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Development of simulation tests to assess the fate of Unilever ingredients under untreated discharge conditions.

Institute of Bioscience and Technology

MPhil

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Supervisor : Prof Phil Warner

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ABSTRACT

Unilever product ingredients are discharged into the environment via a number of routes, in many regions of the world there is a lack of municipal waste water treatment and the discharge of chemicals directly into the environment in the presence of untreated sewage is a major pathway. An absence of data on the behaviour of the fate and effects of chemicals under such conditions requires overly stringent and unrealistic assumptions when assessing risk (e.g. no biodegradation is assumed). Traditional risk assessment fails since water quality is compromised by pollutants associated with raw sewage (e.g. BOD and ammonia) and the relevance of the 'standard' risk assessment approach has thus been questioned. An alternative risk assessment model, based on the 'impact zone' concept, has been proposed for direct discharge conditions. In this model, chemicals are assessed in terms of their predicted environmental concentration (PEC) at the end of an impact zone, within which the ecosystem is impacted by the pollutant, free ammonia, and beyond which it is not. Linear alkylbenzene sulphonate (LAS) was used a model compound to understand the fate of materials classified as readily biodegradable in this scenario. Batch and dynamic test systems simulating conditions associated with untreated discharge, confirmed that LAS was degraded quicker than the general organics present in settled sewage and that beyond the defined 'impact zone' it is extensively removed.

Predicted no effect concentrations (PNECs) can also be generated for chemicals on the inhibition of key microbial processes (biological oxidation and nitrification) which are essential in rivers for self purification. A variety of detergent ingredients (ranging from readily biodegradable to anti-bacterial) were investigated in short term toxicity tests. The tests produced a range PNECs and confirmed that these ingredients can show selective inhibition towards heterotrophic or autotrophic bacterial populations. All of the PNECs generated were above the PEC for these ingredients.

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NOMENCLATURE AND ABBREVIATION

AEs	alcohol ethoxylates
B	width of a single channel (m)
BOD	biological oxygen demand
COD	chemical oxygen demand
Cc	concentration of oxidised nitrogen, N, in milligrams per litre, in the control flask without inhibitor, after incubation
Ct	concentration of oxidised nitrogen, N, in milligrams per litre, in the flask containing the test substance, after incubation
Cb	concentration of oxidised nitrogen, N, in milligrams per litre, in the flask containing the reference inhibitor, after incubation
d	depth of the layer of water above the glass beads metres (m)
dO_2	dissolved oxygen
*D*s	degree of biodegradation percentage
EC	effect concentration (mg/L)
ERA	Environmental risk assessment
g	gram
GCMS	gas chromatography mass spectrometry
HRT	hydraulic residence time
IZ	impact zone
*k*eff	biodegradation rate constant inverse days (d^{-1})
LAS	linear alkylbenzenesulphonate
LCMS	liquid chromatography mass spectrometry
MBAS	methylene blue anionic surfactant

NOMENCLATURE AND ABBREVIATION (cont)

MLSS	mixed liquor suspended solids
mg	milligram
mCi	millicurie
mmol	millimoles
mL	millilitres
µCi	microcurie
µg	microgram
n	number of the final channel
NOEC	no observed effect concentration
OECD	organisation of economic development
PEC	predicted environment concentration
PNEC	predicted no effect concentration
ρ b	biomass mass concentration
ρ 0	is the initial activity, expressed in disintegrations per minute, of the test compound in the inlet of channel 1
ρ b	biomass mass concentration
ρ 0	is the initial activity, expressed in disintegrations per minute, of the test compound in the inlet of channel 1
ρ*n*	is the final activity, expressed in disintegrations per minute, of the test compound in the outlet of channel *n*
ρs	substrate mass concentration

NOMENCLATURE AND ABBREVIATION (cont)

ρ*n*	is the final activity, expressed in disintegrations per minute, of the test compound in the outlet of channel *n*
ρs	substrate mass concentration
qV	volume flow rate cubic metres per day (m3/d
*r*d	rate of biodegradation
Rt	mean oxygen consumption rate at tested concentration of test substance
Rc	mean oxygen consumption rate of controls
S	free flow cross-section of a single channel square metres (m^2)
SEAC	safety & environmental assurance centre
SS	suspended solids (mg/L)
STP	sewage treatment plant
$T_{1/2}$	degradation half-life days (d)
TOC	total organic carbon
vx	axial flow speed metres per day (m/d)
WWTP	wastewater treatment plant
x_n	distance between channel 1 and channel *n* metres (m)

LIST OF FIGURES (cont)

CHAPTER 1

General Discussion

1. Introduction

Unilever is now making a large commitment to expanding its global business into the developing and emerging markets. Lack of sewerage treatment prior to discharge into the receiving waters in these market regions leaves a void of knowledge on the fate of Unilever ingredients. This is a particular problem when determining the environmental risk assessment (ERA) for any ingredient and in the absence of data, it becomes a necessity to assume stringent defaults when predicting the safety margin. This has a significant impact on the resulting safety margins in developing and emerging markets (as well as several developed regions still lacking adequate treatment) in which Unilever operates.

Prediction of the environmental concentration of Unilever ingredients is essential to determine whether or not they pose an unacceptable risk to the environment.

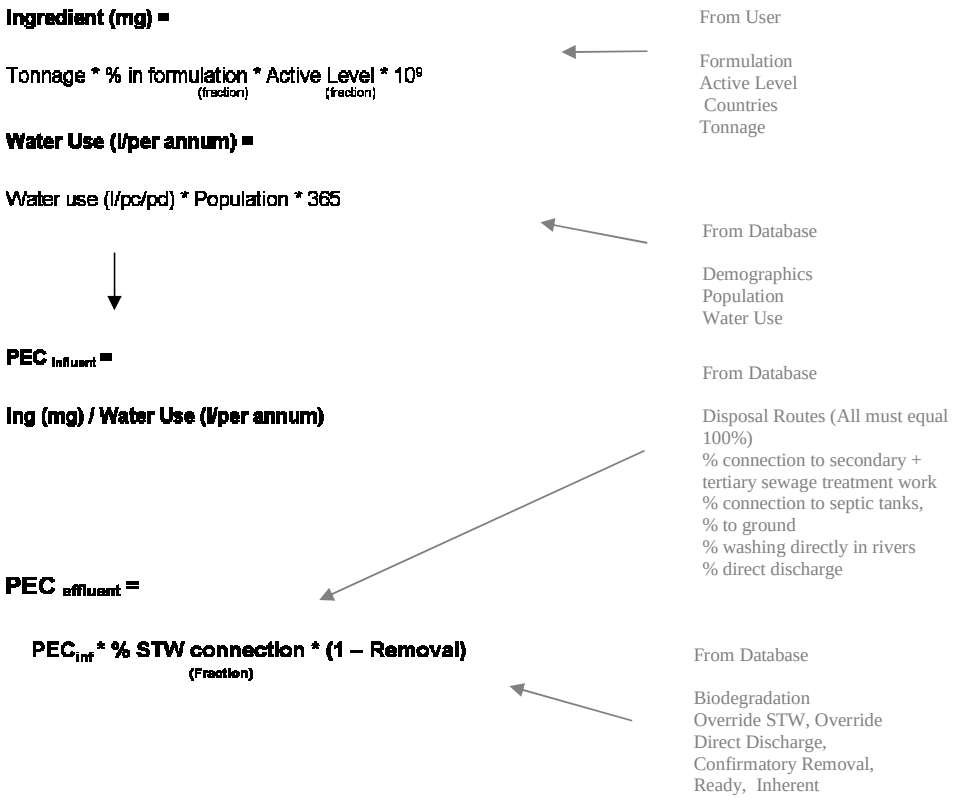
It is also vital to support an improvement in the risk assessment thus allowing the business room for manoeuvre in terms of tonnage with particular ingredients in their respective markets. SEAC (Safety and Environmental Assurance Centre) have now developed a new interface for the Unilever business to interact with to gain safety approval in the form of an intranet application ‘Pelican’. The aim of Pelican is to implement a single SEAC process to provide formal Unilever safety approval to all Innovation Centres and operating companies in a consistent and co-ordinated manner. One of the five domains for which safety approval is sought is Ecotoxicology.

The lack of data available in this domain for the biodegradation of chemicals in the particular environmental compartments associated with direct discharge, results in over conservative estimations being made, e.g. no biodegradation is assumed because there is no sewage treatment prior to release in to the receiving water.

As Pelican tailors a risk assessment to the particular geographic region, in places with installed infrastructure the risk assessment will be of a different order from regions where direct discharge of untreated wastes is commonplace.

The lack of data available for Pelican to source for untreated discharge is in contrast to the large amount of available biodegradation data for chemicals, which are exposed to a wastewater treatment prior to release in to the environment.

The basis of the risk assessment is determined by understanding the fate of the ingredient, the predicted environmental concentration (PEC), and the ratio of this in comparison to the effects of the ingredient, or specifically the predicted no-effect concentration (PNEC). Fig 1.1 shows the algorithm used by Pelican.



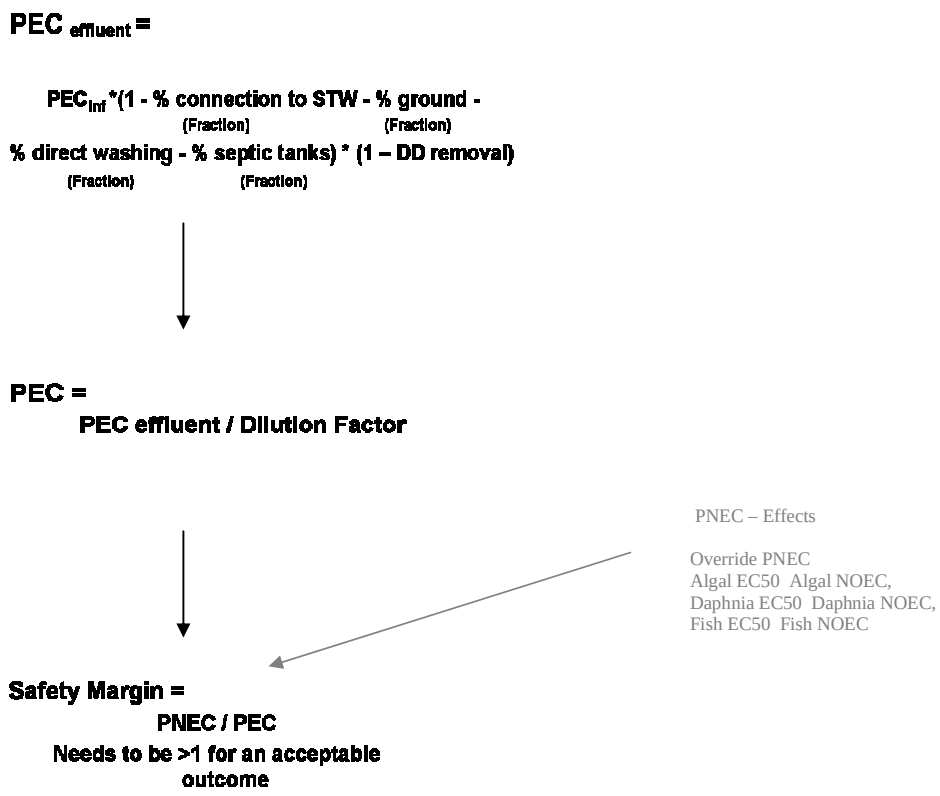


Fig 1.1 The Pelican Algorithm.

1.1 Alternative ERA methodology – the impact zone concept

In terms of behaviour of detergent substances in a direct discharge scenario, AISE / CESIO (1995) summarised that the following principles should be applied.

i) Detergent ingredients should not significantly delay or impair the recovery processes in polluted rivers.

ii) Detergent ingredients should degrade as fast as the general organic chemicals in organic sewage.

iii) Following the recovery of a stream from pollution by general organic chemicals, detergent ingredients should not be present at harmful concentrations.

The principles agreed for detergent behaviour in this scenario allow for the construction of an alternative ERA methodology and provide guidance on the data required and design of screening tests. This methodology is based around the idea of the impact zone, the zone in which water quality is severely impaired by the components of raw sewage (e.g. high free ammonia and nitrite and low oxygen concentrations) illustrated in Fig 1.2.

A whole series of biochemical changes occur in the impact zone of a body of water after receiving a discharge of untreated waste. The receiving water has to complete self-purification against this pollutant loading through a series of physical/chemical and biological processes.

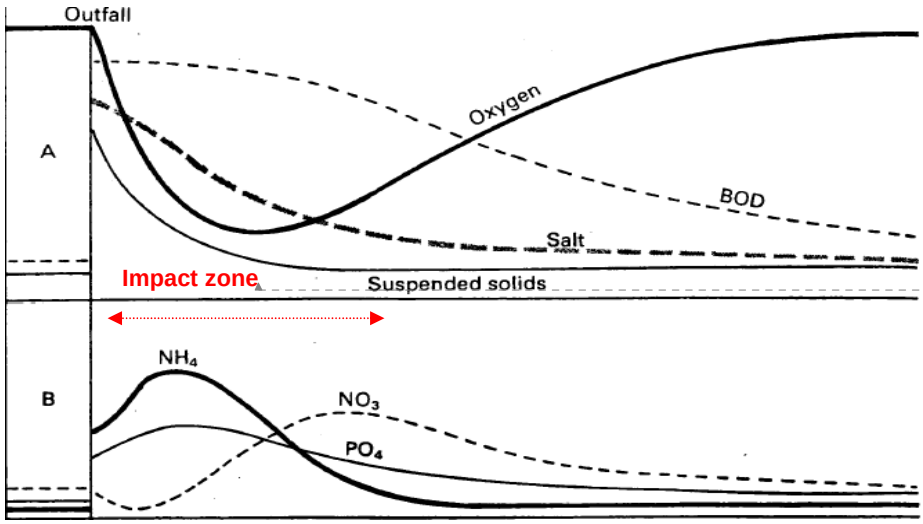


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Fig 1.2 Representation of the typical changes observed in water quality from a point source discharge at the 'impact zone' and further downstream where the wasteload has been assimilated.

Self purification involves physical processes, which will include mixing, dilution, and sedimentation which will occur with suspended solids floculating and forming benthal deposits. It also involves chemical processes, including oxidation of reducing agents such as sulphides, but by far and away the most important process in self purification, is biochemical oxidation through the activity of micro-organisms.

The large volumes of biodegradable organic materials present in sewage discharges contribute to the depletion of the dissolved oxygen (DO) in the receiving waters due to microbial activity utilizing these substrates for growth with the remainder being converted to relatively stable end groups. This is by far the most important process for the stabilisation and removal of a polluting waste load.

Aerobic

Organic matter + Bacteria + O_2 ⟶ New Cells

CO_2, NO_3, H_20

Anaerobic

Organic matter + Bacteria ⟶ New Cells

Alcohols and Acids + Bacteria ⟶ New Cells CH_4,H_2S,NH_3, CO_2,H_20

The DO levels will only recover when this organic substrate has been mineralised sufficiently, and the phenomena is often referred to the 'DO sag'. It is essential that DO does recover, as, this can be the limiting factor for maintaining aquatic life. Another key process during river self purification is the nitrification process. Ammonia is present in large quantities in sewage and is toxic to aquatic life in the un-ionised form. In aqueous solution ammonia forms ions and an equilibrium is reached between ammonia, ammonium and the hydroxide ion, this ratio being dependant on temperature and pH :

$$NH_3 + H_20 \longleftrightarrow NH_4^+ + OH^-$$

The ammonia is converted in to the less harmful nitrate via nitrite during the nitrification process, which, occurs anywhere in the biosphere, provided that the environments are such that the nitrifying bacteria can exist.

1. Organic + $O_2 \rightarrow NH_3 + O_2$
2. $NH_3 + O_2 \rightarrow NO_2^- + 3H^+ + 2e^-$
3. $NO_2^- + H_2O \rightarrow NO_3^- + 2H^+ + 2e^-$

The nitrification process is very important for oxygen conditions in soil, streams, lakes and biological treatment plants. Specific auto-trophic micro-organisms, *Nitrobacter* and *Nitrosomonas* perform this vital function during river self purification. The first stage of proposing an alternative methodology is to define what the impact zone is using agreed criteria. In the case of DO as an indicator, the sensitivity of fish to low concentrations of DO differs between different life stages and processes e.g. growth and reproduction (Alabaster & Lloyd , 1982). Providing other environmental factors are favourable a minimum constant value of 5 mg/L is satisfactory for most life stages of fish.

In the case of un-ionised ammonia the lowest reported lethal concentration for salmonids is 0.2 mg/L but adverse effects by prolonged exposure are absent only at < 0.025 mg/L. Cyprinids are slightly more resistant (Alabaster & Lloyd, 1982). Concentrations of total ammonia containing 0.025 mg/L of unionised ammonia range from 19.6 mg/L at pH 8.50 and 30^{o}C to 0.12 mg/L at pH 7.0 and 5^{o}C, mostly because of the influence of pH. McAvoy *et al.,* (1993) reported a value of 0.01 mg/L as a concentration for toxicity to freshwater fish, derived from a species sensitivity distribution curve. Definition of the impact zone in these experiments will be based on the more conservative water quality criteria for concentrations of un-ionised ammonia for toxicity to freshwater fish of 0.025 mg/L. This also complies with the EC Freshwater Fish Directive 78/659/EEC.

When determining the ERA in the defined impact zone a PEC value for the ingredient can be determined but, alternative PNECs are required. In particular, substance specific data is required for the inhibition of key ecosystem functions, such as inhibition of the degradation of organic matter or nitrification, rather than the standard

species used for PNEC determination in risk assessment such as algae, invertebrates and fish. The standard PNEC used for an ingredient to determine a safety margin becomes meaningless in the large presence of organic materials from sewage, the accompanying increase in biological oxygen demand (BOD), increased levels of ammonia and suspended solids means that the presence of a detergent ingredient to already poor conditions has little effect.

Another alternative approach to ERA would be to determine a PEC for a specific ingredient beyond the defined ‘impact zone’ after an untreated discharge. Still using conventional risk assessment (the ratio PEC: PNEC) but now using a PEC value which allows for any degradation which has occurred to the ingredient during the river ‘self purification’ process.

