $\mu S.cm^{-1}$ before returning to being close to the control pots afterwards. The zinc sulphate produced a very similar response to the bleach with a maximum being experienced immediately after dosing of 80 $\mu S.cm^{-1}$ and returning to being close to the control after 12 hours.

6.3.5 Potential Foulant Analysis

6.3.5.1 Soluble Microbial Product (SMP) Turbidity

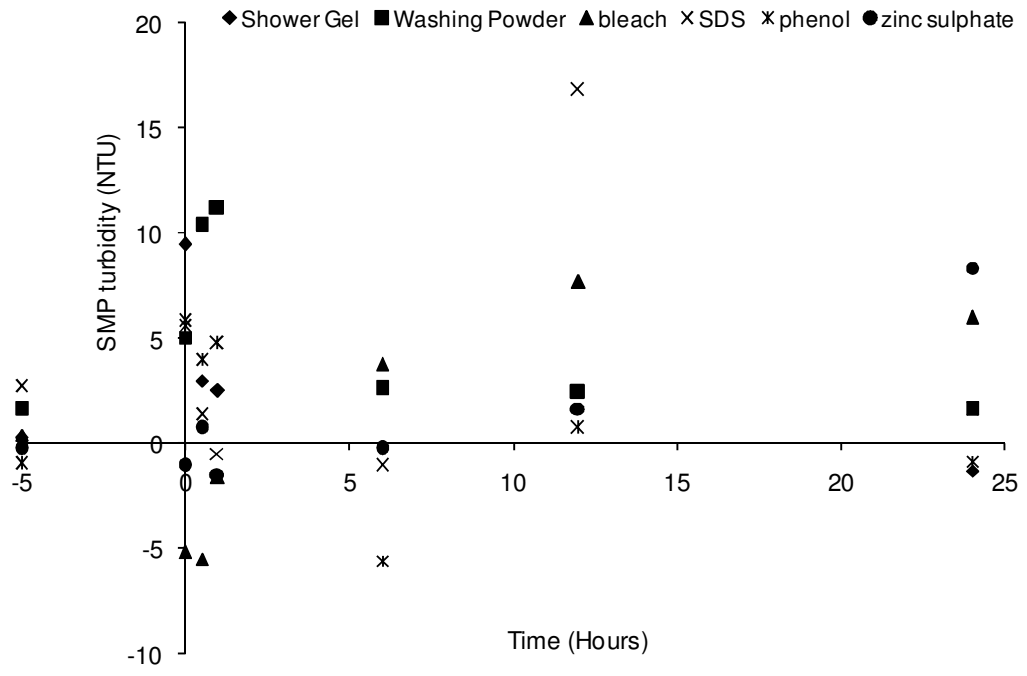


Figure 6-20 – SMP turbidity for six toxins normalised to their respective controls.

The effect on SMP turbidity by the toxins was almost exclusively seen in the first hour after dosing (shower gel, washing powder, SDS and phenol), in contrast to the effluent turbidity where the peaks were observed at 6 hours or greater. The

exceptions were bleach which peaked at 12 hours and zinc sulphate which increased up to the end of the 24 hours observation time. Interestingly, the bleach dose produced a significant decrease in SMP turbidity over the first hour after dosing, with readings of 5 NTU less than the respective control pots but had increased to a maximum of 7.68 NTU above the control after 12 hours.

The zinc sulphate, however produced an inconclusive effect for the first 12 hours of sampling with readings between 1 to 2 NTU above or below the control to then a marked increase at 24 hours to 8.3 NTU above the control. The SDS had a peak at 12 hours with an SMP turbidity of 16.8 NTU above the control.

6.3.5.2 Soluble Microbial Product Proteins and Carbohydrates

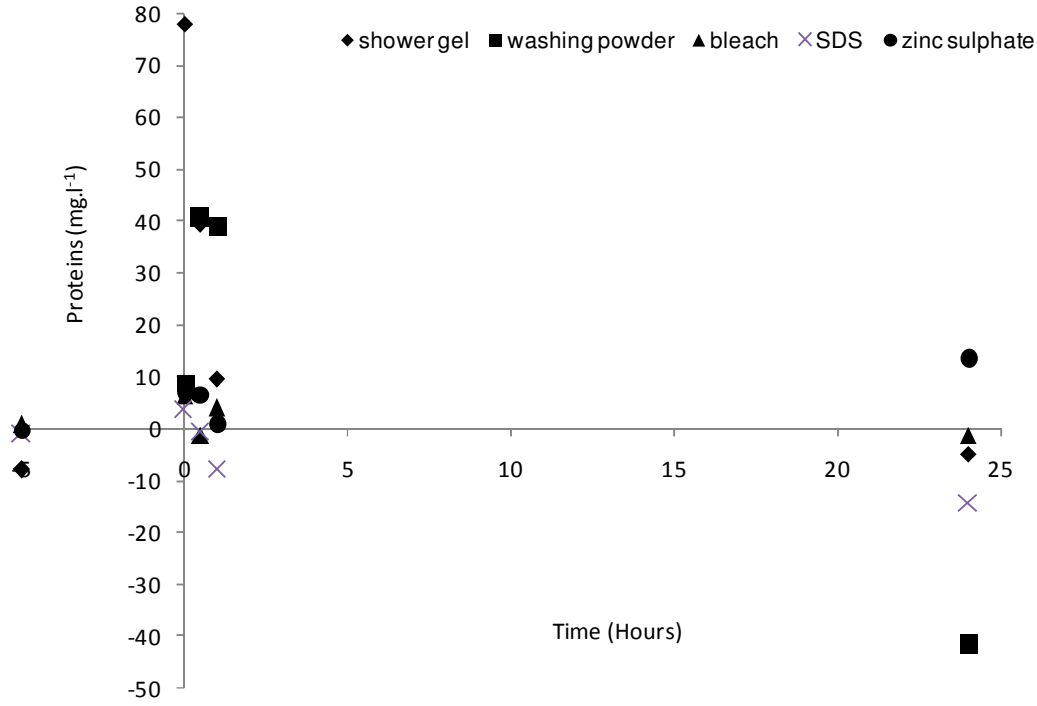


Figure 6-21 – SMP proteins for five toxins normalised to their respective controls.

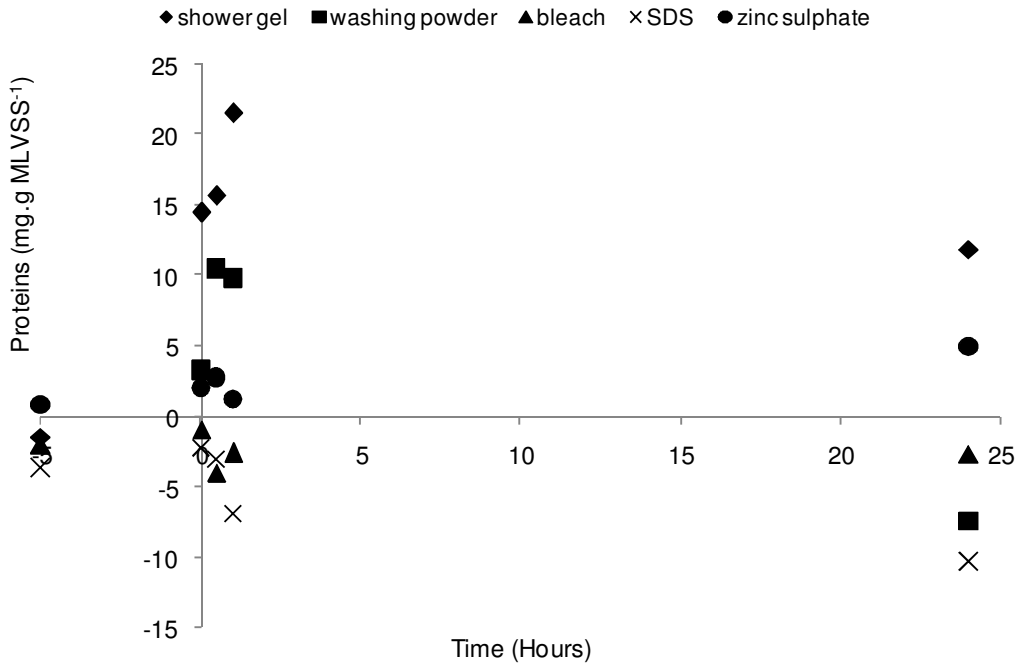


Figure 6-22 – SMP proteins for five toxins normalised to control and to MLVSS.

Following the pattern of the SMP turbidity the SMP proteins for all the five toxins, had a peak immediately after dosing. The phenol dosed samples could not be tested as the phenol interfered with the protein method that is based on phenol addition. Both washing powder and shower gel had the most pronounced effects with shower gel producing an immediate increase of over 70 $mg.l^{-1}$ above the control. This had dropped to less than 10 mg.l-1 difference by the end of the first hour after dosing, however, the amount normalised to the MLVSS increased dramatically as the MLVSS decreased rapidly at this stage of the trial from 5.4 $mg.l^{-1}$ to 1.39 $mg.l^{-1}$ so that each gram of MLVSS was producing 20 mg of protein compared to 5 $mg.gMLVSS^{-1}$ in the control pots. By the end of the 24 hour monitoring period the absolute level of proteins for the pots dosed with shower gel was $5mg.l^{-1}$ less than that of the control pots, however, the MLVSS normalised value was 11.9 $mg.gMLVSS^{-1}$.

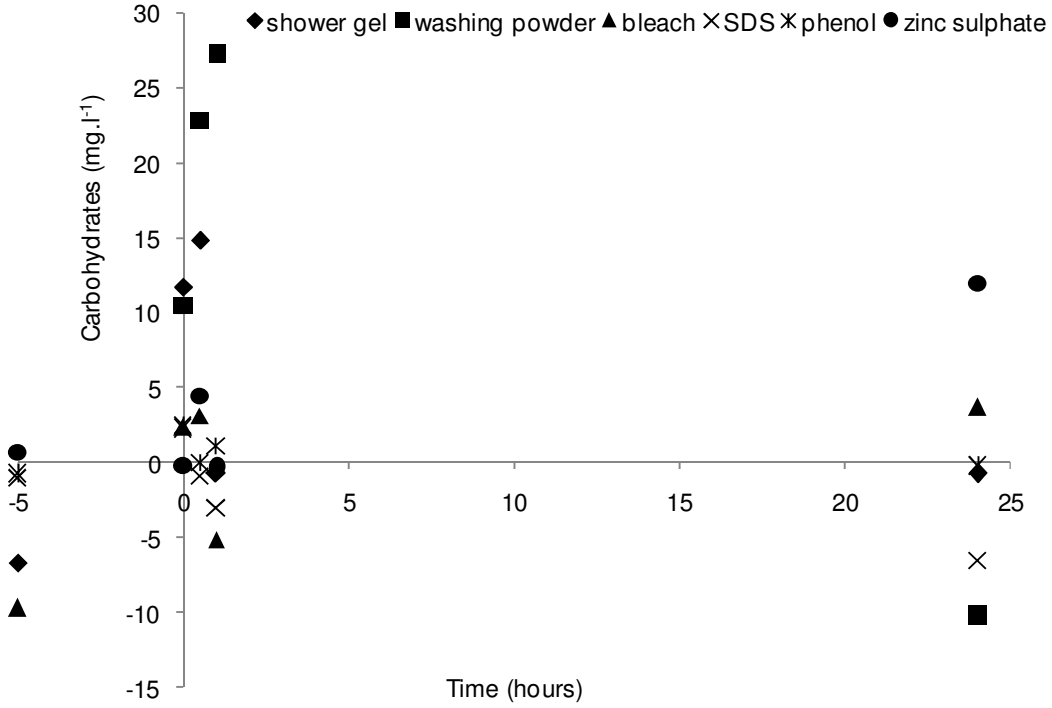


Figure 6-23 – SMP carbohydrates for six toxins normalised to their respective controls.

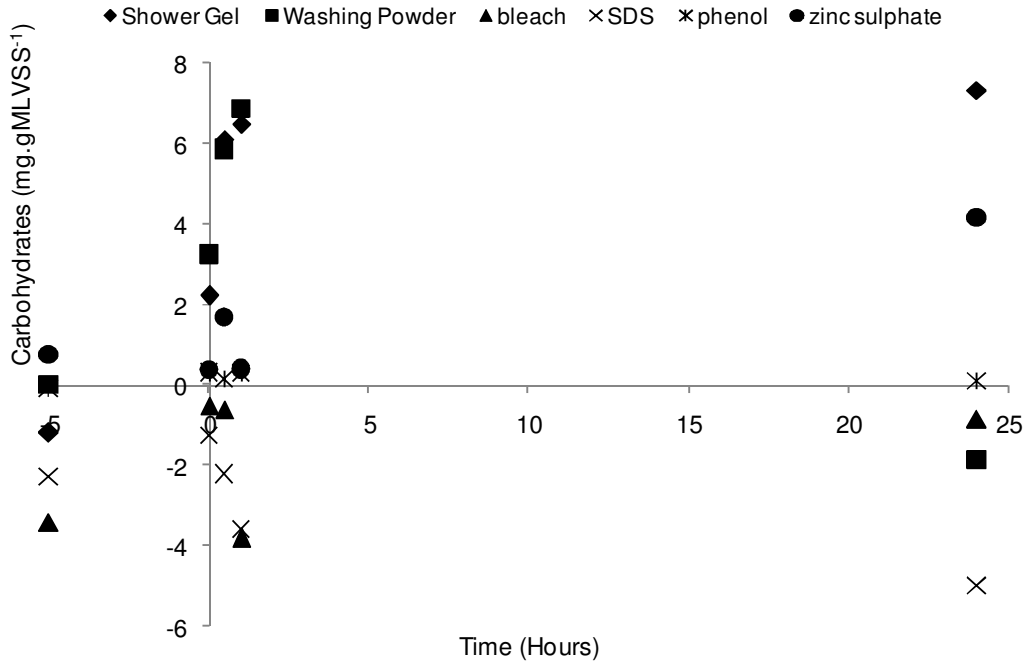


Figure 6-24 – SMP carbohydrates for six toxins normalised to their respective controls and MLVSS.

Similarly the SMP carbohydrates peaked at the hour mark for most of the toxins. SDS was a notable exception where the carbohydrates level increased immediately after dosing and then decreased after 30 minutes. This decrease continued at the hour mark and by 24 hours the reading was 6.5mg.l^{-1} less than the control. Both shower gel and washing powder dosed biomass showed a sustained increase in carbohydrates over the first hour after dosing, reaching maximums of 25.8mg.l^{-1} at 0.5 hours for shower gel and 33.8mg.l^{-1} at 1 hour for washing powder. These were 14.8mg.l^{-1} and 27.2mg.l^{-1} respectively above their controls. The washing powder dosed pots had returned to close to pre dosing levels after 24 hours or 4 HRTs but the effect induced by the shower gel dose was still in evidence after 24 hours.

It is unclear whether the levels of SMP proteins and carbohydrates observed in these trials would cause fouling. There was no provision to monitor any fouling of the membranes used in the porous pots, however, the toxins dosed did provide a change in the parameters measured and could result in fouling in an MBR. SMP carbohydrates have been identified as one of the main parameters associated with fouling (Drews *et al.*, 2006, Lesjean *et al.*, 2005, Rosenberger and Kraume, 2002).

6.4 System performance

The toxins dosed into the porous pots had an effect on the system performance when compared to the control pots. Comparison with the standard compiled in the Aquarec report of 2005 found that the effluent quality was sub standard for several of the toxin dosing trials (Table 6-7). All of the toxins caused a breach in effluent turbidity with washing powder causing the worst breach with a maximum of 18.2 NTU, however, the effluent turbidity for the bleach and SDS dosing were below the mean values of between 5.1 and 5.77 NTU observed for steady state operation. The colloidal fraction in a biomass matrix has been identified as one of the contributing factors to membrane fouling (Bouhabila *et al.*, 2001, Itonaga *et al.*, 2004) and it is likely that the effect seen here in the porous pots will have an impact on the MBR system.

Table 6-7 – Effluent quality criteria and comparison for the porous pots.

Parameter	*Aquarec Standard*[2]	*Toxin*	*Maximum Value*
COD ($mg.l^{-1}$)	<100	Shower Gel	105
		Washing Powder	118
		Bleach	39.5
		SDS	50.5
		Phenol	754
		Zinc sulphate	52.5
Ammonia ($mg.l^{-1}$)	2 - 20	Shower Gel	13.96
		Washing Powder	8.2
		Bleach	8.28
		SDS	3.84
		Phenol	11.38
		Zinc sulphate	8.61
Turbidity (NTU)[1]	<2	Shower Gel	13.5
		Washing Powder	18.2
		Bleach	4.88
		SDS	4.63
		Phenol	11.8
		Zinc sulphate	14.4
pH	6 – 9.5	Shower Gel	5.9 (7.1)
		Washing Powder	6.1 (7.3)
		Bleach	5.7 (6.8)
		SDS	5.2 (6.5)
		Phenol	6.9 (7.8)
		Zinc sulphate	5.2 (6.4)
Conductivity ($\mu S.cm^{-1}$)	<3000	Shower Gel	813
		Washing Powder	1183
		Bleach	890
		SDS	818
		Phenol	991
		Zinc sulphate	838

[1] Accepted level for effective downstream disinfection.
[2] Class 1 Private, urban and irrigation purposes.

Interestingly, none of the toxins caused a breach of the ammonia standard indicating that the increase in the other parameters are most likely to have been caused by interference with the interstitial elements of the biomass matrix to cause deflocculation rather than a true toxic effect on the biomass, as the nitrifiers are the most sensitive of the microbiological community, which would be reflected in an increase in effluent ammonia.

In general toxic effects on the biomass itself were not observed in terms of the MLVSS and the particle size, apart from the dosing trial with the shower gel where a significant drop in biomass was observed and an increase in the median particle size. This was accompanied by an increase in effluent turbidity

levels indicating a wash out of a mixture of the biomass, colloids and solids from the system, however, this still did not cause a breach in the ammonia standard and the COD breach was by just 5 $mg.l^{-1}$ at the maximum observed. This demonstrates the robustness of the biological community and reinforces the point that most of the biomass is shielded from the effects of the substrate variation by the floc structure present in suspended growth biological systems.

All toxins caused an increase in the parameters monitored as potential foulants, although it was impossible to comment as to whether the membranes used in the pots fouled more or less than before the dosing. Quantitative values are quoted in the literature for these parameters, however, the configuration and operation of a biological system contribute to the threshold values necessary to cause fouling.

The dosing trials with the porous pots have indicated that there is some effect on the biological system caused by the toxins and that these will be worth investigating further at MBR pilot scale.

7 Membrane Bioreactor Trials

7.1 Choice of household products

The porous pot trials provided an indication of the effects of the tested toxins on the biological system. One of the toxins that had shown an effect on the porous pot system, washing powder, was then tested further on the pilot scale membrane bioreactor, however, it was also decided to verify those that had unexpectedly shown little effect at the bench scale to see if they would behave similarly at pilot scale with a different property membrane, to eliminate any effects of scale. The toxins that were investigated further at pilot scale were washing powder, hypochlorite based household bleach, sodium dodecyl sulphate and zinc sulphate.

7.2 Flux step tests

7.2.1 Critical flux tests

An online clean was performed before the two critical flux tests that were carried out, prior to dosing with toxins, following the flux-step method described by Le Clech at al., 2003. These were carried out at aeration rates of 1 $l.s^{-1}$ and 2 $l.s^{-1}$. Both these rates were outwith the manufacturers advised operation range of aeration; one below and one above the range, however, this gave some indication of the operating limits of the membrane. The maximum flux that could be obtained at 2 $l.s^{-1}$ was 30 $l.m^{-2}.h^{-1}$ as the pilot plant had been fitted with an automatic shut off at 600 mbar TMP to protect the membranes. With this limitation a critical flux could not be determined at an aeration rate of 2 $l.s^{-1}$, however, the 1 $l.s^{-1}$ aeration rate gave a critical flux of 17 $l.m^{-2}.h^{-1}$ (Figure 7-1). This critical flux is similar to that reported by Pollice *et al.,*2005 of 19 $l.m^{-2}.h^{-1}$ for a hollow fibre submerged MBR with 12-19 $g.l^{-1}$ MLSS, although aeration rates were not reported.

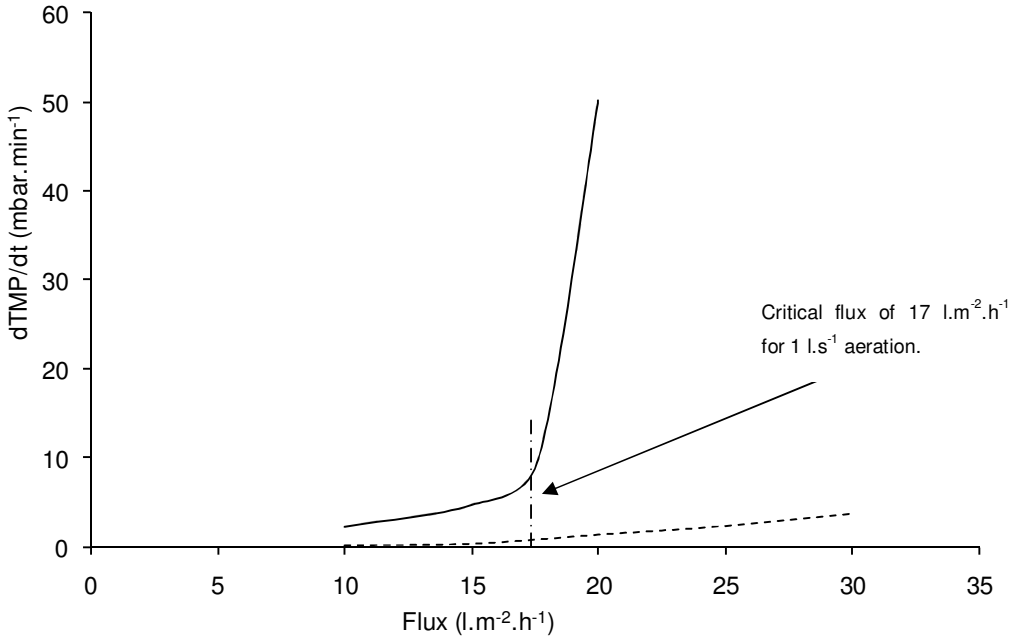


Figure 7-1 – Fouling rates for aeration rates of 1 $l.s^{-1}$ (solid line) and 2 $l.s^{-1}$ (dashed line).

In the course of running these tests it was observed that the permeate pump was cavitating when excessive demands was placed on it and for this reason no more critical flux tests were performed.

7.3 Steady state operation of the MBR

The MBR was run for two months to acclimatise it to the Cranfield sewage feed, with the flow being steadily increased, as advised by the industrial sponsor who provided the pilot plant (KeppelSeghers). After two months it was running with approximately an 8 hour HRT and it was decided to start wasting biomass to give a 15 day SRT. This proved catastrophic to the biomass and wasting was stopped as the biomass level dropped very low to below 1 $g.l^{-1}$ and the MBR was foaming. It was tried several times to establish a longer SRT of 20 days but in the end this was abandoned as the biomass struggled to maintain an acceptable level, due to the low concentration of feed water. It was attempted to run the MBR with an 8 hour HRT but the pump could not maintain a constant flux of 20 $l.m^{-2}.h^{-1}$ over a sustained period (Figure 7-2). When the flux was reduced to 17.5 $l.m^{-2}.h^{-1}$ sustainable operation over a longer period of time was

achieved (Figure 7-3). The MBR was run with an HRT of 11 hours and an infinite SRT (sampling wastage only) and the MLVSS concentration stabilised at around 4 g.l^{-1}.

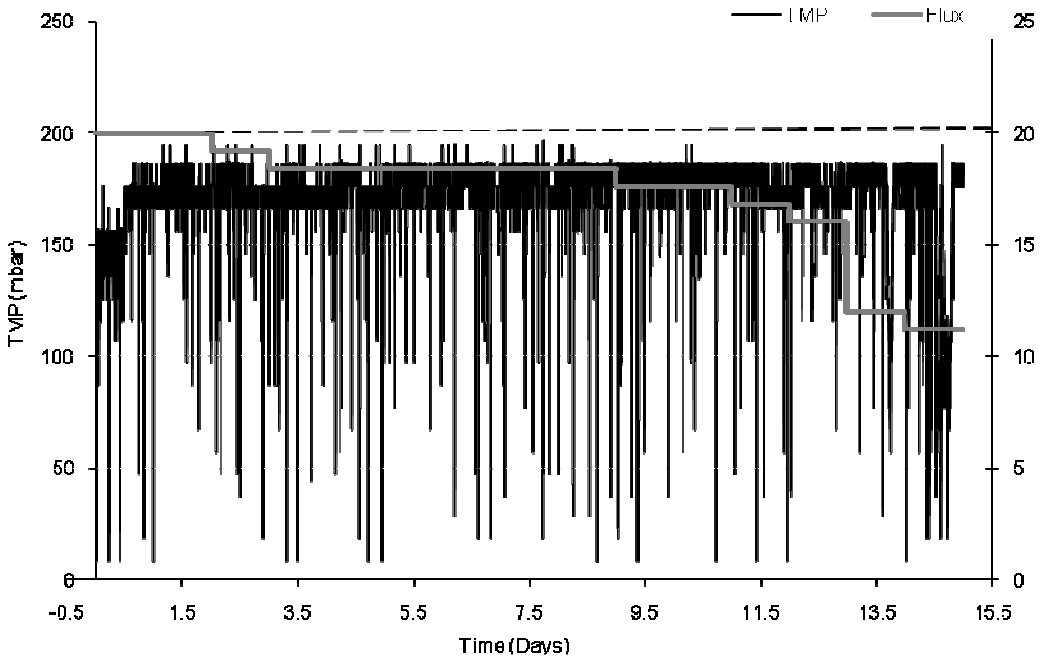


Figure 7-2 – TMP vs flux for a sustainable flux of 20 l.m^{-2}.h^{-1}.

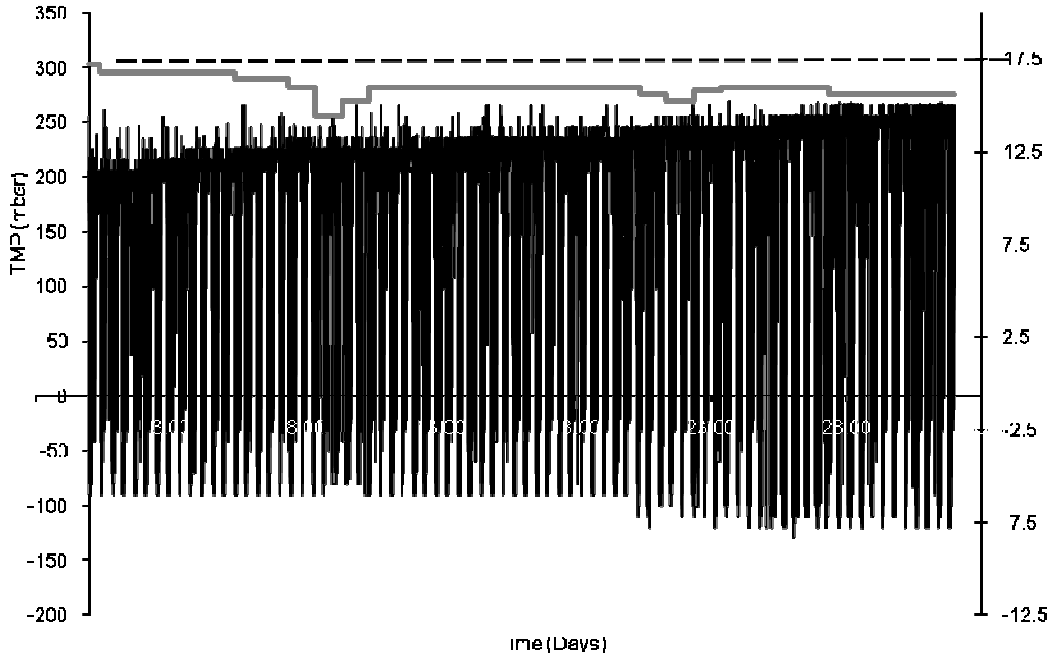


Figure 7-3 – TMP vs flux for a sustainable flux of 17.5 l.m^{-2}.h^{-1}.

7.3.1 Cleaning regime

Cleaning was carried out on the membrane when the trans membrane pressure increased to greater than 250 mbar or if the permeate flow could not be maintained at the required level. Four in situ cleans were carried out before an offline clean was performed when an in situ clean proved ineffective.

7.3.2 Operational problems

Several problems were encountered with the rig both with the auxiliary pumps and with the membrane housing itself. An offline clean resulted in an ingression of biomass into the permeate side of the membrane and no reason could be found for this (Figure 7-4).

Figure 7-4 – Biomass ingress into the permeate side of the membrane.

To try to rectify this, the membrane housing was reassembled several times and the stainless steel piping disinfected but no solution was found. Additionally the permeate flow was decreased for several weeks and then slowly increased, with no improvement. It was decided to carry on with the pilot plant in this state as the effluent quality was not compromised.

The recirculation pump, from the aeration tank to the membrane tank, failed after three dosing trials had been carried out and was replaced with a centrifugal pump. This initially severely affected the biomass (Figure 7-9) and the MBR was left for two months to return to steady state levels for MLSS before the final trial, of dosing with sodium dodecyl sulphate, took place.

7.3.3 Baseline monitoring

Baseline monitoring was carried out over a 16 month period. The effluent quality and biomass analysis was monitored to ensure that the MBR was running at steady state. The operational problems encountered described in Section 7.3.2 had an effect on the parameters monitored, as did the insitu or offline cleans, which have been indicated to further understand their effects.

7.3.3.1 Influent characteristics

The sewage feed to the MBR was the same as that delivered to the porous pots with a range of COD from 132 to 722 $mg.l^{-1}$, ammonia from 10.1 to 34.2 $mg.l^{-1}$ and turbidity from 31.7 to 409 NTU (Table 7-1).

Table 7-1 – Summary of feed characteristics during baseline monitoring for the pilot scale MBR.

Parameter	***Feed***			
	Mean	Range	Standard deviation	n
COD ($mg.l^{-1}$)	295.8	132-722	112.6	41
Ammonia ($mg.l^{-1}$)	25.35	10.1 – 34.2	6.34	30
Turbidity (NTU)	121.1	31.7 - 409	67.6	37

These feed characteristics are similar to those in several other studies treating municipal wastewater with COD levels ranging from 93 – 4292 $mg.l^{-1}$, ammonia from 11.7 – 78.2 $mg.l^{-1}$ and turbidity from 54.6 to 148 NTU (Table 7-2):

Table 7-2 – Literature values of feed characteristics for MBRs treating municipal wastewater.

Parameter	*Range*	*Reference*
COD ($mg.l^{-1}$)	132-722	This study
	150 – 690	Wu *et al.*, 2007
	112 - 389	Yoon *et al.*, 2000
	93 - 217	Fatone *et al.*, 2006
	299 - 4294	Rosenberger *et al.*, 2002
	135	Ravazzini *et al.*, 2005
	482	Côté *et al.*, 1997
Ammonia ($mg.l^{-1}$)	10.1 – 34.2	This study
	17.8-58.2	Wu *et al.*, 2007
	11.7 – 16.2	Fatone *et al.*, 2006
	21.2 – 78.2	Rosenberger *et al.*, 2002
	29.9	Ravazzini *et al.*, 2005
	39	Côté *et al.*, 1997
Turbidity (NTU)	31.7 - 409	This study
	54.6	Ravazzini *et al.*, 2005
	148	Côté *et al.*, 1997

7.3.3.2 Effluent quality analysis

7.3.3.2.1 Effluent COD

The effluent COD for the MBR while operating at steady state was consistently below the proposed effluent COD level of 100 $mg.l^{-1}$ from Aquarec (2005) for European water reuse guidelines (Figure 7-5 with the horizontal dashed line indicating the Aquarec guidelines).