1.2 Overview of Sewage treatment connection

The statistics are both interesting and in some cases surprising when looking at the percentage of the population that is actually served by sewage treatment and provide a reminder that the discharge of untreated wastewater is not an issue solely confined to developing and emerging economies. The OECD looked at the current state of the wastewater treatment connection rates of its member countries at the end of the 1990s and found that the OECD-wide share of the population connected to a municipal wastewater treatment plant had rose from 50 % in the early 1980s to more than 60 % today. Further investigation of the actual level of the quality of the sewage treatment applied reveals that the discharge of inadequately treated waste-loads is also still a problem for many of the developed countries, illustrated in Fig 1.3.

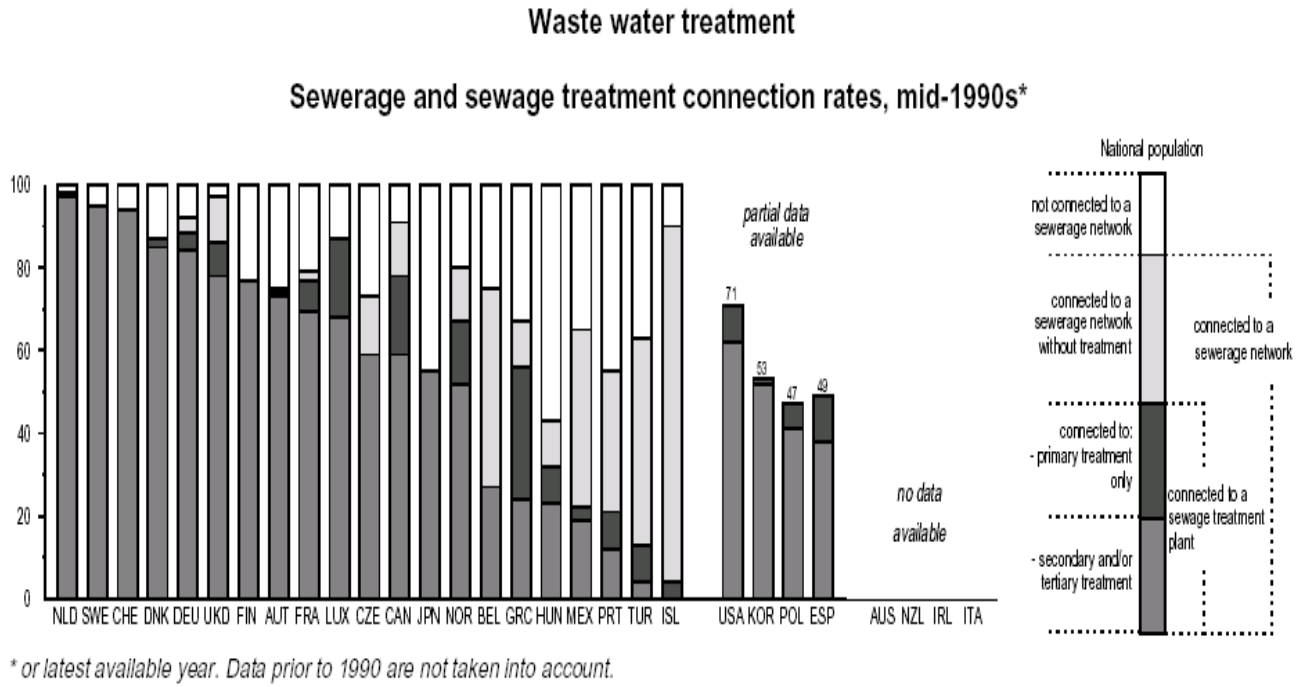

Fig 1.3 Sewerage and sewage treatment connection rates as collected by the OECD at the end of the 1990's. (OECD, 1999).

The levels of treatment vary significantly within the OECD community, the varying economic and environmental conditions and the rate at which countries have addressed their waste water treatment in the past, will all be contributing factors which will have influenced these statistics. Some countries are still completing sewerage networks or first generation treatment plants, whilst other countries will have reached their economic limits in terms of sewerage connection.

Within Europe the implementation of the Urban Wastewater Treatment Directive (91/271/EEC) has stimulated the building of many municipal wastewater treatment plants and new processes being adopted, such as biological treatment stages.

The Urban Directive addresses this problem of urban wastewater pollution by requiring that cities, towns and other population centres meet minimum wastewater collection and treatment standards within deadlines fixed by the directive. The expiry of these deadlines was fixed as the end of 1998, 2000 and 2005, depending on the sensitivity of the receiving water and on the size of the population centre. However, this has been a rather slow process for several countries, recently there has been the report of the case of the European Commissions final legal warning to Italy over failure to ensure proper sewage treatment in Milan. Belgium was another country also condemned by the European Court of Justice for failing to prepare adequate plans for treating sewage in Brussels, its capital city. All of Brussels sewage is discharged directly into the Senne river, which has been culverted in the city centre to avoid the smell. A commission report commented on the Senne downstream of the Belgian capital being "more an open sewer than a river". In the developing regions of the globe, the levels of accurate data available on wastewater treatment is very limited but it is estimated that more

than 90 percent of sewage is discharged directly into rivers, lakes, and coastal waters without treatment of any kind (WRI, 1997).

The World Health Organisations report on Global Water Supply and Sanitation (WHO, 2000) has gathered information on access to sanitation services through household connections and other means for Africa, Asia, Latin America and the Caribbean, which have the largest concentrations of developing regions (see Tables 1.1 and 1.2).

Table 1.1 Sanitation coverage by category of service (Other access includes septic tanks, pour flush systems, ventilated pit latrines, pit latrines).

	% Coverage		
	Sewerage Connection	Other Access	No Access
Africa	13	47	40
Asia	18	30	52
LA&C	49	29	22
Total	20	33	47

Table 1.2 Median percentage of urban wastewater collected through the sewerage systems that are reported to be treated in sewage treatment plants.

	% median of urban wastewater treated
Africa	0
Asia	35
LA&C	14
N America	90
Oceania	-
Europe	65

1.3 Wastewater and Wastewater Treatment

Wastewater can be defined as a contaminated aqueous discharge of domestic or industrial origin, which is unfit for any purpose without being subjected to some form of purification process. It can be described by its flow and quality characteristics as well as it's source, i.e. domestic / municipal or industrial with the latter source having more variability with respect to flow and quality.

Wastewater consists of particles of various sizes suspended in a relatively weak solution of organic and inorganic compounds and its quality can be defined by its physical, chemical and biological characteristics (Table 1.3).

Table 1.3 Physical, Chemical, and Biological Wastewater Characteristics (Metcalf and Eddy, 1991).

Physical	**Chemical**	**Biological**
Solids	*Organics*	Plants
Temperature	Proteins	Animals
Colour	Carbohydrates	Viruses
Odour	Lipids	
Surfactants		
Phenols		
Pesticides	*Inorganics*	
	pH	
	Chlorine	
	Alkalinity	
	Nitrogen	
	Phosphorus	
	Heavy Metals	
	Toxic Materials	
Grit	*Gases*	
	Oxygen	
	Hydrogen Sulfide	
	Methane	

The composition of domestic and municipal wastewater varies significantly both in terms of place and time, this is in part due to variations in the discharged amounts of substances. However, the main reason is variations in water consumption, infiltration and ex-filtration. Concentrated wastewater represents cases with low water consumption and or infiltration, dilute wastewater represents high water consumption and or infiltration. Domestic sewage generally will contain approximately 1000 mg/l of impurities, of which about two thirds are organic. A breakdown of the typical average contents of organic matter present and the typical average contents of nitrogen matter in domestic wastewater are shown in Tables 1.4 and 1.5.
Depending on the concentration of the wastewater, typically detergent concentrations in wastewater measured as linear alkylbenzenesulphonates (LAS) can range from between 1 – 15 mg/L (Henze, 1996). Conventional wastewater treatment is a combination of physical and biological processes aimed at removing the organic matter from solution. Sewage is most commonly treated in a three-stage process including: preliminary treatment, primary treatment, also known as sedimentation, and secondary biological treatment.
In certain circumstances there is a need for tertiary treatment, making treatment a four-stage process, but this is more common where the receiving waters are of a more sensitive nature or are required for abstraction for drinking purposes.
A by-product of wastewater treatment is sludge which requires further treatment or disposal. Preliminary treatment is basically a screening of the wastewater aimed at removing larger floating objects and grit making sewage more amenable to treatment. However, this does not significantly impact on reducing the polluting or pathogenic load of the wastewater.
Therefore if effluents are discharged immediately after preliminary treatment, a significant health and environmental risk will remain in the area of the outfall, though the aesthetic

environmental problems will be minimised. Primary treatment further reduces the polluting load through sedimentation and removal of floating scum formed by fats, oils and greases (FOGs). It uses sedimentation tanks, in which, wastewater is retained for 2-6 hours to permit particulate matter to settle out of suspension. These particles collect at the base of the tank to form a sludge. In total, approximately 55% of suspended solids are removed during primary treatment, with the result that biological oxygen demand (BOD) decreases by approximately 35%. There are two main types of secondary treatment, activated sludge treatment and trickling filter (also known as biological filter or percolating filter). Initially in both cases, effluent from primary treatment undergoes biological processes whereby micro-organisms oxidise the BOD. In activated sludge this is followed by a further sedimentation, which separates the micro-organisms from the final effluent. In trickling filter systems the micro-organisms are left behind on the filter. If this stage is present, more than 95% of biodegradable surfactants would be expected to be removed.

As mentioned earlier some wastewaters require further purification and this may include disinfection, which aims to reduce the number of viable micro-organisms that can cause subsequent infection of people. Nutrient removal is another key cleaning up process required for some wastewaters where eutrophication is an issue for the receiving waters and this is also classified as a tertiary treatment in some literature.

Where none of these treatment stages are involved, then sewerage networks simply act as a transportation system for the wastewater to its receiving waters.

This receiving water then has to utilise its own self-purification processes, physical, chemical and biological, to clean up the waste load. If the receiving body of water cannot self-purify the waste loading, serious and often irreversible changes in its ecology may be induced. The

typical fate and potential pathways for Unilever ingredients in to the aquatic environment and their potential routes for removal are illustrated in Fig. 1.4.

Table 1.4. Typical average contents of organic matter in domestic wastewater (Henze, 1996).

Analysis Parameters	Unit (1)	Wastewater Type			
		Concentrated	Moderate	Diluted	V.Dilute
Biochemical Oxygen demand, BOD	g O_2/m^3				
- Infinite		530	380	230	150
- 7 days		400	290	170	115
- 5 days		350	250	150	100
Chemical Oxygen Demand, COD	g O_2/m^3				
- total					
- *dissolved*		740	530	320	210
- *suspended*		300	210	130	80
		440	320	190	130
Total Organic Carbon					
- Carbohydrate	g C / m^3	250	180	110	70
- Proteins		40	25	15	10
- Fatty Acids		25	18	11	7
- Fats		65	45	25	18
		25	18	11	7
Fats Oil and Grease	g / m^3	100	70	40	30
Phenol	g / m^3	0.1	0.07	0.05	0.02
Phtalates, DEHP	g / m^3	0.3	0.2	0.15	0.07
Phtalates, DOP	g / m^3	0.6	0.4	0.3	0.15
Nonylphenols, NPE	g / m^3	0.08	0.05	0.03	0.01
Detergents, anion (2)	g LAS / m^3	15	10	6	4

1) g / m3 = mg/L = ppm
2) LAS = Lauryl Alkyl Sulphonate

Table 1.5 Typical average contents of nitrogen matter in domestic wastewater (Henze ,1996)

Analysis Parameters	Unit (1)	Wastewater Type			
		Concen-trated	Moderate	Diluted	V.Dilute
Total Nitrogen	g N / m^3	80	50	30	20
Ammonia Nitrogen [1]	g N / m^3	50	30	18	12
Nitrite Nitrogen	g N / m^3	0.1	0.1	0.1	0.1
Nitrate Nitrogen	g N / m^3	0.5	0.5	0.5	0.5
Organic Nitrogen	g N / m^3	30	20	12	8
1) $NH_3 + NH_4^+$					

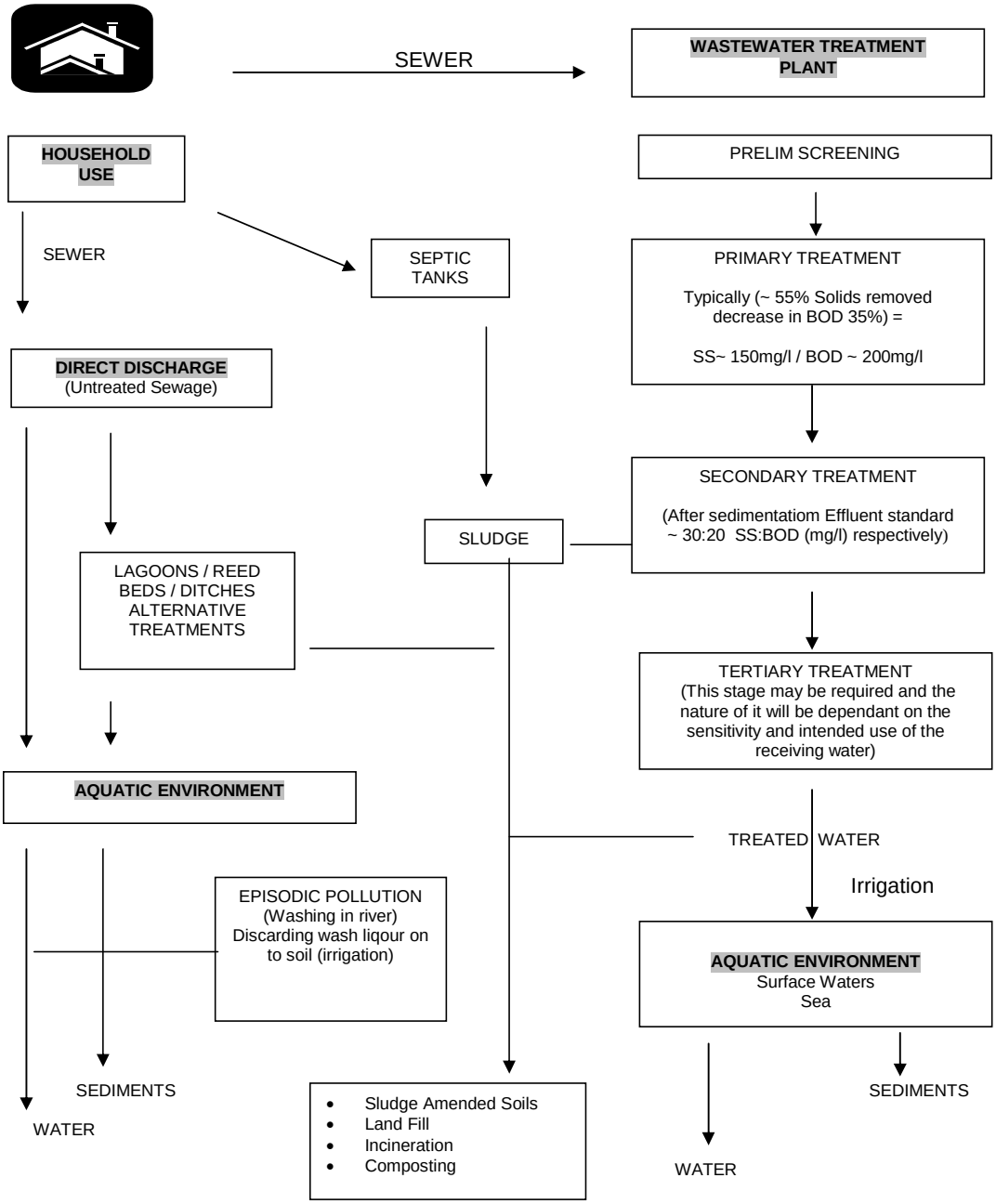

Fig 1.4 Typical fate / pathways for Unilever ingredients into the aquatic environment and potential routes for removal.

1.4 Previous Work

The relationship between results obtained for classifications of biodegradability of chemicals tested in laboratory tests and those from sewage treatment simulation tests is very well established. Current standard biodegradation tests determine the general biodegradation potential of specific chemicals and a material which passes a ready screening test has been shown to also rapidly degrade in both waste water treatment plants (Gerike and Fischer, 1979) and the environment. The EU Technical Guidance Document (TGD Commission Directive 93/67/EEC) assigns default values for each situation, which are first order rate constants of 1 h^{-1} for use in risk assessment modelling in WWTP's and 0.047 d^{-1} in surface waters. This knowledge has been used for environmental risk assessment, primarily in Western Europe and the US or specific regions where treatment occurs prior to release. Several tests have developed over time to evaluate the biodegradability of chemicals in environmental waters from simple batch systems to more complex simulation tests. A variety of different conditions have been proposed for simulating this scenario with varying composition of test media (natural and synthetic), micro-organism sources, test conditions, test substance concentrations and analytical techniques. These factors raised many questions on how to evaluate and apply the results generated for risk assessment purposes with confidence.

Several test methods involving batch systems were initially developed for determining biodegradability in surface waters (Means *et al.,* 1981; Wylie *et al.,* 1982) who proposed die-away systems in a 2.5L flask using river water as both test media and source of micro-organisms. $^{14}CO_2$ analysis was used to detect test concentrations at 0.1mg/l. Adequate repeatability was obtained with the reference material (phthalic acid) but less so with the test

materials which showed greater variation. A die away system using methylene blue anionic surfactant (MBAS) analysis and a test concentration of 25 mg/L was explored by Anderson *et al.*, (1990). This is a convenient indirect method for monitoring the biodegradation of surfactants when a specific analysis is not available or is too costly.

A lot of proposed test systems for simulating biodegradation used large open systems such as model streams. Oba *et al.*, (1977) looked at systems using chlorinated water as the test medium and the supernatant of activated sludge as the source of micro-organisms. An artificial stream of 10.8 m in length was constructed with a 50 hr residence time and LAS biodegradation monitored with MBAS and total organic carbon (TOC) analysis. Adaptation to quaternary ammonium surfactants by suspended microbes in a model stream was investigated by Shimp *et al.*, (1989).

A 20 m length was constructed simulating a river with sediment (depth 1-2 cm) and under controlled light (10 h/day) and realistic test concentrations (0.2 - 1 ppm) using $^{14}CO_2$ analysis. An overflow system using 500 x 50ml vessels was developed by Engellman *et al.*, (1978), with a residence time of 5 h. These studies all suggested that continuous systems of this nature required at least four weeks to achieve steady state conditions. The overflow system was further developed by Scholz & Muller (1991), to create a more complex riverine model using an aquatic staircase system. The test system consisted of test units in cascades run in parallel, each containing seven channels made from photographic washing tanks (50 x 39 cm).