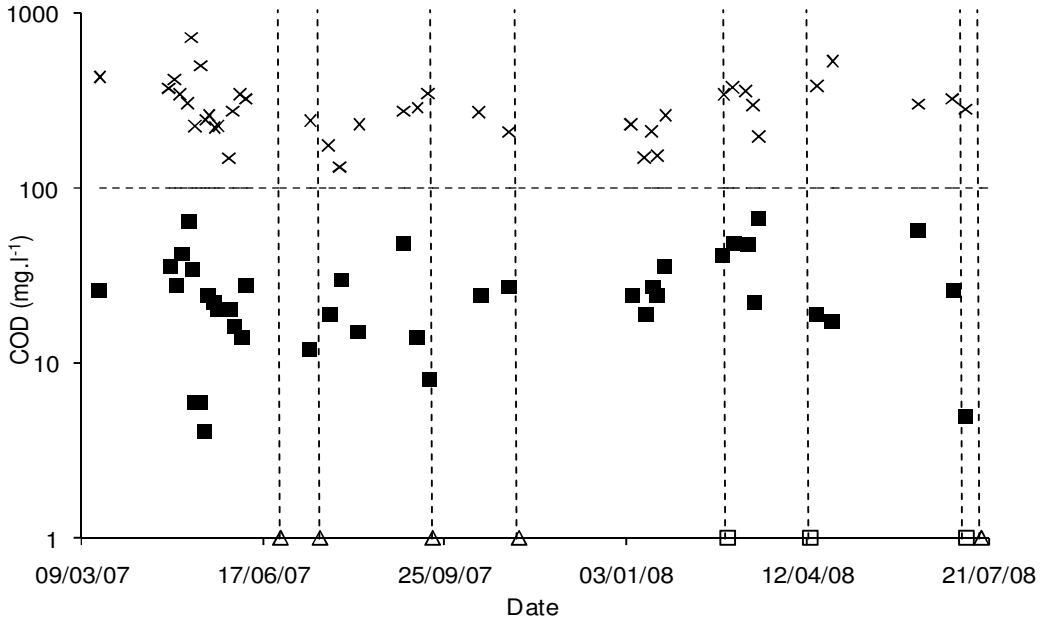


Figure 7-5 – Baseline influent and effluent COD for the pilot scale MBR (x = influent, ■ = effluent, Δ = insitu clean, □ = offline clean).

This output level was maintained even through the operational problems (biomass ingress into permeate after the second offline clean and failure of recirculation pump). Insitu and offline cleans are indicated to monitor any effect these may have on the performance of the MBR, however, no adverse affects were observed. This gave an average removal rate of 90% for COD over the 518 days monitored.

7.3.3.2.2 Effluent Ammonia

The pilot MBR was below the upper end of the range of the Aquarec guidelines for ammonia, of 2 to 20 $mg.l^{-1}$ (range for Class I private and urban reuse), for the whole monitoring period, with only six incidences over the 2 $mg.l^{-1}$ level in the sixteen months of steady state operation (Figure 7-6 with the horizontal dashed lines representing the upper and lower limit for the Aquarec guidelines for Class 1 private, urban and irrigation reuse).

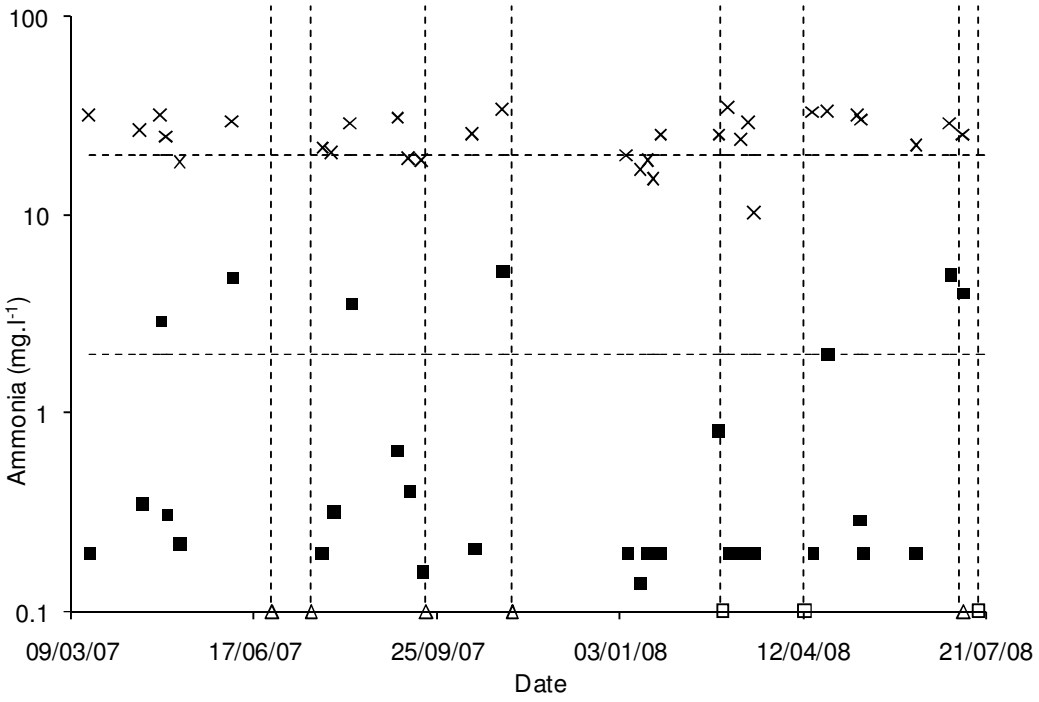


Figure 7-6 – Baseline influent and effluent ammonia for the pilot scale MBR (x = influent, ■ = effluent, Δ = insitu clean, □ = offline clean).

7.3.3.2.3 Effluent Turbidity

The effluent turbidity at steady state was consistently below 2.5 NTU for the first year of operation, however, it was severely affected by the biomass ingress into the permeate. Despite this, it remained < 6 NTU apart from 3 samples, directly after an offline clean of 14.5, 17.7 and 24.6 NTU (Figure 7-7). Effluent turbidity is not listed specifically as one of the parameters for water reuse by Aquarec. However, it is generally acknowledged that the effluent turbidity should be <2 NTU (horizontal dashed line in Figure 7-7) to prevent problems with downstream disinfection (Crook, 1998). The MBR performed satisfactorily at this level for over a year. Most of the pre dosing measurements were at an acceptable level of around 2 NTU, the exception being the bleach dosing trial where the pre dosing level was close to 6 NTU.

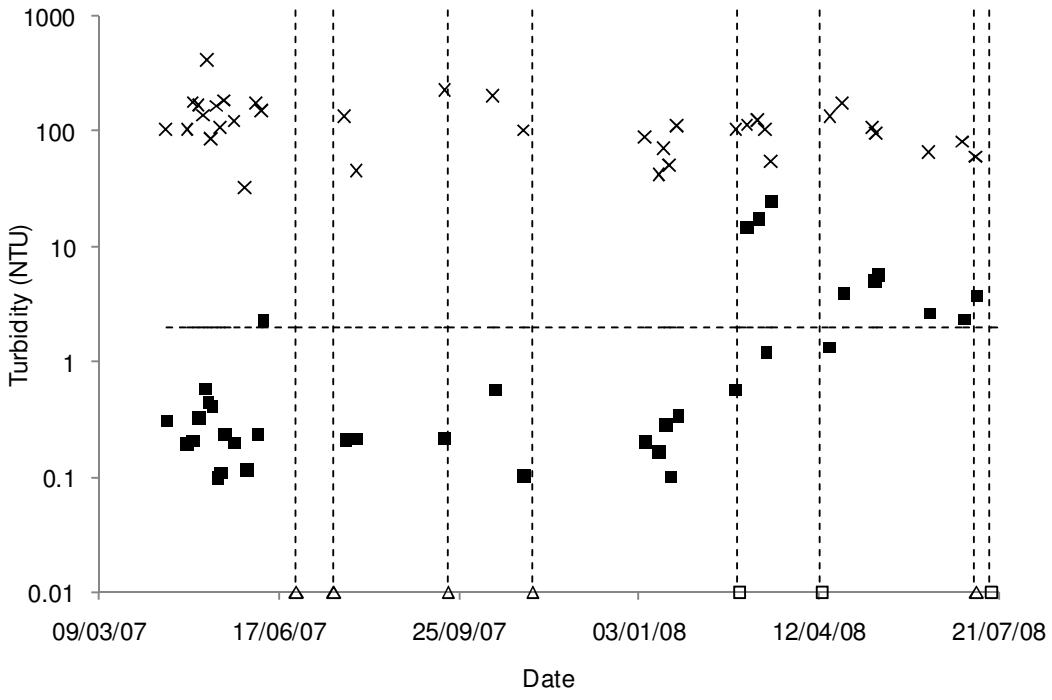


Figure 7-7 – Baseline influent and effluent turbidity (x = influent, ■ = effluent, Δ = insitu clean □ = offline clean).

7.3.3.3 Baseline biomass analysis

7.3.3.3.1 Capillary Suction Time

The CST increased in line with the increase in MLSS as was expected (Houghton *et al.*, 2001) (Figure 7-8). The mean CST over the 518 days monitored was 187 seconds with a standard deviation of 207.6 seconds. However, if the first five samples are disregarded then the mean was 103.6 seconds with a standard deviation of 42.5 seconds. A mean of 103.6 seconds is higher than that for a similar sized, but flat plate MBR, operated at a much shorter HRT of 2.2 hours and SRT of between 10 and 40 days.by Wu *et al.* (2007) who recorded CST readings of less than 50 s. Khongnakorn *et al.*, (2007) also observed low CST readings of >30 seconds for a pilot MBR of 50 litres volume with an HRT of 2 days and an infinite SRT. On the other hand Pollice *et al.*, (2005) reported CSTs of between 11.9 and 1542 seconds for a range of pilot and full scale MBRs.

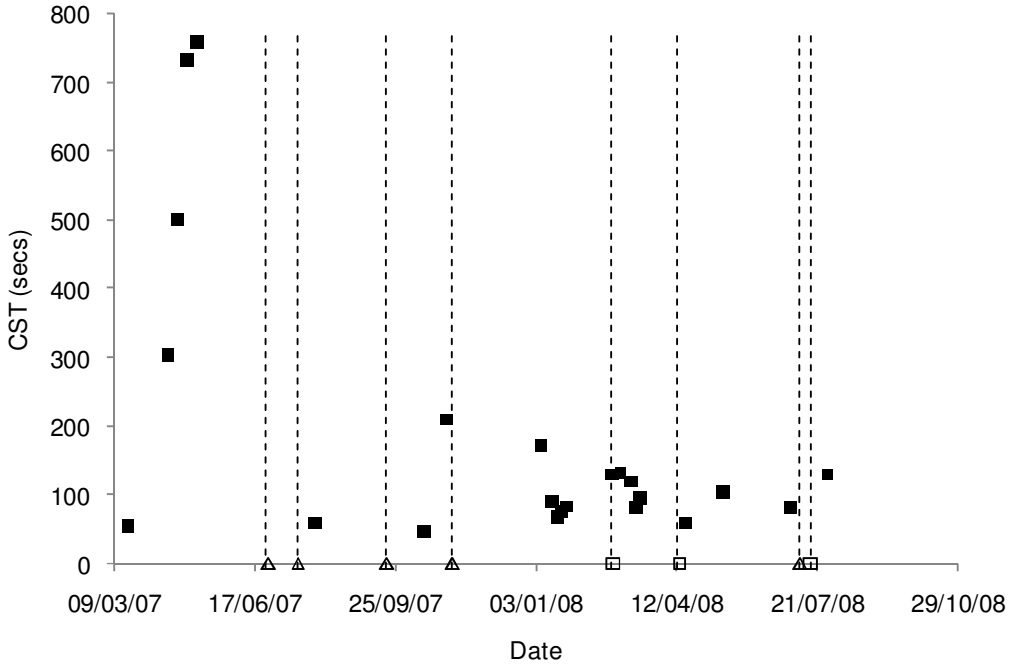


Figure 7-8 – Baseline capillary suction time for the pilot scale MBR (Δ = insitu clean ☐ = offline clean).

7.3.3.3.2 Mixed Liquor Suspended Solids and Mixed Liquor Volatile Suspended Solids

The MLSS broadly stabilised in line with the HRT: the longer the HRT, the lower the MLSS concentration (Figure 7-9). At an HRT of 11 hours the MLSS stabilised at around 5 g.l^{-1}. As the MBR was fed with real sewage the variation in influent COD will effect the level of MLSS and MLVSS so that a true steady state is never reached.

The MLVSS concentration stabilised at around 90% of the MLSS concentration which is high compared to other municipal wastewater treatment systems where a value of 70% is considered common (Rosenberger *et al.*, 2002).

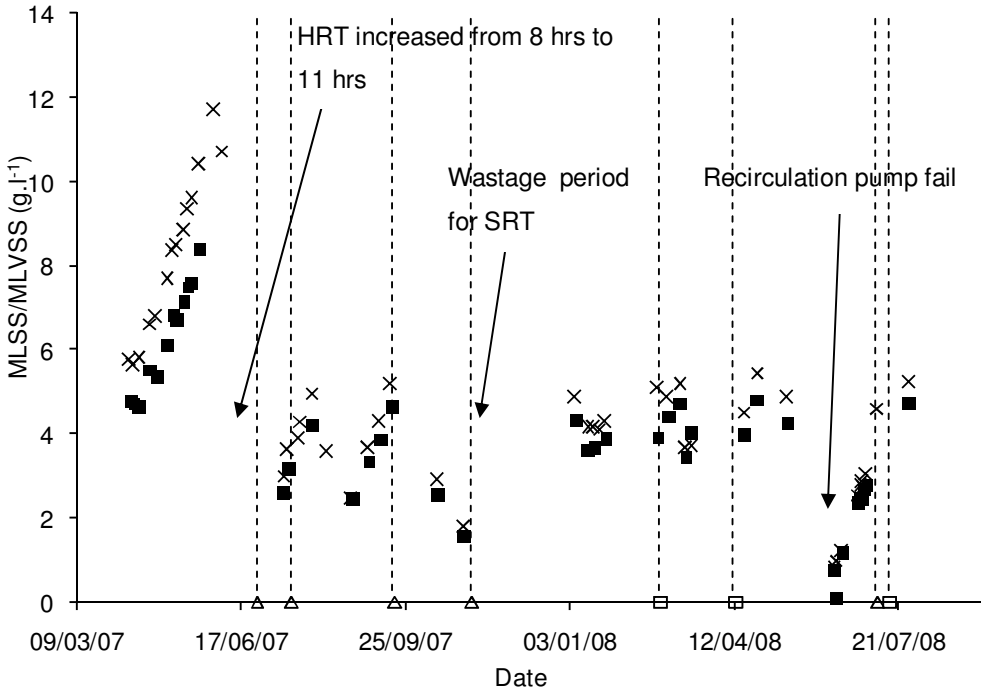


Figure 7-9 – Baseline mixed liquor suspended solids (x) and mixed liquor volatile suspended solids (■) for pilot scale MBR (Δ = insitu clean, □ = offline clean).

7.3.4 Steady state performance

Despite these issues the MBR performed well and the effluent quality was good with a maximum effluent COD of 67 mg.l^{-1}, a maximum effluent ammonia of 5.21 mg.l^{-1}. As explained earlier the effluent turbidity was disturbed by the cleaning regime and a maximum of 24.6 NTU was recorded, however, the

mean was 2.77 NTU over the fourteen months monitored (Table 7-3). The biomass stabilised at approximately 5 $g.l^{-1}$ and the pH was in the limits for aerobic systems (6-9) (Tchobanoglous *et al.*, 2003).

Table 7-3 – Summary table of parameters analysed during baseline monitoring for the pilot scale MBR.

Parameter	***Mean***	***Range***	***Standard deviation***	***n***
COD ($mg.l^{-1}$)	27.01	4-67	15.45	41
Ammonia ($mg.l^{-1}$)	1.00	0.14 – 5.21	1.59	30
Turbidity (NTU)	2.77	0.10-24.6	5.46	37
Conductivity ($\mu S.cm^{-1}$)	752.9	574.0 – 911.0	97.3	29
pH	6.40	4.45 – 7.28	0.75	37
CST (secs)	187.0	48.5 – 761.1	207.6	22
MLSS ($g.l^{-1}$)	4.97	0.8 – 11.68	2.55	49
MLVSS ($g.l^{-1}$)	4.13	0.09 – 8.41	1.91	39

The MBR gave an average of 90% COD removal, 96% ammonia removal and 97% turbidity removal while running at steady state. This performance is in accordance with several other studies with MBRs treating municipal or domestic wastewater which reported >88% COD removal and 82% ammonia removal (Table 7-4).

Table 7-4 – Literature values for pollutant removal for steady state MBRs.

Parameter	***% removal***	***Reference***
COD ($mg.l^{-1}$)	90%	This study
	>93%	Brindle *et al.*, 1996.
	>88%	Brindle *et al.*, 1996.
	95%	Rosenberger *et al.*, 2002
Ammonia ($mg.l^{-1}$)	96%	This study
	82%	Rosenberger *et al.*, 2002

7.4 Acute toxicity dosing results

7.4.1 Foaming

To ensure that foaming was not the limiting factor for the dosing trials the same doses were used as had been used for the porous pots (Table 7-5).

Table 7-5 – Dose of toxins for the MBR

Toxin	***EC_{50} by respirometry***	***Actual amount dosed***
Washing powder	1.2g.l^{-1}	0.5g.l^{-1}
Bleach	0.48ml.l^{-1}	0.4ml.l^{-1}
SDS	NT	28mg.l^{-1}
Zinc sulphate	85mg.l^{-1}	85mg.l^{-1}

This strategy was successful in all but the case of the washing powder which caused excessive foaming in the MBR (Figure 7-10). This is most likely to be caused by the increased aeration in the MBR; 1.5 $l.s^{-1}$ compared with the pots aeration rate of 0.9 $l.s^{-1}$. The foaming caused some loss of biomass from the membrane tank but the results are presented and the foaming taken into account as one of the effects. In comparison the bleach caused very little foaming, as had been experienced with the porous pot dosing trials (Figure 7-11).

Figure 7-10 – Foaming as a result of 0.5 g.l^{-1} dose of washing powder.

(a) (b)

Figure 7-11 – (a) aeration tank before (b) aeration tank after, 0.4 ml.l^{-1} bleach dosing.

7.4.2 Effluent analysis

7.4.2.1 Hydraulic characteristics of the MBR

It was assumed that the MBR had the same hydraulic characteristics as the porous pots, with the system being a completely mixed reactor (Chapter 6 Section 6.3.3.1). Any delay seen in parameters monitored for the system are assumed to be due to interaction with the biomass in some way.

7.4.2.2 Effluent COD

The effluent COD before dosing was <20 mg.l^{-1} for all the experiments carried out demonstrating that the MBR was running at steady state with a COD removal rate of >94 % for each run. The shock dose of COD varied for each of the toxins (Table 7-6) with washing powder and sodium dodecyl sulphate contributing the most to the chemical oxygen demand.

Table 7-6 – COD shock dose from each toxin

Toxin	*COD (g)*
Washing powder	291
Bleach	86
Sodium dodecyl sulphate	232
Zinc sulphate	87

There was no clear pattern to the effects of the toxins on the effluent COD, but none of the toxins produced a breach of the Aquarec guideline levels for water reuse of 100 mg.l^{-1} (Aquarec, 2005) (Figure 7-12).

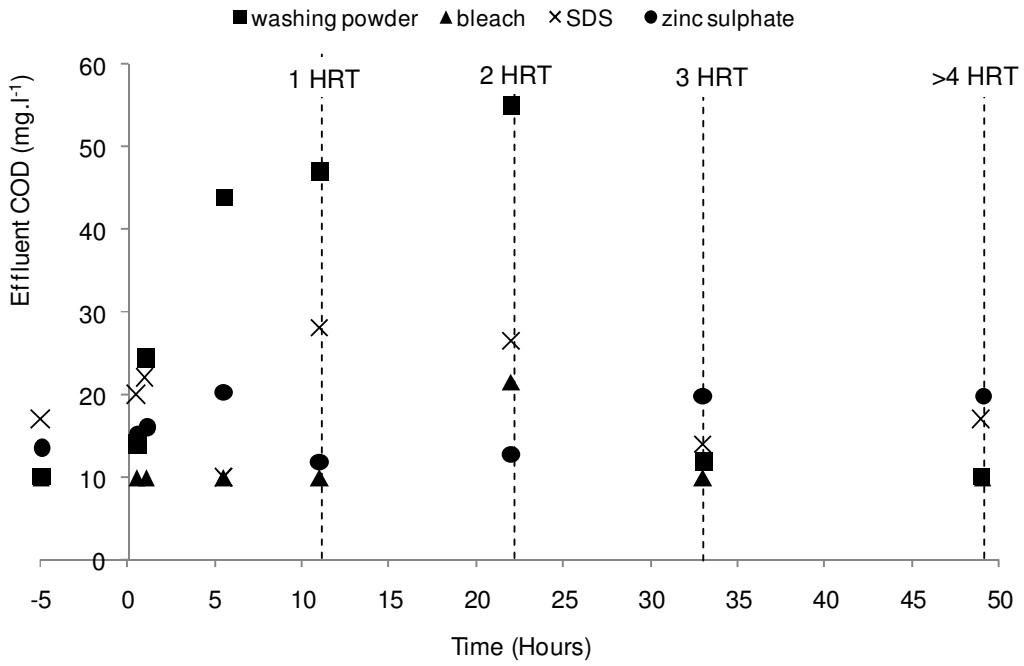


Figure 7-12 – Effluent COD for all four toxins (steady state operation at t = -5 hrs and toxin dosed at t = 0 hrs).

The zinc sulphate dose initially caused an increase for the first 0.5 HRT of observation from 13.5 mg.l^{-1} to 20.5 mg.l^{-1} then decreased to 12-13 mg.l^{-1} at the 1 and 2 HRT mark before levelling out at 20 mg.l^{-1} at 3 HRTs and beyond. These small variations are most likely to be the variation in the system and are unlikely to be attributed to the zinc sulphate dose.

Similarly the bleach had little effect on the effluent COD, as was previously seen with the porous pots. An increase to 21 mg.l^{-1} was seen at 2 HRTs, but otherwise the COD measured was at the lower limit of detection for the range, at 10mg.l^{-1}.

The most pronounced affect of the toxin dosing was caused by the washing powder with a gradual increase over the first 2 HRTs (22 hours) to a maximum

of 55 mg.l^{-1}, with a rapid decrease to pre dosing levels after 3 HRTs (33 hours). There is no evidence of deflocculation from the particle size distribution measurements (Section 7.4.3.3), however, there was a substantial increase in effluent turbidity at the same time so there may be some interaction with the washing powder and the membrane that has decreased the effectiveness of the membrane barrier or the washing powder has increased the colloidal fraction in the aqueous solution that has then passed through the membrane but has not had an effect on the biomass flocs.

The SDS produced an erratic response in the effluent COD: a slight increase after 1 hour to 22 mg.l^{-1} was followed by a decrease at 0.5 HRT (5.5 hours) to below pre dosing level (10 mg.l^{-1} compared to 17 mg.l^{-1} respectively), at 11 hours it increased to 28 mg.l^{-1} then by 33 hours (3HRTs) it had returned to close to pre dosing levels at 14 mg.l^{-1}. As there was no increase in effluent turbidity in line with the effluent COD it is likely that these are normal variations over the cycle of the HRT.

After all four of the toxin dosing experiments the MBR had returned to pre dosing levels after 4 HRTs.

7.4.2.3 Effluent Ammonia

The toxins dosed did not contribute to the ammonia loading of the system. In general there was little effect on the ammonia removal in the system with the bleach and SDS dosing having no effect at all on the effluent ammonia (Figure 7-13). Zinc sulphate had the most effect on the effluent ammonia with a rapid increase to >6mg.l^{-1} after 1 HRT followed by a return to pre dosing levels after 2 HRTs. The only other toxin to have any effect on the effluent ammonia was the washing powder which increased to 2.5 mg.l^{-1} after 1 HRT. In fact this is similar to the response shown by the porous pots where the washing powder actually improved the ammonia removal from the system by increasing the pH of the reactor. This is true for the washing powder dose in the MBR, however, the initial pH was at the optimum for nitrifiers of 7 and the increase to 7.2 does not seem to have had any detrimental effect.

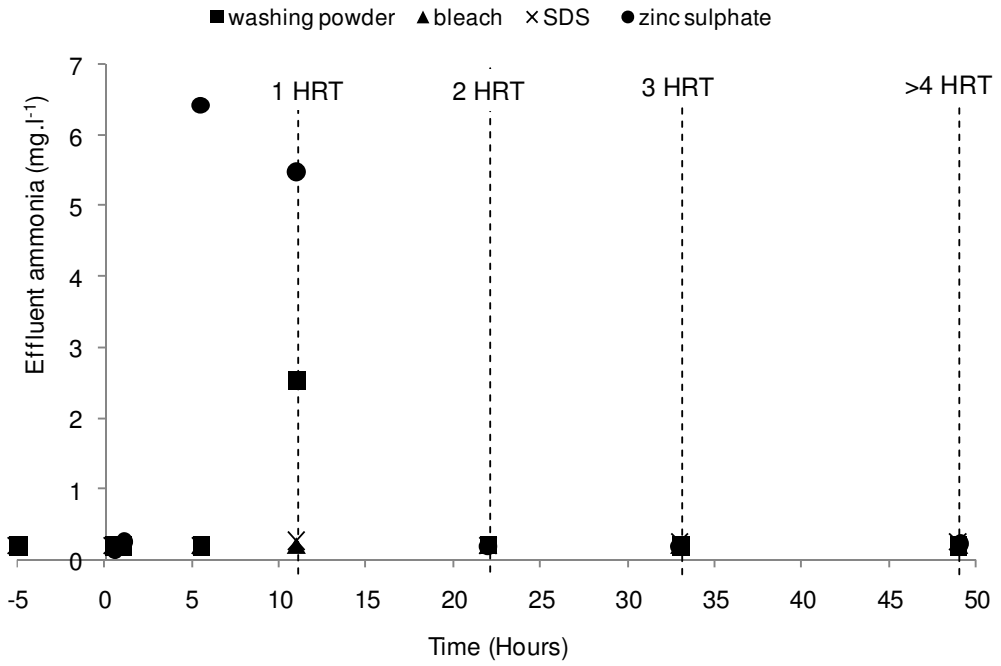


Figure 7-13 – Effluent ammonia for all four toxins (t = -5 hrs was steady state operation before dosing and toxin was dosed at t = 0 hrs).

Only the washing powder and zinc sulphate produced effluent ammonia levels above the lower range of the Aquarec standard of 2 mg.l^{-1} but none of the toxins dosed breached the upper range of the standard of 20 mg.l^{-1}.

7.4.2.4 Effluent Turbidity

No clear pattern was apparent for the effluent turbidity with four different responses for the four different toxins. The washing powder produced a steady increase in effluent turbidity from 1.7 NTU before dosing to 18.57 NTU after 22 hours (2 HRT). Over the final two HRTs the effluent turbidity returned to pre dosing levels. The effluent turbidity followed the profile of the effluent COD almost exactly for the washing powder showing that the effluent COD was particulate (Figure 7-12). Washing powder is designed to lift and separate dirt from clothing and, as such, seems to be performing the same function on the biomass (Madsen *et al.*, 2001).

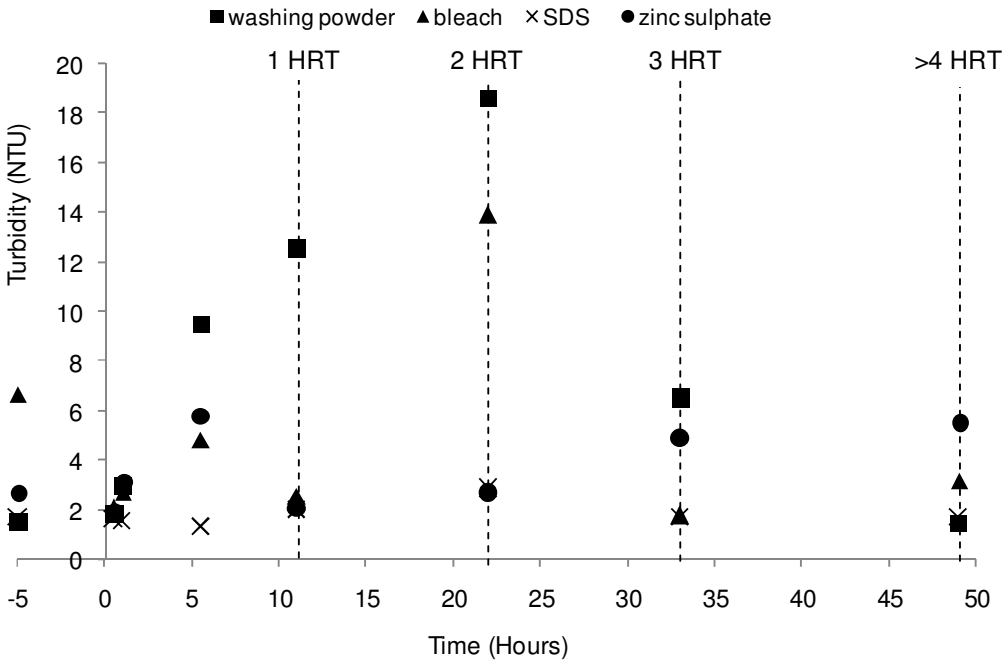


Figure 7-14 – Effluent turbidity for all four toxins (t = -5 hrs was steady state operation before dosing and toxin was dosed at t = 0 hrs).

The bleach dose started with a high turbidity, before dosing, of 6.66 NTU which was reduced after dosing to 2.07 NTU but increased again to a maximum of 13.9 NTU at 2 HRT before reducing again to steady state levels of around 2 NTU. It is unclear that the fluctuations in effluent turbidity can be attributed to the bleach dosing alone, because of these fluctuations. Zinc sulphate produced an initial increase from 2.74 NTU to 5.4 NTU in the first 5.5 hours (0.5 HRT) after dosing. This decreased again to around 2 NTU before increasing to around 5 NTU after 3 HRTs. It was still at this level after 4 HRTs. The sodium dodecyl sulphate had no discernible effect on the effluent turbidity with a minimum of 1.31 NTU and a maximum of 2.88 NTU occurring at 2 HRTs.

7.4.3 Biomass Analysis

7.4.3.1 CST

Neither the bleach nor the zinc sulphate had any effect on the CST over the 4 HRTs monitored (Figure 7-15). The washing powder caused an immediate

increase in the CST from 156 seconds to 255 seconds in 0.5 HRTs. This then decreased over the next three HRTs from 188 seconds to 137 seconds, below the pre dosing levels, in contrast to the effects observed in the porous pots where the washing powder caused a decrease in the CST readings (Chapter 6 Section 3.4.1).

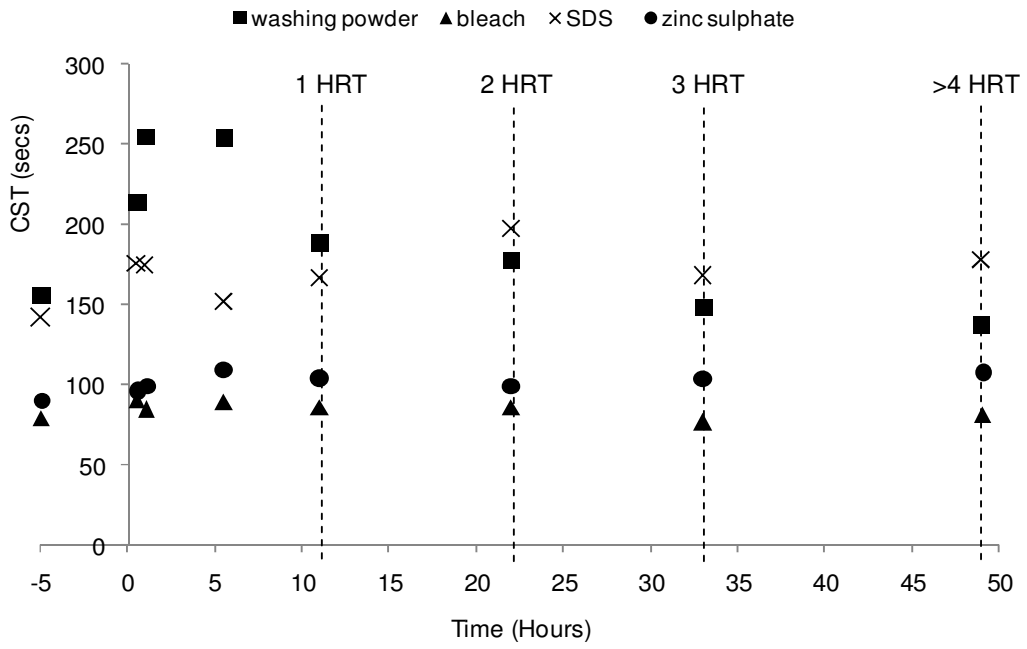


Figure 7-15 – CST for all four toxins (t = -5 hrs was steady state operation before dosing and toxin was dosed at t = 0 hrs).

In the case of the pots it is likely that the surfactant action separated some of the smaller particles which were then washed from the system (evidenced by a concomitant increase in effluent turbidity) through the relatively large pore size membranes (60 – 90 µm) whereas in the MBR system these were not expelled from the system due to the much smaller pore size in the hollow fibre membranes of 0.1 µm and hence added to the decrease in dewaterability. The washing powder caused a lot of foaming over this same time resulting in loss of biomass which will have contributed to the later lower CST readings. In light of this, the lower readings cannot be solely attributed to the effects of the washing powder.

The SDS had an intermittent effect on the CST. An initial increase from 142 seconds to 175 seconds immediately after dosing was followed by a decrease to 150 seconds before another increase to 197 seconds and a final decrease to 178 seconds. It is likely that these results are due to the variation introduced from sampling rather than a definite effect of the SDS as there is only a 20% difference between each successive reading. CST readings from a variety of pilot and full scale flat plate, hollow fibre and tubular MBRs ranged from 11.9 to 1542 seconds, showing the vast differences that are encountered (Pollice *et al.*, 2005). The operating parameters for the MBRs and method for the CST measurement were not included in the review, by Pollice *et al.*, but it is assumed that the same method was used throughout.

7.4.3.2 MLSS and MLVSS

Washing powder was the only toxin to have an effect on the MLVSS level and this was most likely caused by foaming and overspill, rather than a specific toxic kill (Figure 7-10). None of the other toxins had a significant effect on the biomass levels (Figure 7-16). Bleach had a range of 5.72 – 5.96 $g.l^{-1}$, zinc sulphate had a range of 4.56 – 5.3 $g.l^{-1}$ and SDS from 5.45 – 5.81 $g.l^{-1}$. Clearly there has been no detrimental effect on the levels of MLVSS and the predicted toxins have not shown a toxic effect, resulting in cell death, on the bacterial community.

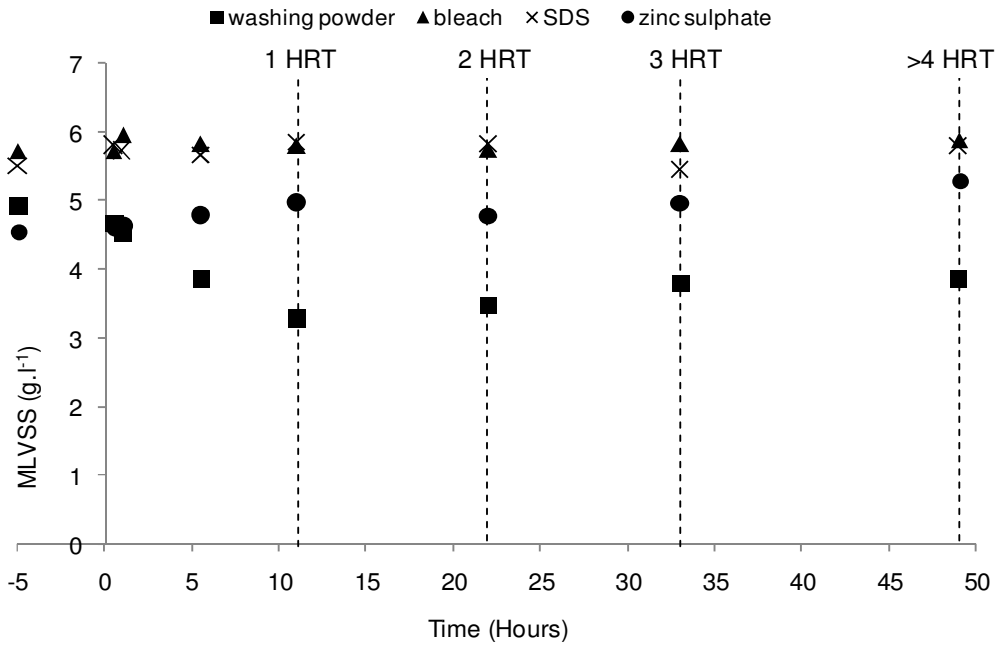


Figure 7-16 – MLVSS for all four toxins (t = -5 hrs was steady state operation before dosing and toxin was dosed at t = 0 hrs).

7.4.3.3 Particle Size Distribution

The baseline median diameter for the MBR biomass of 20 – 22 µm (Figure 7-17) was much lower than that for the pots of approx 100 µm. This is due to the increased stress on the biomass from aeration and pumping in the bioreactor. The smaller flocs are more susceptible to toxic effects by reducing mass transfer limitations (Henriques *et al.*, 2005), however, this does not seem to have affected the performance of the biomass in the MBR.

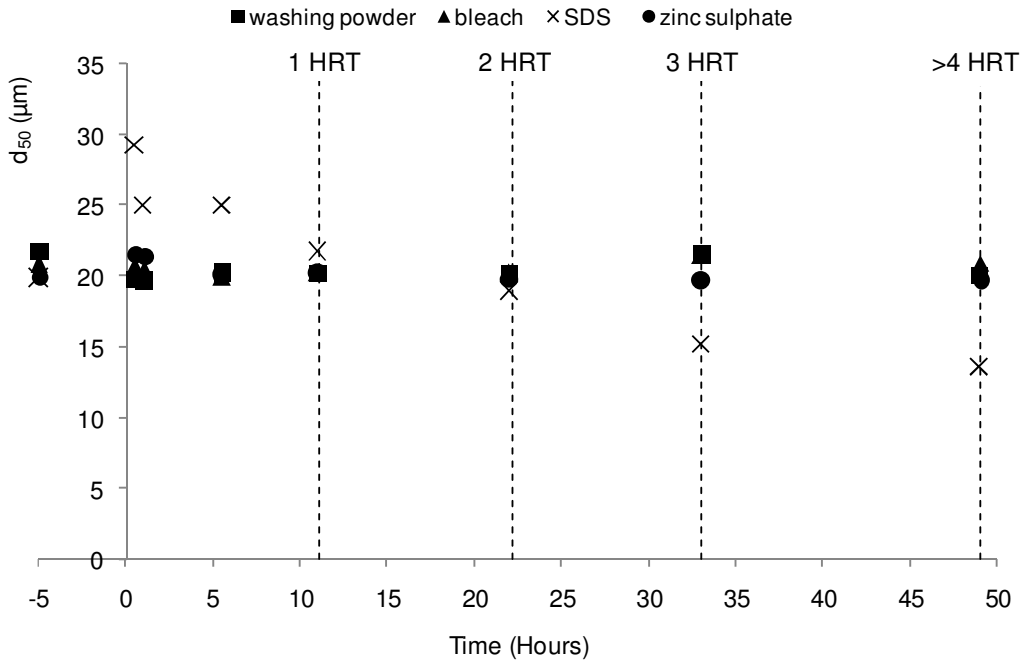


Figure 7-17 – d_{50} for all four toxins (t = -5 hrs was steady state operation before dosing and toxin was dosed at t = 0 hrs).

For the SDS dosing, a detailed breakdown of the particle size distribution (Figure 7-18) reveals the extent of the aggregation over the time monitored. Interestingly, there is a peak at 0.8 µm, which also appears in all the other dosing trials, which represents the colloidal element within the mixed liquor. An initial aggregation of the biomass in the first 30 minutes after dosing (the peak of the distribution shifted from 26.3 µm before dosing to 34.6 µm 30 minutes after dosing) was followed by a return to pre dosing levels at 22 hours (the peak occurred at 22.9 µm). The 48 hour sample, however, reveals a new peak at approximately 140 µm with the peak of the distribution being smaller and at 10 µm rather than 30 µm, indicating that the SDS has caused flocculation, of some of the medium sized flocs (around 20 – 30 µm). The smaller peak at <1 µm has remained unchanged over the entire monitoring period indicating that the colloidal element has been unaffected by the surfactant addition.

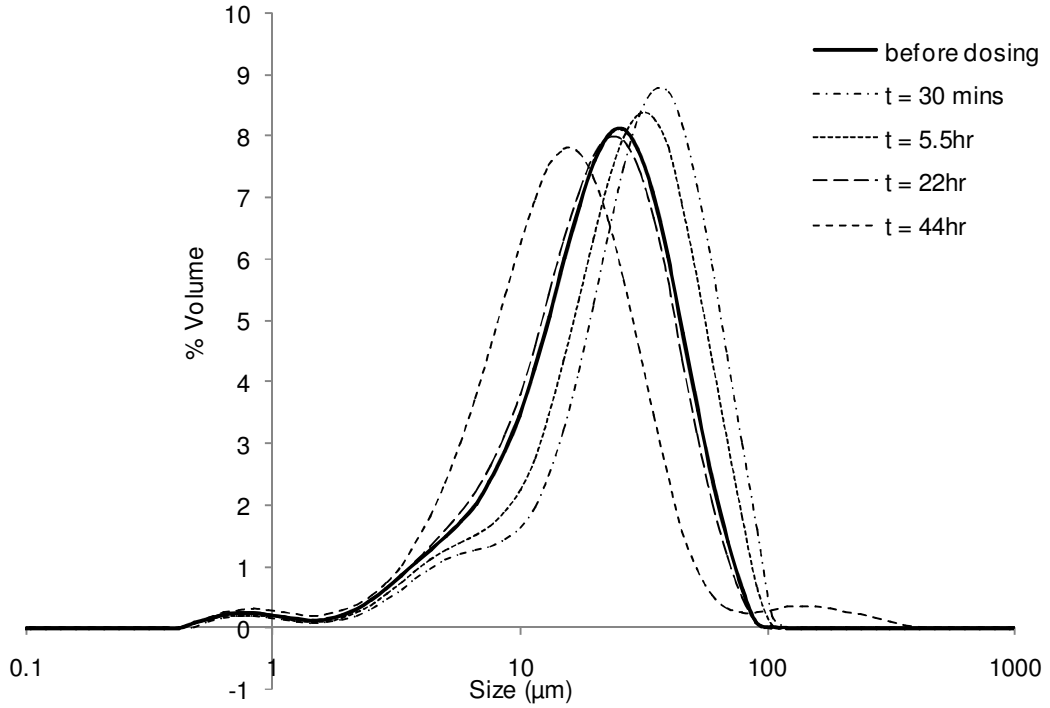


Figure 7-18 – Detailed breakdown of particle size distribution over time for sodium dodecyl sulphate (all data not included).

7.4.3.4 pH

Neither the bleach nor the zinc sulphate had any significant effect on the pH of the system. SDS had the most significant effect on the biomass pH level causing a significant drop in pH from 6.1 to 5 over 4 HRTs (Figure 7-19). As this effect was not seen in the porous pot trial it is assumed that this was due to outside environmental factors rather than the action of the surfactant dosing.

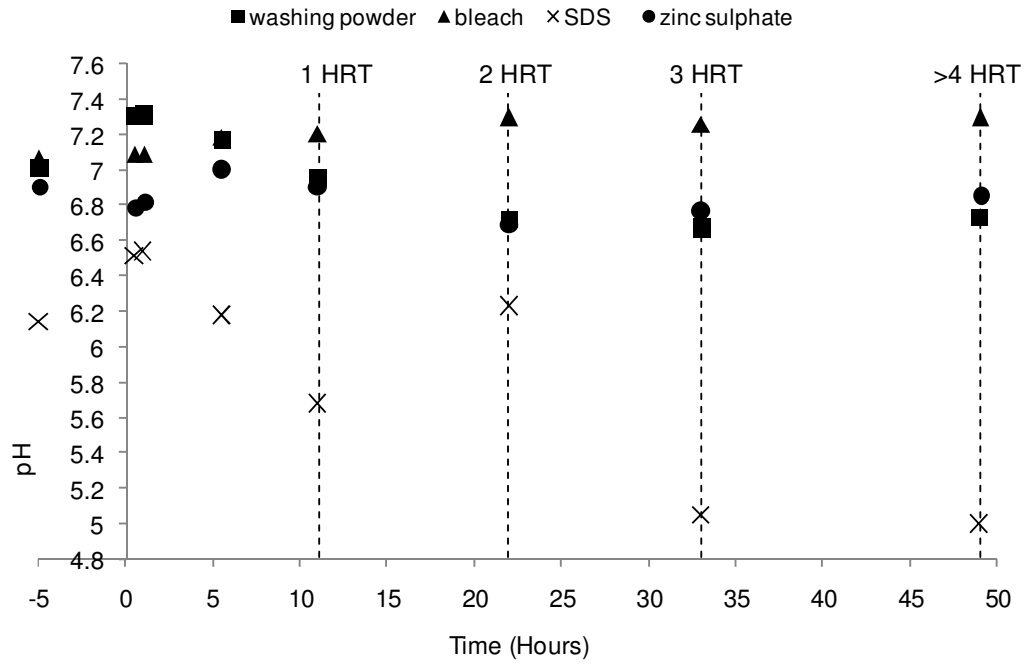


Figure 7-19 – pH of biomass for all four toxins (t = -5 hrs was steady state operation before dosing and toxin was dosed at t = 0 hrs).

7.4.3.5 Conductivity

The washing powder showed a classic dose response for an ideal completely mixed reactor with a non reactive tracer passing through the system. It seems to have no long term effect on the biomass as the conductivity has passed through the MBR after 3 HRTs and returned to pre dosing levels (Figure 7-20).

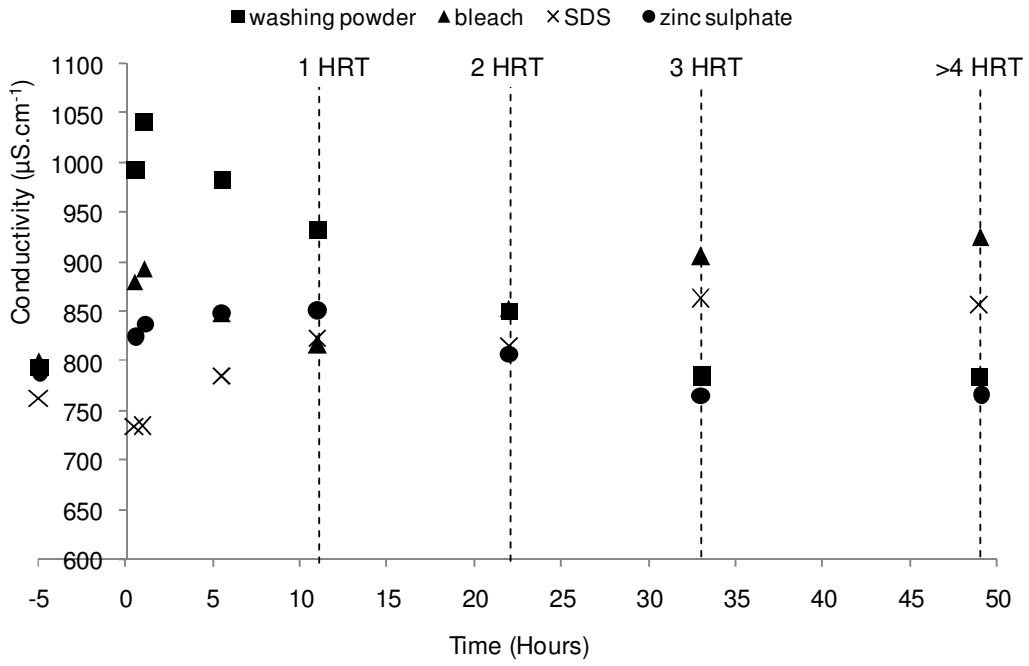


Figure 7-20 – conductivity of biomass for all four toxins (t = -5 hrs was steady state operation before dosing and toxin was dosed at t = 0 hrs).

7.4.4 Foulant Analysis

7.4.4.1 SMP turbidity

Three out of the four toxins had pre dosing levels of 1 -2 NTU which is similar to that found by Ramesh *et al.*, 2006, and Rosenberger *et al.*, 2006 who observed an SMP turbidity of between 2 and 9 NTU for aerobic return sludge and pilot scale MBRs.

All four toxins had differing effects on the SMP turbidity (Figure 7-21). The bleach had no detrimental effect and the turbidity remained at <3 NTU for the entire observation period. In fact, the bleach had a positive effect lowering the turbidity from a pre dosing level of 2.05 NTU to a post dosing level at >4 HRTs (48 hours) of 0.92 NTU.

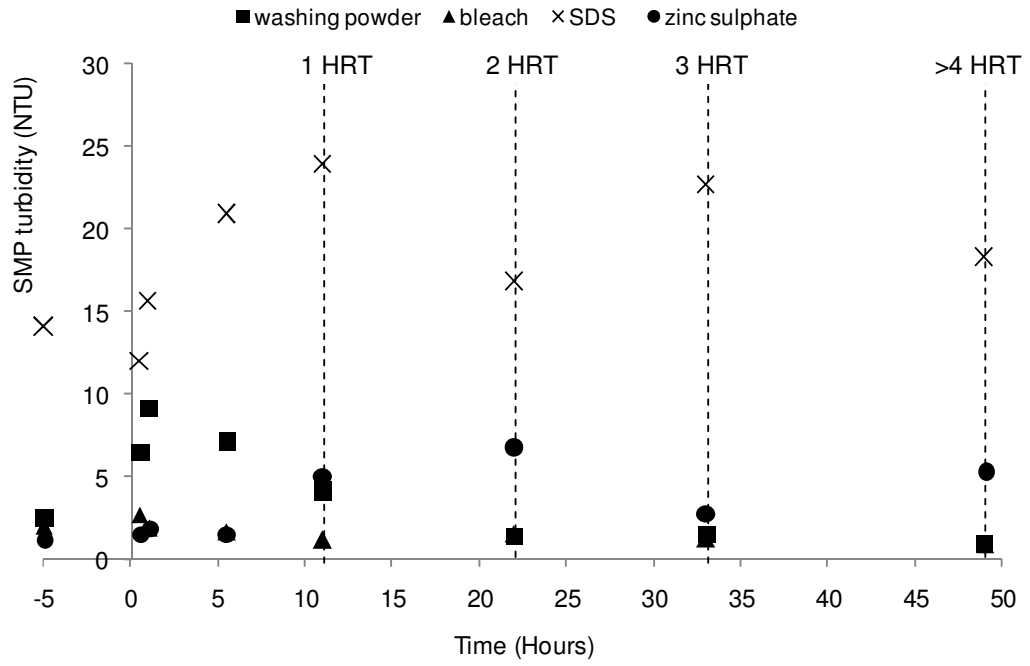


Figure 7-21 – SMP turbidity for all four toxins (t = -5 hrs was steady state operation before dosing and toxin was dosed at t = 0 hrs).

The other three toxins had varying effects. The zinc sulphate showed a linear increase from 1.92 NTU at 0.5 HRTs (5.5 hours) to 5.1 NTU at 2 HRTs (22 hours). By 3 HRTs (33 hours) this had decreased again to 2.63 NTU but was followed by an increase at the >4 HRT sample. This shape of response was not echoed in any of the other parameters measured for the zinc sulphate dosing which indicates that the zinc sulphate is not interacting with the biomass in the system. It is possible that the metal salt is interacting with other compounds in the feed matrix to produce more colloidal substances in the supernatant and it is unlikely to be due to a response from the biomass.

The washing powder produced a very similar response to that observed with the porous pots (Chapter 6 Section 3.5.1). This appears to be the washing powder passing through the system and affecting the colloidal element within the mixed liquor, without having any effect on the microbial actions of the biomass. As there was severe foaming at the time this might have affected the SMP turbidity, however, as the response was so close to that seen in the pots that did not

have foaming, it can be assumed that this is not the primary influence. The washing powder separates loosely bound colloidal elements from the flocs and causes the increase in SMP turbidity. These are either rebound when the washing powder is degraded, washed from the system or are degraded easily, as the SMP turbidity returns to pre dosing levels after 2 HRTs (22 hours). As there was a parallel increase in the total effluent COD it is more likely that these are washed from the system.

The SMP turbidity for the sodium dodecyl sulphate (SDS) trial was initially very high. This could have been due to the introduction of a centrifugal pump as the recirculation pump. This exchange of pumps will have disrupted the biomass by introducing a different form of shear than that experienced with the positive displacement pump (Kim *et al.*, 2001). There is evidence that the SDS did increase the SMP turbidity (from 12 NTU to 24 NTU over 1 HRT) and as this effect was also seen in the porous pot trials it can be assumed that it is the effect of the SDS destabilising the flocs by increasing the negative charge in the reactor.

7.4.4.2 SMP proteins and carbohydrates

None of the toxins dosed into the system contributed to the proteins or carbohydrates measured themselves. The carbohydrates and proteins were at a similar level of 2 -4 $mg.gMLVSS^{-1}$ or 9 -12 $mg.l^{-1}$ before all of the dosing trials, apart from the sodium dodecyl sulphate where both proteins and carbohydrates were approximately 4 $mg.gMLVSS^{-1}$ or 20 $mg.l^{-1}$ (Figure 7-22 and Figure 7-23). This high level before the SDS trial is due to the emergency change in recirculation pump, to a centrifugal pump, as all other parameters were the same as the other trials. The centrifugal pump exerts a higher shear force on the biomass, compared to the previous positive displacement pump, resulting in stress to the biomass (Kim *et al.*, 2001) which in turn increases the amount of SMP excretions (Rosenberger and Kraume, 2002).

Other studies have found varying ratios of carbohydrates to proteins depending on the operating conditions and feed matrix (Table 7-7). One study (Klatt and

LaPara, 2003) found much higher levels of proteins at steady state (90 mg.l^{-1} proteins, but this was using synthetic wastewater which has been shown to produce more proteins and carbohydrates (Le Clech *et al.*, 2003). The low levels measured before dosing in this study are due to a combination of factors: the long HRT, infinite SRT and the slow start up procedure employed (Grelier *et al.*, 2006, Pollice *et al.*, 2005).

Table 7-7 – Literature values of proteins and carbohydrates for differing system configuration and feed matrix compared to steady state values for this study.

Proteins (mg.l^{-1})	***Carbohydrates (mg.l^{-1})***	***Operating parameters***	***Reference***
9-12	6-10	Flux = 17 l.m^{-2}.h^{-1} SRT = no wastage HRT = 11 h Feed = municipal + toxin	This study
12	32	Flux = 5 l.m^{-2}.h^{-1} SRT = 106 days HRT = 15 h Feed = municipal	Spérandio *et al.*, 2005
90	20	Flux = ~6 l.m^{-2}.h^{-1} SRT = ∞ HRT = 8.5 h Feed = synthetic	Klatt and LaPara, 2003
16.8	13.1	Flux = 4.5 l.m^{-2}.h^{-1} SRT = 13 days HRT = ~8 h Feed = municipal	Ernst *et al.*, 2007

Neither the bleach nor the zinc sulphate had any impact on the protein or carbohydrate levels, with neither producing levels above 4 mg.gMLVSS^{-1} over the entire 4 HRT observation period. The washing powder and sodium dodecyl sulphate did have an effect on the protein and carbohydrate levels with both toxins producing an increase in levels. The washing powder had the more pronounced effect, with a sharp increase in protein levels observed directly after dosing, from 2.5 to 8.7 mg.g MLVSS^{-1} with a peak of 12.2 mg.gMLVSS^{-1} after one hour. A more moderate increase in carbohydrate levels was also recorded from 2 to 5.6 mg.gMLVSS^{-1} with a peak of 6.4 mg.gMLVSS^{-1} after one hour.

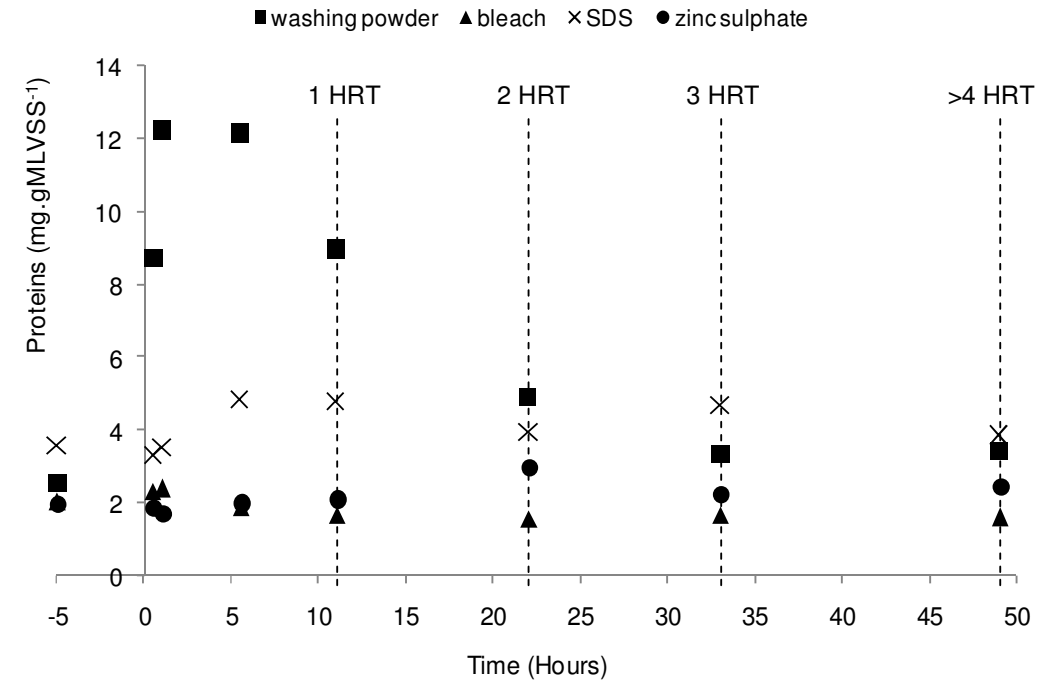


Figure 7-22 – SMP proteins normalised to MLVSS (t = -5 hrs was steady state operation before dosing and toxin was dosed at t = 0 hrs).

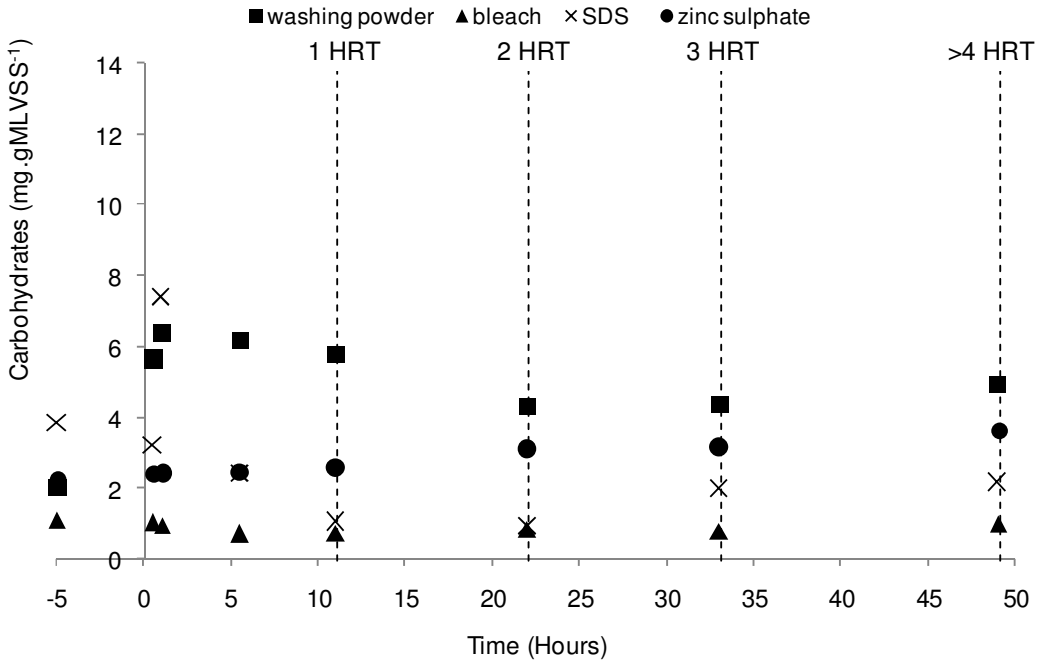


Figure 7-23 – SMP carbohydrates normalised to MLVSS (t = -5 hrs was steady state operation before dosing and toxin was dosed at t = 0 hrs).

In contrast to the washing powder, the SDS caused a larger increase in the carbohydrate concentration (3.2 to 7.4 mg.gMLVSS^{-1} from 0.5 hours to one hour after dosing) compared to the protein concentration (3.5 to 4.8 mg.gMLVSS^{-1} from one to 5.5 hours after dosing). Again these levels did not cause any significant fouling during the dosing trial. The lack of fouling is likely to be due to the operational setup of the MBR – a long HRT (Meng *et al.*, 2007) and SRT (Lee *et al.*, 2003), real sewage (Le Clech *et al.*, 2003), and a relatively low MLVSS (Li *et al.*,2008) are all conducive to a low fouling rate.

7.4.4.3 Trans membrane pressure

None of the toxins had any effect on the TMP of the membrane and no fouling was observed. This could be due to the fact that the system was operating as more of a static TMP system and the flow could not be automatically logged. The flow was checked at each sampling time and it was ensured it was at the correct level, however, towards the end of the monitoring period this meant that there were long periods when the flow was not monitored. For example, during the washing powder experiment there was a substantial decrease in TMP, but this occurred when the flow wasn't monitored and the TMP had increased again by the next sampling time (Figure 7-24). The flow could have decreased at this point, due to fouling and increased again when the scouring effect of the aeration had removed the temporary fouling. A similar effect was seen for the bleach dosing, however, no other parameter showed any indication of fouling or a detrimental effect on the biomass and it is assumed that this was due to operational difficulties rather than any specific effect on the fouling rate (Figure 7-25).

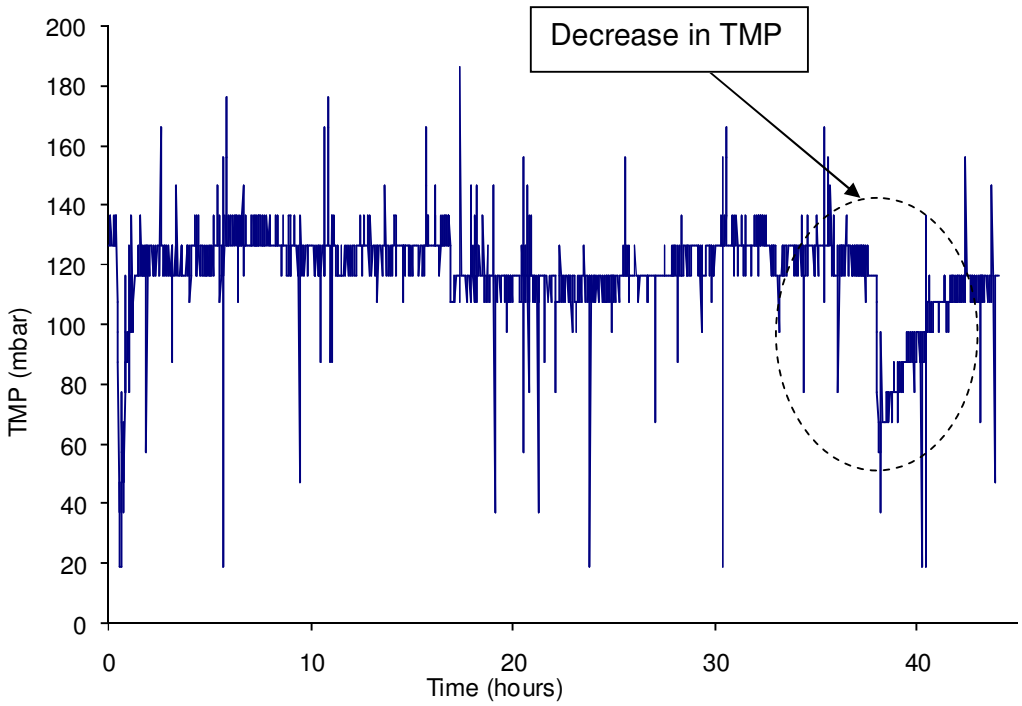


Figure 7-24 – TMP trace for washing powder

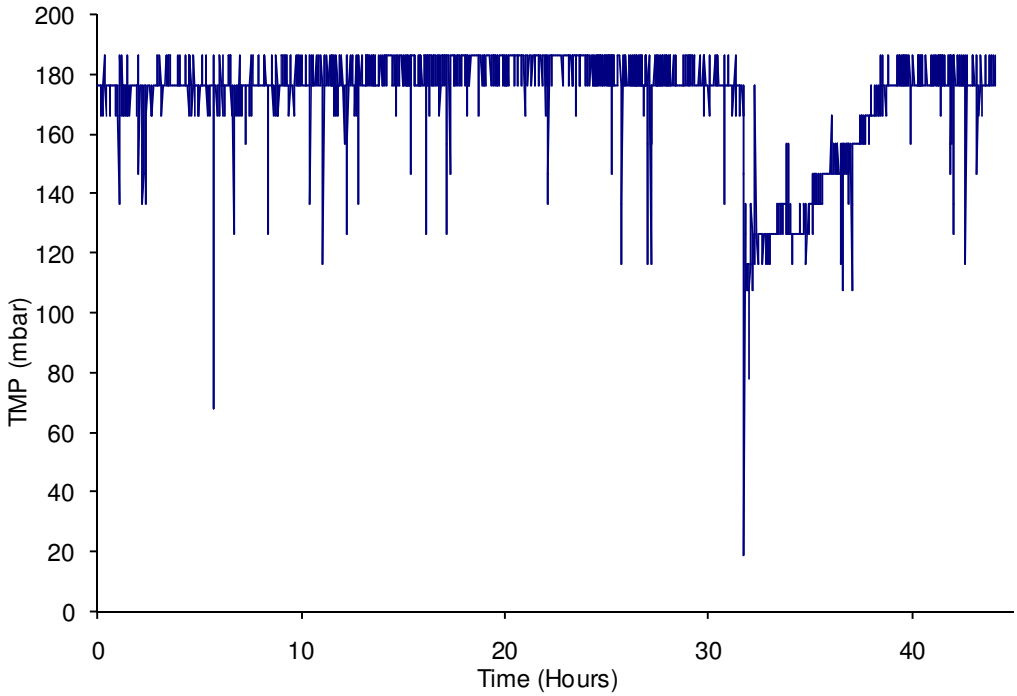


Figure 7-25 – TMP trace for bleach dosing.