This ‘riverine’ system was fed with the outflow of an OECD confirmatory test system containing no test substance and being dosed with a synthetic feed. The outflow from the confirmatory test was then diluted with dechlorinated tap water in a ratio of 1 : 4.
This diluted outflow was then fed at 15 L / d into the topmost tank of the cascade, an outflow from this tank was then fed in to the next tank and so on. One test system acted as a control, whilst, the other cascade was exposed to the test substance.
The system (Fig. 1.5) was based on a idea by (Guhl, 1987), to simulate a stable part of a riverine system and was designed to close the gap between standardised single species tests and the situation observed in the field. This study compared the species of organisms detected in his model in various laboratories with those found by him in the lower reaches of the Rhine. Good correlation and representation of the species in surface waters was found.

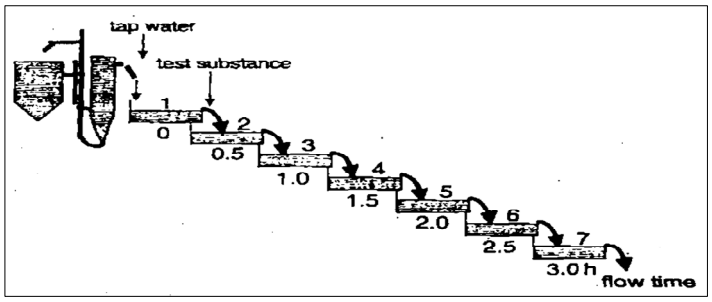

Fig. 1.5 Diagram of cascade system for the surface water simulation method.

This comparison improved confidence in the applicability of the results obtained from the model to actual events occurring in surface waters in the real world. Scholz and Muller F, (1991) used the test system for determining ecotoxicity and biodegradation.

Several biological species, including, producers, bacterial predators, algivores and carnivores, were measured to determine ecotoxicity and the effects of LAS on them.

The biodegradation was monitored using MBAS analysis. Further work using this system was published by Koziollek *et al.*, (1996) who presented it as a suitable dynamic system river model for biodegradability studies. A series of non-volatile and non-sorbing model compounds (2,4-dinitrophenol, naphthalene-1-sulphonic acid and sulphanillic acid) were tested in the cascade system and compared with two standardised batch shake flask tests. The modified OECD screening test (MOST, OECD 301E) and the dissolved organic carbon die away test, (DAWT, OECD 301A). These tests are both batch 'static' systems with very similar conditions, the main difference that the DAWT test allows the use of higher microbial cell densities.

The compounds were tested at the standard test concentrations and lower, to get closer to the very often low concentrations observed in the environment. ^{14}C labelled compounds were measured at 50 µg/L, unlabelled compounds by capillary electropheresis at 5000 µg/L and the removal of dissolved organic carbon (DOC) at 50000 µg/L. This study concluded that the river model produced reliable test results of high predictive value in an environmentally realistic range of concentrations for test substances which are non volatile and not sorbing specifically on biomass. It also concluded the use of specific analytical techniques or radio labelled compounds is required to investigate at low test concentrations. The results from the DAWT batch test were reliable and could be compared with the river model. The MOST test was found to be inadequate in comparison, particularly at low inoculum concentration and was not a suitable test for predicting biodegradation in surface waters.

A similar exercise was completed by Seel *et al.,* (1993) who measured the degrees of elimination of test substances in this test system and compared them with MOST and DAWT systems and suggested that both batch systems were comparable with the river model, so some discrepancy seems to exist over the MOST test. Koziollek *et al.,* (1996) also concluded that although this was not a simple test, it could be standardised and be used as a tool to investigate substances on a high simulation level and to compare the results of simpler batch style systems to increase their predictive value. A range of surfactants were monitored in the riverine system by Schoberl *et al.,* (1997) to try and predict the distance downstream of a wastewater point source within which degradation / elimination of a discharged substance would take place.

This investigation found that the low flow rate of the river model (maximum 1 metre per hour) did not allow the measured distance to be applied directly to the real world conditions. However, the time during which a certain percentage of degradation / elimination of a particular substance takes place in the model can be applied to any surface waters with a water quality comparable with that in the model irrespective of their flow rates. So if in the model an ingredient is 50% biodegraded within a distance of 1 m and it takes 1hr for the model surface water to travel this distance, then in any comparable surface water in the real world 50% of the ingredient would expected to be removed within the distance covered by the surface water in 1 hr. These experiments were not designed for determining biodegradation but for investigating chronic toxicity, so the surfactants were selected for these purposes. Biodegradation was followed by non specific techniques, MBAS and bismuth active substances (BiAS) for non-ionic surfactants so the surfactant concentrations were also higher than would be expected in surface waters.

The results for degrees of degradation from the river model did not differ significantly from those observed in the DAWT and MOST tests.

However, the degrees of degradation / elimination were reached in hours in the river model simulation studies after completion of the lag phase and only after days in the static tests. The surfactant half lives calculated in the surface water simulation model also compared well with monitoring data on the surfactants from the environment, which further validated the applicability of this test system.

Boeije *et al.,* (2000) constructed a laboratory scale artificial river system (Fig 1.6) which also considered the incorporation of biofilm activity in river biodegradation using LAS as the case study. A mathematical model was constructed which considered both biofilm and suspended biomass activity in relation to the biodegradation of chemicals in rivers. The artificial river model was constructed again as a cascade but using 5 U-shaped gutters each 2 m in length with a total volume of 36 L. A hydraulic residence time (HRT) of ~3 h was set requiring a flow rate of

0.2 L / min.

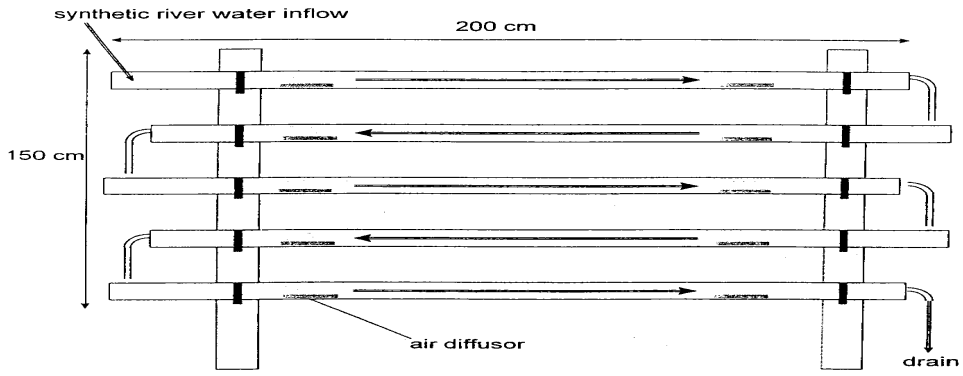

Fig 1.6 Artificial river design, Boeije *et al.,* (2000)

Air diffusers were placed in each piece of guttering to encourage oxygenation and to counteract sedimentation. A synthetic river water was used to reduce variability which consisted of a laboratory scale trickling filters effluent mixed 50/50 with tap water. The chemical oxygen demand (COD) concentration was in the order of 40 mg/L, LAS levels were measured in the order of 1-2 mg/L. The trickling filters influent was a synthetic sewage (containing LAS) based on a synthetic sewage developed by Boeije *et al.*, (1998). Biofilm was allowed to develop on the edges of the gutters and on an artificial material to mimic bed material in a river or vegetation present in natural rivers, (polypropylene truncated cones with fins). LAS was then measured using a specific Azure-A analytical method.

The artificial river model design used by Boeije *et al.*, (2000) seems to be less demanding on laboratory floor space and of a more compact design than some of the previous lab scale river models discussed.

Three experiments looked at the effects of no biofilm presence, presence of biofilm on guttering edges and suspended biomass, and, finally biomass on carrier material, guttering edges and suspended biomass. The results showed no significant removal took place in the absence of biofilm but in the presence of biofilm significant removal was observed. This indicated that in rivers with a high surface area to volume ratio, biodegradation by biofilms was more important than that by suspended biomass.

The absence of removal in the ‘no biofilm’ case also confirmed that biodegradation was the process occuring in the ‘with biofilm’ cases. The biodegradation model which was proposed was successfully corroborated with a field study completed in Red Beck, a small river in the Calder catchment (Yorkshire, UK) for which LAS removal measurements were available, Fox *et al.*, (2000). Investigations of the removal of LAS by biofilm in an urban shallow

stream has also been published by Takada *et al.*, (1994). Field observations in the Nogawa river, a polluted shallow stream in Tokyo, were made from 1987-1990. LAS concentrations decreased from 1 mg/L in the upper stream receiving untreated domestic wastewater to less than 0.05 mg/L at 6 km downstream. Within the upper portion of a 1.8 km concrete open channel (travelling time of water, 1-3 h), more than 80% of the LAS was removed. Considerably shorter half lives were observed (approx 1 h) than those obtained from biodegradation experiments using river water only (tens of hours).
Biodegradation experiments using biofilm collected from the stream bed indicated that LAS is much more rapidly degraded in the presence of biofilm. Negligible LAS measurements in the biofilm of the stream bed indicated that biodegradation by the stream bed biofilm was the predominant removal mechanism of LAS in an urban shallow stream. The culmination of these experiments and different systems was that test guidelines were established for the simulation of biodegradation in environmental waters, described in ISO 14592 (2002) - Water quality, Evaluation of the aerobic biodegradability of organic compounds at low concentrations :

ISO 14592 *Part 1*: Shake-flask batch test with surface water or surface water/sediment suspensions specifies a test method for evaluating the biodegradability of organic test compounds by aerobic micro-organisms by means of a shake-flask batch test. It is applicable to natural surface water, free from coarse particles to simulate a pelagic environment ("pelagic test") or to surface water with suspended sediments added to obtain a level of 0,1 g/l to 1 g/l dry mass to simulate a water body with suspended sediment. It is applicable to organic test compounds present in lower concentrations (normally below 100 µg/l) than those of natural

carbon substrates also present in the system. Under these conditions, the test compounds serve as a secondary substrate and the kinetics for biodegradation would be expected to be first order ("non-growth" kinetics). This test method is not recommended for use as proof of ultimate biodegradation which, is better assessed using other standardized tests. It is also not well suited to studies on metabolite formation and accumulation which require higher test concentrations. The test is specifically designed to provide information on the biodegradation behaviour and kinetics of test compounds present in low concentrations, i.e. sufficiently low to ensure that they simulate the biodegradation kinetics which would be expected to occur in natural environmental systems.

ISO 14592 *Part 2:* Continuous flow river model with attached biomass specifies a method for evaluating the biodegradability of organic test compounds by aerobic micro-organisms in natural waters by means of a continuous flow river model with attached biomass. As Part 1, it is applicable to organic test compounds present in lower concentrations than those of natural carbon substrates also present in the system. Under these conditions, the test compounds serve as a secondary substrate and the kinetics for biodegradation would be expected to be first order ("non-growth" kinetics). The ISO paper suggests the cascade system using trays (aquatic staircase) as discussed earlier as one system which is suitable but suggests it is also possible to use other test systems (e.g. different size and shape of the trays, other sediments or different surface-volume relations) and other test conditions (e.g. flowrate of water, hydraulic load, illumination, inoculation). In this case, all the relevant parameters of a different test systems have to be documented and taken into consideration for the test performance and the calculation of the test result. The inoculum (source of micro-organisms) used in test studies of this nature should be selected to accurately represent the micro-

organisms present in the environmental compartment that the test ingredient will be present in on release.

Micro-organisms have mainly been collected from rivers and lakes, Wylie *et al.*, (1982) or suitable surface waters such as epilithic microbial communities or from sediments, Larson *et al.*, (1981) for determination of biodegradation in this compartment. Care needs to be taken that adaptation is considered (i.e. if the source of organism has previously been exposed to the test material in the environment) as this can impact lag times and degradation rates, Shimp *et al.*,(1989).

As with the source of micro-organisms the test media should also represent the environmental water into which the test chemical is going to be released. This can present difficulties because of the variability and composition of environmental samples. This has led to studies using artificial media in an attempt to reduce variability. However, to truly simulate an environmental compartment of interest the natural water from this environment should be used and this is were the difficulty of measuring chemicals discharged without any treatment occurs. The majority of the test systems discussed have modelled biodegradation in fairly 'clean' environmental matrices, such as, river water alone or in the presence of effluent. The surface water simulation models discussed earlier used an effluent feed from a confirmatory plant then diluted this feed in a 1: 3 or 1: 4 ratio with tap water. This has led to extensive biodegradation kinetics data being available for major surfactants under conditions when wastewater has passed through a treatment plant prior to discharge, (Waters et al., 1995; Rapaport *et al.*,1990) but, limited work has been published looking at a 'true' discharge scenario where potentially high levels of sewage will be present.

The ‘worst case scenario’ in terms of dilution assumed in a direct discharge scenario for risk assessment purposes is a 1 : 2 (settled sewage : river water) ratio, referred to as a dilution factor of 3. Batch tests conducted by Peng *et al.*, (2000) evaluated the decay rates of MBAS and COD under conditions simulating untreated discharge with 1 : 2 ratio of sewage / river water and concluded that under anaerobic conditions neither MBAS or COD decreased in concentration while under aerobic conditions the decay rate of MBAS was consistently faster than the COD.

^{14}C LAS was also tested in 100% raw sewage and 33% raw sewage in river water. Half lives were 8-10 h for loss of the parent material and 11-12 h for complete mineralisation, with dilution in river water having no effect. This indicated that LAS would degrade more rapidly than COD under these conditions and LAS would be expected to be at very low levels once a stream had recovered from the addition of untreated sewage. Peng *et al.*, (2000), also suggested that these studies could form the basis for developing standardized tests for assessing the fate of chemicals under untreated discharge conditions.

The test chemical should be ideally tested at the concentration that would be predicted to be present in the real environment because the test concentration of the chemical can affect the rate of biodegradation, Larson *et al.*, (1981).

The predicted concentrations of chemicals in the environment are usually extremely low and this generates problems in terms of the analytical limits of detection,

particularly when measuring biodegradation in untreated discharge conditions because of the nature of the environmental matrix. Radiolabelled test materials allow the measurement of biodegradation at more realistic environmental concentrations or alternatively, specific analysis with HPLC can be used although this only confirms primary biodegradation unless

metabolites are also followed. Non specific methods are also applicable although their use is limited as they usually require concentrations higher than would be expected in the environment.

1.5 Aims of the Project

The aim of this research is to review and develop predictive screening tools, which will provide a clearer understanding of the fate of detergent ingredients when exposed to an untreated discharge scenario and hence provide more realistic and valuable data for the Unilever environmental database to utilize, when, assessing the environmental risk. A tiered screening test approach designed around an alternative ERA methodology concept to demonstrate the extent of removal of some key detergents through biodegradation, in particular, the rate of biodegradation relative to other key self purification parameters will be explored.

The focus will be on chemicals, which are of considerable environmental importance because of their high volume consumption and widespread use as essential chemicals in most home and personal care products, such as linear alkylbenzenesulphonates (LAS).

The tiered screening test approach will include investigating short term inhibition studies, static batch systems to compare biodegradation rates of key ingredients and water quality parameters, through to a higher tier dynamic simulation system. The aim is to apply the data generated from the screening tests to the alternative risk assessment model for untreated discharge.

1.6 Good Laboratory Practice

SEAC (Safety and Environmental and Assurance Centre) operates in accordance with SEAC Policy on Good Laboratory Practice based on the UK Good Laboratory Practice Regulations 1999, Statutory Instrument No. 3106 and OECD Principles on Good Laboratory Practice (as revised in 1997) ENV/MC/CHEM(98)17.

The studies described in this report were conducted in accordance with this Policy and to the Standard Operating Procedures (SOP) in force in the testing facility at the time of the study. In consequence the studies were subject to periodic audit and evaluation by Quality Assurance.

CHAPTER 2

Biodegradation of linear alkylbenzenesulphonate and alcohol ethoxylates in river water simulating untreated discharge conditions

2.1 Introduction

In many developing and emerging markets (as well as several developed regions), lack of sewage treatment prior to discharge into the receiving waters means that traditional risk assessment assumptions cannot be used, and in the absence of data, it becomes necessary to assume stringent defaults when predicting the safety margins. A series of batch screening tests were conducted to simulate and help understand the fate of detergent chemicals under these conditions.

An impact zone will occur after an untreated discharge, as the receiving water has to complete self-purification against this pollutant loading through a series of physical/chemical and biological processes. If evidence can be provided that the decay rates of key surfactants are quicker than that of the general organics, or the free ammonia present in untreated sewage, it would indicate that these surfactants would be at very low levels after the receiving body of water had recovered from the discharge.

The study concentrates on two of the most widely used surfactants in all detergent and cleaning products, LAS and alcohol ethoxylates (AEs). The biodegradation kinetics of LAS and AEs within sewage treatment are very well understood, but this is not the case for untreated discharge scenarios where reported data are very limited. The primary aim of this study was to assess the suitability of a batch die away system for simulating direct discharge conditions and obtain biodegradation kinetics of the native levels of LAS and AEs in the settled sewage. A comparison could then be made with the kinetics of the general organics, measured as COD, and ammonium.

2.2 Materials and Methods

An initial study was completed looking at the decay rates of anionic surfactants by measurement of MBAS in comparison to the general organic loading present as COD in sewage, using a batch test system. This study was repeated again measuring COD and MBAS, but with the inclusion of specific analysis for LAS by LC/ESI/MS (liquid chromatography electrospray ionisation mass spectrometery), and AEs using derivatisation with phthalic anhydride followed by LC/ESI/MS . Ammonium (NH_4^+) was also measured in both studies.

Study 1

A batch reactor system (Plate 1) was prepared and dosed with 2 L of a settled sewage / river water mixture (dilution factor =3, 1 part settled sewage to 2 parts river water) simulating a heavily polluted system and assuming a worst case scenario for direct discharge.
Settled sewage was collected from Broadholme sewage treatment works, Ditchford, Northants (Anglian Water). Broadholme STW treats predominately domestic waste, with trade flow at 6.2% and trade organic load at 12.4%.The settled sewage was coarsely filtered with glass wool prior to dilution with river water. The river water was obtained from the River Great Ouse at Felmersham Bridge, Felmersham, Bedfordshire. The sample was taken mid channel and again coarsely filtered using glass wool prior to addition to the settled sewage. The test system was operated for a five-day period and was temperature controlled at 20^{o}C throughout the duration of the test. A slow stream of air was continually pumped through the test system to ensure sufficient air was available in the headspace to keep the test

media saturated with dissolved oxygen (dO_2) so that the test system simulated an environment of continual re-aeration. A mixing speed of 125 rpm was maintained throughout the duration of the test, which caused sufficient agitation to the system without encouraging foaming. Periodically samples were removed and analysed for COD, MBAS and NH_4^+ over the 5-day period. Suspended solids measurements (SS) were made of the initial samples based on measuring the dry weight after 24 h at 105^{o}C of a known volume of water sample.

Plate 1. Batch reactor system.

Temperature, pH and dissolved oxygen was continuously monitored. Water quality analysis, COD, NH_4^+ and MBAS were all measured using the cuvette test methods (Hach Lange) on the Xion 500 Spectrophotometer (Hach Lange GMBH, Dusseldorf, Germany).

These methods employ photometric analysis of colour complexes formed in chemical reactions with the analyte. In the COD test, oxidisable substances in the water samples react with a sulphuric acid – potassium dichromate solution in the presence of silver sulphate as a catalyst to form the coloured ion Cr^{3+}. In the NH_4^+ test, ammonium ions react at pH 12.6 with hypochlorite ions and salicylate ions in the presence of a sodium nitroprusside catalyst to form indophenol blue

Comment [S1]: Not sure what you mean by this?

Comment [Unilever2]: Added all this text to explain cuvette test analysis chemistry

Comment [S3]: I don't understand what you mena by "masked"

Study 2

This study provided kinetic data on the disappearance of native levels of LAS and AEs naturally present in the sewage. As in study 1, the batch reactor system was prepared and dosed with 2.6 L of a settled sewage / river water mixture (1 part settled sewage to 2 parts river water) simulating a heavily polluted system. The increased volume in comparison to the first study was required to allow sampling for LC/ESI/MS analysis, which permitted a comparative study of the rate of decay of the various homologues. All other aspects were as study 1. At various time points samples were removed and analysed for COD, MBAS and NH_4 and 30 ml duplicate samples were also taken during the day at three time points from day 0 to day 3 for specific analysis of LAS and AEs by LC/MS. As before. suspended solids (SS) were measured in the initial samples and temperature, pH and dissolved oxygen were continuously monitored.

Analytical Methods

Duplicate 30 mL samples were taken per time point analysis. One sample was then extracted for LAS and the other for AEs using an automated solid phase extraction (SPE) procedure, Sparham *et al*., (2005) but with different pretreatment and extraction cartridge.

For AEs, 70 mL of ultrapure water was added to the samples followed by 66 mL of methanol. The samples were then loaded onto the Zymark Autotrace with the C_8 SPE cartridges (Isolute SPE cartridges, C_8 1g/6 mL (p/n 290-0100-C), Jones Chromatography). The samples were then dryed under nitrogen and eluted with MeOH:MTBE:DCM (2:1:1) and evaporated. The final extracts taken from the Zymark were concentrated under a gentle stream of nitrogen (room temperature) until just less than 2 mL was left. At this point the extract was transferred to a 2 mL HPLC vial and carefully concentrated to incipient dryness. Derivitisation reagent (990µL), made up of (3g phthalic anhydride / 50 mL pyridine) and 10 µL of the internal standard ($C_{16}D_{33}OH$) was added, the vial was then capped and derivatised for 1 hour at 85°C. A series of calibration standards were prepared in pyridine: Genopol C100 (C_{12} and C_{14} alkyl chain), Genapol T110 (C_{16} and C_{18} alkyl chain) ex-Clariant and Lutensol AO7 (C_{13} and C_{15} alkyl chain) ex-BASF. The derivatised AE extracts were then analysed by LC ESI MS, in negative ionisation mode, utilising the negative charge imparted by the derivatisation, using a HP1100 series LC-MSD.

For LAS, 70 mL of Ultrapure water was added to the samples, which were then loaded onto the Zymark Autotrace with the Isolute SPE cartridges, C_{18} 1g/6mL (p/n 220-0100-C), Jones Chromatography. The samples were then dried under nitrogen and eluted with MeOH:MTBE:DCM (2:1:1) and evaporated. The final extracts taken from the Zymark were concentrated under a gentle stream of nitrogen (room temperature) until just less than 1 mL

Comment [S4]: I don't understand. If they have been evaporated to dryness how is 2ml left? (Removed reference to dryness)

Comment [S5]: What does this mean?

Comment [S6]: Is this correct? If not give the right name for the LC-MS you used (This is correct reference)

Comment [S7]: Same comment as before – what is this evaporation to dryness?

was left. At this point the extract was transferred to a 10 mL volumetric flask and made up to volume with methanol, 990µL of the derivitisation reagent (3g phthalic anhydride/ 50mL pyridine) and 10 µL of the internal standard ($C_{16}D_{33}OH$), this was then derivatised for 1 hour at 85°C. A LAS standard (Na LAS Paste) – Unilever, was prepared in methanol (1000 µg/mL) and a series of calibration standards prepared from this. The derivatised LAS extracts were analysed by LC ESI MS, in negative ionisation mode utilising the anionic properties of the analyte The HP1100 series LC-MSD conditions were the same as for the AE analysis except that the mass range was 200 to 400 m/z.

Comment [S8]: You've lost me here, when did it get into the vial? (Removed reference to vial

Comment [S9]: You haven't told me the mass range used for AE!

2.3 Results

Study 1

Appendix 1 details the experimental results of the MBAS versus COD die away experiment and the measured parameters throughout the study. Initial suspended solids levels were measured (75mg/L). After 5 days 58.1% of the COD had been removed, 84.9% of the MBAS and 47.0% of the NH_4.

The die-away curves of the COD, MBAS and NH_4 measured concentrations against time are illustrated (Fig. 2.1). The same data is then illustrated as a semi-log plot used to calculate the decay rates (Fig 2.2.). In these studies the decay is assumed to be a first order reaction and the integrated rate law can be used for determination of k (decay rates) and $t_{1/2}$ (half-life). The integrated rate law expresses the concentration of a reactant as a function of time.

[A]= the concentration of A at time t

$[A]_0$= the initial concentration of A at t=0

$$\ln [A] / [A]_0 = - k t$$

$$[A] / [A]_0 = \exp (-kt)$$

And the half-life $t_{1/2}$ can be calculated.

$t_{1/2} = (-\ln 0.5) / k$

$t_{1/2} = 0.693 / k$

The semi-log plot was made over the range of maximum decay, and later points (when decay had levelled out) were not used.

Comment [Unilever10]: NA commented on Fig 2.3 why is last points missing, Response the decay curves had levelled out, it is common to calculate decay ratees fusing this approach explained in more detail further down in this chapter

Comment [S11R10]: I've brought the explanation forward, because it's necessary for a full understanding of what's going on here

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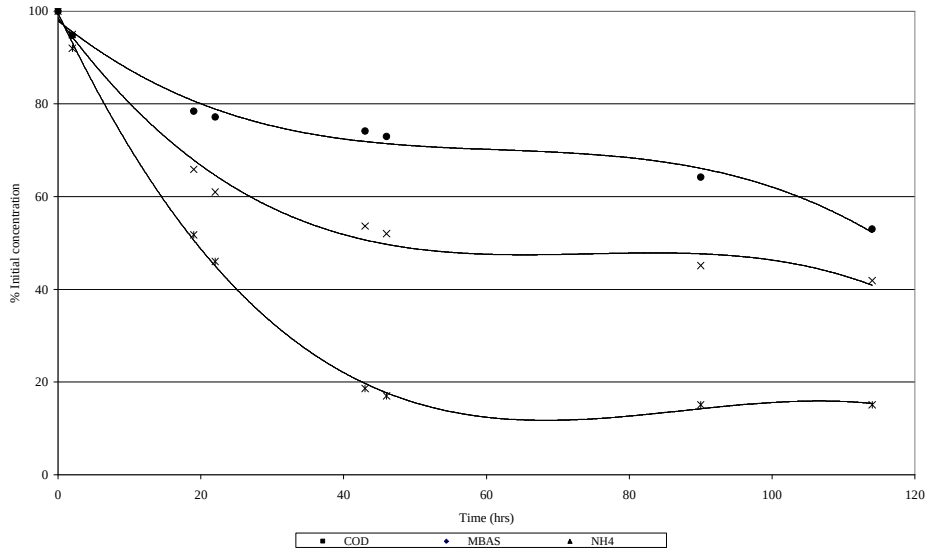

Fig 2.1 Die-away of COD/MBAS/NH_4 under aerobic conditions (100% dO_2 saturation).

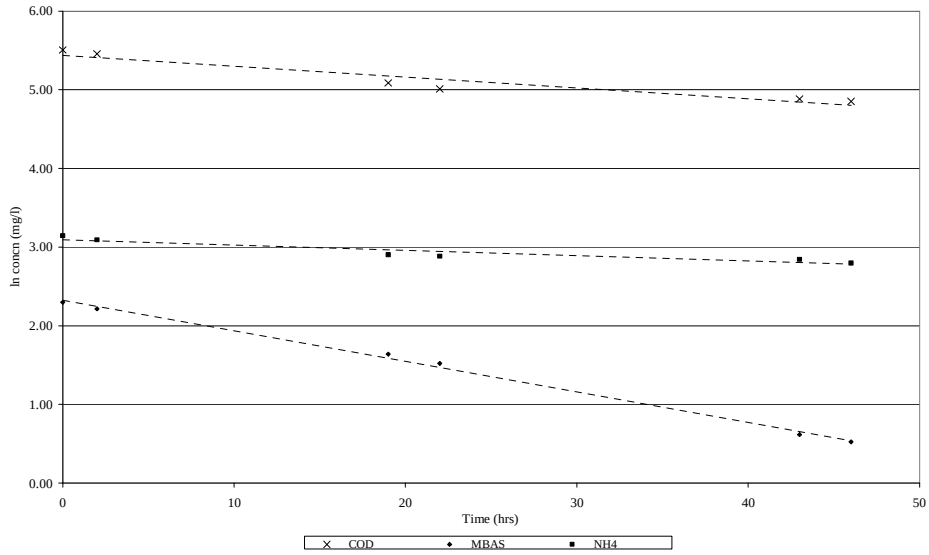

Fig 2.2 Semilog plot of the same data as in Fig 2.2 used to determine k (the decay rate constant).

The decay rate of MBAS was calculated to be 0.92 d^{-1} with half-life ($t_{1/2}$) = 18 h, while the COD decay rate was 0.34 d^{-1} with ($t_{1/2}$) = 48 h. The decay rate of MBAS removal was thus 2.7 times faster than that of the COD ($k_{MBAS} / k_{COD} = 2.7$). The rate of decay NH_4 was 0.19 d^{-1} with ($t_{1/2}$) = 85.9 h. The rate of removal of MBAS was thus 4.8 times faster than that of the NH_4^+ ($k_{COD} / k_{NH4} = 4.8$). NH_4^+ (12.3 mg/L) was remaining at the end of the study of which 3.82% was calculated (from temperature and pH) to be in the form of NH_3, equivalent to 0.46 mg/L

Study 2

Appendix 1 details the experimental results of the batch die away experiment and the measured parameters throughout the study. In this case the initial suspended solids measurement was lower (48mg/L). After 5 days 65.8% of the COD had been removed, 86.0% of the MBAS and 52.7% of the NH_4 (Fig 2.3).

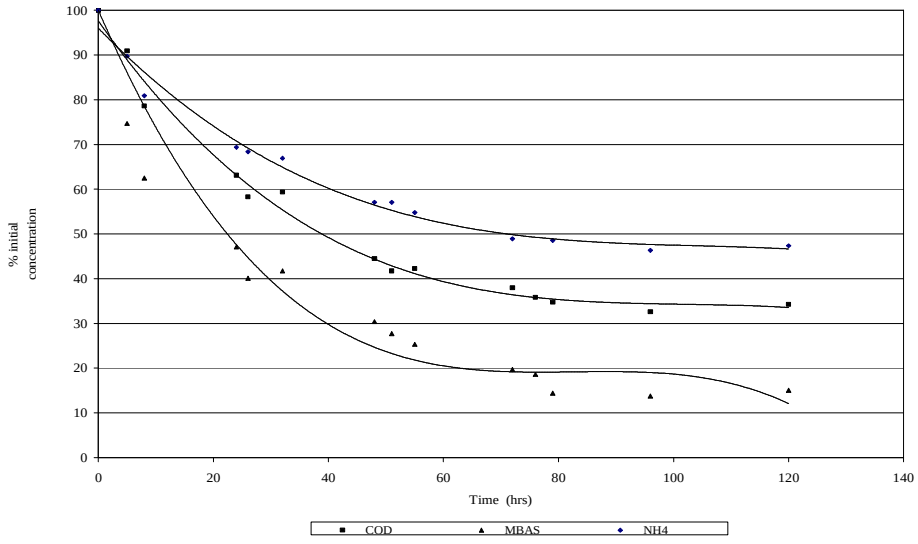

Fig 2.3 Die-away of COD/MBAS/NH_4 under aerobic conditions (100% dO_2 saturation)

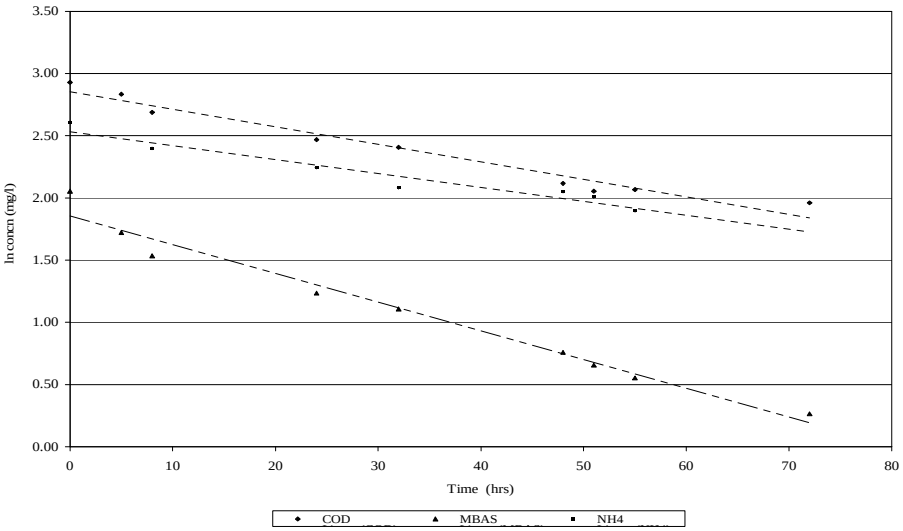

Fig 2.4 Semilog plot of the same data as in Fig 2.4 used to determine k (the decay rate constant)

The decay rate of MBAS was 0.60 d^{-1} with half-life ($t_{1/2}$) = 27.8 h. The COD decay rate was 0.32 d^{-1} with ($t_{1/2}$) = 51.5 h, the decay rate of MBAS was 1.8 times faster than that of the COD (k_{MBAS}/k_{COD} = 1.8). The decay of rate NH_4^+ was 0.24 d^{-1} with ($t_{1/2}$) = 70.5 h, the rate of removal of MBAS was 2.5 times faster than that of the NH_4 (k_{COD} / k_{NH4} = 2.5).

Calculation of the levels of un-ionised ammonia (NH_3) remaining after 5 days showed that 6.4 mg/L of NH_4^+ was remaining at the end of the study of which 7.5% would be in the form of un-ionised NH_3 equivalent to 0.48 mg/L

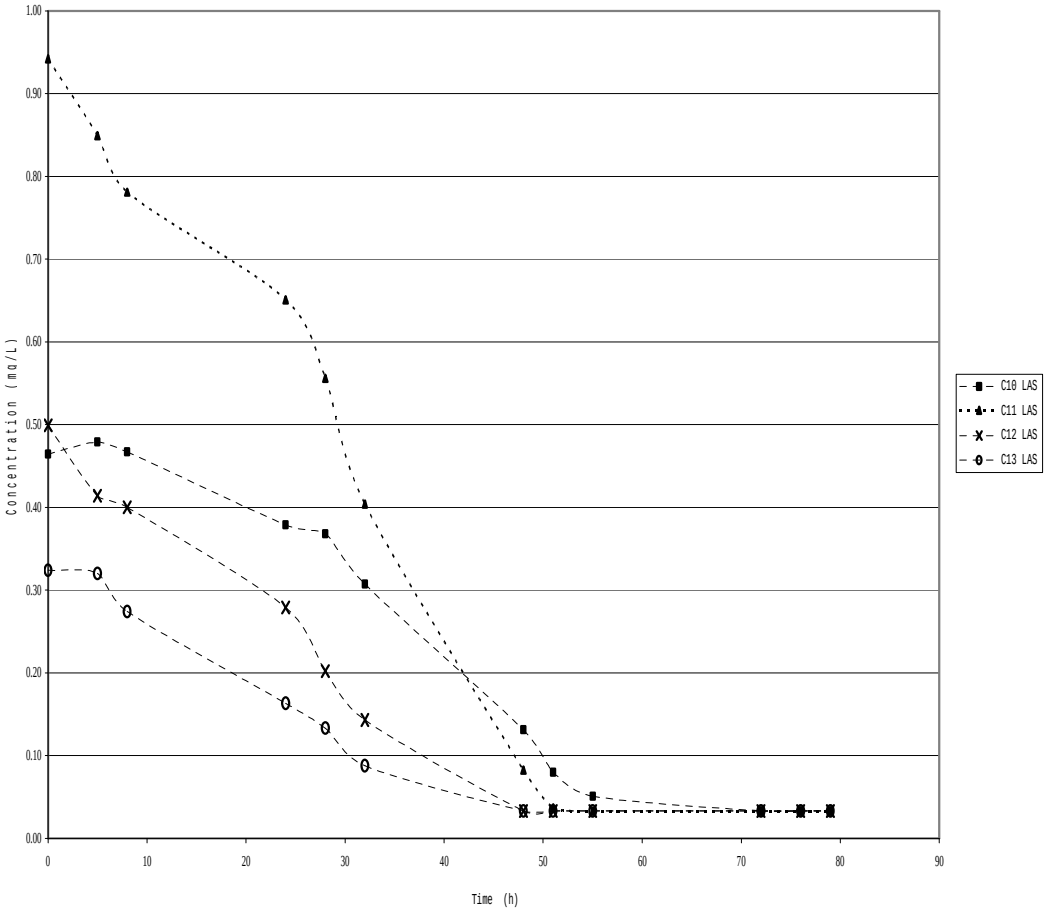

Fig 2.5 Plot of C_{10}-C_{13} LAS removal in batch die away system

Rapid removal of C_{10}-C_{13} homologues of LAS determined by LC-MS was observed (Fig 2.5). As before, the disappearance of LAS and AEs is assumed to be a first order reaction and the integrated rate law was used to determine k (decay rates) and $t_{1/2}$ (half- life). The approach applied to each homologue is illustrated in Fig. 2.6 and 2.7.