After all the dosing trials had been carried out the membrane was removed from the MBR and evidence of clogging can be seen at the base of the module (Figure 7-26). Again from the analysis carried out this did not appear to have a detrimental affect on the performance of the MBR.

Figure 7-26 – Membrane after dosing trials had been carried out.

7.5 System performance

7.5.1 Effluent Quality

Overall the effluent quality was good with the effluent COD being below the levels put forward in guidelines for water reuse in Europe (Aquarec, 2005). These levels are 100 $mg.l^{-1}$ for effluent COD and 2-20 $mg.l^{-1}$ for ammonium nitrogen, for reuse in Class 1 for private and urban reuse. It has been advised that effluent turbidity should be ≤2 NTU and absolutely less than 5 NTU, to ensure effective downstream disinfection (Crook, 1998). This would be a problem with the current configuration of the MBR as the effluent turbidity increased to above 2 NTU for each of the dosing experiments with only SDS

maintaining an effluent turbidity of less than 5 NTU for the duration of the dosing experiment.

7.5.2 Toxic effects on the biomass

None of the toxins chosen for the dosing trial appeared to have a toxic effect on the biomass according to the parameters monitored. Most importantly the effluent ammonia was unaffected, as nitrifying bacteria are well known to be the most sensitive of the bacterial community to environmental changes. There was no effect on the biomass in terms of MLVSS and the CST was not adversely affected in the long term by any of the doses. The pH and conductivity remained in the same range as for the baseline monitoring and the particle size distribution was only affected by the SDS dosing.

7.5.3 Fouling potential

From the data gathered for the four dosing trials carried out it is likely that no fouling took place, however, as the flow could not be automatically monitored the fouling of the membrane may have resulted in a decrease in flux rather than a recorded decrease in TMP. Fouling is dependant on many factors including aeration rate, feed matrix and membrane type. Many studies have been carried out, as discussed previously in the literature review, with correlations being identified with SRT, the colloidal fraction of the biomass matrix, SMP proteins and carbohydrates, MLSS concentration and HRT. The effects of SRT and HRT can be discounted as both were observed to increase fouling at low values (SRT of 8 days Lesjean *et al.,* 2005 and HRT < 6h Meng *et al.,* 2007), neither of which were relevant for the pilot MBR. The MLSS concentration was at the lower end of the range for MBRs and this can also be discounted. This leaves the SMP fraction and the colloidal fraction as the most likely to cause fouling.

The reported role of proteins and polysaccharides in membrane fouling is contradictory with some studies reporting a direct correlation and others disputing the role (Drews *et al.*, 2006, Marshall *et al.*, 1993). Although quantitative values above which fouling will occur are reported, these are very dependant on the set up of the MBR, the feed matrix and the method of

measurement. For example, Evenblij *et al.*, (2005) did not find a correlation with fouling for the carbohydrates measured in the biomass supernatant for concentrations below 10 $mg.l^{-1}$, whereas Rosenberger *et al.*, (2006) found the fouling rate at a concentration of 10 $mg.l^{-1}$ carbohydrates to be twice that at 5 $mg.l^{-1}$. In the case of this study, neither the carbohydrates nor the proteins seem to have reached a critical level to cause fouling (Section 7.4.4.3).

The colloidal fraction present in the biomass matrix was measured using SMP turbidity. This fraction has been shown to influence fouling in the membrane with Bouhabila *et al.,* (2001) observing that the colloids present in the supernatant caused half of the loss of permeability over a 180 minute filter time. On the other hand Itonanga *et al.*, (2004) found that the colloidal fraction contributed as much as the suspended solids fraction to the overall resistance to filtration (~18 %) in a conventionally run MBR at steady state whereas it contributed more than 60% in an MBR with coagulation and sedimentation as a pretreatment stage, in a rapid fouling phase. The SMP turbidity values of >20 NTU for the SDS dosing compared to <15 NTU pre dosing, indicates a considerable fraction of colloidal particles is present, however, this does not seem to effect the membrane. The steady state levels of 1-2 NTU for the pilot MBR (apart from the SDS pre dosing level) are similar to those found by Rosenberger *et al.*, (2006) of 2 – 9 NTU.

Overall the MBR performed extremely well, with no effect on any of the system parameters measured, demonstrating the robustness of the system. The combination of a long hydraulic retention time, infinite sludge age and the use of a membrane as a barrier to stop any perturbations affecting the effluent have been an effective combination in negating any effects from the increase in toxic loading from the domestic products and industrial substances.

8 Chemical Mitigation of Fouling

The role of the parameters analysed for fouling potential were not fully explained by the trials on the MBR reported in Chapter 7. Although fouling was not observed for the four toxins tested, the effect of these toxins on the SMP turbidity and SMP proteins and carbohydrates was noticeable. These effects were mainly observed within the first hour after dosing and observing the response of these to the toxin dosing in more detail would lead to a better understanding of whether this was an instantaneous reaction or whether there was some time delay associated with it and when the peak occurred. It was also hoped to determine some quantitative measurement of these parameters to predict the boundary conditions that would cause fouling.

In order to counteract the release of foulants the use of chemical addition could be used to either coagulate or adsorb the colloidal particles and microbial secretions to minimise the risk of fouling and flux decline. A preliminary investigation was undertaken to find the most efficient chemical at producing this effect, using SMP turbidity and zeta potential as indicative parameters of the reduction of fouling potential and efficient coagulation or adsorption.

8.1 Jar testing with toxin only

Jar testing was carried out for four different toxins, washing powder, zinc sulphate, sodium dodecyl sulphate and bleach (Figure 8-1). Samples were taken for the first 60 minutes after dosing, at ten minute intervals, as this was when the most perturbation had been observed previously in the dosing trials (Chapters 6 and 7) and to ensure that the jar tests provided a biological system with a similar response to that seen in the porous pots and MBR. None of the toxins dosed contributed directly to the parameters measured.

The biomass used for the SDS, bleach and zinc sulphate jar tests had similar pre dosing CSTs of 100 seconds whereas the biomass used for the washing powder trial had an unusually low CST of 50 seconds, however, no explanation

was found for this as the origin of the biomass was the same and no changes had been made to the operational parameters of the MBR.

Three different responses were observed for the four toxins with respect to the CST measurements taken (Figure 8-1). The response to the sodium dodecyl sulphate (SDS) and zinc sulphate dosing was minimal with only small variations of less than 20%, from the pre dosing levels. This was expected as neither of these toxins had had any effect on CST in the previous MBR dosing trials. The washing powder dose produced an immediate 20 second increase which then remained stable over the remaining 60 minutes monitoring period. In contrast, the bleach dose produced a more delayed and steady increase in CST of approximately 5 seconds for each ten minute interval until a peak of 130 seconds was observed at the 40 minute sample, an overall increase of 20 seconds from pre dosing levels. This effect had not been observed in the MBR dosing trials, but is likely to be influenced by the oxidation action of the bleach on any organic content in the mixed liquor.

All of the jar tests had a similar SMP turbidity before dosing of approximately 2 NTU. As previously, the bleach, sodium dodecyl sulphate and zinc sulphate doses had little effect on the SMP turbidity. The washing powder dose, however, caused an immediate increase of turbidity from 2.2±0.02 NTU before dosing to 10.5±0.09 NTU ten minutes after. The peak turbidity was at 20 minutes after dosing at 14.8±0.03 NTU, thereafter the level settled to around 10 NTU (10.9±0.1 NTU, 9.5±0.1 NTU and 10.4±0.1 NTU at 30, 40 and 50 minutes respectively). This effect is the same as that experienced in the porous pot and MBR trials where the washing powder dose produced an instantaneous increase in SMP turbidity, however, as the jar test is a closed system the reduction in SMP turbidity after 20 minutes must have been due to a reflocculation of some of the colloidal element within the biomass matrix.

All the pre dosing samples had similar SMP protein levels of 10 to 12 $mg.l^{-1}$. Again the bleach, sodium dodecyl sulphate and zinc sulphate doses had little

effect on the SMP proteins measured. The washing powder, however, caused an immediate increase from 13.0±0.2 $mg.l^{-1}$ before dosing, to 44.8±0.3 $mg.l^{-1}$ after 10 minutes stirring time. This increase continued until a peak, after 50 minutes stirring, of 58.5±0.2 $mg.l^{-1}$.

The four toxins dosed produced a varied response in terms of SMP carbohydrates. The biomass used for both the zinc sulphate and the SDS had a high level of SMP carbohydrates of 17±0.3 and 16±0.4 $mg.l^{-1}$ pre dosing, respectively, whereas the biomass for the washing powder and bleach jar tests had pre dosing levels of 3.7±0.3 and 8.2±0.5 $mg.l^{-1}$.The washing powder dose showed only a slight increase over the first twenty minutes from the pre dose level of 3.7±0.3 $mg.l^{-1}$ to 4.4±0.4 $mg.l^{-1}$, however, the SMP carbohydrates increased steadily over the remaining forty minutes monitored, to a peak of 12.1±0.1 $mg.l^{-1}$ at 50 minutes. The bleach dosing produced a more erratic response in the SMP carbohydrates with levels being around 8 $mg.l^{-1}$, apart from at 30 minutes where there was an increase to 12.6±0.3 $mg.l^{-1}$.

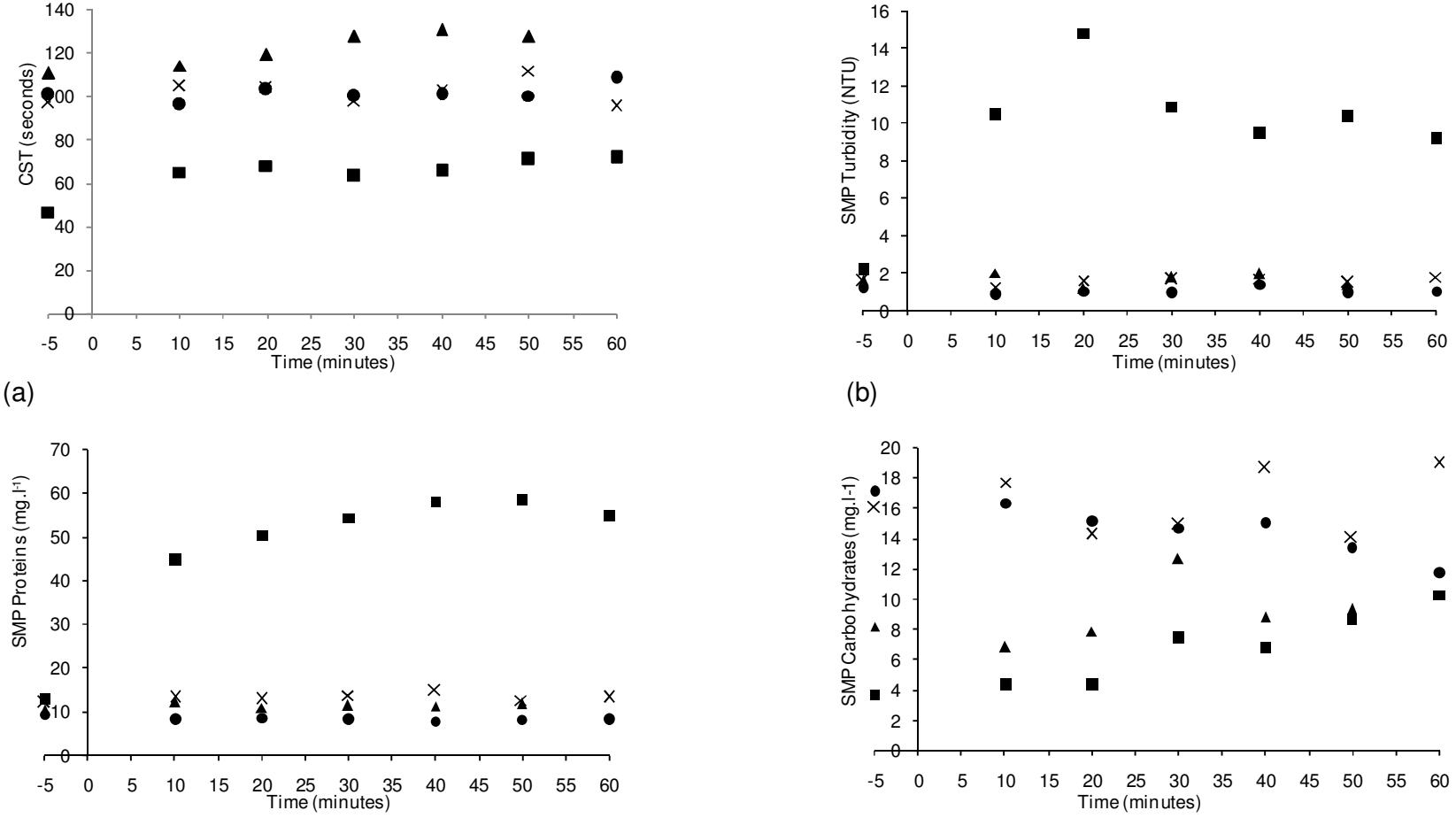


Figure 8-1 – (a) CST (b) SMP Turbidity (c) SMP proteins and (d) SMP carbohydrates for biomass dosed with washing powder (■), bleach (▲), SDS (x) and zinc sulphate (●) (t at -5 minutes = biomass only before toxin testing).

A summary of the effects observed in the first hour of the porous pot, MBR trials and the jar tests is given below (Table 8-1). The washing powder produced the most pronounced effects over the parameters measured with changes observed across the three systems used. For example, an increase of at least 7 NTU in SMP turbidity was observed across the three dosing trials. The other three toxins produced more varied responses with changes in parameters seen in some dosing trials but not others (Table 8-1).

In general, the effects observed in the jar tests reflected those in the other dosing trials, with the results following the MBR trials more closely than the porous pot trials. This is understandable as the biomass used for the jar testing was MBR biomass. An explanation of the effects observed have been described and explained in the previous trials chapters, the same being true for the jar tests. There were some exceptions, however, for example, in the first hour after the washing powder dose the CST increased by 40 seconds in the porous pot trial and by 100 seconds in the MBR trial, whereas for the jar testing the increase was only 30 seconds over the hour monitored.

Table 8-1 – Summary of the effects observed in the first hour after dosing for the porous pot and MBR trials.

Parameter	***Toxin dosed***	***Porous pot trial***	***MBR trial***	***Jar testing***
CST (secs)	WP[1]	60 to 106	156 to 255	47 to 72
	SDS[2]	40 to 47 to 44	142 to 175	97 to 112 to 95
	Bleach	68 to 86 to 82	80 to 85	111 to 130
	$ZnSO_4$	68 to 133 to 50	91 to 100	100 to 108
SMP turbidity (NTU)	WP	1.61 to 11.2	2.5 to 9	2 to 14 to 9
	SDS	6 to 8	14.1 to 15.6	1.6 to 1.8
	Bleach	4 to 8.5 to 5.9	2 to 2.7 to 2	1.7 to 2
	$ZnSO_4$	4 to 7.4 to 5	1.2 to 2	No change
SMP proteins ($mg.l^{-1}$)	WP	14 to 50	13 to 56	13 to 58 to 55
	SDS	No change	No change	No change
	Bleach	19 to 28 to 26	No change	No change
	$ZnSO_4$	17 to 24 to 18	No change	No change
SMP carbohydrates ($mg.l^{-1}$)	WP	7 to 34	10 to 29	4 to 10
	SDS	No change	21 to 42	16 to 19
	Bleach	5 to 14	No change	8 to 13
	$ZnSO_4$	12 to 13 to 8	No change	No change

[1]WP = washing powder, [2]SDS = sodium dodecyl sulphate.

Although the jar testing set up did not mirror the conditions or environment of the two dosing trials exactly, it would provide an opportunity to observe trends in the behaviour of the release of foulants and in the mitigation effects of the coagulants added.

8.2 Dosing with toxin and chemical.

8.2.1 Baseline analysis of the biomass.

For each jar test carried out a sample was taken of the biomass before any toxin or ancillary chemical was added. The distribution of the zeta potential and SMP turbidity of these samples are both normal with means of -13.1±0.2 mV and 13.5±1.8 NTU and the standard deviations are 1.207 and 9.8 respectively (Figure 8-2 and Figure 8-3). The zeta potential is similar to the range of -13 to -15 mV reported by Wu *et al.*, (2006) for activated sludge with an MLSS content of 3.9 – 4.3 $g.l^{-1}$. The SMP turbidity has a much larger spread of data for the baseline state with a shallower more widespread distribution of data compared to the zeta potential illustrating that the SMP turbidity is highly variable and dependant on many more variations within the biological system.

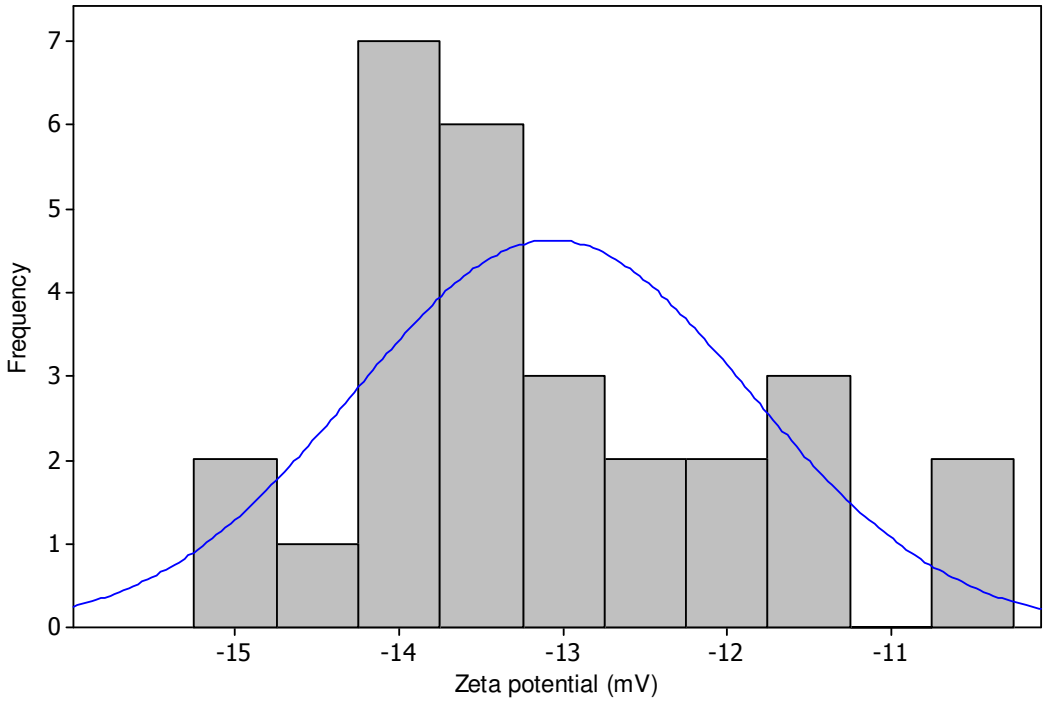


Figure 8-2 – Distribution for biomass zeta potential.

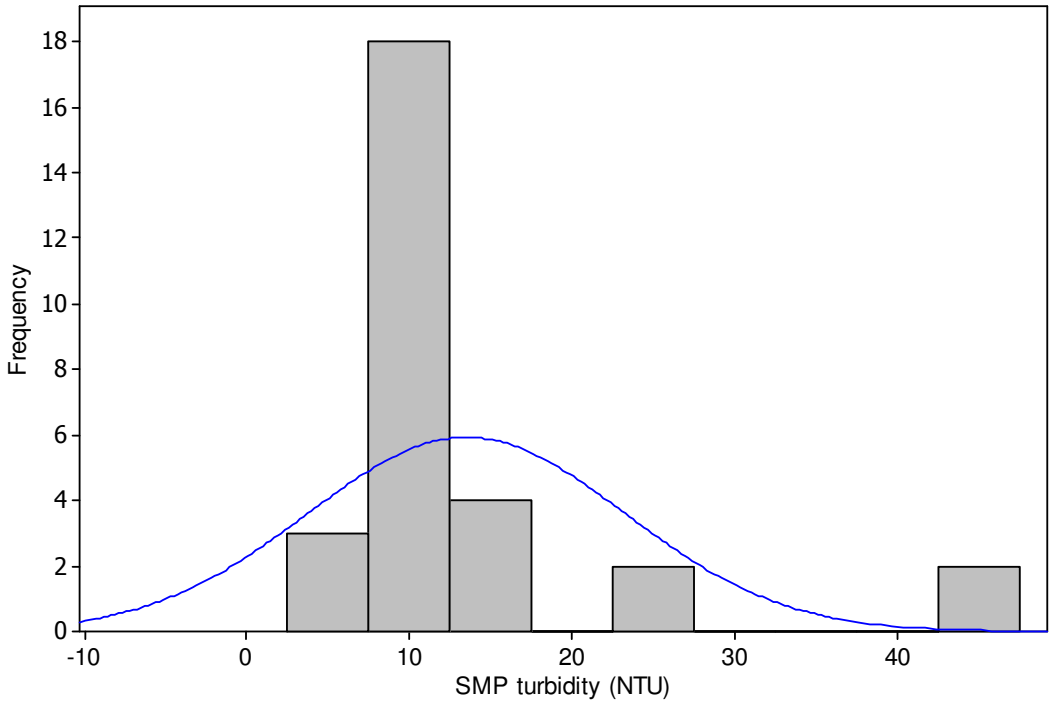


Figure 8-3 – Distribution for biomass SMP turbidity.

A plot of zeta potential against SMP turbidity for the 28 baseline samples taken reveals the extent of the spread of the data (Figure 8-4). The SMP turbidity has a range of 5.02±0.09 NTU to 44.3±0.5 NTU or 39.28 NTU whereas the zeta potential has a range of -15.1±0.1 mV to -10.6±0.6 mV or 4.5 mV. Any perturbation caused to the system by a toxin will be more apparent in the zeta potential value rather than the SMP turbidity.

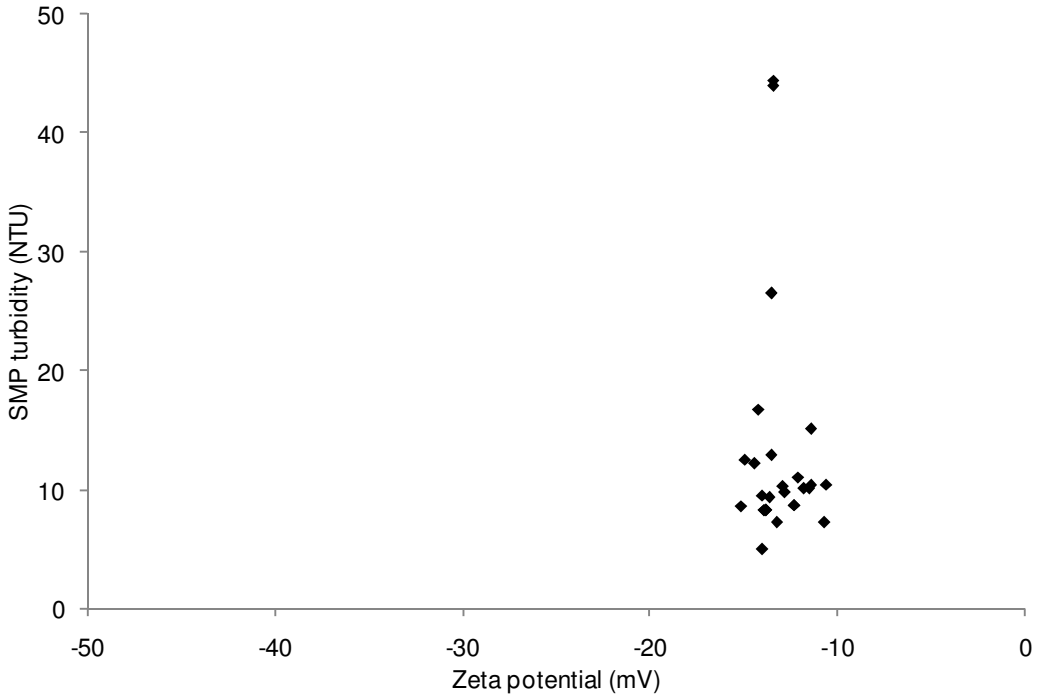


Figure 8-4 – Graph of zeta potential vs SMP turbidity for MBR biomass.

8.2.2 Effect of toxin on zeta potential and SMP turbidity.

The addition of the toxin to the biomass further changes the system and illustrates further the volatility of the SMP turbidity (Table 8-2). The maximum change to zeta potential caused by the addition of a toxin to the biomass is 3.4 mV whereas a change (decrease) of 32.6 NTU was observed for the SMP turbidity.

Table 8-2 – Change of zeta potential and SMP turbidity caused by toxins.

Toxin	***Change in zeta potential (mV) (max,min)***	***Change in SMP turbidity (NTU) (max,min)***	***n***
Washing Powder	-2.3,-0.5	-32.6,0.5	7
SDS	-1.2,-0.1	-13.3,-0.6	7
Bleach	-1.4,-0.1	-12.1,-0.3	7
$ZnSO_4$	3.4,0.3	-6.9,-1.22	7

8.2.3 Effect of polymer molecular weight on efficiency of coagulation.

Initially a jar test was carried out only using the different molecular weight range polyDADMAC as a coagulant to investigate the effect of the molecular weight of a polymer on the dose needed to reach a neutral zeta potential and any corresponding effect on the SMP turbidity, as a measure of fouling potential (Figure 8-5, Figure 8-6 and Figure 8-7). Data points at a concentration of -100 $mg.l^{-1}$ represent biomass only, while data points at 0 $mg.l^{-1}$ represent biomass plus toxin.

In general, as the molecular weight increased the concentration of polymer needed to reach a neutral zeta potential decreased, although each toxin dosed interacted differently with the polyDADMAC and produced different responses in terms of both the zeta potential and the SMP turbidity.

The sodium dodecyl sulphate and zinc sulphate dosed biomass showed a linear zeta potential response to the polyDADMAC dose, with the molecular weight having a clear effect (Figure 8-5). For example, the biomass dosed with 85 $mg.l^{-1}$ zinc sulphate had a zeta potential, at a dose of 500 $mg.l^{-1}$ of polyDADMAC, for very low molecular weight, low molecular weight, medium molecular weight and high molecular weight of 4±0.2 mV, 15±0.3 mV, 25±2.3 mV and 32±2.7 mV respectively. This response was mirrored for the SDS trial with the corresponding zeta potentials being 4±0.4 mV, 19.5±0.5 mV, 31.6±1.6 mV and 35.4±1.2 mV illustrating that for both toxin doses the difference between the very low, low and medium molecular weight doses are considerably larger than the difference between the medium and high molecular weight. It would appear that the effect is less pronounced as the molecular weight increases above 200,000 Daltons and, in the case of SDS, as the concentration increases; at 500$mg.l^{-1}$ the difference in zeta potential between the medium and high molecular weight polyDADMAC is 3.8 mV compared to 1.2 mV at a concentration of 1000 $mg.l^{-1}$. Both the medium and high molecular weight doses showed a plateauing of the effect after 500 $mg.l^{-1}$ concentration.

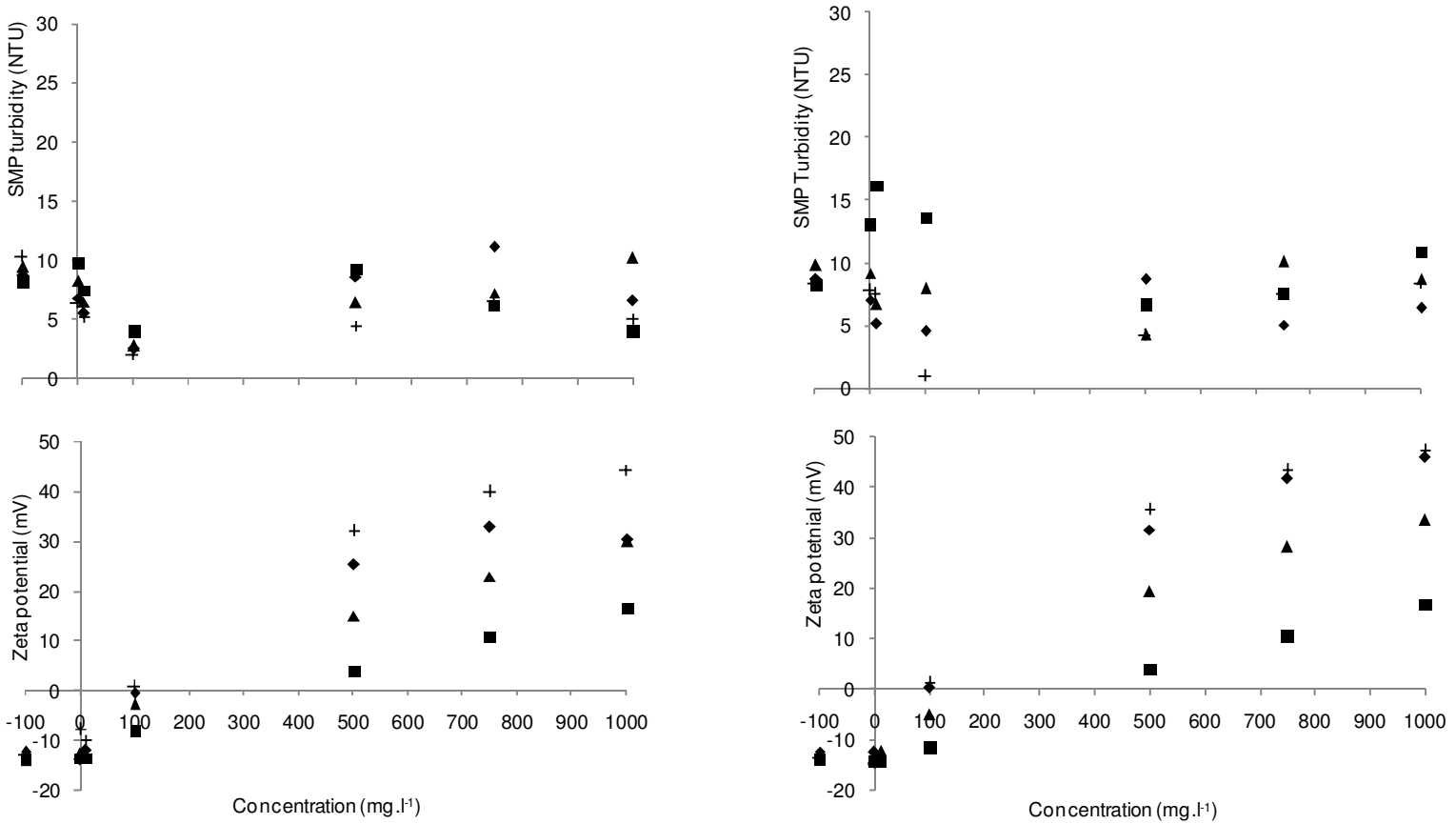


Figure 8-5 – Zeta potential and corresponding SMP turbidity for (a) biomass and $ZnSO_4$ and (b) biomass and SDS dosed with (+) high, (♦)medium, (▲) low and (■) very low MW polyDADMAC.