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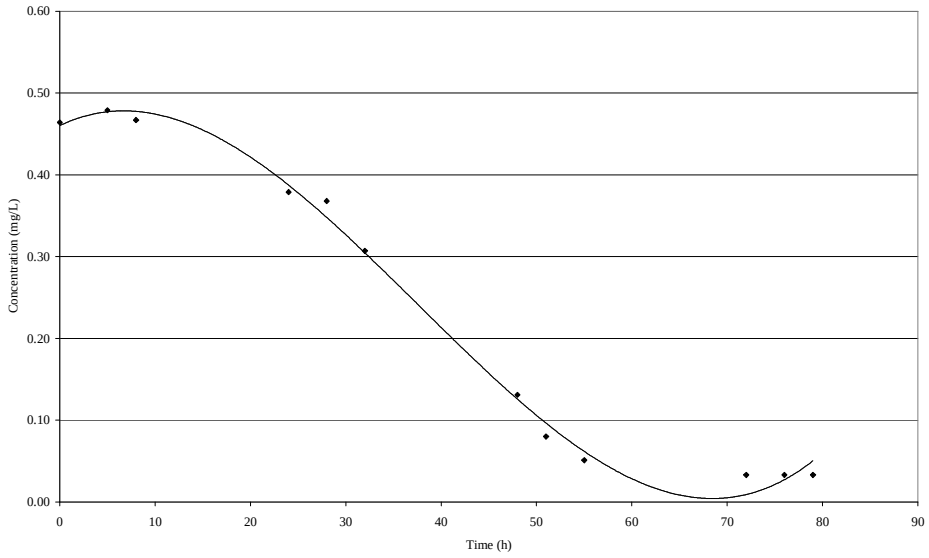

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Fig 2.6. Plot of C_{10} LAS biodegradation

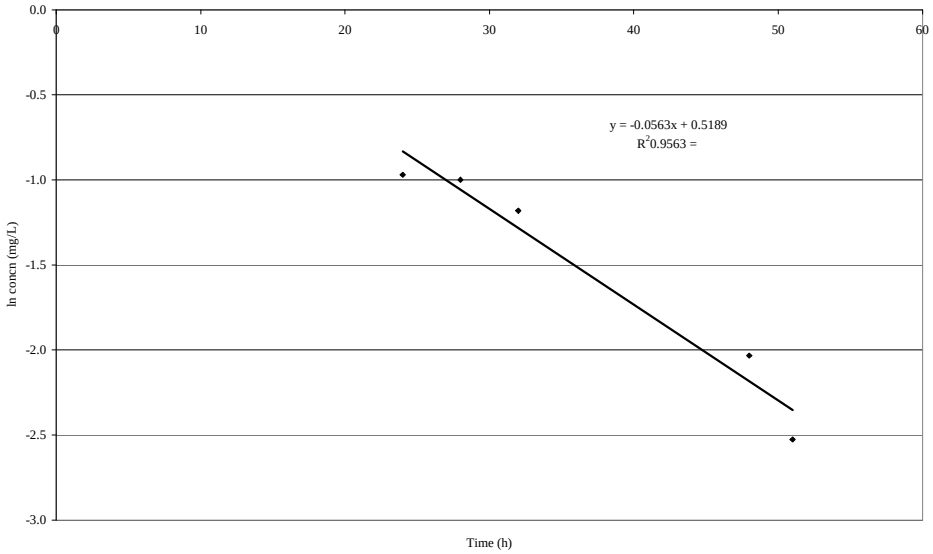

Fig 2.7. Semilog plot of C_{10} LAS concentration versus time against time for determining k (decay rate constant)

The LAS decay rate constant and half-life determination was calculated from the average slope of the approximately straight portion of the resulting line of a semi-log plot of concentration versus time. One of the artefacts of using a batch system is that a lag phase is sometimes observed, prior, to biodegradation of a test material, Larson *et al*., (1989) suggested the use of two extra constants to account for the lag phase and also the levelling of the reaction asymptotic to values shorter than the theoretical amount. Liu *et al*., (1981) suggested similar means to fit biodegradation data into first order form by plotting the logarithm of A/A_0 against time with k then calculated as the average slope of the approximately straight portion of the resulting line. This eliminates then the lag time, if any, from the calculations as it will have occurred before the linear portion. This approach was also adopted for the determination of all of the AE homologues with varying chain lengths measured. A complete summary for all of the calculated decay rates and half-lives for the LAS homologues is presented in Table 2.1 and similarly for the AEs in Table 2.2.

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Table 2.1. Calculated decay rates and half-lives for LAS homologues in batch die away system.

	k decay rate				
	k (h^{-1})	K (d^{-1})	$t_{1/2}$ (h)	$k_{(LAS)}/k_{(COD)}$	$k_{(LAS)}/k_{(NH4)}$
C_{10}	0.06	1.35	12.3	4.2	16.9
C_{11}	0.1	2.49	6.7	7.7	9.2
C_{12}	0.09	2.15	7.7	6.7	10.6
C_{13}	0.07	1.61	10.3	5	14.1

Table 2.2. Calculated decay rates and half-lives for AE homologues, in batch die away system.

	C_{12}		C_{13}		C_{14}		C_{15}		C_{16}		C_{18}	
	$k(d^{-1})$	$t_{1/2}$ (h^{-1})	$k(d^{-1})$	$t_{1/2}$ (h^{-1})	$k(d^{-1})$	$t_{1/2}$ (h^{-1})	$k(d^{-1})$	$t_{1/2}$ (h^{-1})	$k(d^{-1})$	$t_{1/2}$ (h^{-1})	$k(d^{-1})$	$t_{1/2}$ (h^{-1})
EO_0	7.78	2.10	2.86	5.82	6.80	2.44	1.67	9.94	2.41	6.9	6.8	6.85
EO_1	4.15	4.42	1.96	8.49	2.34	7.12	4.83	3.44	1.76	9.47	-	-
EO_2	4.37	3.83	1.95	8.52	2.47	6.74	4.33	3.84	1.38	12.0	-	-
EO_3	4.22	3.94	1.84	9.01	1.74	9.55	1.44	11.57	-	-	-	-
EO_4	3.82	5.00	1.88	8.84	1.84	9.02	1.26	13.20	-	-	-	-
EO_5	3.26	5.92	1.89	9.14	2.17	7.67	1.39	11.93	-	-	-	-
EO_6	3.19	6.00	1.92	8.67	1.71	9.73	1.50	11.12	-	-	-	-
EO_7	3	5.58	1.74	9.57	2.16	7.71	1.28	13.03	-	-	-	-
EO_8	2.52	7.29	1.94	8.58	2.10	7.93	1.81	9.19	-	-	-	-
EO_9	2.26	8.34	1.76	9.44	2.19	7.60	1.89	8.78	-	-	-	-
EO_{10}	2.02	9.34	1.63	10.19	1.83	9.09	1.18	14.09	-	-	-	-
EO_{11}	1.97	9.55	1.68	9.87	1.75	9.49	1.56	10.63	-	-	-	-
EO_{12}	1.27	13.15	1.57	10.61	2.30	7.23	-	-	-	-	-	-

(- denotes that detection limits had been reached and that decay rates and half lives could not be derived)

Comment [S15]: This table wants to be expanded to 12 pt, which means turning it 90° and having it landscape, perhaps on its own page.

2.4 Discussion

Comment [S16]: Hang on! We seem to have got here without mentioning the AE results. (Have mentioned above that same approach used to determine decay rate and expanded further in this discussion).

The percentage remaining of the initial concentrations of MBAS, COD, and NH_4^+ in each study after 5 days was very similar, and the decay rates of MBAS and COD in both studies showed faster removal of the MBAS.

Comment [S17]: But your results show higher SS in Study 1! (Response Error changed this)

The difference in rates observed between the studies could be due to the increased initial suspended solids present in Study 1. Peng *et al.*, (2000) reported effects of initial suspended solids concentrations on the decay rate of COD in studies where ratios of *k* MBAS to COD varied from 1.7 to 7.4, although, in these particular studies the effect seems more pronounced on the MBAS and NH_4^+ removal.

Initial concentrations of MBAS, COD, NH_4^+ were also different in the two studies and this may have had an influence on the observed decay rates. Potential interference effects are also possible when using methylene blue analysis in the environmental media used in these studies.

Methylene blue is a non-specific analytical technique and is responsive to not only anionic surfactants but also any materials containing a strong anionic centre. Pitter *et al.*, (1972) has suggested that errors are possible in the presence of sewage, although, most studies have concluded the uncertainty of detection of biodegradation using MBAS is far smaller than the uncertainty associated with the viability of organisms in a sample collected from the environment.

Determination of the remaining levels of un-dissociated ammonia was also similar in both studies. The levels after 5 days were in excess of the PNEC (predicted no-effect concentrations) reported by North American EPA (0.02 mg/L) and the European EIFAC (0.025 mg/L) toxicity levels to freshwater fish.

In study 2, specific analysis was also completed using LC/ESI/MS. Native levels of LAS and AE homologues were measured and the primary biodegradation rates and half-lives determined. A total LAS concentration in the media of 2.23 mg/L was measured. Primary biodegradation of C_{10}-C_{13} LAS was complete after 55 h, based on reaching the limit of detection (0.033mg/L), with decay rates ranging from 1.35 to 2.49 d^{-1}, half-lives 6.7 to 12.3 h^{-1}. The ratio of decay rates of the C_{10}- C_{13} LAS to COD varied from 4.2 to 7.7 and decay ratios of C_{10}- C_{13} LAS to NH_4^+ ranged from 9.2 to 16.9 times faster. In all cases the varying LAS chain lengths were rapidly removed in comparison to the COD and NH_4^+.

Comment [S18]: What do you mean by native levels? Response – the settled sewage samples collected from the treatment works have LAS and AE present so these materials are not being spiked in to the test media they are already present, native to the sample.

The biodegradation of LAS in this system compares favourably with that observed by Peng *et al.*, (2000), who measured primary biodegradation completion of ^{14}C-LAS after 48 h and calculated half-lives of 8-10 h for primary biodegradation and 11-12 h for complete mineralization in raw sewage and in raw sewage and river water.

AE biodegradation rates of removal were quicker again than the LAS, with all homologues removed after 24 h (based on reaching limits of detection (Table 7).

The removal rates were in the orders proposed by the Japanese SDA (HERA, 2007) for AE removal in river water. In these studies, a range of AE homologues were monitored in river water die away tests. Half lives ranging from between 4 – 24 h for chain lengths C_8 – C_{18} with groups from EO_{0-20} were agreed.

The batch die away test system therefore seems a suitable approach for assessing the rates of removal of chemicals in comparison to the COD under direct discharge conditions.

These studies have conclusively shown that MBAS, LAS, and AE primary biodegradation in an aerobic environment is more rapid than the general organic loading

present from the sewage, measured as COD. This suggests that these chemicals would be at very low concentrations when discharged in to a receiving body of water, prior, to the removal of the general organic materials present from sewage. It also showed that after 5 days, levels of ammonia were still present at concentrations in excess of the PNEC of ammonia to freshwater fish. Possibly, with very little SS present and no encouragement of biofilm formation, the consortia of bacteria responsible for nitrification may not have been present in sufficient numbers.

Comment [S19]: Doesn't make sense

In comparison, LAS was extensively removed (all homologues after 55 h) and the concentrations after 5 days were lower than the PNEC in water (0.27 mg/L for C_{12} homologue) reported by HERA (2004).

Future work could include the addition of established biofilms in to the test system as well as investigating the effects of dissolved oxygen and temperature on the rates of removal.

CHAPTER 3

Investigation of short term toxicity tests for micro-organisms in the aquatic environment under direct discharge conditions

3.1 Introduction

A key principle applied to direct discharge is that detergent ingredients should not significantly delay or impair the recovery processes in polluted rivers (AISE / CESIO, 1995), and this concerns their impact on the community of micro-organisms.
Micro-organisms have vital ecological roles in the aquatic environment. They assimilate and re-introduce dissolved organic compounds back in to the food chain thus supporting the growth of higher organisms and play a major role in the biogeochemical cycling of carbon, hydrogen, oxygen, nitrogen, phosphorus, iron and sulphur. Micro-organisms are of a diverse nature ranging from simple prokaryotic organisms such as bacteria, to more complex eukaryotic micro-organisms such as fungi and algae. Their growth rates and hence activity are heavily influenced by their environmental conditions, including temperature, oxygen availability, pressure, pH, nutrient availability and the presence of toxic or inhibitory substances.
The large amounts of biodegradable organic materials present in sewage discharges, are stabilised by heterotrophic micro-organisms utilizing these organic substrates for growth and converting them to relatively stable end products via aerobic or anaerobic processes, depending on oxygen availability. Another key biological oxidation process performed by micro-organisms is nitrification, which, stabilizes the large amounts of nitrogenous matter in domestic wastewater. Nitrification is an extremely important autotrophic microbiological process in soil, streams and lakes as well as in biological waste water treatment plants that converts ammonia into nitrite and ultimately nitrate.

It takes place in two stages , firstly, ammonium is converted to nitrite by a group of bacteria known as *Nitrosomonas*. Nitrite is then subsequently oxidised to nitrate by another group of organisms known as *Nitrobacter*.

Nitrogen exists in four main forms in the water cycle ;

- Organic nitrogen - nitrogen in the form of protein, amino acids and urea
- Ammonia (NH_3) nitrogen – nitrogen as ammonium salts, e.g. $(NH_4)_2CO_3$ or as free ammonia
- Nitrite (NO_2) nitrogen – an intermediate oxidation stage
- Nitrate (NO_3) nitrogen – final oxidation product of nitrogen

The stabilisation of nitrogen matter, and in particular the removal of ammonia, is an essential process because of its toxicity, particularly when in the un-ionised form.
It is therefore vital that the microbial populations present in the aquatic environment are protected and maintained to provide this self- purification process. A nitrification stage is now common practice in many sewage treatment plants (STP), in this situation, microbial populations can be closely monitored and controlled and environmental conditions can be maintained to improve wastewater quality.
The processes taking place during biological wastewater treatment are efficient and reliable, but they may be susceptible to disturbances and toxic loadings and for this reason it has been important to know the potential inhibitory effects of substances to prevent disturbances (Juliastuti *et al.*, 2003; Pagga *et al.*, 2006).
Aerobic inhibition tests have been established for determining the effects of chemical compounds on micro-organism respiration during sewage treatment by OECD, (OECD 209 – Activated sludge respiration inhibition test) and ISO (ISO 8192 – Test

for inhibition of oxygen consumption by activated sludge). Nitrification inhibition tests in activated sludge have also been developed (ISO 9509 : Water quality - Method for assessing the inhibition of nitrification of activated sludge micro-organisms by chemicals and waste waters). These tests have been specifically developed to protect wastewater treatment plants from toxic shock loadings and to also determine non-inhibiting concentrations for biodegradation testing in aquatic test systems. Each of these tests measures a different end-point which is based on different microbial populations with different metabolic characteristics. The nitrification inhibition test measures the toxicity of a test material to a mixed population of autotrophic bacteria that use inorganic sources of nitrogen as their source of energy. The respiration inhibition test measures the toxicity of the test material to the respiration of both the heterotrophic and autotrophic populations present in the media. However, these tests are not directly applicable to the aquatic environment which has continual fluctuations in environmental conditions resulting in population shifts. There is a requirement for new respiration inhibition and nitrification inhibition test systems for determining the toxicity of chemical compounds to micro-organisms which play a key role in self purification in a river after discharge of untreated sewage.

Comment [REA20]: Title in full first time

In the studies discussed in this chapter, respiration inhibition was looked at in a 'worst case' direct discharge scenario, using a media were a sewage discharge has a dilution factor of 3 applied to it (one part sewage : two parts river water). An Oxitop respirometric test system was evaluated in these studies, for its applicability for the determination of the inhibition of respiration by micro-organisms under these defined conditions. Nitrification inhibition was measured by following the principles of ISO 9509, the international standard prepared for determining nitrification inhibition in

activated sludge treatment plants. In activated sludge plants, controlled processes mean suspended solids are usually at a concentration of approximately 3 g/L to maximise the removal of pollutants from the influent waste load. The ISO 9509 method suggests using these concentrations for determining inhibition to nitrifying bacteria. However, these suspended solids concentrations are unrealistic to apply to a untreated discharge scenario, which would have much lower suspended solids concentrations. Typical concentrations of suspended solids in a moderate to concentrated wastewater would range from approx. 300 – 450 mg/L. With the dilution factor of 3 applied, a suspended solids of 125 mg/L would seem a more appropriate concentration for simulating a direct discharge scenario.

3.2 Materials and Methods

Nitrification Inhibition

A portion of activated sludge was obtained from a nitrifying sewage treatment plant and maintained aerobically in a semi-continuous activated sludge unit prior to use. Before use in each test, the activated sludge was washed by centrifuging the activated sludge for 2 mins at 5000 rpm, the supernatant was then discarded and the residue washed with an equal volume of water. This was repeated twice before re-suspending and taking a sample for mixed liquor suspended solids (MLSS) determination.
The remainder was kept aerated prior to addition to the test vessels, the sludge was then added to an appropriate volume of test medium to give the required concentration of mixed liquor suspended solids (125 mg/L).

The test medium was prepared by dissolving 5.04 g of sodium hydrogen carbonate ($NaHCO_3$) and 2.56 g of ammonium sulphate $(NH_4)_2SO_4$ in 1 L of UPW water. This medium when diluted 1 : 10, contains 56 mg/L of N and has a pH value of about 7.6 and allows the production of at least 25 mg/L of oxidised nitrogen without changing the pH. A reference inhibitor stock solution was prepared by dissolving 1.16 g of allylthiourea (ATU) in 1L UPW. Conical flasks (500 mL capacity) were used as the test vessels and equal volumes of washed, nitrifying sludge, was added to a series of test vessels so that an MLSS of 125 mg/L was achieved (Plate 2). Test medium (25 mL) was added to each vessel and a range of volumes (usually five) of test ingredient solution and sufficient distilled water to make the final volume up to 250 mL. A control flask was added with sludge, medium and water and 2.5 mL of ATU.

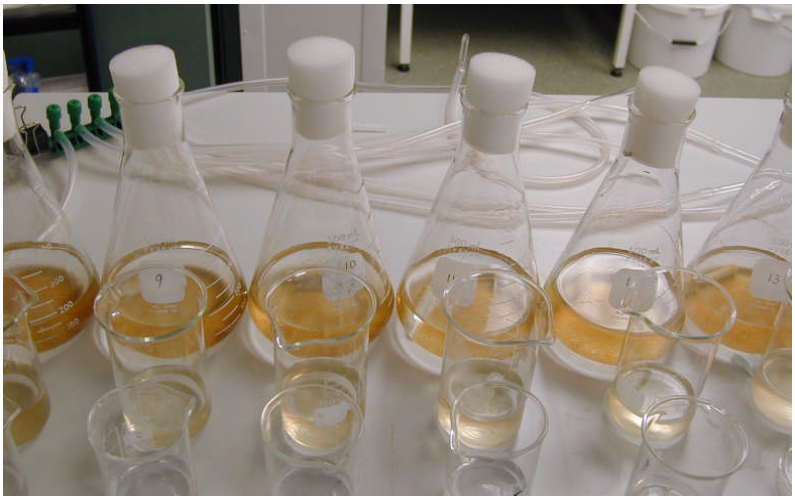


Plate 2 : Conical flasks (500 mL) used as the test vessels with equal volumes of washed nitrifying sludge added to a series of test vessels so that an MLSS of 125 mg/L was achieved.

All test vessels then had a stirrer bar added and were placed on a stirrer bed in a temperature controlled room at 20^{o}C to keep the sludge in suspension and maintain the dissolved oxygen concentration above 2 mg/L. The test vessels were then covered and incubated in the dark for 4 h.

After 4 h, a suitable volume of sample was taken from each vessel for oxidised nitrogen analysis. The products of oxidised nitrogen, nitrite nitrogen (NO_2-N) and nitrate nitrogen (NO_3-N) were determined using cuvette test analysis on a Xion 500 spectrophotometer.

Measurement of nitrite and nitrate was preferred to ammonium as the disappearance of ammonium is not always attributable to nitrification but may be due to volatilisation. Before analysis the samples were filtered using 0.45 µm glass fibre filters.

Calculation and expression of results

The percentage of inhibition of formation of oxidised nitrogen N was determined as follows :

(*Equation 1*)

$$\% \text{ Inhibition} = \frac{Cc - Ct}{Cc - Cb} \times 100$$

Where,

Cc is the concentration of oxidised nitrogen, N, in milligrams per litre, in the control flask without inhibitor, after incubation;

Ct is the concentration of oxidised nitrogen, N, in milligrams per litre, in the flask containing the test substance, after incubation;

Cb is the concentration of oxidised nitrogen, N, in milligrams per litre, in the flask containing the reference inhibitor, after incubation;

A plot of the percentage inhibition against the log of the concentration of the test material was used to interpolate the effect concentration (EC) . A linear trend line was applied to the plot over the relevant concentrations required to obtain the desired EC. The tests were considered valid if a nitrification rate between 2 mg of N / per gram of suspended solids per hour (g.h) and 6.5 mg of N / (g.h) were observed in the control vessel. It was essential that ammonium salt was left at the end of the test period to ensure that the substrate had not become rate limiting.

Determination of the nitrifying activity of an activated sludge

Prior to using the activated sludge source, the nitrifying activity of the sludge was determined together with the influence of using lower suspended solids levels than the ISO 9509 recommendation. Equal volumes of washed activated sludge of a known suspended solids concentration (500, 250 and 125 mg/L) were added to two 500 mL conical flasks per suspended solid concentration. 25 mL of test medium was added to each test vessel and 2.5 mL of reference inhibitor to one flask only. This was then made up to volume in each flask (250 mL) with distilled water. The flasks were then stirred for 4 hrs in a temperature controlled environment (20^{o}C). After 4 hrs a suitable volume of sample was taken from each vessel for oxidised nitrogen analysis,

prior to analysis the samples were filtered using 0.45µm glass fibre filters..
The specific nitrification rates in milligrams of nitrogen per gram per hour was determined as follows :

(*Equation 2*)

$$\frac{Ct - Cb}{MLSS \times 4}$$

Where,

Ct is the concentration of oxidised nitrogen, N, in milligrams per litre, in the reaction mixture after 4 h, incubation.

Cb is the concentration of oxidised nitrogen, N, in milligrams per litre, in the mixture plus reference inhibitor after 4 h, incubation.

MLSS is the concentration, in grams per litre, of mixed liquor suspended solids in the test flask.

Cuvette test analysis

The NO_2-N and NO_3-N analysis was measured using cuvette test methods on the Xion 500 Spectrophotometer (Hach Lange GMBH, Dusseldorf, Germany). This is a rapid measurement technique for screening these parameters, which uses photometric analysis of colour complexes formed in chemical reactions with the water sample in the cuvette.

Respiration Inhibition

A measured volume of test media (settled sewage and river water) in a 1:2 ratio (dilution factor = 3) was dispensed to the Oxitop glass bottles (154 mL), this test media volume was used to ensure sufficient headspace was available to complete the oxidation process. The respiration rate of the micro-organisms present in the media was then compared with that of various spiked concentrations of the test substance under otherwise identical conditions. The effect from a range of concentrations were monitored (0-100 mg/L of the test substance).

Comment [S21]: I'm not sure what this means

The inhibitory effect of the test substance could then be expressed at a desired percentage effect (e.g. EC_{50} = concentration at which 50% of oxygen uptake is inhibited in comparison to the control). Test substance (10 mL) at the various concentrations or the control (ultra pure water) was added to the vessels to make a total test media volume of 164 mL.

Comment [S22]: Doesn't this contradict the earlier statement "the sample volume was determined by the expected BOD measurement"? **CF response this was the sample volume of test media being discussed earlier this refers to the test substance volume being added. I have altered previous sentence at start of page to make clearer.**

Tests were performed in at least duplicate or triplicate depending on the availability of the test system. An alkali trap was placed in the headspace (containing 2 sodium hydroxide pellets) in each vessel and the vessel sealed with an Oxitop measurement head (Plate 3). The vessels were then incubated on stirrer plates at a constant temperature (20^{o}C). As the micro-organisms actively respired, oxygen was used by the micro-organisms to assimilate the organic material present from the settled sewage and the carbon dioxide evolved as a by product of this process was absorbed by the alkali traps. This lead to a pressure drop which is directly proportional to the oxygen consumed. The Oxitop measurement heads recorded these pressure changes continuously for the duration of the test, with the Oxitop heads effectively acting as manometers.

Plate 3. Oxitop BOD system.

A measurement controller downloaded these measurements during the experiment through an infra red sensor in the Oxitop heads. The inhibitory effect of the test substance was then calculated and expressed as a percentage of the mean of the control respiration rates.

The percentage inhibition at each concentration was then plotted against the log of the concentration and the EC (effect concentration) was derived.

Calculation and expression of results

(*Equation 3*)

$$1 - \frac{R_T}{R_c} \cdot 100 = \text{per cent inhibition}$$

Where,

Rt = mean oxygen consumption rate at tested concentration of test substance

Rc = mean oxygen consumption rate of controls

A plot of the percentage inhibition against the log of the concentration of the test material was used to interpolate the effect concentration (EC). A linear trend line was applied to the plot over the relevant concentrations required to obtain the desired EC.

Test items and preparation

The test materials selected are a range of common ingredients in Unilever products (Table 3.1) with some known to have antimicrobial effects.

In both the respiration and nitrification inhibition tests, stock solutions of the test items were prepared at a concentration of 1 g/L and serial dilutions were then prepared from this stock solution. Concentration ranges for each test item in the specific tests are detailed in tabular form (Appendix 2).

In the case of Triclosan and 3,5 Dichlorophenol, dropwise addition of 1M NaOH was required to get the test material into solution prior to serial dilution.

Table 3.1 : Test item information.

Name	**Commercial Name**	**Activity (%)**	**Formula**	**Supplier**
3,5 Dichlorophenol	3,5 Dichlorophenol	>97	$C_6H_4Cl_2O$	Sigma-Aldrich
Triclosan	Irgasan DP300	100	$C_{12}H_7Cl_3O_2$	Ciba Geigy
Benzalkonium Chloride	Catigene LM80	79.4	$C_6H_5CH_2N(CH_3)_2RCl$	Stepan Europe
Polyethylene polyamine	Tinofix CL	33	-	Ciba
Alcohol Ethoxylate	Lutensol TO20	100	$C_{13\text{-}15}EO_{20}$	BASF
Aniline	Aniline	99	$C_6H_5NH_2$	Sigma Aldrich
Phenol	Phenol	>99	C_6H_5OH	Sigma Aldrich
Na LAS Paste	Linear alkylbenzenesulphonate	51	$(C_{12}H_{24})C_6H_4SO_3Na$	Unilever Port Sunlight

3.3 Results

Nitrification Inhibition

The nitrifying activity of the activated sludge was determined prior to use in these studies to ensure it contained sufficient nitrifying bacteria and also to examine the influence on nitrification rates of the relatively low levels of suspended solids in comparison to those suggested in ISO 9509. Determination of the nitrifying activity of the suspended solids was measured at 125, 250 and 500 mg/L. The MLSS concentration of the activated sludge was determined in duplicate and a mean value of 4292 mg/L was obtained. An appropriate volume of sludge was then added to the test vessels to give suspended solids levels of 125, 250 and 500 mg/L respectively. A nitrification rate of 4.5 mg of N (g.h) was observed at 125 mg/l SS, 4.0 mg of N (g.h) at 250 mg/L SS and 4.0 mg of N at 500 mg/L SS. The nitrification rates observed at all SS levels were thus within the rates suggested for validity of the test in ISO 9509. These results also confirmed that using relatively low levels of suspended solids to simulate a direct discharge scenario did not effect the ability to detect nitrification and subsequently its inhibition. Following these observations a suspended solids level of 125 mg/L was used in the nitrification inhibition studies. Results of these studies are presented in Table 3.2 showing the concentrations required to produce 20%, 50% and 80% inhibition.

Comment [S23]: What's this unit?

Table 3.2 Summary of nitrification inhibition results for all test materials after 4 h.

Test Material	Concentration range measured of test material (mg/L)	EC_{20} (mg/L)	EC_{50} (mg/L)	EC_{80} (mg/L)
Aniline	0 – 100	1.09	3.51	11.3
Phenol	0 – 100	0.65	3.47	48.0
3,5 Dichlorophenol	0 – 50	0.12	0.54	4.00
Alcohol Ethoxylate $C_{13\text{-}15}EO_{20}$	0 – 100	78.0	-	-
Alkyl dimethyl benzyl ammonium chloride	0 – 74	1.35	3.02	6.75
Linear alkylbenzene sulphonate	0 – 51	-	-	-
Polyethylene polyamine based polymer	0 – 33	0.93	31.04	-
2,4,4'-Trichloro-2'-hydroxy-diphenyl ether	0 – 100	0.67	1.27	12.8

(- denotes that a insufficient inhibition was obtained to determine an EC value)

Respiration Inhibition

Respiration inhibition was determined at 4 hours and 5 days and the results are presented in Tables 3.3 and 3.4, the concentrations required to produce 20%, 50% and 80% inhibition have been determined.

Table 3.3 Summary of respiration inhibition results for all test materials after 4 h.

Test Material	Concentration range measured of test material (mg/L)	EC_{20} (mg/L)	EC_{50} (mg/L)	EC_{80} (mg/L)
Aniline	0 – 100	-	-	-
3,5 Dichlorophenol	0 – 100	1.28	3.24	8.22
Alcohol Ethoxylate $C_{13\text{-}15}EO_{20}$	0 – 100	3.81	16.7	73.1
Alkyl dimethyl benzyl ammonium chloride	0 – 74	3.10	5.21	8.76
Linear alkylbenzene sulphonate	0 – 100	4.02	16.1	64.25
Polyethylene polyamine based polymer	0 – 100	3.29	8.76	23.31
2,4,4'-Trichloro-2'-hydroxy-diphenyl ether	0 – 100	1.22	1.82	2.73

(- denotes that a insufficient inhibition was obtained to determine an EC value)

Table 3.4 Summary of respiration inhibition results for all test materials after 5 d.

Test Material	Concentration range measured of test material (mg/L)	EC_{20} (mg/L)	EC_{50} (mg/L)	EC_{80} (mg/L)
Aniline	0 – 100	-	-	-
3,5 Dichlorophenol	0 – 100	7.17	23.59	-
Alcohol Ethoxylate $C_{13\text{-}15}EO_{20}$	0 – 100	-	-	-
Alkyl dimethyl benzyl ammonium chloride	0 – 74	1.4	-	-
Linear alkylbenzene sulphonate	0 – 100	12.75	-	-
Polyethylene polyamine based polymer	0 – 100	6.89	46.6	-
2,4,4'-Trichloro-2'-hydroxy-diphenyl ether	0 – 100	1.89	25.02	-

(- denotes that a insufficient inhibition was obtained to determine an IC value)

Comment [S24]: Make sure that tables do not go across pages in the final version

3.4 Discussion

The different inhibition endpoints measured in these tests have confirmed that test materials can show selective inhibition towards heterotrophic or autotrophic bacterial populations. As an example, aniline showed no inhibition in the respiration inhibition test (EC_{50} > 100 mg/L). This was not surprising as it is a readily biodegradable material regularly used as a soft standard in biodegradation screening tests. However, it was toxic to autotrophic nitrifying populations at low concentrations (IC_{50} = 3.51 mg/L). Observations from these short term inhibition studies on key 'self purification' processes also suggest that substances that are known to inhibit microbes still require concentrations in excess of the PECs to cause significant inhibition. Some of the highest inhibition effects in both tests was observed from 2,4,4' Trichloro-2'-hydroxy-diphenylether (Triclosan). Effects observed in both tests after 4 h ($EC_{50\ Nitrification}$ = 1.27 mg/L , $EC_{50\ Respiration}$ = 1.82 mg/L). Actual concentrations

entering waste water treatment plants has been reported, based on a range of UK waste water treatment plants (WWTP's) values of 0.7-22 µg/L, Thompson *et al.*, (2005) are typical. Triclosan has also been measured in the influent of selected WWTP's in the United States. Monitoring of Triclosan included two activated sludge treatment plants (located in Columbus, Ohio and Loveland, Ohio) and two trickling filter treatment plants (located in Glendale, Ohio and West Union, Ohio).

The concentrations of Triclosan in the influent waste water ranged between 3.8 and 16.6 µg/L (McAvoy *et al.*, 2002).

In the case of linear alkylbenzene sulphonate (a high volume surfactant) an EC_{50} for nitrification >51 mg/L was observed and an EC_{50} for respiration of 16.1 mg/L after 4 h and >100 mg/L after 5 d. This also correlates well with the finding of Feijtel *et al.*, (1995) that respiration inhibition NOEC for *Pseudomonas putida* was 35 mg/L.

Comment [S25]: ?

Expected concentrations of LAS in sewage in extreme cases would be approximately 15 mg/L prior to any form of dilution, so this suggests at the expected environmental concentrations that it would not be inhibitory to these key ecosystem functions when released under direct discharge conditions.

In many cases in these tests, short term inhibitory effects observed in the respiration inhibition tests after 4 h, were no longer observed when respiration rates in the same vessels were measured after 5 d, which, suggests after several generations of the microbial populations, the inhibitory effects from the test item no longer occur due perhaps to either adaptation or biodegradation. This longer incubation period of 5 d is probably the more valid measurement point when applying these tests to a direct discharge scenario, as the short term incubation and measurement times suggested in

the ISO and OECD guidelines (4 h) are based around the typical retention times an ingredient would spend within a treatment plant. This could also be applied to the nitrification test although the effects observed after 4 h for all the chemicals in these studies was still in excess of their PEC's.

Comment [S26]: Don't understand

The use of micro-organisms as test organisms in toxicity testing is not only valid for the protection of microbial populations in wastewater treatment processes but also the protection of microbial populations present in the aquatic environment. The nitrification test examined in this study was a simple variation on the established ISO method in which the activated sludge levels were reduced to make it more applicable to a direct discharge scenario. The considerable reduction in suspended solids made the test more conservative and more valid in an untreated discharge scenario. The respiration inhibition method explored in these studies also used a medium more relevant to direct discharge. There are no established guidelines for measuring inhibition in this particular medium, so the results from these tests have been derived by measuring a range of test concentrations compared to control vessels containing no test material, and no other validation requirements were applied (e.g. a reference inhibitor). Further validation could form an appropriate extension to this work. After 4 h the respiration rates were fairly low in this particular medium, due to the fact that the suspended solids levels were extremely low (~30 mg/L). After 5 days active respiration ensured this was no longer a problem and as mentioned above this measurement time-point is probably more valid to the scenario being investigated.

Comment [S27]: Where is this data? In the appendix?

CHAPTER 4

Biodegradation of an anionic surfactant in a continuous flow simulation of untreated discharge conditions

4.1 Introduction

In many developing countries, lack of sewage treatment prior to discharge into receiving waters means assumptions used for traditional risk assessment of 'down the drain' chemicals (i.e. removal in sewage treatment plant) cannot be used.

Traditional risk assessment fails since water quality is compromised by pollutants associated with raw sewage (e.g. BOD and ammonia) and the relevance of the 'standard' risk assessment has thus been questioned. An alternative risk assessment model, based on the "impact zone" concept, has been proposed for direct discharge conditions (Limlette III Workshop, 1995; McAvoy *et al.*, 2003). In this model, chemicals are assessed in terms of their predicted concentration at the end of an "impact zone" (IZ), within which the ecosystem is impacted by pollutants such as free ammonia and BOD, and beyond which it is not.