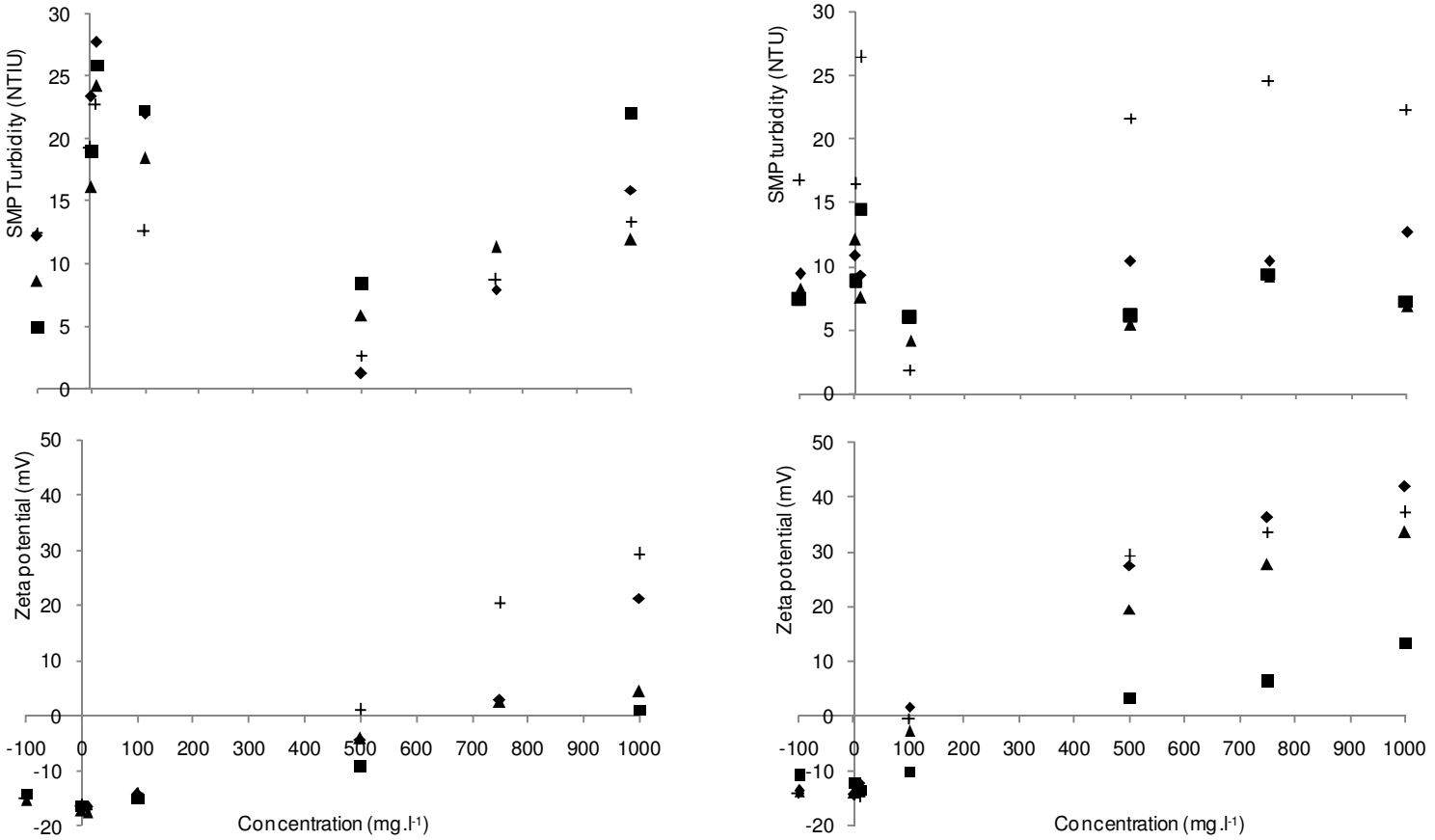


Figure 8-6 - Zeta potential and corresponding SMP turbidity for (a) biomass and washing powder and (b) biomass and bleach dosed with (+) high, (♦)medium, (▲) low and (■) very low MW polyDADMAC.

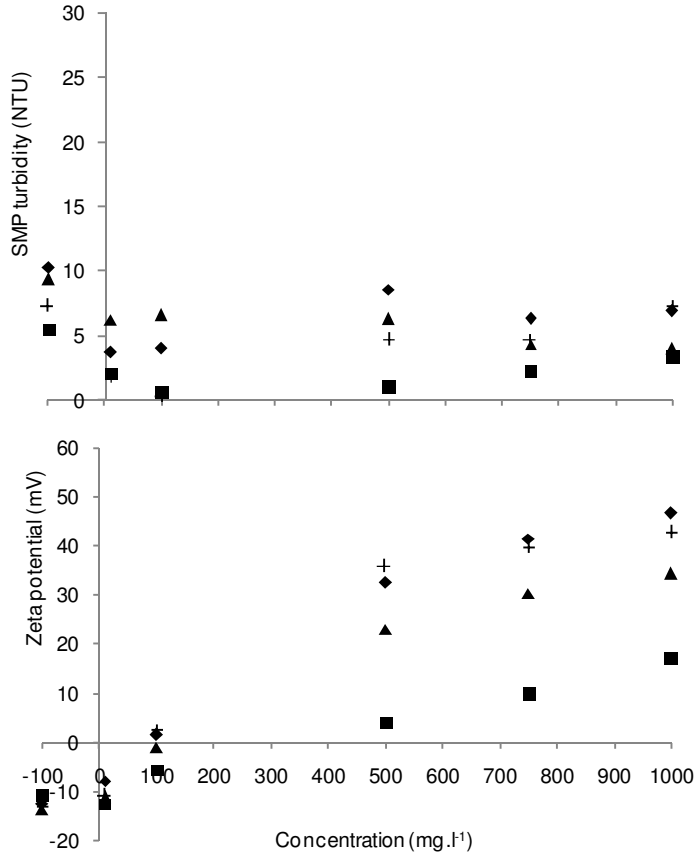


Figure 8-7 - Zeta potential and corresponding SMP turbidity for biomass dosed with (+) high, (♦)medium, (▲) low and (■) very low MW polyDADMAC.

Similar linear responses for zeta potential were observed for the biomass dosed with washing powder and bleach, however the distinction between the molecular weight ranges was not as clear (Figure 8-6). For bleach dosed biomass there was no distinction between the high and medium molecular weight polyDADMAC doses through the whole concentration range and the effect of the polyDADMAC plateaued at the higher concentrations for both high and medium molecular weight ranges. Moreover, the biomass dosed with washing powder showed an erratic response. At doses of ≤100 $mg.l^{-1}$ of polyDADMAC, at all molecular weight ranges, for the biomass dosed with 0.5 $mg.l^{-1}$ washing powder, no effect was observed on the zeta potential and at 500 $mg.l^{-1}$ of polyDADMAC the range was just 10 mV from very low to high molecular weights (-9±1 mV, -4±0.5 mV, -4.2±1.8 mV, 1.1±2.4 mV respectively). Compare this with a range of 28 mV for the zinc sulphate dosed biomass, 25.8 mV for bleach dosed biomass and 31 mV for the SDS dosed biomass. At 1,000 $mg.l^{-1}$, the highest concentration tested, the range was back to a comparable 28 mV.

This effect was also observed for the jar test with the biomass only dosed with polyDADMAC (Figure 8-7), however, there was some discernible effect on the zeta potential with as little as 100 $mg.l^{-1}$ polyDADMAC. There was little difference between the medium and high molecular weight doses, although the high molecular weight dose plateaued before the medium molecular weight, at a concentration of 500 $mg.l^{-1}$ and the medium molecular weight dose produced a slightly greater effect for the 750 and 1000 $mg.l^{-1}$ doses with a zeta potential of 41.4±0.4 mV and 46.5±1.7 mV respectively compared with 39.5±2.9 mV and 42.5±1.7 mV for the high molecular weight dose.

The dose required to reach a neutral zeta potential was dependant on the toxin dosed and the molecular weight of the polyDADMAC added (Table 8-3). For example, for the very low molecular weight polyDADMAC the estimated dose required for a neutral zeta potential for the biomass dosed with washing powder (1013 $mg.l^{-1}$) was almost three times that required for the biomass only (371 $mg.l^{-1}$), in comparison the zinc sulphate, SDS and bleach only needed around 1.2 times (419, 436 and 472 $mg.l^{-1}$ respectively). As the molecular weight

increased the dose needed to reach a neutral zeta potential decreased across all the toxins dosed. In the case of the biomass dosed with washing powder this decrease was close to three times less between the very low and high molecular weight doses (1013 $mg.l^{-1}$ and 360 $mg.l^{-1}$).

Table 8-3 – Analysis of dosing of polyDADMAC with respect to zeta potential.

Molecular weight range	***Toxin dosed***	***Estimated dose for neutral zeta potential ($mg.l^{-1}$)***	***Gradient ($mV.(mg.l^{-1})^{-1}$)***	***R^2 value***
very low	biomass	371	0.026	0.979
	$ZnSO_4$	419	0.029	0.988
	SDS[1]	436	0.030	0.987
	WP[2]	1013	0.015	0.933
	bleach	472	0.024	0.966
low	biomass	159	0.047	0.954
	$ZnSO_4$	240	0.042	0.977
	SDS	210	0.048	0.969
	WP	749	0.021	0.960
	bleach	215	0.048	0.965
medium	biomass	83	0.077	0.993
	$ZnSO_4$	176	0.047	0.904
	SDS	134	0.061	0.951
	WP	519	0.031	0.917
	bleach	132	0.074	0.953
high	biomass	86	0.085	0.981
	$ZnSO_4$	111	0.057	0.956
	SDS	139	0.064	0.935
	WP	360	0.044	0.966
	bleach	138	0.079	0.958

[1]SDS = sodium dodecyl sulphate
[2]WP = washing powder

The gradient of the dose response curve was calculated (before the plateau in the case of bleach and biomass for medium and high molecular weight polyDADMAC) which gives the mV change in zeta potential per $mg.l^{-1}$ change in concentration of polyDADMAC (Table 8-3). The gradient increases as the molecular weight increases, illustrated by the biomass only jar test, with a gradient for very low molecular weight addition of 0.026 $mV.(mg.l^{-1})^{-1}$ which doubled for low molecular weight and again for medium molecular weight doses with gradients of 0.047 and 0.077 $mV.(mg.l^{-1})^{-1}$. This effect is then slightly diminished with a gradient of 0.085 $mV.(mg.l^{-1})^{-1}$ for the high molecular weight dose. Therefore, as the molecular weight increases the dose required to neutralise the zeta potential decreases. The biomass dosed with the four toxins showed similar effects as the molecular weight of the polyDADMAC dosed increased.

A simultaneous minimum in SMP turbidity was observed with the neutral zeta potential point, across all the molecular weight ranges, for twelve out of the twenty jar tests completed, moreover, there was not a general response to the polyDADMAC dosing (Figure 8-5 to Figure 8-7 and Table 8-4). In the case of the biomass only jar test, the polyDADMAC had a rapid effect at 10 $mg.l^{-1}$ with decreases of 3.53, 3.08, 6.62 and 5.54 NTU for very low, low, medium and high molecular weights, respectively. Thereafter only the very low and high molecular weight polyDADMAC caused a further decrease to a minimum of 0.645±0.05 NTU and 0.288±0.01 NTU respectively, which coincided with the neutral zeta potential point, whereas the low and medium molecular weight polyDADMAC caused a slight increase at this point and in fact the minimum SMP turbidity for low molecular weight occurred at 10 $mg.l^{-1}$, while for medium molecular weight this did not occur until 1,000 $mg.l^{-1}$, the highest concentration tested, far outwith the neutral zeta potential point. Low molecular weight polymer will cause charge neutralisation reducing the repulsive forces between the particles and result in an aggregation of these particles, however, too much polymer addition will reverse the charge from negative to positive and the same repulsive forces will be present again (Ebeling *et al.*, 2005). For example, the biomass only jar test had a zeta potential of -12.5 mV with 10 $mg.l^{-1}$ of very low MW polyDADMAC with a corresponding SMP turbidity of 2.02 NTU and at a zeta potential of 9.8 mV with 750 $mg.l^{-1}$ the SMP turbidity was roughly the same at 2.22 NTU. A high MW polymer will cause charge neutralisation as well as bridging between the particles producing larger, more loosely packed flocs. Again, if too much polymer is dosed then the long tail of the polymer attaches to too many sites on the target particle and wraps itself around, leaving no free sites to bridge to other particles – the “hair ball” effect (Ebeling *et al.*, 2005). For example for the biomass jar test a minimum SMP turbidity of 0.288 NTU was reached at the neutral zeta potential and then this increased as the dose increased to a maximum of 7.33 NTU.

The washing powder dosed biomass showed a similarly erratic response; a small dose of 10 $mg.l^{-1}$ of the polyDADMAC across the molecular weight ranges caused a large increase in the SMP turbidity of between 3.5 NTU (high molecular weight polyDADMAC) and 8.1 NTU (low molecular weight

polyDADMAC). The minimum SMP turbidity, across the molecular weight range, only coincided, in this case, with the neutral zeta potential point for the high molecular weight polyDADMAC, and the minimum SMP turbidities observed were greater than those for the other three toxins apart from the medium molecular weight polyDADMAC where it was considerably lower (1.27±0.19 NTU for washing powder compared with 3.68±0.04 NTU for biomass only, 2.49±0.16 NTU for zinc sulphate, 4.52±0.09 NTU for sodium dodecyl sulphate and 5.9±0.16 NTU for bleach). The surfactant action of the washing powder caused a greater increase in SMP turbidity initially compared to the other toxins (normally 7 – 8 NTU compared to 2-4 NTU for the other toxins) and this had an effect on the action of the polymer, coupled with the vigourous stirring present for the duration of the test.

The biomass dosed with bleach responded differently to the different molecular weight polyDADMACs (Figure 8-6). At 10 $mg.l^{-1}$ concentration the very low and high molecular weight doses caused an increase in the SMP turbidity (from 8.73±0.02 NTU to 14.5±0.02 NTU for very low MW and 16.4±0.12 NTU to 26.4±0.34 NTU for high MW), whereas, the low and medium molecular weight doses caused a decrease (12.1±0.09 NTU to 7.53±0.02 NTU low MW and 10.8±0.03 NTU to 9.3±0.3 NTU for medium MW). In spite of this initial response the high molecular weight polyDADMAC dose produced the minimum SMP turbidity (Table 8-4) of 1.85±0.09 NTU before increased doses forced a large increase to levels of around 20 NTU.

Table 8-4 – Minimum observed SMP turbidity, maximum SMP turbidity removal and corresponding concentration of polyDADMAC.

Molecular weight range	***Toxin***	***Minimum SMP turbidity (NTU±S.E.)***	***Max. SMP turbidity removal (%)***	***Coincident with neutral zeta potential point?***	***Concentration ($mg.l^{-1}$)***
very low	biomass	0.645±0.05	89	✓	100
	$ZnSO_4$	4.05±0.06	59	x	100
	SDS[1]	6.68±0.09	49	✓	500
	WP[2]	8.45±0.13	56	x	500
	bleach	5.93±0.15	32	x	100
low	biomass	4.05±0.03	57	x	1000
	$ZnSO_4$	2.8±0.09	66	✓	100
	SDS	4.22±0.04	54	x	500
	WP	5.87±0.1	64	x	500
	bleach	4.1±0.01	66	✓	100

Molecular weight range	*Toxin*	*Minimum SMP turbidity (NTU±S.E.)*	*Max. SMP turbidity removal (%)*	*Coincident with neutral zeta potential point?*	*Concentration (mg.l^{-1})*
medium	biomass	3.68±0.04	64	x	10
	$ZnSO_4$	2.49±0.16	64	✓	100
	SDS	4.52±0.09	36	✓	100
	WP	1.27±0.19	95	x	500
	bleach	5.9±0.16	45	✓	100
high	biomass	0.288±0.01	96	✓	100
	$ZnSO_4$	2.01±0.15	69	✓	100
	SDS	0.937±0.11	88	✓	100
	WP	2.67±0.39	86	✓	500
	bleach	1.85±0.09	89	✓	100

[1]SDS = sodium dodecyl sulphate
[2]WP = washing powder

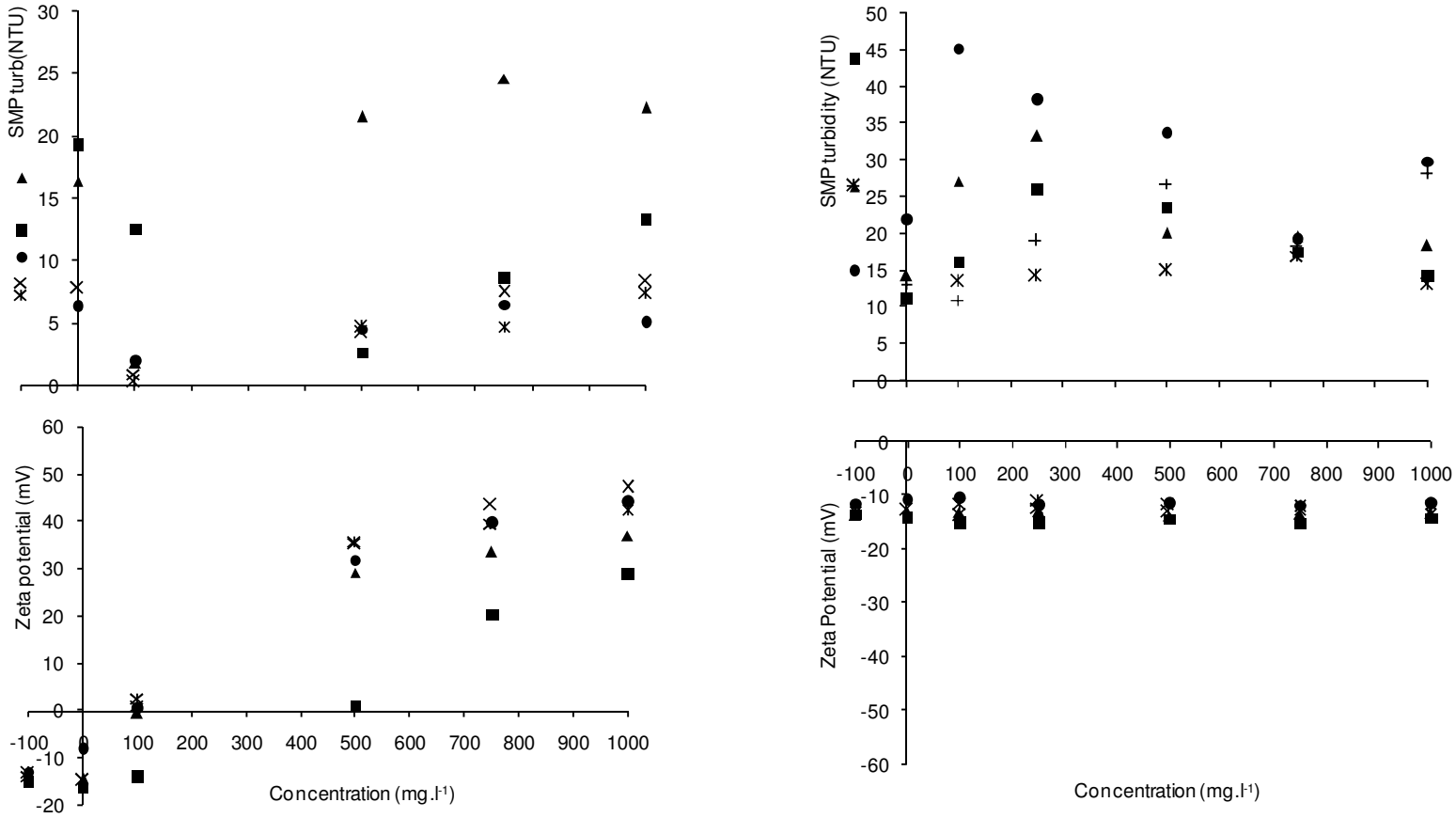
8.2.4 Effect of type of chemical on removal of fouling potential.

In terms of the parameters monitored, zeta potential and SMP turbidity the polymers produced a better overall result than the metal salt or powdered activated carbon. The powdered activated carbon had no effect on the zeta potential as was expected as the action is not conducive to destabilising the electrostatic effect but rather an adsorption of colloids. Once the Ferripol XL reached a neutral zeta potential there was no further increase and in fact the metal salt started to precipitate out and increase the SMP turbidity.

At the initial concentrations tested the biomass dosed with washing powder did not reach a neutral zeta potential with 1,000 mg.l^{-1} concentration of MPE50 therefore further tests were carried out to ensure that the neutral zeta potential point had been passed (Table 8-5).

Table 8-5 – Additional concentrations for MPE50 to gain a neutral zeta potential with biomass dosed with washing powder.

Concentration	*Zeta potential (mV)*	*SMP turbidity (NTU)*
1500	4.3±0.8	0.577±0.04
2000	1.8±1.0	0.766±0.04
2500	3.7±0.4	0.437±0.1



(a) (b)

Figure 8-8 – Zeta potential and corresponding SMP turbidity for (a) high MW polyDADMAC and (b) powdered activated carbon as coagulant for the four toxins (■) washing powder, (▲) bleach, (x) SDS, (●) $ZnSO_4$ against a control: (∗) biomass.

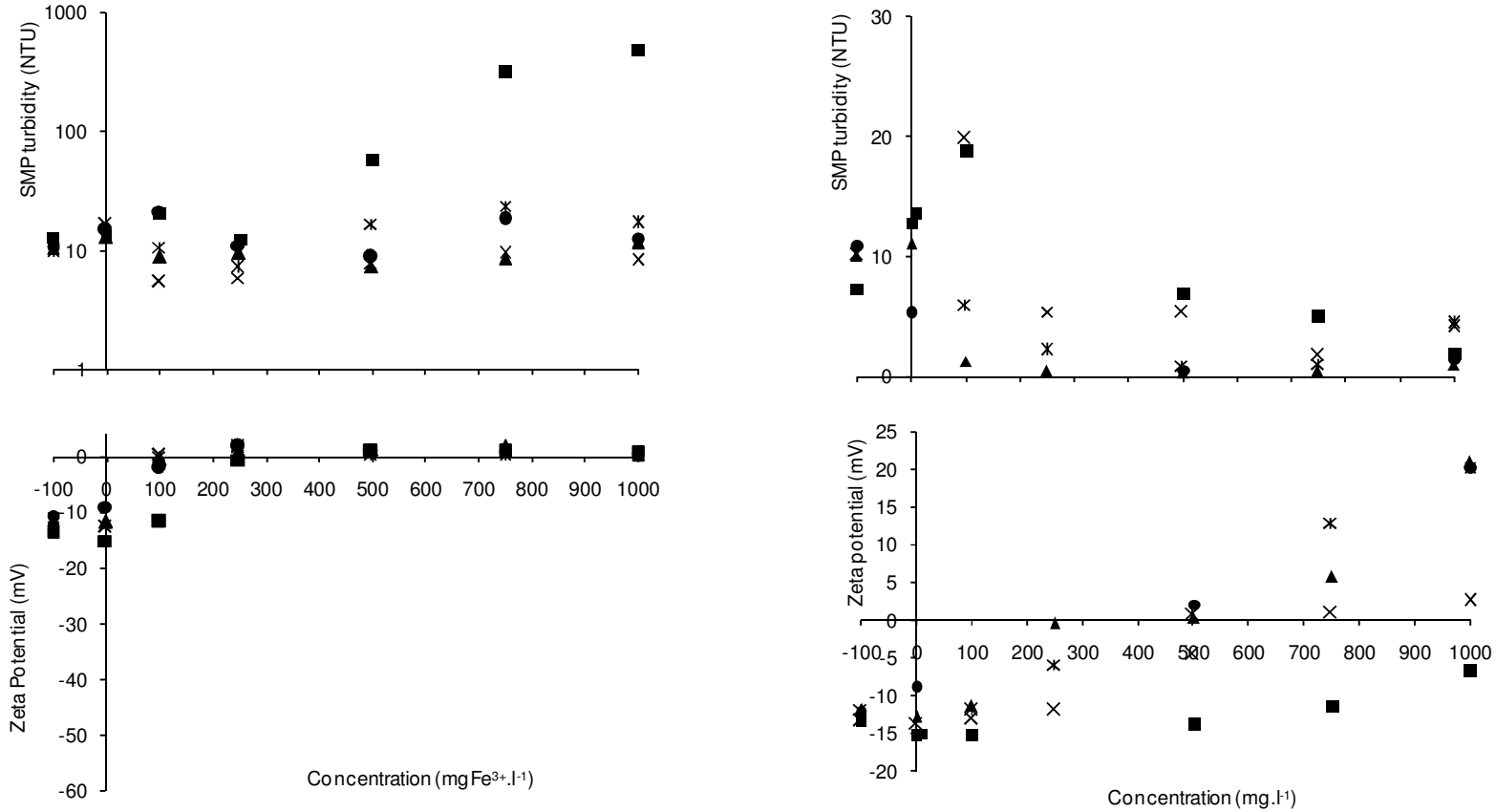


Figure 8-9 - Zeta potential and corresponding SMP turbidity for (a) Ferripol XL and (b) MPE50 as coagulant for the four toxins washing powder(■), bleach(▲),SDS(x) and $ZnSO_4$ (●) against (✳) biomass.

Analysis of the dose required to reach a neutral zeta potential and the gradient of the dose response curves reveals that there is some difference between the chemicals (Table 8-6). In general the zeta potential versus concentration was a linear response apart from the biomass dosed with washing powder and MPE50 which produced a quadratic response. The maximum gradient was taken over the steepest part of the curve in each instance, over a minimum of three data points. A steeper gradient translates into a potentially lower concentration of chemical needed to destabilise the colloids present in the biomass matrix, to allow their capture in the resultant larger flocs, in turn minimising the fouling potential in an MBR.

Table 8-6 - Analysis of dosing of chemicals with respect to zeta potential.

Chemical	***Toxin dosed***	***Estimated dose for neutral zeta potential ($mg.l^{-1}$)***	***Max Gradient ($mV.(mg.l^{-1})^{-1}$)***	***R^2 value***
High MW. polyDADMAC	biomass	86	0.085	0.981
	$ZnSO_4$	111	0.057	0.956
	SDS [1]	139	0.064	0.935
	WP [2]	360	0.044	0.966
	bleach	138	0.079	0.958
Ferripol XL	biomass	100	0.042	0.880
	$ZnSO_4$	194	0.041	0.921
	SDS	183	0.054	0.737
	WP	270	0.059	0.964
	bleach	200	0.046	0.719
MPE50	biomass	403	0.031	0.961
	$ZnSO_4$	343	0.028	0.985
	SDS	809	0.017	0.946
	WP [3]	1370	0.018	0.993
	bleach	413	0.028	0.921

[1] SDS = sodium dodecyl sulphate
[2] WP = washing powder
[3] Including additional data points

The washing powder increased the dose of chemical needed to reach a neutral zeta potential in all tests, compared with the biomass. In the case of high MW polyDADMAC the dose for biomass only was 86 $mg.l^{-1}$ compared with 360 $mg.l^{-1}$ with washing powder addition, for Ferripol XL the dose for biomass dosed with washing powder was over twice that for biomass alone (270 $mg.l^{-1}$ versus 100 $mg.l^{-1}$) and with MPE50 as the additive chemical the dose was increased by over three times from 403 $mg.l^{-1}$ to 1370 $mg.l^{-1}$. The other toxins increased the respective doses needed but never by more than double, apart from the biomass dosed with zinc sulphate which had a lower concentration of MPE50 compared to that of the biomass of 343 $mg.l^{-1}$ compared to 403 $mg.l^{-1}$.

SMP turbidity removal rates varied greatly over the tests performed with the best removal rate being 96% for both the biomass with high MW polyDADMAC added and biomass dosed with sodium dodecyl sulphate with MPE50 added, and the worst being 7% for biomass dosed with washing powder with Ferripol XL added (Table 8-7).

Table 8-7 - Minimum observed SMP turbidity, maximum SMP turbidity removal and corresponding concentration of added chemical.

Chemical	***Toxin***	***Minimum SMP turbidity (NTU±S.E.)***	***Max. SMP turbidity removal (%)***	***Coincident with neutral zeta potential point?***	***Concentration ($mg.l^{-1}$)***
high MW polyDADMAC	biomass	0.288±0.01	96	✓	100
	$ZnSO_4$	2.01±0.15	69	✓	100
	SDS[1]	0.937±0.11	88	✓	100
	WP[2]	2.67±0.39	86	✓	500
	bleach	1.85±0.09	89	✓	100
Ferripol XL	biomass	7.21±0.20	28	x	250
	$ZnSO_4$	8.74±0.57	41	✓	500
	SDS	5.40±0.3	67	✓	100
	WP	12.4±0.3	7	✓	250
	bleach	7.28±0.07	44	x	500
PAC	biomass	13.0±0.1	51	N/A	1000
	$ZnSO_4$	19.5±0.3	11	N/A	750
	SDS	11.0±0.2	17	N/A	100
	WP	>control	N/A	N/A	N/A
	bleach	>control	N/A	N/A	N/A
MPE50	biomass	0.927±0.03	91	✓	500
	$ZnSO_4$	0.565±0.06	90	✓	500
	SDS	1.76±0.43	96	✓	750
	WP	0.437±0.10	94	x	2500
	bleach	0.359±0.02	97	✓	500

In two cases (washing powder and bleach with powdered activated carbon added) the SMP turbidity increased above that of the control (biomass with toxin added). On the whole, the two polymers (high MW polyDADMAC and MPE50) added to the biomass performed better than ferric sulphate or PAC. However, the high MW polyDADMAC reached removal rates of >85% in three instances with a concentration of just 100 $mg.l^{-1}$ compared to the MPE50 which needed doses of 500 – 2500 $mg.l^{-1}$ for >90% removal. The levels of MPE50 for three out of the four toxins are similar to those reported by Collins *et al.* (2006), who found concentrations of between 400 – 600 $mg.l^{-1}$ were needed to enhance the flux by twofold, in a membrane bioreactor running at steady state. Iversen *et*

al., (2008) and Koseoglu *et al*., (2008), reported an optimum dose of 500 mg.l^{-1} of MPE50 for MLSS concentrations of 15 and 8-10 g.l^{-1} respectively. In the tests carried out in this study the washing powder has increased the concentration needed to reach a neutral zeta potential to around five times these levels.

9 Discussion

Key observations from the work were:

1. The highest level risks identified from the Microtox and respirometry were identified for the bleach based household products, the hydrogen peroxide and sodium hypochlorite. Secondary risks from washing up liquids containing specific antimicrobial agents or essential oils were also identified. Overall, 11 out of 19 of the household products were likely to be discharged at levels that would exceed the EC_{50} values on a weekly basis.
2. No identifiable pattern was observed between the toxicity within a product group and the ingredients of the products and/or the cost or environmental status of the products. In particular, complete product toxicity could not be inferred from the individual ingredients and thus makes whole product testing critical for any assessment. Microtox represented a more sensitive system giving more conservative EC_{50} values than the respirometry by two orders of magnitude and so potentially acts as a conservative screening tool.
3. The impact of the toxic shocks varied depending on the scales of the system considered. In the case of the porous pots, the impacts were principally observed in relation to effluent turbidity, effluent COD, effluent ammonia, and the potential foulants of SMP turbidity and SMP carbohydrates and proteins. Whereas in the case of the MBR shocked with washing powder the impacts were principally observed in relation to an increase in effluent COD, effluent turbidity, conductivity, SMP turbidity and SMP carbohydrates and proteins.
4. Remediation of the impact of increase in turbidity could be effectively managed through polymer addition. MPE50 (cationic polymer) was observed to be more effective than polyDADMAC and the efficacy increased in general as the MW of the polymer increased. Application of MPE50, for instance, enabled a 90% removal of turbidity irrespective of the toxin added.

The overall implications of these observations on the operation of an MBR for urban water reuse are that chemical shock loads represent a key risk to both effluent compliance and sustainable membrane operation.