Estimation of the concentration of the chemical at the end of the IZ depends on its removal rate. Taking LAS as an example, several field studies have reported in-stream removal rates in rivers (e.g. Whelan *et al.*, 1999; Fox *et al.*, 2000; McAvoy *et al.*, 2003; Whelan *et al.*, 2007), wetlands (Inaba *et al.*, 1988) and estuaries (Amano *et al.*, 1991).

Low half life values of typically 1 - 3 hours have been reported for shallow rivers and streams (e.g. Takada *et al.*, 1994; Fox *et al.*, 2000; McAvoy *et al.*, 2003) with contact with stream bed biofilm implied as a predominant removal mechanism (Boeije *et al.*, 2000). Longer half lives have been reported for deeper rivers in temperate zones (e.g. 15 h: Whelan *et al.*, 1999) and in the tropics (7 h: Whelan *et al.*, 2007).

Typically LAS concentrations in wastewater can range between 1 and 15 mg/l (Henze *et al.*,1990).

However, studies of this magnitude are often difficult and expensive to conduct and it is not feasible to assess the behavior of new chemicals using such methodologies. A laboratory-based simulation system is required which will enable the realistic behaviour and associated ecological risks of a range of different chemicals to be evaluated under direct discharge conditions. Batch test systems, such as those described by Peng *et al.*, (2000) undoubtedly have some screening-level value but they are unlikely to generate rate constants which are comparable with those observed in the field.

This chapter describes a laboratory simulation study using a continuous flow river model with attached biomass, based on systems described by Boeije *et al.*, (2000) and IS0 14592 under direct discharge conditions with a single point source at the upstream end. These two systems have, thus far, only been applied to relatively clean systems (i.e. without significant fraction of raw wastewater). In order to allow a comparison with field observations (McAvoy *et al.*, 2003; Whelan *et al.*, 2007), LAS was selected as the model chemical. Commercial LAS is not a single compound, but, ideally, a mixture of approx 20 homologues and isomers, all sub- terminally substituted, linear, alkyl chains C_{10}-C_{13} (Schleheck *et al.*, 2003), but in this study a radiolabelled sample of LAS, ^{14}C sodium dodecane-6-benzene sulphonate (Phenyl –^{14}C 6-DOBS) was used. This is C_{12} LAS with the phenyl ring in the 6-position. The material was radio-labeled on the phenyl ring and was originally synthesized for determination of LAS biodegradation. By measuring the native levels of LAS in the settled sewage and then assuming that removal followed that of the Phenyl –^{14}C 6-DOBS, it was possible to compare LAS levels to that of the general organic loading (COD) and ammonical N (NH_4^+ and NH_3) present in the test medium. A radiolabelled material, ^{14}CAniline HCl,

was used as a reference material prior to dosing Phenyl –^{14}C 6-DOBS, to confirm that a viable biofilm had been developed and steady state conditions had been reached in the system.

The test system consisted of a cascade containing five channels, designed to operate with a mean hydraulic retention time of 26 h under steady state conditions. Biomass growth was supported on artificial sediment (glass beads). The test medium containing organic carbon in the form of settled sewage was used as the major carbon and energy source (primary substrate) for the microorganisms, and the test compound was added to the influent of the cascade as a secondary substrate.

4.2 Material and Methods

Artificial river system

The test system (artificial river) consisted of one test unit (cascade) containing five UPVC gutters (channels) constructed to form an aquatic "staircase". The channels were shallow and rectangular, 1.8 m in length, 0.8 m width and arranged horizontally at a vertical distance of about 18 cm below the previous one (Plate 4). The volume of water in each channel was 2 L ± 0.1L, with a water depth of approx 2-3 cm.
The bottom of each channel was covered with glass beads, 5 mm diameter, each bead weighed 0.17g and 724 g in total (approx 4250 beads) were added to each channel to completely cover the bed surface to act as an artificial surface serving as a support for the growth of biofilm in the test system.

The total surface area of bead per channel could be calculated *viz*:

Radius (glass bead) = 5mm = 0.5cm = 0.005m

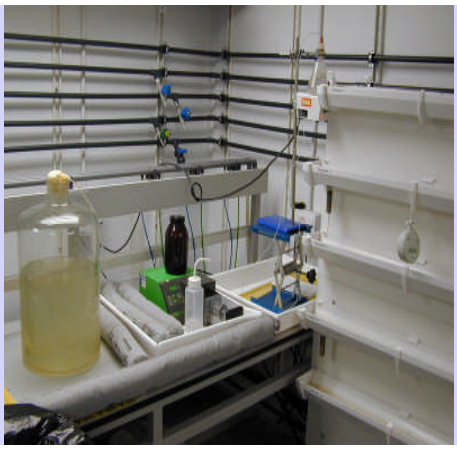
Surface Area = 4 .Π.r^2 = 0.0003142 m^2 =1.335 m^2 surface area

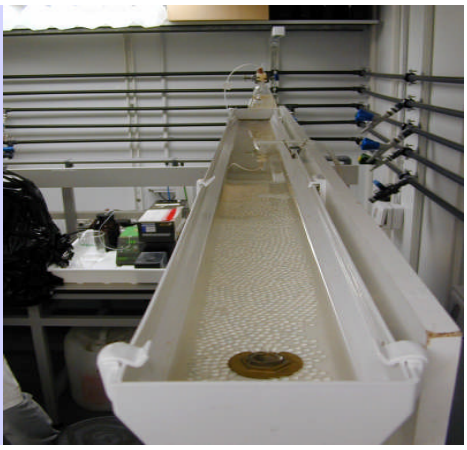
At the downstream end of each channel in the middle, was a hole fitted with a small tube for leading the test media from one channel to the next in the cascade. The hole was fixed in such a way that the depth of the water was about 1 cm above the glass beads. The system was operated in a temperature controlled environment at 20^oC under controlled illumination of 8 h per day, measured at 1800 lx .

Plate 4. Artificial river model system, consisting of five channels.

The test medium storage vessel was connected to the top of the first channel of the cascade (Plate 5 and 6). Due to the nature of the test media in this particular case with a dilution factor = 3 (1 part settled sewage: 2 part river water), a relatively short acclimation phase was expected.

Plate 5 Plate 6





Comment [S28]: I think these would be better bigger

Plate 5. Test media feed to cascade system.

Plate 6. Mixing vessel leading in to channel 1.

The development of a biofilm was further accelerated by the addition of activated sludge (20 mL) in to the first channel on day 2, this was also acted to broaden diversity of bacteria in the test system. Previous studies investigating LAS in systems of this nature have tended to use a synthetic river water and synthetic sewage, e.g. Boeije *et al* (1998), using these media to aid composition and consistency but the resulting COD from these types of synthetic media do not really achieve the levels of COD expected in an untreated discharge scenario with low dilution. In these studies river water and settled sewage were collected fresh every other day from a river and sewage treatment works. River water was collected from the River Ouse and settled sewage from Broadholme treatment works (Plates 7 and 8).

Plate 7

Plate 8

Plate 7. Collection point for river water, River Ouse, Felmersham bridge, Felmersham, Bedfordshire (OS grid ref : 991578).

Plate 8. Collection point for sewage, settled sewage channel, Broadholme STW (Anglian water), Ditchford, Wellingborough.

To help reduce variability in the test medium, 15 L were prepared daily and mixed with the remainder of the residual medium prepared the day before, which had not been dosed over the HRT (normally approx 5 L). Water quality measurements were continually performed throughout the duration of the study on the test media.

Comment [S29]: Not clear. Do you mean 15L was prepared daily and added to any residual medium in the reservoir?

The radiolabelled organic test compound (LAS) and reference compound (Aniline) was added to the influent of the cascade as a secondary substrate at a low concentration, once, sufficient biomass had developed. Under these conditions, with the test or control substance serving as a secondary substrate, the kinetics for biodegradation would be expected to be first order (growth independent).

The HRT of the test system was established to be 24 hrs. Although the biodegradation half life of LAS was expected to be less than this, changes in the other water quality parameters of interest to define the impact zone in the system were unknown and could potentially be longer in the artificial river system

Hydraulic Characterisation

Prior to dosing the test system the hydraulics were characterised using a NaCl tracer test (the glass beads were in the system during the tracer tests). A solution was prepared of 58 g/L of NaCL in 10L of ultrapure water, giving a conductivity of 10.46 mS. This was then dosed in to each channel and measured continuously until the conductivity in the outlet of each channel was the same. Each channel had a water volume of 2 L and the flow was fixed at 7 mL / min, which theoretically achieves a total residence time of 24 h.

The mean HRT through channels 1 to 5 was measured (peak travel time) at 300, 342, 309, 330, 294 min giving a total retention time of 26.6 h. Measured rather than theoretical HRT's were used in all calculations and actual measured flow was also continually recorded during the study.

The mixing characteristics were assumed to remain similar during the experimental phase. Further confirmation of system hydraulics was obtained by measuring the remaining test medium after dosing and volume of collected effluent from the cascade.

Measurements

From the start of the test, samples were withdrawn from the inlet and outlet of each channel, (i.e, at 0, 1.8, 3.6, 5.4, 7.2, 9.0m) and analysed for total ammonium ($NH_4^+ + NH_3$) N, NO^-_2, NO^-_3, and COD three times per week. Water quality analysis was measured using colorimetric cuvette test methods on the Xion 500 Spectrophotometer (Lange). The channels were continuously monitored for

temperature, pH (allowing the free ammonia in the system to be determined) and dissolved oxygen concentration. (dO_2).

The water quality parameters measured and the dO_2 measurements were used as indicators to determine when the system had achieved steady state conditions and when it was suitable to dose in the labelled materials. When the radio-labelled materials were dosed in to the test system the mid-point in each channel was also sampled. The native levels of LAS in the settled sewage were analysed using an Agilent 1100 LC/ESI/MS (liquid chromatography electrospray ionisation spectrometery) to calculate total LAS C_{12} in the system and its subsequent removal. For both the ^{14}C aniline and the ^{14}C phenyl 6-DOBS, the analysis of samples collected at different points in the cascade represent the ^{14}C remaining, regardless of whether this is parent molecule or metabolites. Reductions in the concentration of ^{14}C, therefore, represent complete mineralisation (conversion to CO_2) rather than primary degradation. This is an important distinction between the results of this study and many field studies which often employ specific analysis (e.g. Fox *et al.*, 2000; McAvoy *et al.*, 2003; Whelan *et al.*, 2007) and therefore investigate the fate of the parent molecule (i.e. primary degradation).

Comment [S30]: Name of instrument

Each sampling point was analysed in triplicate for the determination ^{14}C and $^{14}CO_2$. The ^{14}C was determined by counting in triplicate, 1ml of the media, in 25 mL plastic vials, with 10ml Starscint (Packard) and counting. The $^{14}CO_2$ was determined by counting in triplicate, 1 mL of the media in triplicate with 10 ml Starscint, and 100 uL of concentrated sulphuric acid. (The acidified samples were then left over night in a fume cupboard prior to analysis the following day).

Comment [S31]: Name of supplier

Sodium phenyl – [U ^{14}C] dodecane-6-benzene sulphonate

The test compound was added to the influent of the cascade as a secondary substrate after sufficient biomass had developed and steady state conditions had been achieved. Steady state was defined as when the concentration of the water quality parameters and test compounds remain constant at any place and time.

Phenyl –^{14}C 6-DOBS (Scynexis), was supplied as 99 % radiochemically pure, with a specific activity of 19 mCi/mmol (58.18 µCi/mg) and the material radio labeled in the phenyl ring. Radiochemical purity of the sample was determined using radio HPLC (Spectra Physics Radio HPLC System), performed on a Prodigy C18 column (250 x 4.6mm) supplied by Phenomenex.

Mobile phase was prepared by dissolving 3.4043g Potassium dihydrogen phosphate, 4.3483g di Potassium hydrogen phosphate and 1.6955g of Tetrabutylammonium hydrogen sulphate in 1L of water giving nominally 25mM phosphate buffer / 5Mm TBAS.

The mean purity of the sample was calculated to be 99%. A stick solution of Phenyl – ^{14}C 6-DOBS (3.7 mg / mL) was prepared by dissolving 7.4 mg in 2 mL of methanol (HPLC grade). Aliquots of 3 x 50 µl of the concentrated stock were each made up to 10 mL with methanol and 3 x 100 µl aliquots from each stock were mixed with Starscint (Perkin Elmer) for liquid scintillation counting.

A working stock solution was prepared so that a count of approximately 5000 DPM / mL was obtained after dilution in the test system. Under steady state conditions, the difference between the inlet and outlet concentrations in the cascade was used to determine the degree of biodegradation and to plot degradation curves.

The degradation rate constant and the degradation half-life of the test and the reference compounds in this test system were calculated using the measured data derived under steady-state conditions. This data, the degradation curves and any other available information were used to evaluate the biodegradability of the test compound. The native levels of LAS C_{12} were determined in the settled sewage / river water medium by liquid chromatography / mass spectrometry (LC/MS).
An alternative method of measuring LAS concentration using derivatisation and gas chromatography / mass spectrometry (GC/MS) to analyse individual LAS isomer concentrations was also investigated, specifically to measure the amount of 6 Phenyl-C_{12} in the samples.

[^{14}C]Aniline HCl / Aniline HCl standard

The formation of a viable biomass before the addition of the ^{14}C sodium dodecane-6-benzene sulphonate (LAS) to the test system was confirmed with the use of a radio-labelled reference material ^{14}C aniline hydrochloride. [U-^{14}C] Aniline Hydrochloride (ARC), was supplied as 99 % radiochemically pure with a specific activity of 77 mCi/mmol (594µCi/mg), the material was radiolabelled in the ring structure.
Aniline was used as reference material as it is commonly used as standard in ready biodegradability tests (OECD) and is also suggested as a reference material in IS0 14592.
The actual radiochemical purity of the sample was determined using radio HPLC (Spectra Physics Radio HPLC System) performed on a Prodigy C18 column (250 x 4.6mm) supplied by Phenoenex. Mobile phase was acetonitrile : phosphate buffer (aq)

1:1 by volume. 3.4013 g of potassium dihydrogen phosphate (Fisher Chemicals, AR grade) was dissolved in 500 mL of millipore water to give a 50mM solution.

Comment [S32]: MilliQ?

A volume of 500 mL of 50mM KH_2PO_4 + 500 mL acetonitrile were mixed to give the mobile phase 9.4 mg of Aniline HCl were dissolved in 10 mL of methanol (HPLC grade) to give a 0.9 mg / mL solution. Concentrated stock [^{14}C]Aniline HCl (10 µL) were mixed with 100 µL of the 0.9 mg / mL aniline HCl solution and 900 µl of Millipore water. The resulting solution was analysed by radio HPLC to give the purity of the [^{14}C] Aniline HCl. to be 95.78%. The contents of the labelled sample (nominally 1.7mg) were taken up in methanol and a ^{14}C assay on this solution determined to confirm the actual concentration. This then determined the volume to be added to the working stock solution so that a count of approx. 3000 DPM/ml was obtained. A cold solution of Aniline Hydrochloride was prepared (approx.4.3mg/L) and the appropriate amount of radiolabelled [U-^{14}C] aniline hydrochloride added to this.

Analysis of LAS in dosing test media

A 1000µg/mL LAS Stock Solution was prepared by adding 0.1955g ± 0.0005g of Na LAS paste (linear alkyl benzene sulphonate, 51.14 % active) to a 100 mL volumetric flask and making to the mark with methanol, samples were prepared in duplicate. The sample (20 mL) was pipetted to a 500 mL Duran bottle and made to 500 mL with Ultrapure water. To this, methanol (20 mL) was added and mixed well. 'Spiked' samples were also prepared by adding a 100 µL of a 1000 µg/mL LAS stock solution in methanol to the medium before treating as above. This spike was equivalent to

5mg/L added to the sample. Blank samples were also prepared as above without addition of the sample.

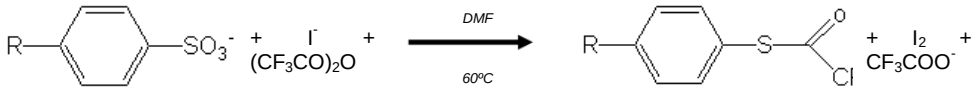
Solid Phase Extraction (SPE)

SPE was used as the separation process to extract the LAS homologues from the test media. Each SPE cartridge (IST 1g/6mL C_{18}) used was conditioned with methanol and water, by allowing them to pass through under gravity. The sample was then loaded on to the cartridge at about 10 mL/min. The cartridges were dried under vacuum for 1 h and eluted with methanol (approximately 20 mL) and made up quantitatively to 20 mL.

LC/MS analysis

LAS was quantified by liquid chromatography / mass spectrometry (LC/MS).
The solutions were analysed by reverse phase LC, injecting 10 µL onto a Varian Pursuit C_{18} column (5µm, 150 x 2.0mm) held at 30°C. A gradient elution with 5mM ammonium formate (pH 3.0) (Solvent A) and acetonitrile (Solvent B) was used.
The gradient started at 25% B and increased to 100% B over minutes and was held for 10 minutes. The system was allowed to equilibrate with the initial conditions for 20 minutes after each analysis. Reagents wherever possible were HPLC grade, AR grade or equivalent. Single ion monitoring for ions *m/z* 297, 311, 325 and 339 was used for C_{10}, C_{11}, C_{12} and C_{13} LAS respectively. Calibration standards were run at concentrations of 1, 2, 5, 10 and 15 µg/mL of total LAS, of which the homologue distribution for C_{10}, C_{11}, C_{12} and C_{13} was 13.2, 32.8, 31.3, 22.8 % w/w, respectively.

Derivatisation of LAS Isomers For GC/MS Analysis



A range of volumes (0, 1, 5, 10, 15 and 20mL) of a 1000 µg/mL LAS solution in methanol were blown down to dryness under a stream of Nitrogen at 60°C. The dried sample was reacted with sodium iodide (1mmol, 166mg) and trifluoroacetic acid anhydride (1mmol, 140µl,TFAA) in 2ml of dimethylformamide (DMF) for 2 hours at 65°C. The reaction was stopped by the addition of 700µl of 5% sodium thiosulphate in water solution. The derivatised products were then extracted into 2ml of hexane. The hexane layer was dried with anhydrous sodium sulphate prior to analysis.