The toxic effects predicted by the assessment using Microtox and respirometry were not observed in the biological systems. For illustration the case study comparing washing powder and bleach will be considered. The washing powder had an EC_{50} value of 1400 ppm, obtained by respirometry, which was far greater (less toxic) than the EC_{50} of 480 ppm for bleach. However, the impact of the washing powder on the system was evident even at the much lower dose of 500 ppm compared with 400 ppm dose for bleach, which showed little or no effect. The differences are related to the nature of biomass where the susceptibility to toxins is reduced due to the protective nature of excreted extracellular polymeric substances. In conjunction, the impact of toxins needs to be considered in relation to the amount of substrate available. For instance, an investigation into the effects of substrate concentration revealed that, with a constant toxin concentration (in this case 0.4 $ml.l^{-1}$ bleach), the higher the concentration of substrate, the lesser the effect on the relative oxygen uptake rate (Figure 9-1). The consequence of this is that in continuously fed reactors (porous pot, MBR) the constant supply of substrate is reducing the impact of the toxic shock, whereas, the respirometry is a batch fed system with a fixed amount of substrate and hence more sensitive to the shock load. Ultimately this means the translation of toxicity from batch to continuous systems is a complex and difficult process and potentially limits the importance of batch toxicity tests over and above simple ranking.

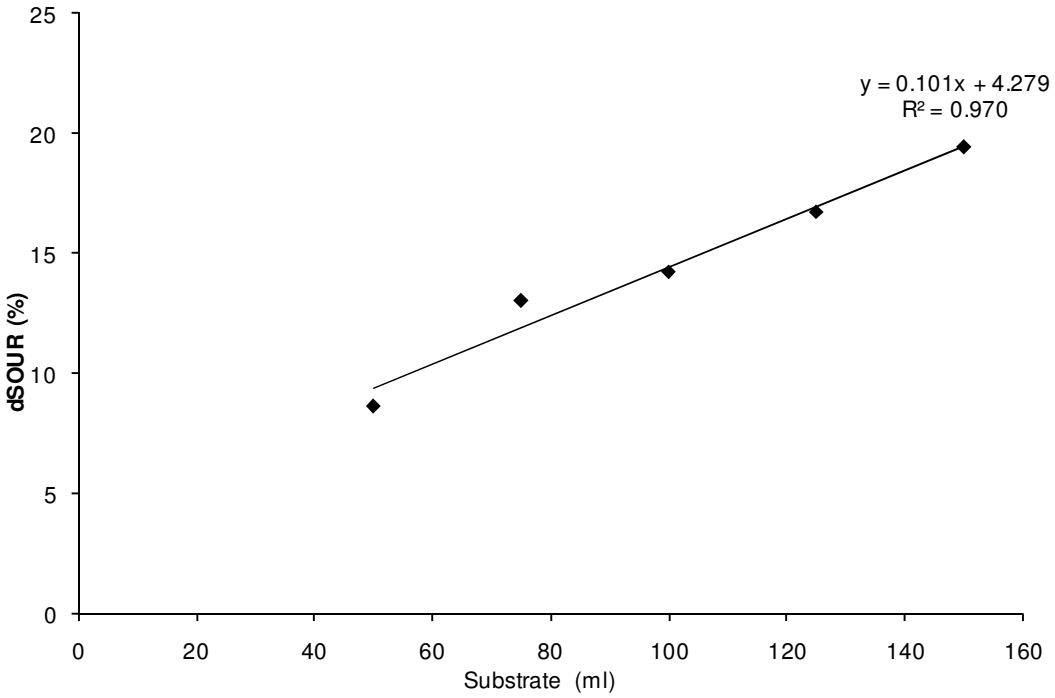


Figure 9-1 – Effect of varying substrate concentration on oxygen uptake with a constant toxin concentration.

Comparison of the impact of the toxic shocks on effluent quality at both porous pot (bench scale) and MBR (pilot scale) demonstrated similar rankings of effected variables but with different magnitudes. No significant correlation between changes in effluent quality and SMP were observed. For instance, the SMP turbidity did not follow the effluent COD or turbidity response for the porous pot trials or the MBR trials, but increased rapidly within the first hour after dosing, in line with the SMP carbohydrates and proteins (Figure 9-2 to Figure 9-1). Reid (2005) demonstrated a positive correlation of SMP turbidity with SMP carbohydrate (Pearsons coefficient of 0.81) and a negative correlation of SMP carbohydrate with permeability (Pearsons coefficient of -0.42) for MBR biomass experiencing high salinity. The release of potential foulants peaked one hour after dosing for both the porous pots and the MBR even though both had different HRTs of 6 hours and 11 hours respectively, whereas the effluent COD and effluent turbidity peaked after 1 HRT for the porous pots and after 2 HRTs for the MBR. Proteins have been shown to be instrumental in floc formations in activated sludge systems (Dignac *et al.,* 1998, Bura *et al.,* 1998)

with extracted extracellular polymer substances being composed of 75% proteins. The rapid increase seen after the washing powder dose is likely to be the result of the deflocculation or break up of the biomass. The increase in effluent turbidity that followed several hours after is likely to be these smaller particles being washed from the system.

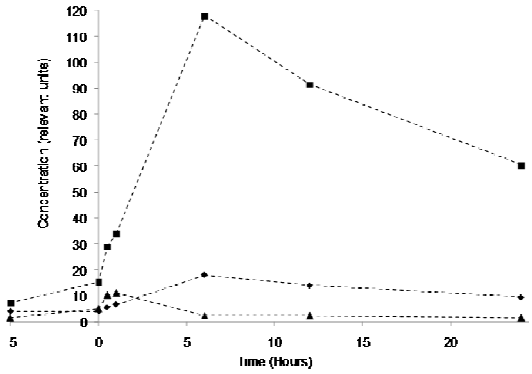


Figure 9-2 – comparison of effluent COD (■, mg.l-1) effluent turbidity (♦, NTU)
and SMP turbidity (▲, NTU) for washing powder dosed into the porous pots.

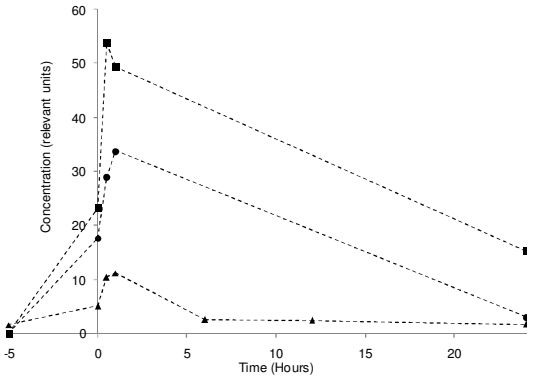


Figure 9-3 – comparison of proteins (■,mg.l^{-1}), carbohydrates (●, mg.l^{-1}) and SMP turbidity (▲, NTU) for washing powder dosed into the porous pots.

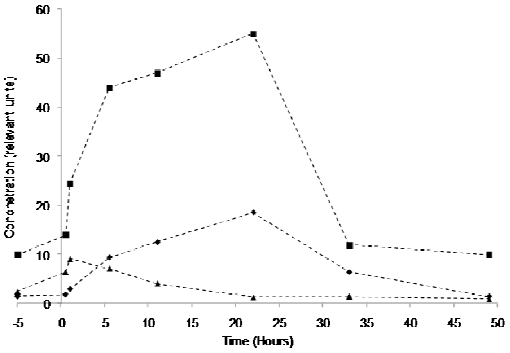


Figure 9-4 – comparison of effluent COD (■, mg.l^{-1}) effluent turbidity (♦, NTU) and SMP turbidity (▲, NTU) for the pilot MBR.

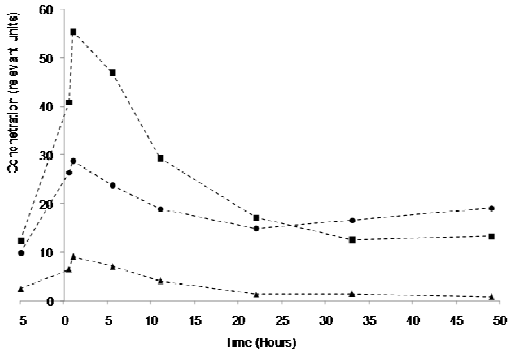


Figure 9-5 – comparison of proteins (■,mg.l^{-1}), carbohydrates (●, mg.l^{-1}) and SMP turbidity (▲, NTU) for washing powder dosed into the pilot MBR

Fouling was not observed in this particular pilot plant for the maximum SMP carbohydrate concentration observed of 28 mg.l^{-1} (washing powder trial), however, carbohydrates have been identified as the major component in membrane fouling (Le-Clech *et al.*, 2006) and a linear relationship was observed for carbohydrate concentration and fouling rate in an MBR treating municipal wastewater with an SRT of 8 days from 5 – 15 mg.l^{-1} carbohydrates

(Lesjean *et al*., 2005). This relationship was generally replicated for an MBR operating at unsteady state, in terms of organic loading, and a 15 day SRT, (Drews *et al*., 2006), but the data collected showed a large scatter around the linear correlation, with some deviations of more than 100%. Concentrations in the range of 20-30 $mg.l^{-1}$ resulted in a wide range of fouling rates of between 10 -90 $x10^{10}$ $m.d^{-1}$, with the differences being attributed to varying fractions of carbohydrates resulting from the unsteady state operation which have varying impacts on fouling.

Overall the significance of such events is seen through a reduction in the reuse applications that can be considered. At steady state, the MBR would be suitable for Classes 1, 2 and 4 for COD, turbidity, ammonia and conductivity. The average pH of 6.4 would have been in the lower limits for Classes 1 and 2 but is too low for Class 4 (industrial cooling water). After dosing with the toxins the effluent would only be suitable for Class 1 use, however, the effluent turbidity would increase the chlorine demand required for downstream disinfection with a maximum effluent turbidity of 18.5 NTU. As a consequence, applications that identify a potential risk of chemical shock need to include preventative or remediative solutions to maintain the application to classes 2 and 4. This suggests the need for risk assessments to incorporate potential chemical shocks within their consideration if the technology is to be used for water reuse applications.

10 Conclusions

10.1 Microtox and respirometry

- 32 household products and industrial substances were tested. 10 household products and 6 industrial substances were identified that present a risk to biological systems.
- Both Microtox and respirometry were most sensitive to sodium hypochlorite and hydrogen peroxide.
- No clear pattern of effect was observed for household products between type of product, cost differential or environmentally friendly or non environmentally friendly.
- The Microtox method was more sensitive than the respirometry by two orders of magnitude.
- A consistent ranking of toxicities was not possible between Microtox and respirometry.

10.2 Porous pot trials

- Foaming was identified as a risk to the system.
- Dosing of some of these toxins into a bench scale biological reactor caused degradation of the effluent quality and increases in potential foulants.
- No acute toxicity was observed, however there was a physical interaction with the biomass matrix.
- Assessment of the toxicity by respirometry did not predict the impact on the biological system.
- SMP turbidity proved to be a good indicator of SMP carbohydrate and SMP protein concentrations.

10.3 MBR trials

- Dosing of four of the toxins into a MBR caused degradation of the effluent quality and an increase in potential foulants.

- No fouling was observed in the specific pilot plant used.

10.4 Chemical Mitigation of Fouling

- Mitigation of the effects of the increase of SMP turbidity caused by toxin addition were possible with the addition of cationic polymers.
- MPE50 and high MW polyDADMAC performed better compared to ferric sulphate and PAC in terms of SMP turbidity reduction, with >90% and >85% reduction respectively.

11 Further Work

11.1 Bench scale

Further investigation of the effects of a range of toxins from both a household and industrial origin should be carried out on a bench scale filtration rig to understand the impact on critical flux that the toxins have. Quantification of the resultant levels of SMP turbidity and SMP carbohydrates and proteins are required to understand if there is a threshold level above which the fouling rate increases exponentially.

The jar tests identified that the cationic polymers MPE50 and polyDADMAC were efficient at removing SMP turbidity. Bench scale filtration trials would reveal if this is still valid in a continuous system.

11.2 Pilot scale

As dosing trials, of a small range of toxins, have been shown to cause perturbations in the system, further investigation, with different toxins or higher concentration of the toxins tested, would enhance the understanding of the limits under which the system can perform satisfactorily.

Repetition of the dosing trials carried out on a pilot plant that is able to operate at higher fluxes, would further enhance understanding of the system.

Addition of MPE50 and polyDADMAC at pilot scale would reveal the true efficiencies of these chemicals at mitigating the effects of chemical shock loads.

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APPENDIX A – Household products ingredients list

ALL PURPOSE CLEANER

Mr Muscle APC	***Morrisons orange APC***	***Ecover APC***	***Nest APC***
Water, C11-15 pareth-7, Decyl glucoside, Tetrasodium EDTA, Perfume, PEG-4, Colourant.	Aqua, Sodium xylenesulfonate, Industrial methylated spirits, Hexylene glycol, Alcohol ethoxylate, C9-11 alcohols, Tetrapotassium pyrophosphate, Parfum, Hydroxy ethyl cellulose, Formaldehyde, BHT.	Water, Ethanol, Alkylpolyglycoside C10-16, Potassium soap of coco and olein fatty acids, Sodium octyl sulphate, Perfume, Limonene, Xanthum gum.	Water, Soda crystals, Lavender essential oils, Bergamot essential oils.

SHAMPOO

Pantene Pro V	***Morrisons Bettabuy***	***Naked Volumising Shampoo***	***Henna Plus Natural Plus***
Water Ammonium laureth sulphate Ammonium lauryl sulphate Sodium chloride Glycol distearate dimethicone ammonium xylenesulfonate citric acid cetyl alcohol sodium citrate cocamide MEA polyquaternium 10 parfum, hydrogenated polydecene, sodium benzoate, disodium EDTA, PEG-7M, Trimethylolpropane tricaprylate/tricaparte, DMDM hydantoin, Hexyl cinnamal, Tetrasodium EDTA, Panthenol, Panthenyl ethyl-ether, Benzyl salicylate, Butylphenyl methylpropional, Lysine HCL, Methyl tyrosinate HCL, Linalool, Limonene, Citronellol, Geraniol, Hydroxyisothexyl 3-cyclohexene, Carboxaldehyde, Histidine, Methylchloroisothiazolinone, Methylisothiazolinone, Tocopherol.	Water, sodium chloride, sodium laureth sulfate, cocamidopropyl betaine, disodium EDTA, parfum, citric acid, sodium hydroxide, triethylene glycol, benzyl alcohol, propylene glycol, sodium benzoate, magnesium choride, magnesium nitrate, methylchloroisothiazolinone, methylisothiazolinone, hexylene glycol, CI 19140, CI 42090.	Water, cocamidopropyl Betaine, Lauryl glucoside, Sodium Cocyl Apple Amino Acids, PEG-120 Methyl Glucose Diolate, Sodium Laurel Sarcosinate, Polyquarternium-16, Betaine, Inulin (Chicory), Parfum (Fragrance), Lactic acid, Butylene Glycol, Linalool, Sodium Lactate, Benzoic Acid, Helianthus Anuus (Sunflower) Seed Extract, Citronellol, Sodium Chloride, Dimethicone Copolyol, PEG/PPG-20/6, Dimethicone, Wheat Amino Acids, Geraniol, Magnesium Nitrate, Phenoxyethanol, Potassium Sorbate, Methylchloroisothiazolinone, Magnesium Chloride, Methylisothiazolinone.	Water, cocamidopropyl betaine, isostearamide Mipa, citric acid, laureth-10, sodium laureth-11 carboxylate, sodium cocyl glutamate, sodium cocamphoacetate, decyl polyglucose, sodium magnesium silicate, betaine, sucrose laurate, paullinina cupana, humulus lupulus, lawsonia inermis, simondsia chinensis, prunus dulcis, panthenol, caprylyl glycol, xanthan gum, behenoyl Pg-trimonium chloride, maltodextrin, glycerin, levulinic acid, sodium levulinate, terpineol, sodium chloride, parfum.

SHOWER GEL

Ecover	***Lavera***	***Morrisons Shower Crème***	***Original Source Mint and Tea Tree***
Water	Water	aqua	aqua
Fatty Alcohol Sulfate C10-16	Glycerin	sodium laureth sulfate	sodium laureth sulfate
Alkyl Poly Glycoside C10-16	Coc Glucoside	sodium chloride	cocamide DEA
Sodium Cocoamphoacetate	Caprylyl/Capryl Glucoside	cocamide DEA	mentha arvensis leaf oil
Sodium Octyl Sulfate	Sodium Lauryl Sulfoacetate	glycerin	melaleuca alternifolia (tea tree) leaf oil
Alkyl Poly Glycoside C8-14	Citrus Aurantium Dulcis (Orange) Flower Water	cocamidopropyl betaine	sodium chloride
Glycol Distearate	Hippophae Rhamnoides Extract	parfum	PEG-150 distearate
Protein Hydrolysate	Xanthum Gum	glycol distearate	sodium lactate
Lactic Acid	Alcohol	cocamide MEA	lauryl betaine
Perfume	Citrus Aurantium Amara (Bitter Orange) Oil	laureth 10	disodium EDTA
Guar Hydroxypropyl Trimonium Chloride	Fragrance	citric acid	styrene/acrylates copolymer
Aloe Barbadensis Extract	Limonene	benzyl alcohol	sodium lauryl sulfate
Citric Acid		magnesium nitrate	lactic acid
Sodium Hydroxymethyl Glycinate		methylchloroisothiazolinone	lavandula angustifolia (lavender) oil
Limonene		methyllisothiazolinone	limonene
Eugenol		magnesium chloride	linalool
		hexylene glycol	magnesium nitrate
		linalool	methylchloroisothiazolinone
		hexyl cinnamal	magnesium chloride
		butylphenyl methylpropional	methyllisothiazolinone
		CI 16035	CI 42090
		CI 19140	CI 19140

WASHING POWDER

Ecover	*Persil*	*Cyclon*	*NEST*
Sodium Carbonate	Pentasodium Triphosphate	Pentasodium Triphosphate	soda crystals (sodium carbonate)
Zeolite	Sodium Silicoaluminate	Sodium carbonate Peroxide	vegetable flakes
Sodium Cocoate	Sodium Carbonate Peroxide	Sodium Alkyl Sulphate	
Sodium Bicarbonate	Sodium Dodecylbenzenesulfonate	Sodium Carbonate	
Sodium Citrate	Aqua	Sodium Silicate	
Coco Glucoside	Sodium Carbonate	Microcrystalline cellulose	
Sodium Sulfate	C12-15 Pareth-7	Sodium Carbonate	
Sodium Poly Asparaginate	Tetraacetyl Ethylene Diamine	C12-15 Pareth-2	
Sodium C12-18 Alkyl Sulfate	Sodium Acetate	TAED	
Sodium Disilicate	Ceteareth-25	Parfum	
Capryl Glucoside	Sodium Silicate	Polyaromatic ester and sodium sulfate	
Lauryl Polyglucose	Sodium Stearate	Optical Brightener	
Sodium Cetearyl Sulfate	Ethylene Diamine Tetra Methylene Phosphonic Acid Ca/Na salt	Diethylenetriamine Pentamethylene Phosphonic acid	
Cellulose Gum	Sodium Bentonite	Aqua	
Trisodium Ethylenediamine Disuccinate	Maize Starch	C12-15 PARETH-2	
Methyl Cellulose	Cellulose Gum	CI 74160	
Magnesium Sulfate	Sodium Sulfate	Hexyl Cinnamal	
Ethoxylated Fatty Alcohol C12-18	Parfum		
	Dimorpholinopyridazinone		
	Sodium Acrylic Acid/MA Copolymer		
	Simethicone		
	Sodium Chloride		
	Sodium Polyacrylate		
	Glyceryl Stearate		
	Sodium Polyaryl Sulfonate		
	Aloe Barbadensis		
	CI 74260		

WASHING UP LIQUIDS

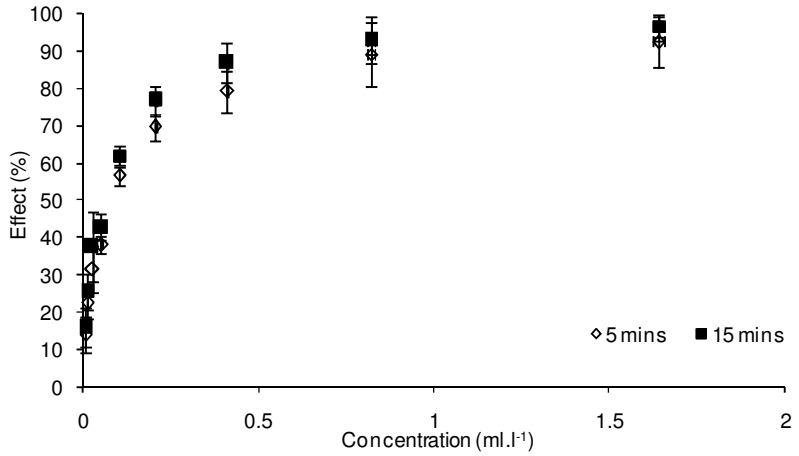
Morrisons Ultra	***Persil***	***Ecover***	***NEST***
Aqua	Aqua	Water	vegetable based soap
Sodium Lauryl Sulfate	Magnesium Laureth Sulfate	Sodium Lauryl Ether Sulfate	malt solution
Sodium Laureth Sulfate	Sodium Laureth Sulfate	Alkyl Poly Glycoside C10-16	fruits
Lauryl Glucoside	Cocamidopropyl Betaine	Sodium Chloride	plants
Sodium xylenesulfonate	Sodium Sulfate	Citric Acid	essential oils
Alkyldimethyl Amine Oxide	Sodium Chloride	Perfume	
Cocamidopropyl Betaine	Disodium Citrate	Limonene	
Undeceth-4	Parfum	Protein Hydrolysate	
5-Chloro-2-methyl-2H-isothiazol-3-one /2-methyl-2H-isothiazol-3-one	Limonene	Aloe Barbadensis Extract	
(Ethylenedioxy)-diamethanol	Monosodium Citrate	Citral	
Parfum	Linalool	2-bromo-2-nitropropane-1,3-diol	
Formaldehyde	Citral		
2-Bromo-2-Nitropropane-1,3-Diol	Methylchloroisothiazolinone		
Citric Acid	Methylisothiazolinone		
CI 19140	CI 19140		
CI 35780	CI 15985		

BLEACHES

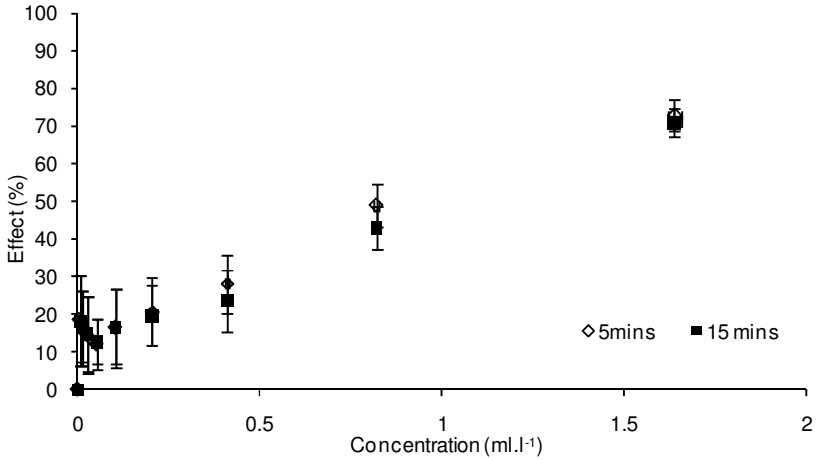
Domestos	***Morrisons thick***	***NEST***
Aqua Sodium Hypochlorite Sodium Chloride Cocamine Oxide Sodium Hydroxide Sodium Laurate	less than 5% sodium hypochlorite less than 5% anionic surfactants less than 5% limescale deterent	6% hydrogen peroxide water

APPENDIX B – Microtox dose response curves.

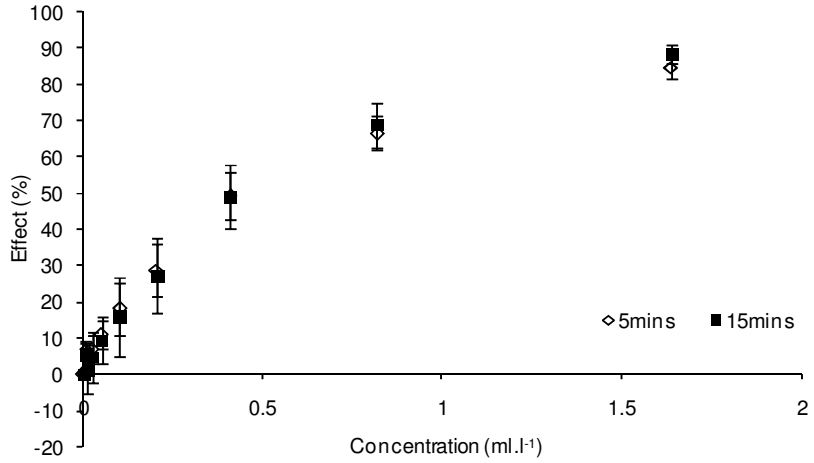
ALL PURPOSE CLEANERS



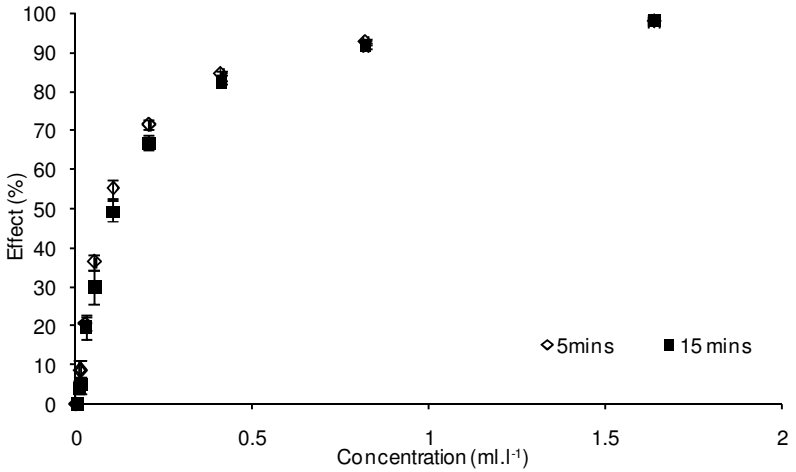
Ecover APC



Nest APC

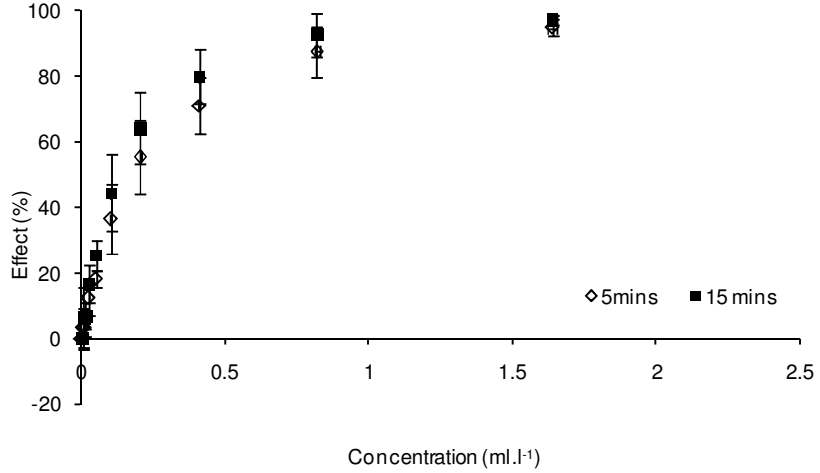


Mr Muscle Orange APC

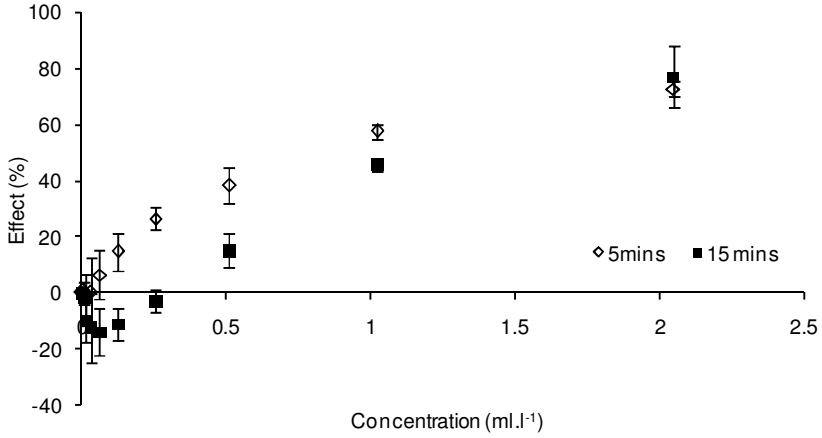


Morrisons Orange APC

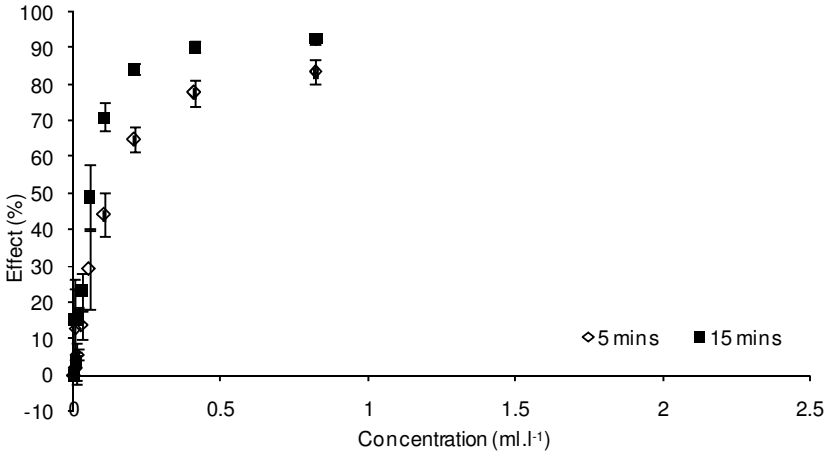
SHAMPOO



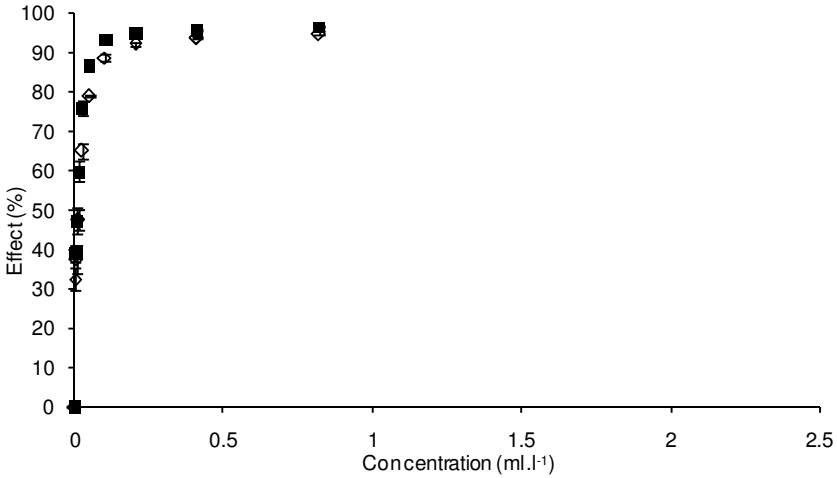
Naked Volumising



Henna Plus Natural

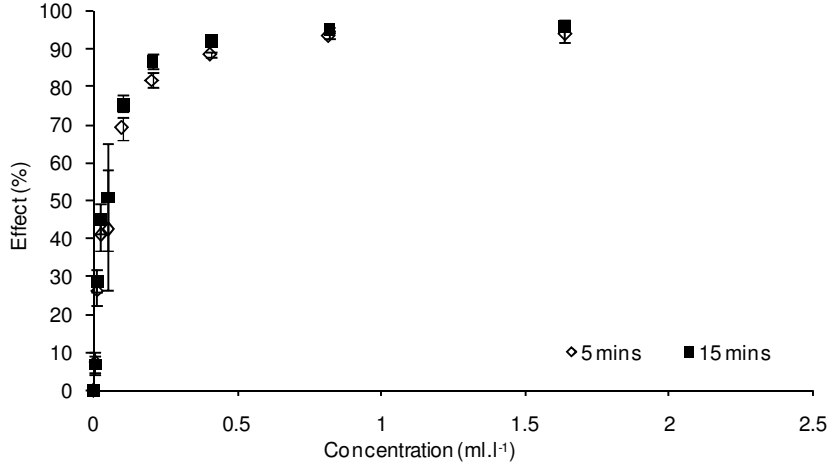


Morrisons Bettabuy Shampoo

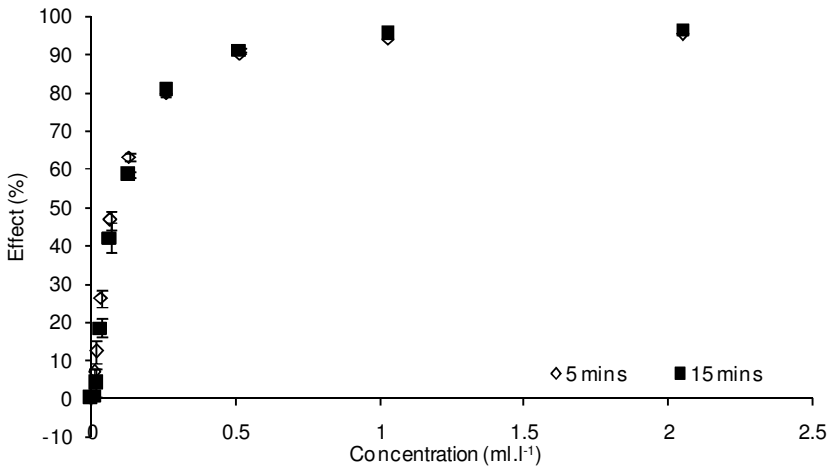


Pantene Pro V

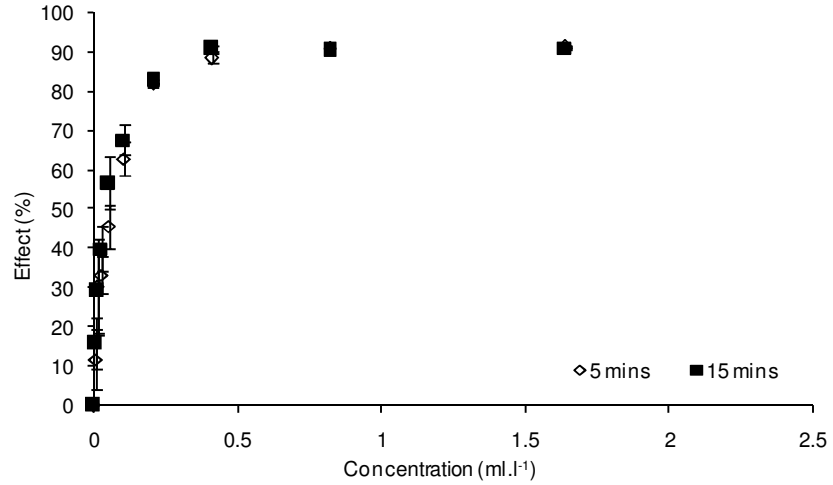
SHOWER GEL



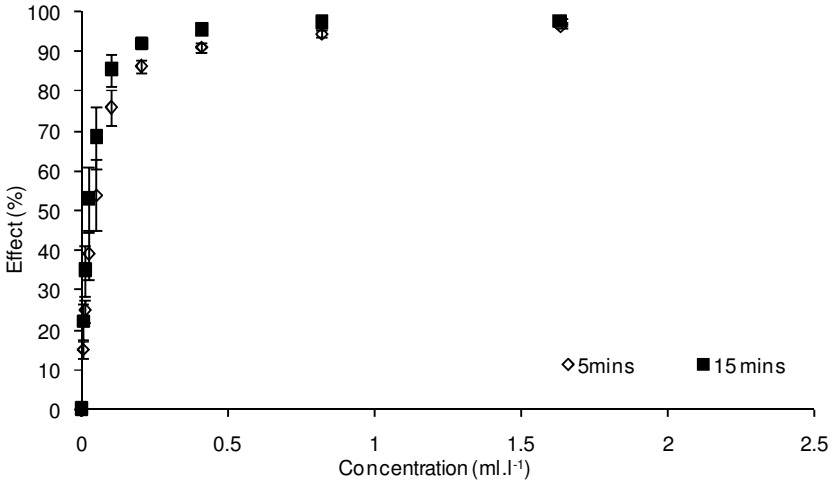
Ecover Shower Gel



Lavera Shower Gel

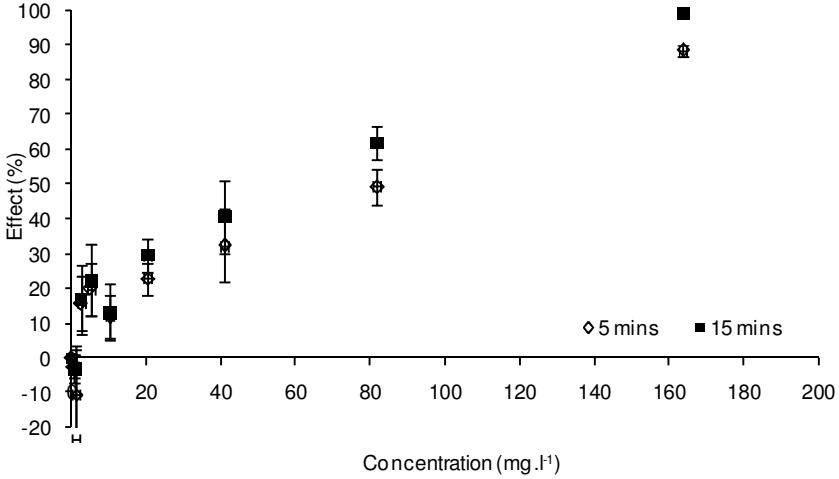


Morrisons Shower Crème

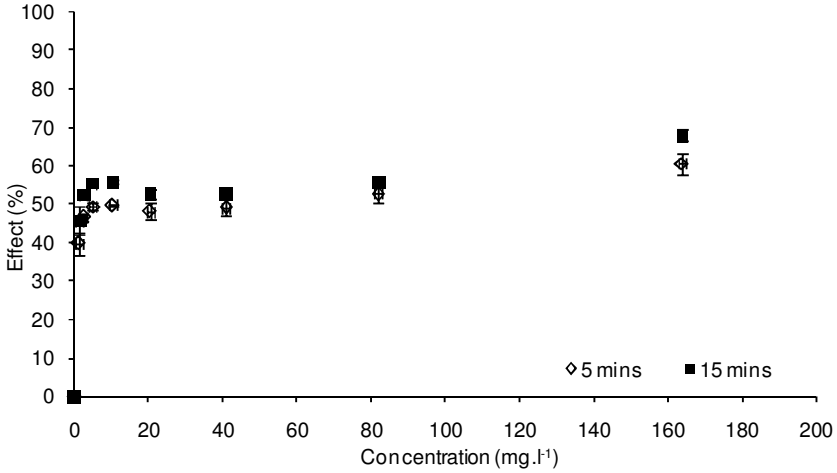


Original Source Tea Tree and Mint

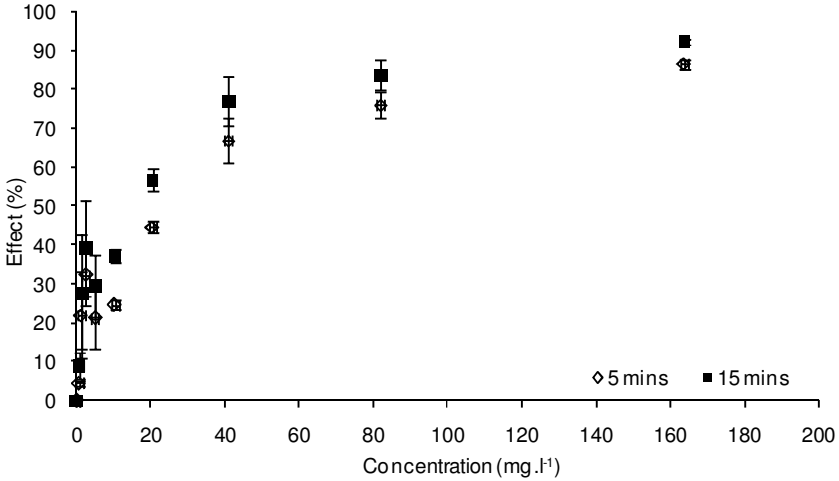
WASHING POWDERS



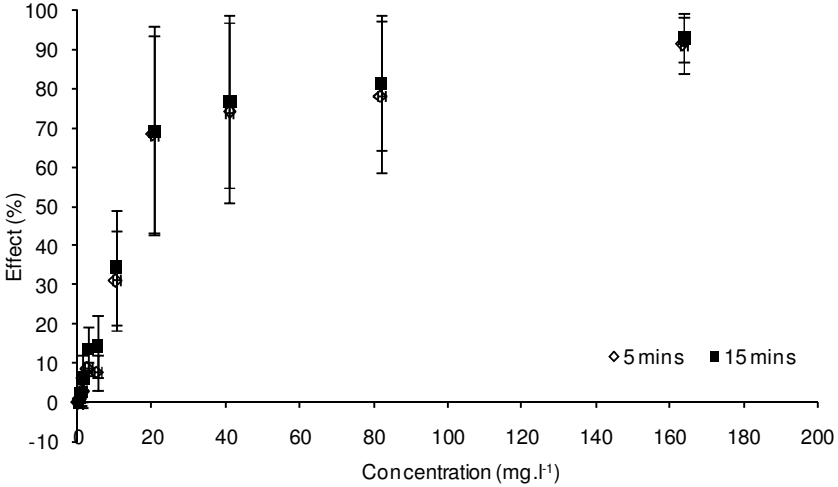
Ecover Washing Powder



NEST washing powder

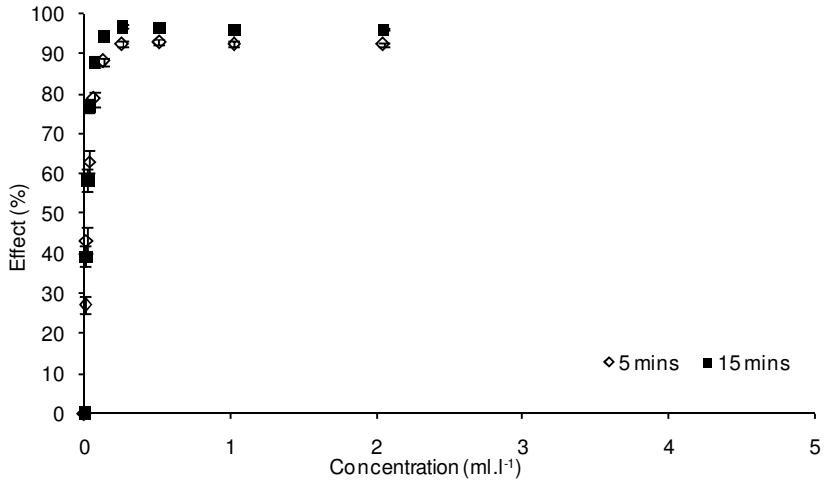


Morrisons Cyclon

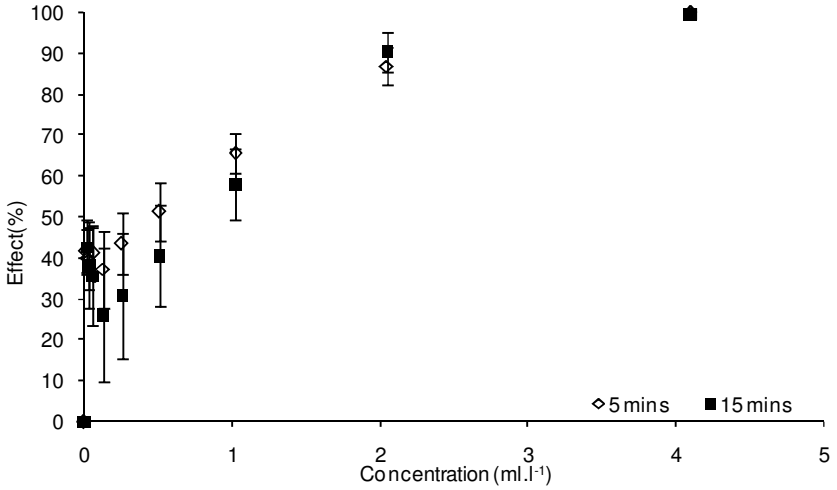


Persil Aloe Tablets

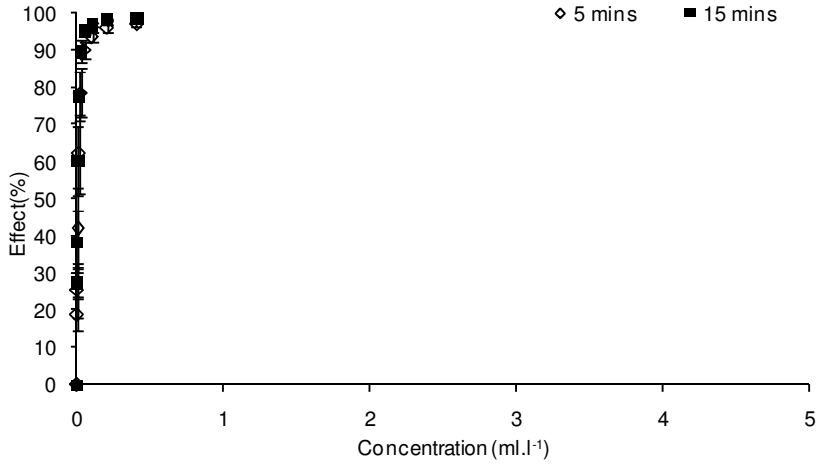
WASHING UP LIQUID



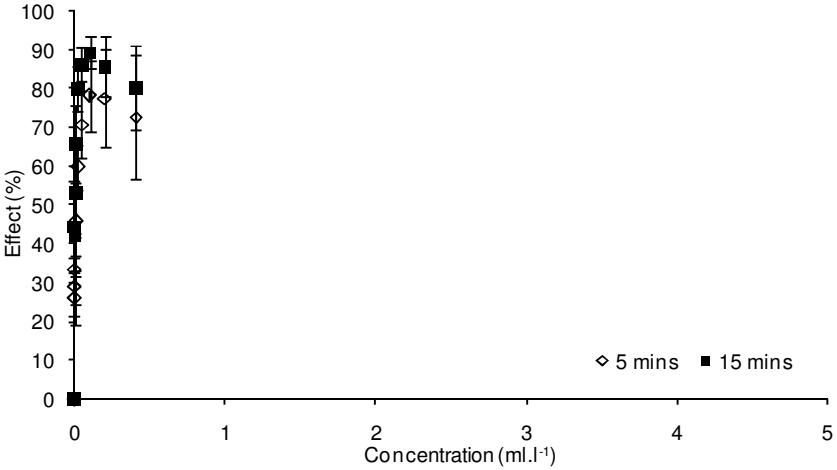
Ecover washing up liquid



NEST washing up liquid

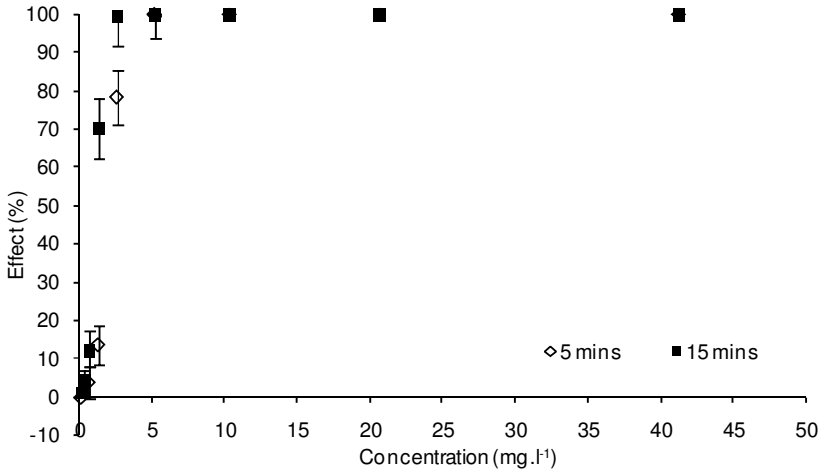


Morrisons Ultra Washing Up Liquid

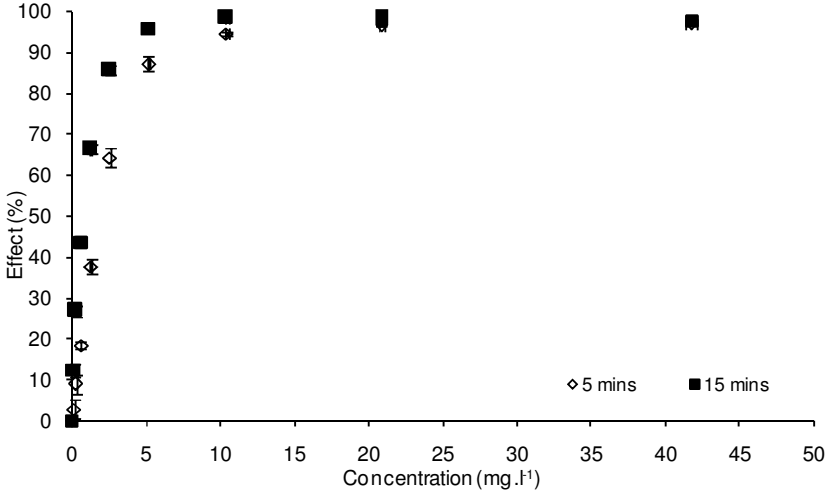


Persil Washing Up Liquid

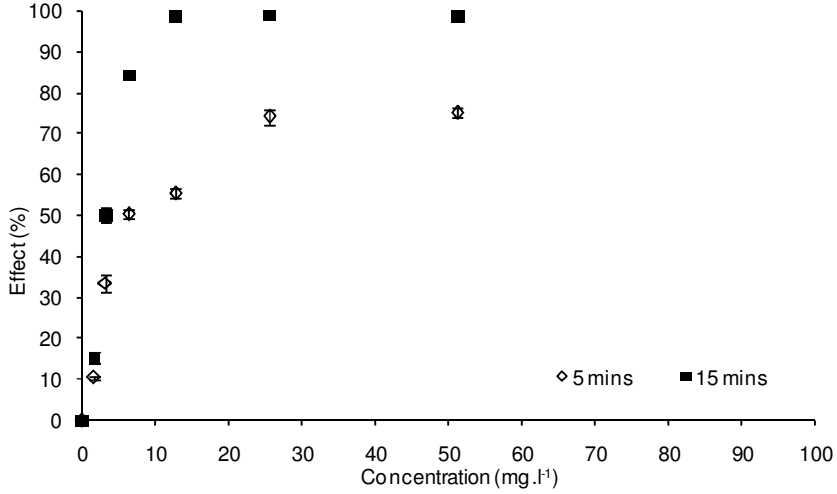
INDUSTRIAL COMPOUNDS



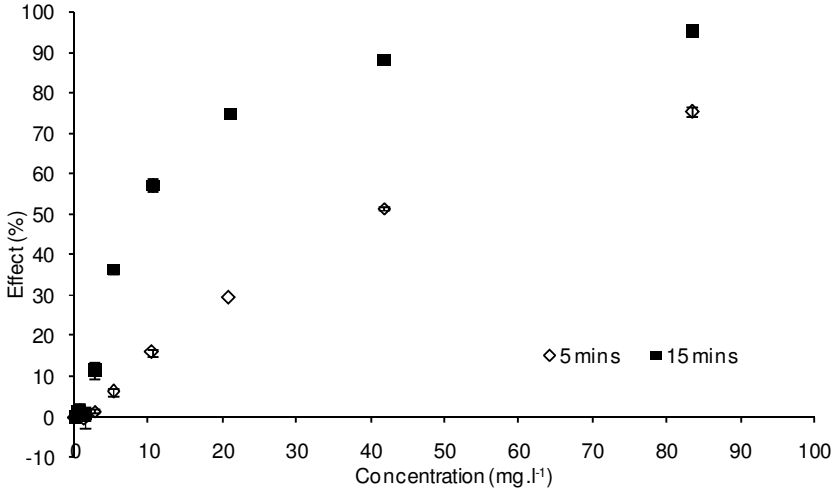
Cetyl trimethyl ammonium bromate (CTMAB)



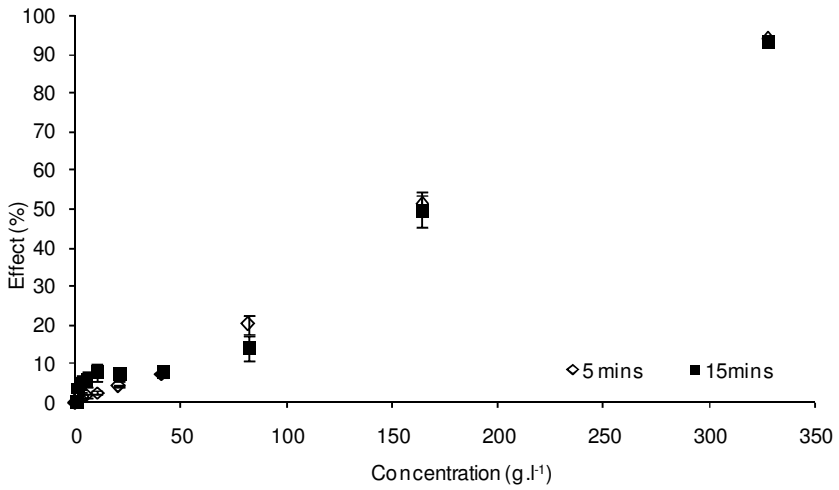
Sodium dodecyl sulphate (SDS)



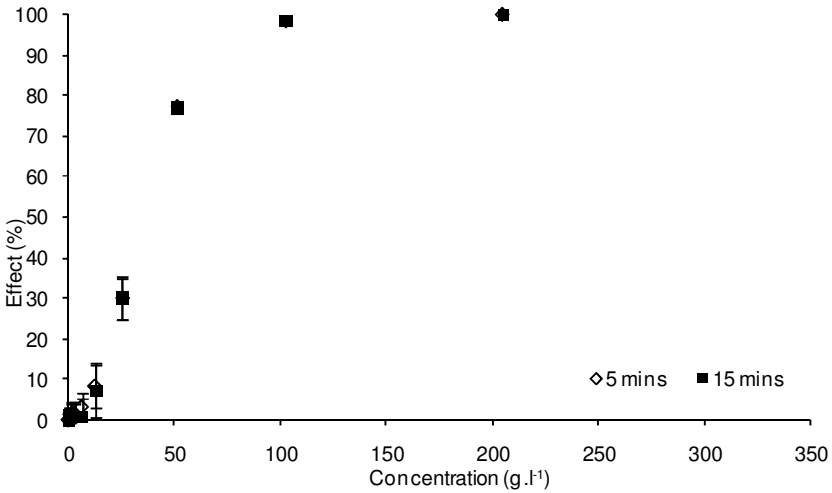
Copper sulphate



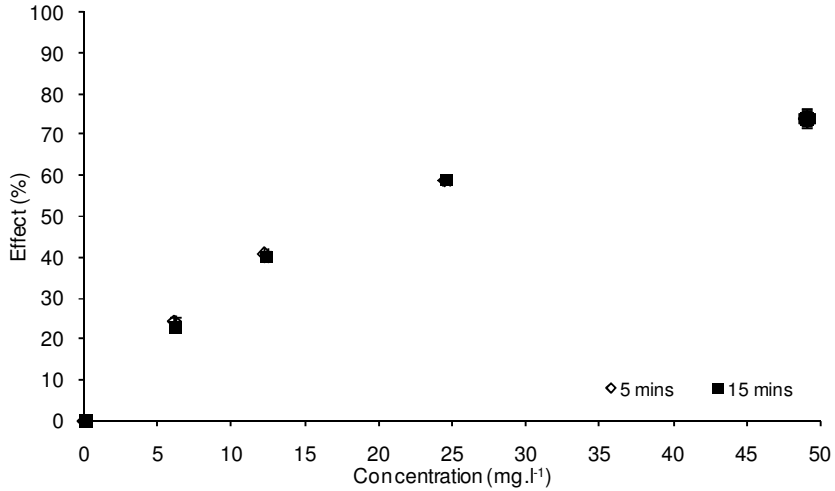
Zinc sulphate



Magnesium sulphate



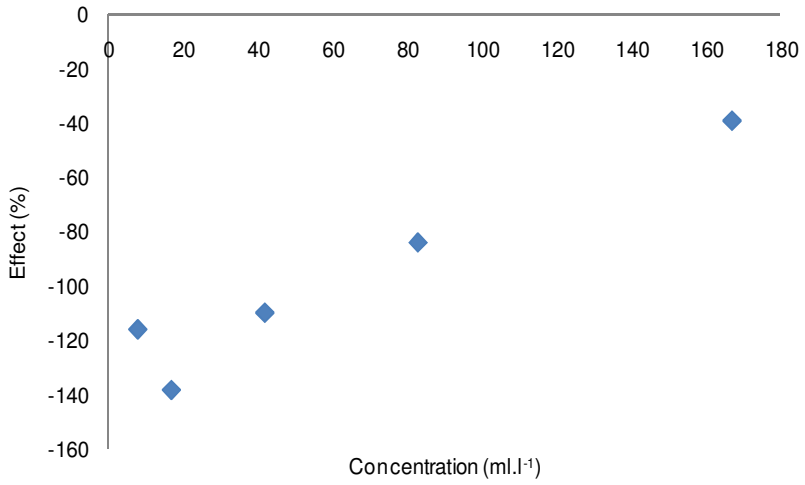
Sodium chloride



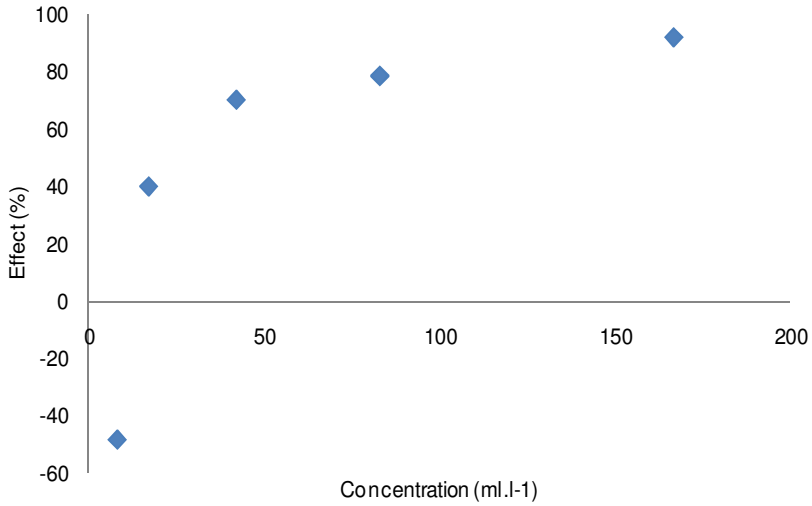
Phenol

APPENDIX C – Respirometry dose response curves.

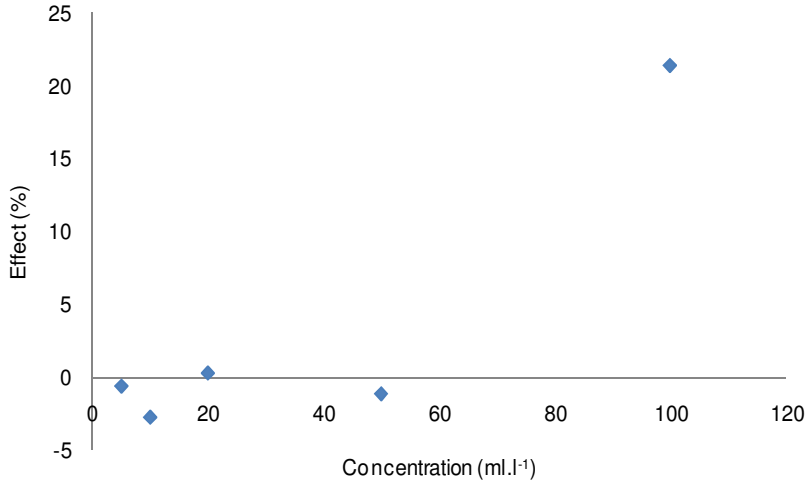
ALL PURPOSE CLEANER



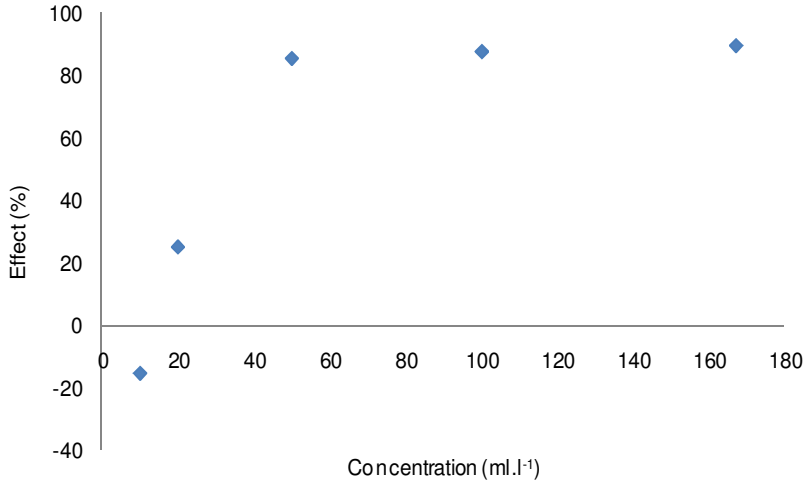
Ecover APC



Morrisons APC

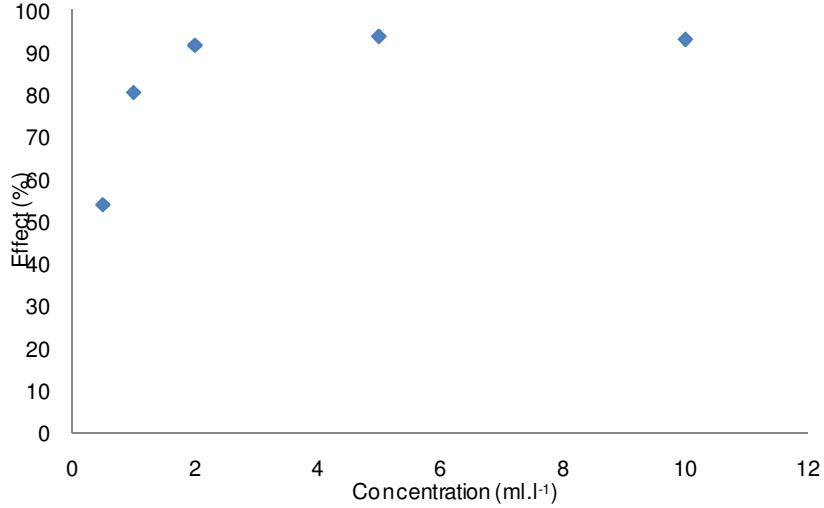


Mr Muscle APC

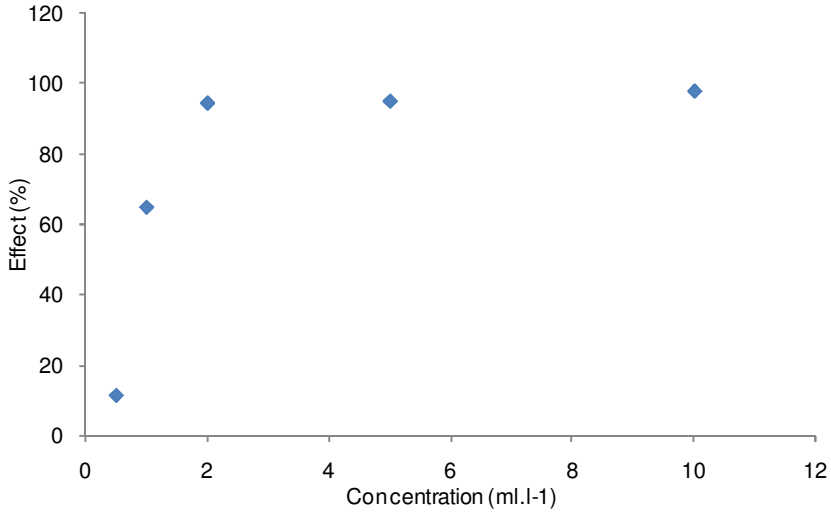


Nest APC

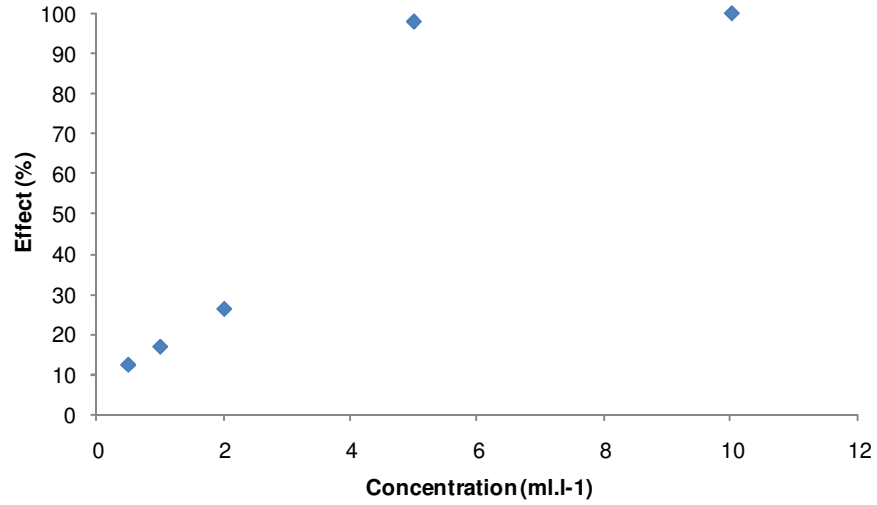
BLEACH



Domestos Bleach

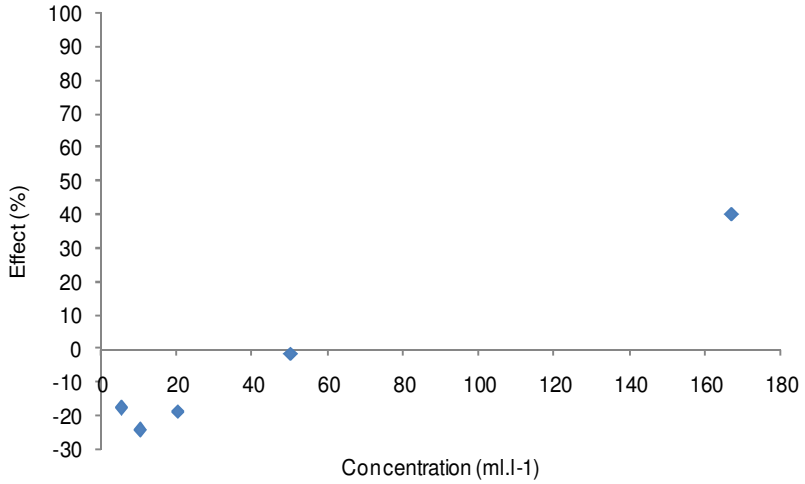


Morrisons Bettabuy

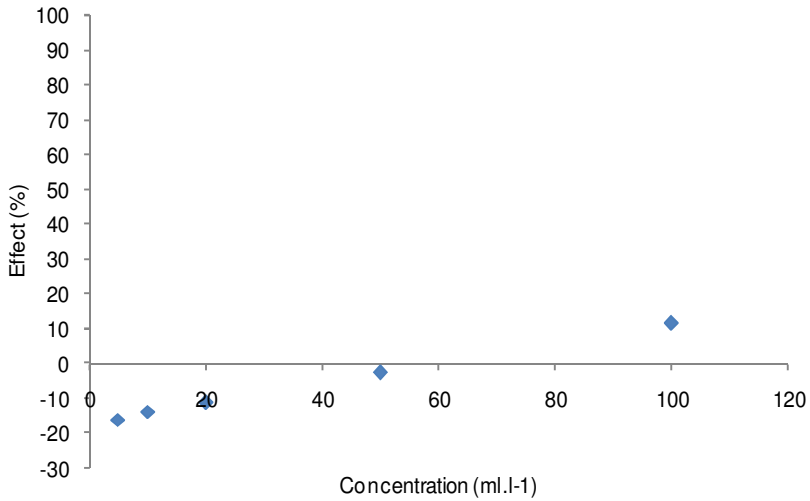


Nest Bleach

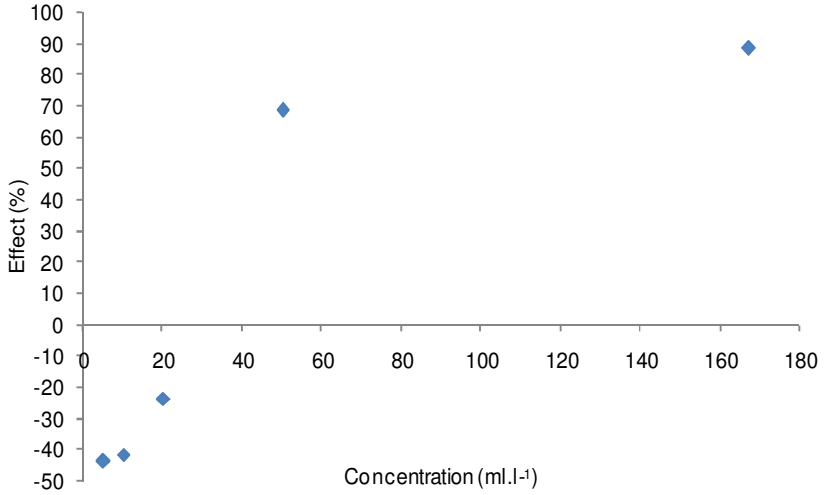
SHAMPOO



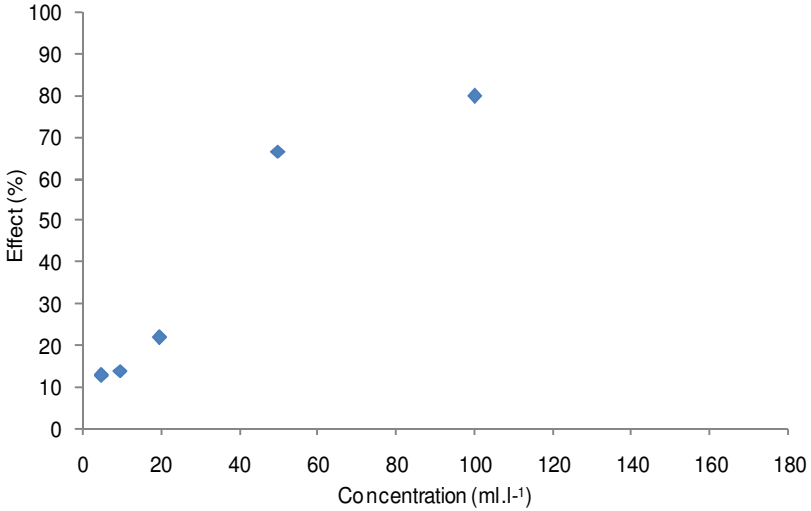
Henna Plus Shampoo respirometry.



Morrisons Bettabuy shampoo.

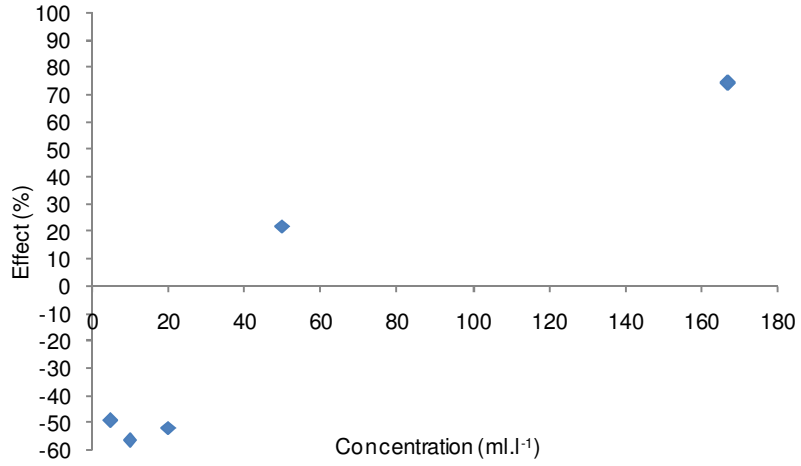


Naked shampoo respirometry.

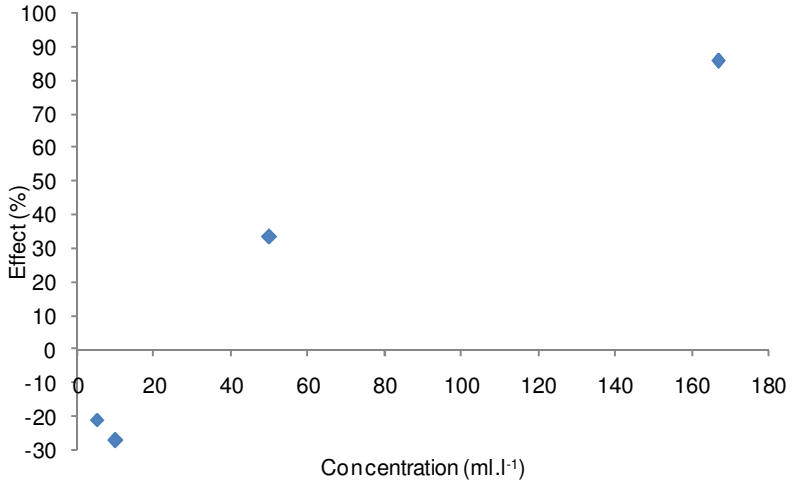


Pantene Pro V shampoo respirometry.

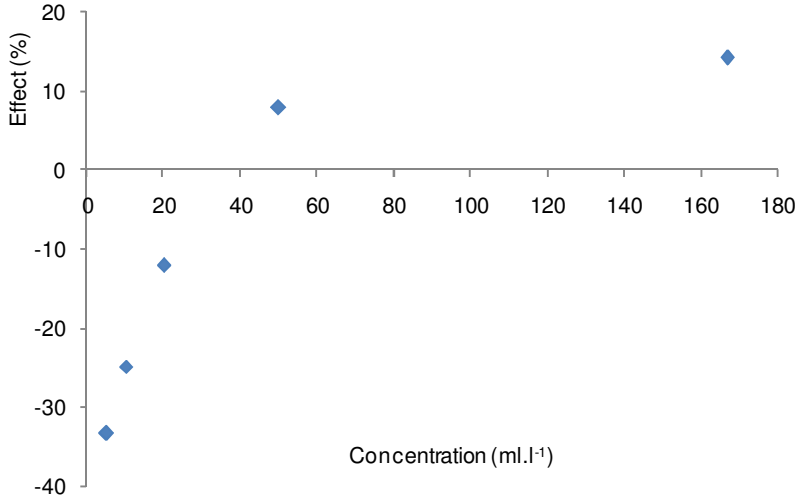
SHOWER GEL



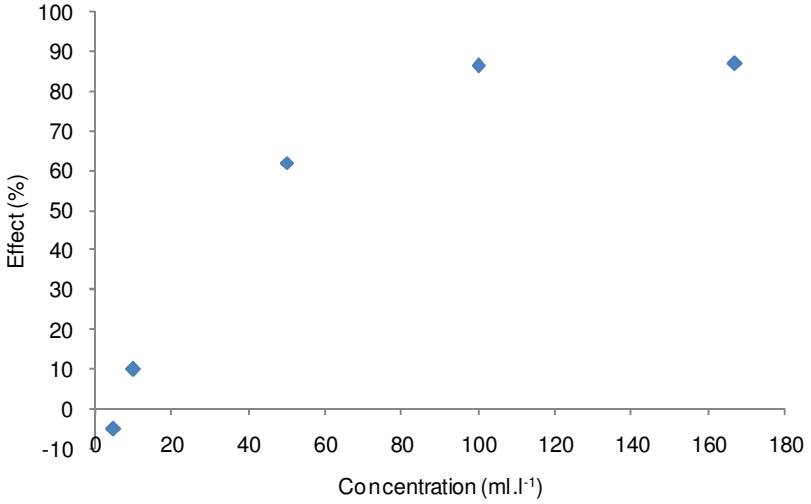
Ecover shower gel.



Lavera shower gel.

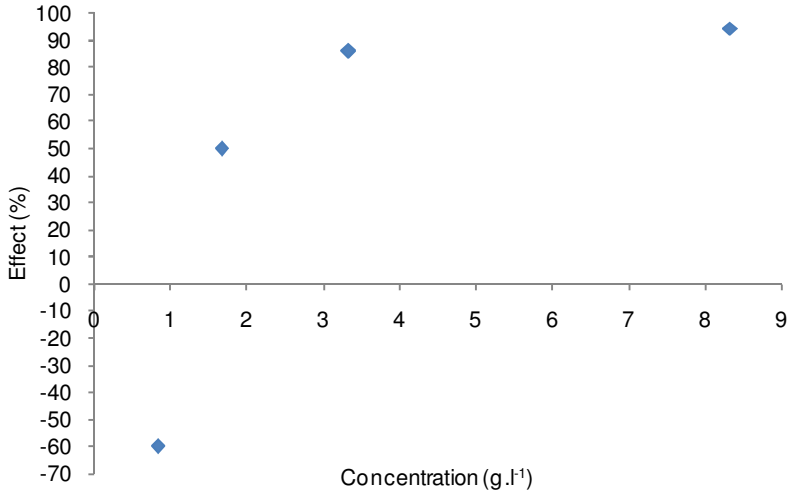


Morrissons Shower Creme.

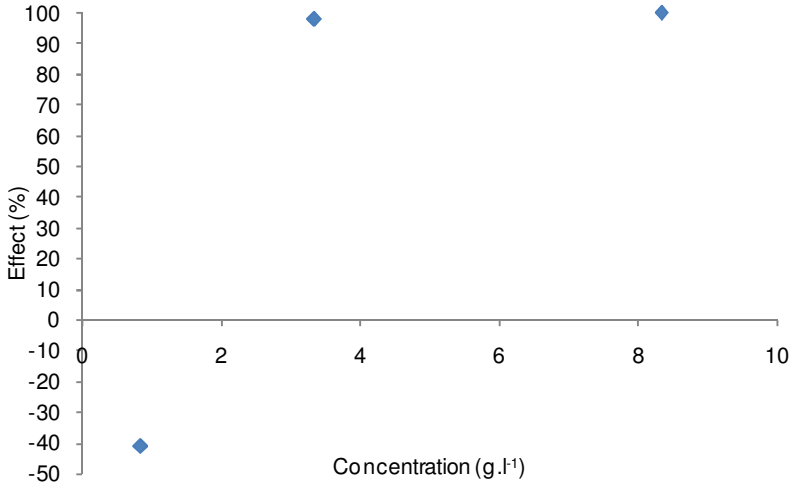


Original Source shower gel.

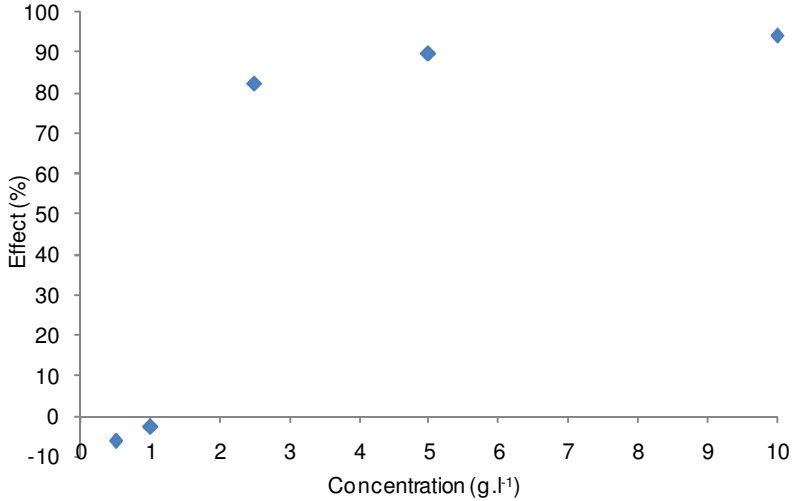
WASHING POWDER



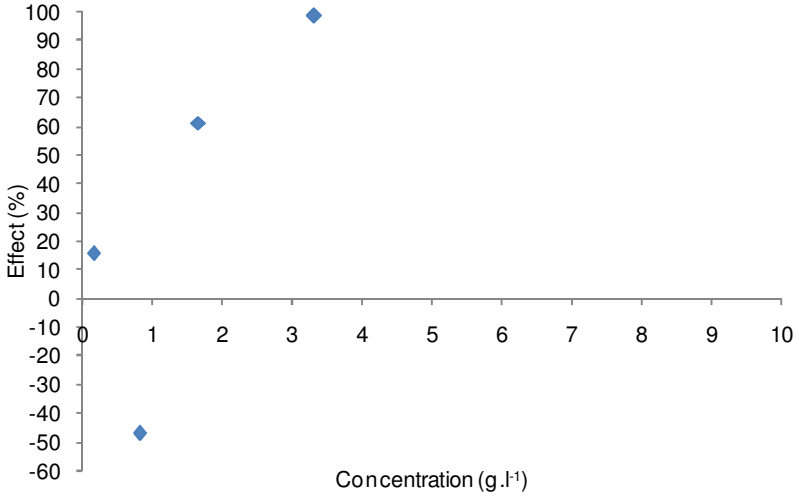
Ecover washing powder.



Morrisons Cyclon washing powder.

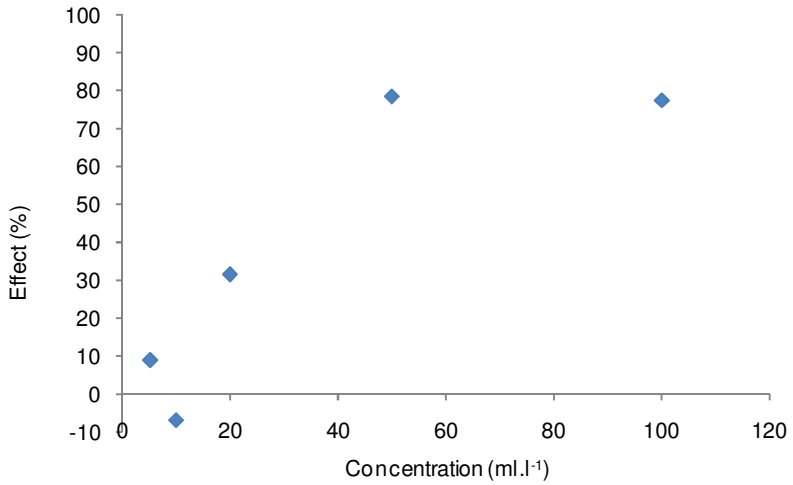


Nest washing powder.

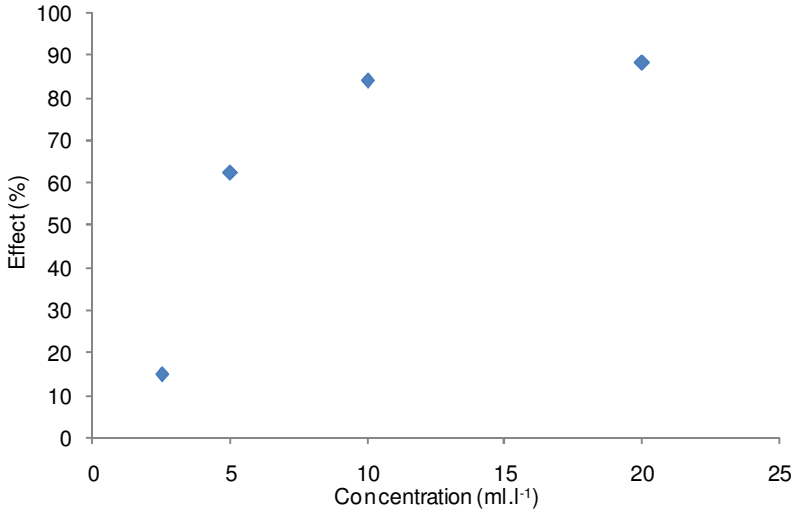


Persil washing powder.

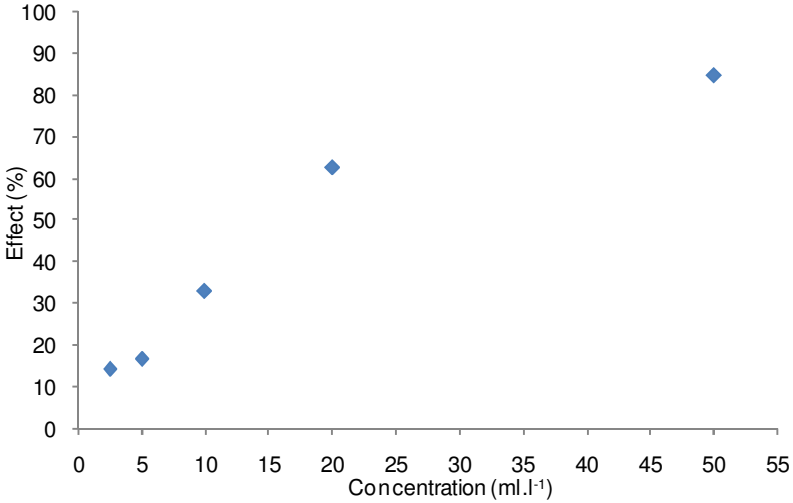
WASHING UP LIQUID



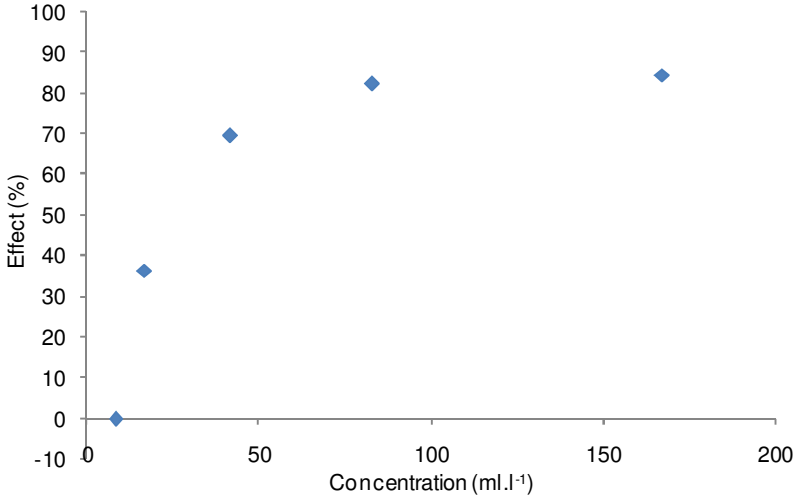
Ecover washing up liquid.



Morrisons Ultra washing up liquid.

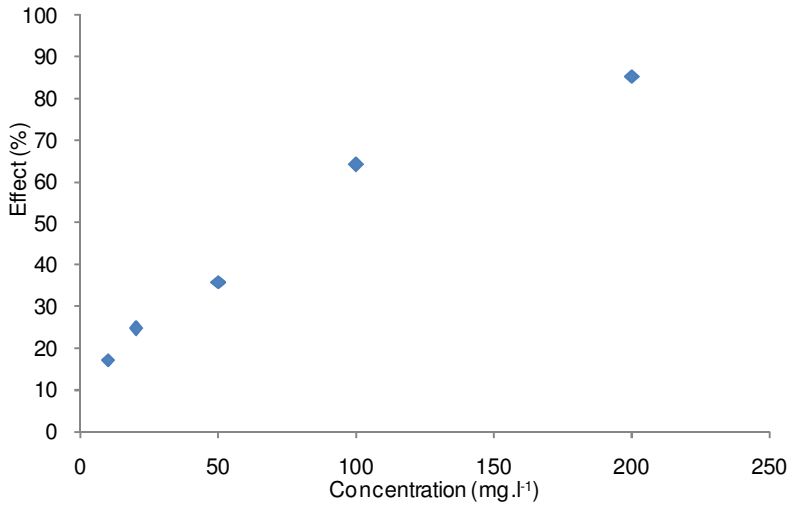


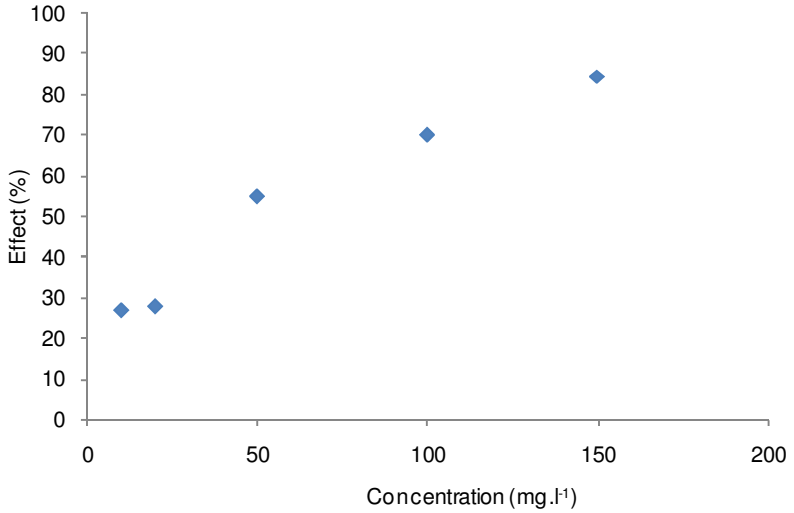
Nest washing up liquid



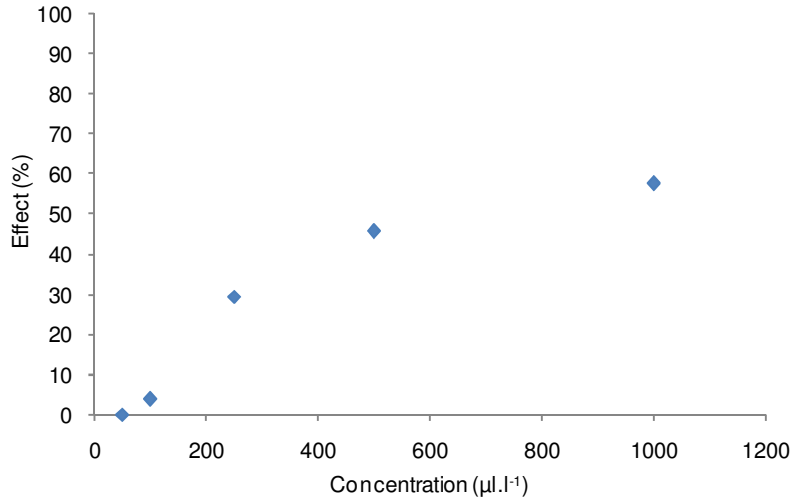
Persil washing up liquid

INDUSTRIAL



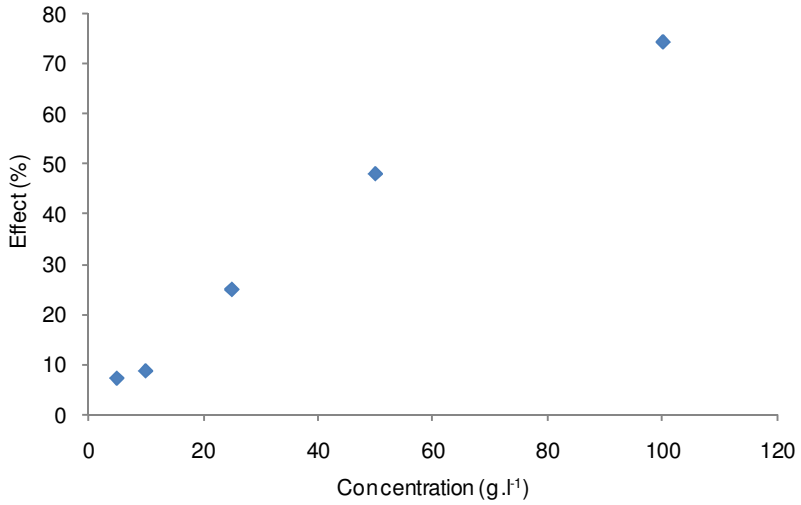


CTMAB

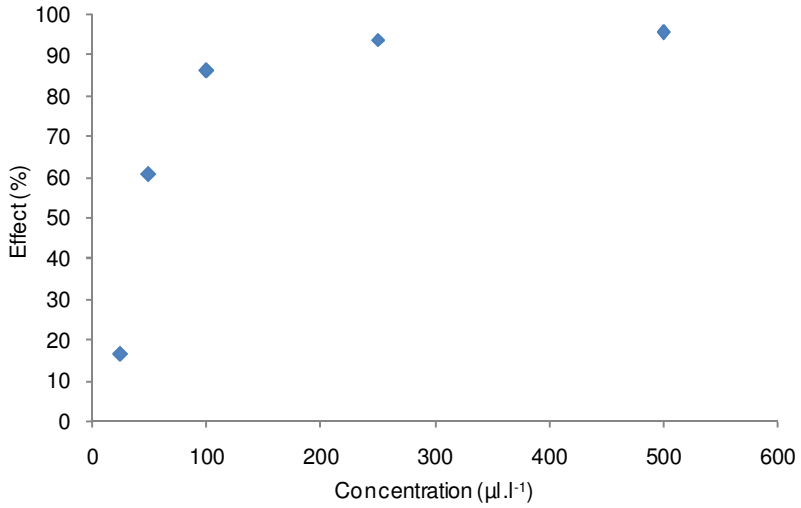


30% H_2O_2

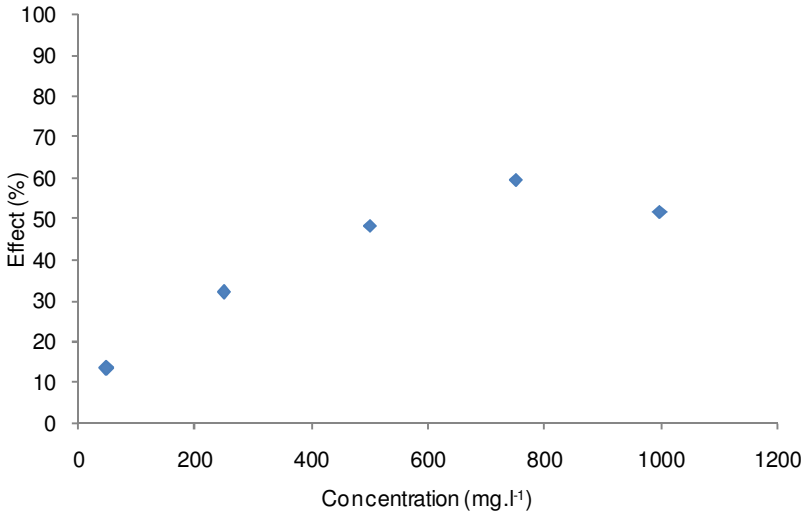
$CuSO_4$



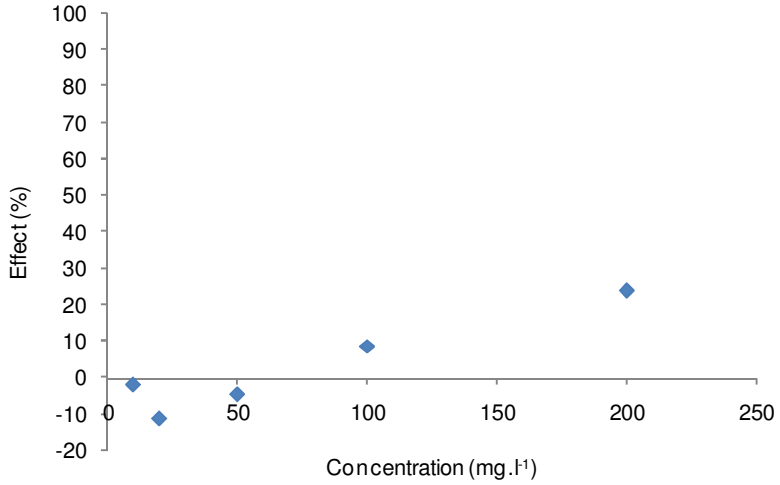
$MgSO_4$



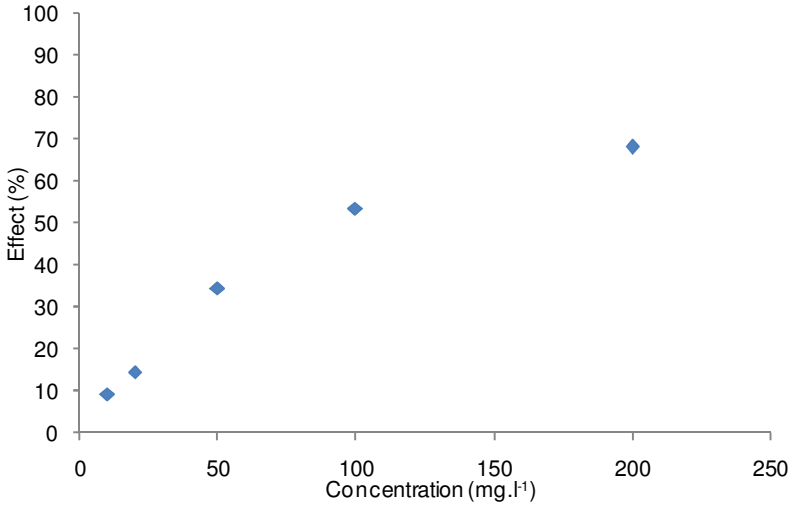
NaOCl



Phenol



SDS



$ZnSO_4$