GC/MS Analysis Parameters

For the analysis, an Agilent 6890/5973 Gas Chromatograph / Mass Spectrometer with an Agilent DB-5MS (30m x 0.25mm with a 0.25µm film thickness) capillary column was used with a constant flow of 1ml/min Helium through the column. A 1µL splitless injection at 250°C was made with a purge flow of 100mL/min after 1 minute. The oven temperature was initially set at 120°C which was held for 1 minute and then ramped up to 280°C at 2°C/min and held for 10 minutes. The transfer line temperature was 325°C. The mass spectrometer was in electron impact (EI) mode scanning from 35 to 500 m/z with a source temperature and quadrupole temperature of 200°C and 150°C respectively, with a 3 minute solvent delay.

4.3 Calculations

Degree of biodegradation

The initial activity of the radio-labeled reference and test compound ($\rho 0$) in the inlet of the first channel and the activity in the outlet of each channel was measured by scintillation counting. The final activity of the test compound was calculated as the mean value of the scintillation counts in the outlet of the last channel after steady state was reached (ρ_n).

The degree of biodegradation, D_s, was expressed as a percentage, calculated using, *(Equation 4)*

$$D_s = \frac{\rho 0 - \rho_n}{\rho 0} \times 100$$

Where;

$\rho 0$ is the initial activity, expressed in disintegrations per minute, of the reference / test compound.

ρ_n is the final activity, expressed in disintegrations per minute, of the reference / test compound.

4.4 Assumptions and boundary conditions of the river model

Biodegradation was assumed to follow first order kinetics. The continuous flow river model is regarded as a plug flow reactor with the following assumptions and boundary conditions :

- the cascade with five channels is a flowing watercourse extending between the co-ordinates $x1 = 0$ and $x2 = L$, where $x1 = 0$ refers to the inlet to the first channel and $x2 = L$ the outlet from the final channel section. Where L is the total length of the channel in the cascade.

Comment [S33]: ?

- The transportation of the primary and secondary substrate in the flowing media and to the biofilm takes place exclusively by convection.

For a constant input rate and at steady state conditions it can be shown theoretically that diffusion and hydrodynamic dispersion has a negligible role in controlling solute concentrations (Gandolfi *et al.*, 2001).

- The biodegradation of the test compound (secondary substrate) can take place without growth of biomass, for example by means of co-metabolism, and takes place on the surface of the biofilm.

- The biodegradation is substrate-limited but not biomass-limited, the relationship applies :

(Equation 5)

$$rd = k_{eff} \times \rho s$$

*r*d	is the rate of biodegradation;
k_{eff}	is the biodegradation rate constant;
ρs	is the substrate concentration;

- The growth of biofilm achieved with the test compound (secondary substrate) is negligible and is therefore attributable solely to the organic medium of the test water (primary substrate).

- Due to this behaviour of the primary substrate the biomass mass concentration (ρb), together with the area specific activity of the biofilm are approximately constant locally over the flow length of the cascade *x* :

(Equation 6)

$$\frac{\rho b}{dx} = 0$$

- The test system is at steady state during the measuring period

The river model is regarded as a plug-flow reactor. This is not entirely correct, it is actually a set of mixed tanks in series, but for the purpose of this test the assumption is acceptable. Knowledge of the hydraulic retention time (*t* hr) of the test system was obtained by tracer studies. With this knowledge and assuming first order kinetics, a biodegradation rate constant can be calculated by *:*

(Equation 7)

$$keff = -\ln \left(\frac{\rho n}{\rho 0} \right) \times t_{hr}, n$$

Where,

$\rho 0$ is the initial activity, expressed in disintegrations per minute, of the test compound in the inlet of channel 1;

ρn is the final activity, expressed in disintegrations per minute, of the test compound in the outlet of channel *n*;

Degradation rate constant

A three-dimensional plot of the measured activity (*y*-axis, disintegrations per minute) versus flow distance (*x*-axis, m) and versus time (*z*-axis, d) can be prepared. From the plots the duration of the lag-phase, the steady state phase and the section of maximum degradation of the test compound can be estimated. Axial flow speed, v_x, expressed in metres per day (m/d), was calculated according to :

(*Equation 8*)

$$v_x = \frac{qv}{S}$$

Where ,

q_V is the volume flow rate, expressed in cubic metres per day (m^3/d);

S is the free flow cross-section, expressed in square metres (m^2), of a single channel.

The free flow cross-section area is estimated using, S :

(Equation 9)

$$S = bxd$$

b is the width, expressed in metres (m), of a single channel;

d is the depth, expressed in metres (m), of the layer of water above the glass beads.

The biodegradation rate constant, k_{eff}, (d^{-1}), in each channel was determined according to :

(Equation 10)

$$k_{eff} = -\ln \left(\frac{pn}{\rho 0} \right) \times \frac{v_x}{x_n}$$

Where,

ρ0 is the initial activity, expressed in disintegrations per minute, of the test compound in the inlet of channel 1;

ρ*n* is the final activity, expressed in disintegrations per minute, of the test compound in the outlet of channel *n*;

x_n is the distance, expressed in meters (m), between channel 1 and the end of channel *n;*

v_x is the axial flow speed, expressed in meters per day (m/d).

Degradation half-life

The degradation half-life, $T_{1/2}$, expressed in days (d), of the test and the reference compound was calculated according to :

(Equation 11)

$$T_{1/2} = \frac{\ln 2}{k_{eff}}$$

where k_{eff} is the biodegradation rate constant (d^{-1}).

4.5 Results and Discussion

The artificial river system (cascade) was an appropriate test system for simulating self purification in an impact zone after an untreated discharge. No aeration was provided to the system and a clear dissolved oxygen 'sag' curve was observed relatively quickly as the biofilm became established in the channel carrier material. Steady state conditions became established after approximately 22 days, this was then used as the calculation period for the removal of the key water quality parameters. Changes in the dO_2 concentration on selected days with distance downstream of injection can be clearly observed (Fig 4.1).

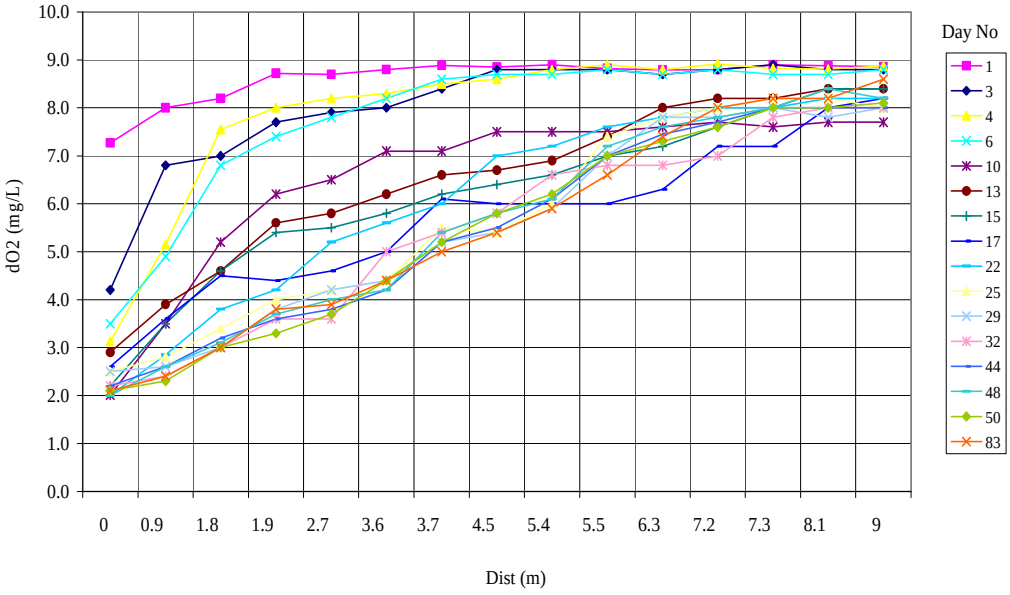


Fig 4.1. Plot of selected dissolved oxygen concentration measurements in the artificial river model, during the acclimation period and calculation period.

Comment [S34]: This might be better a bit bigger?

The use of the radiolabelled reference material ^{14}C aniline hydrochloride confirmed the presence of a viable biofilm by showing extensive removal of the chemical (84%). This was also further supported by observations which showed the establishment of a biofilm through the acclimation phase prior to steady state. Plates 9, 10, and 11 show the biofilm formation observed in the test system.

To further support that the mechanism of removal was biodegradation rather than sorption to solids or sedimentation, some of the cascade carrier material (5 glass beads) containing biofilm were removed from the channel (after the radio labelled analysis had been completed) and used as an inoculum in a respirometric test with LAS. The oxitop respirometric test system contained a standard LAS paste and mineral salts media, with a control vessel containing no LAS operated in parallel. LAS was added to the test vessels at a concentration of 75 mg/L ThOD (theoretical oxygen demand), after 5 days an oxygen demand measurement of over 57 mg/L (test – control) had been measured (Fig 4.2). The importance of physio-chemical processes in LAS removal has been pointed out by Rappaport and Eckhoff (1990) who, suggested mechanisms such as adsorption on to biofilm or suspended particles may play a major role in the removal of ingredients of a hydrophobic nature, such as, anionic surfactants. In this study no specific analysis was used to monitor the biodegradation intermediates of LAS (such as sulphenyl carboxylates) which would have been another method for confirming biodegradation.

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Comment [S36]: ?

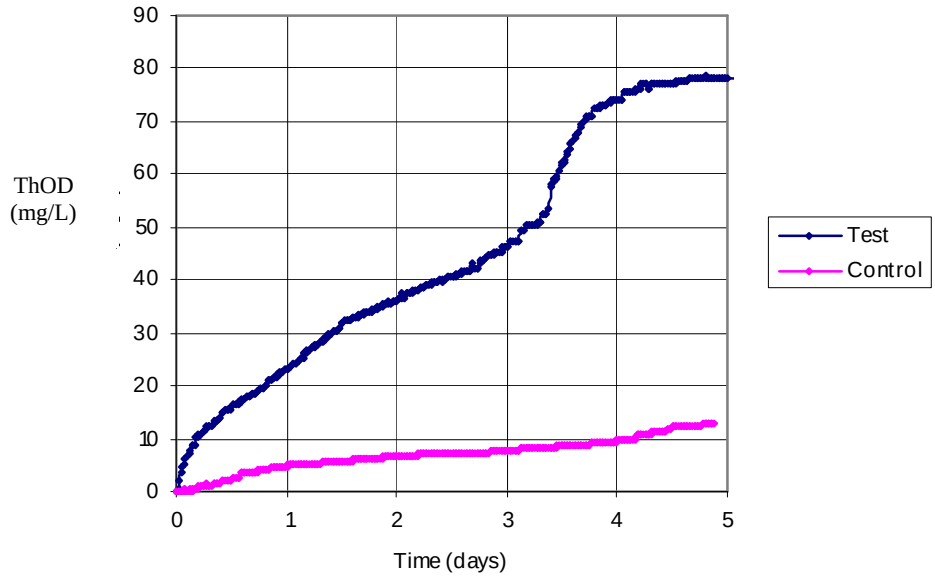

Fig 4.2. Oxygen demand measurement for LAS paste in an oxitop respirometric test system using carrier material (glass beads) from cascade as inoculum source.

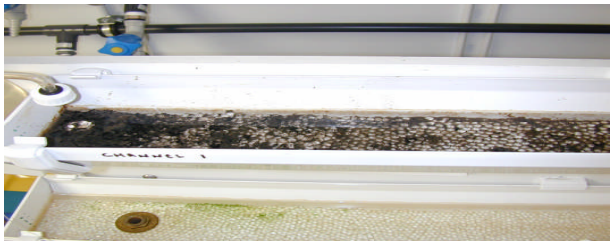

Plate 9. The first section of channel 1 after 6 weeks dosing with test medium. The lower dissolved oxygen (~2 mg/L) in this region produced a darker biofilm. Some algal formation can be seen in the channel below which became more prevalent in the lower channels as the system developed.

Plate 10. The second section of channel 1 (downstream) after 6 weeks dosing with test medium. The biofilm had started to change colour at this point as dissolved oxygen began to increase.

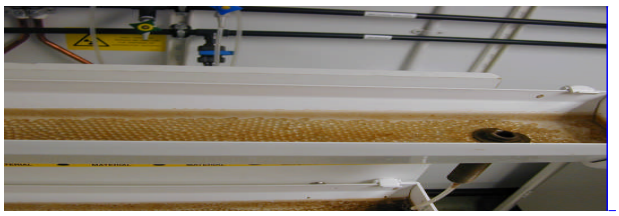

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Plate 11. The final section of channel 1 (downstream) after 6 weeks dosing with test medium. The feed system in to channel 2 can be observed.

4.6. Results for ^{14}C Aniline Hydrochloride

The data show a very significant and clear decreasing trend in ^{14}C aniline activity with travel time. Changes in ^{14}C aniline activity with distance in the cascade at different times are clearly observed (Fig 4.3). The system was initially dosed with ^{14}C aniline to achieve approximately 2500 DPM / mL in the influent.

An overall mean degradation rate constant k = 0.112 h^{-1}, and half life $t_{½}$ = 6.36 h^{-1}, was observed in the test system, cv = 20%. Rate constants were determined by calculating the axial flow speed and distance. The mean percent biodegradation of aniline observed in the system was 84%.

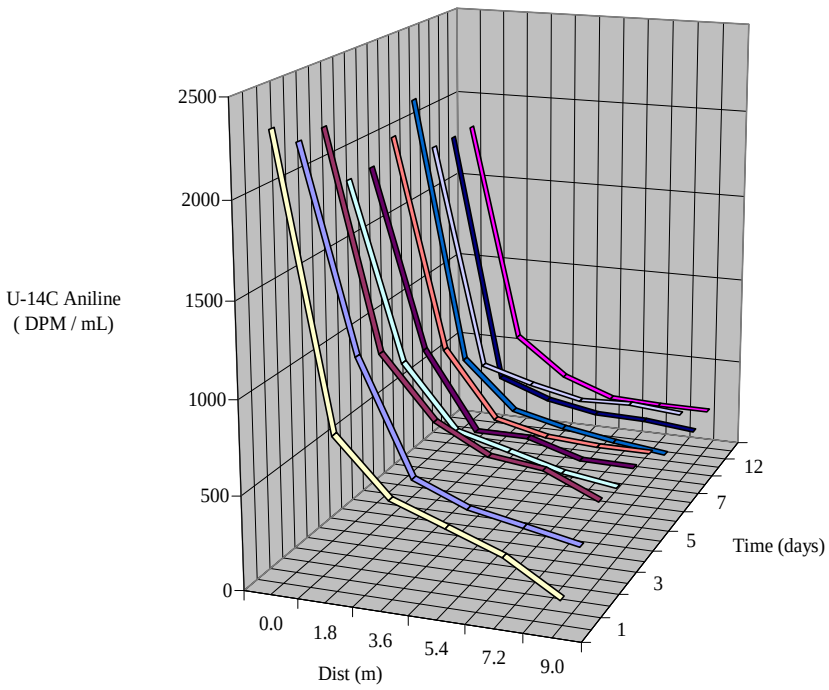
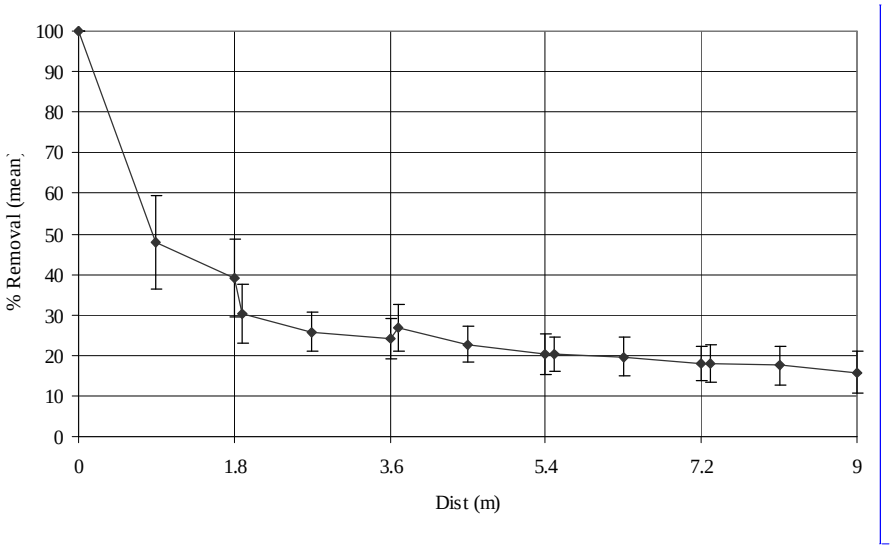


Fig 4.3. U-^{14}C Aniline hydrochloride degradation curve in the continuous flow river model with attached biomass.

Aniline had been selected as reference material because it is a recognised standard material used in ready biodegradability tests (OECD 301) for confirming a viable inoculum. It is also recommended in ISO 14952 as a potential chemical to be considered as a reference material. Mean calculated rate constant (k) was determined as 0.11 h^{-1} and half life $t_{½}$ = 6.36 h^{-1}.



Comment [S38]: I assume that this data is derived from data in Fig 4.2, in which case state that explicitly

Fig 4.4. Plot of loss of 14C Aniline (mean measured values) assumed mainly due to biodegradation in the test system operated under steady state conditions (derived from data in Fig 4.3). Error bars indicate ± one standard deviation.

4.6.1 Results for ^{14}C Phenyl 6 DOBS

Again the data (Fig 4.5) showed a very significant and clear decreasing trend in ^{14}C Phenyl 6-DOBS activity with travel time. The system was dosed with ^{14}C Phenyl 6-DOBS to achieve an activity of approximately 5000 DPM / mL. Figure 4.5 shows the same data for the calculation period expressed against distance which allowed calculation of a degradation rate constant (k) = 0.17 h^{-1} , and half life $t_{½}$ = 5.11 h^{-1} , cv = 5%. This is slightly slower than losses of LAS reported by Takada *et al.*, (1994) and McAvoy *et al.*, (2003), who report half lives between 1-3 h, but, faster than that reported by Whelan *et al.*, (2007) half life = 7 h. The degradation rate constants were calculated using the axial flow speed and distance. The mean percent biodegradation in the test system was 89%.

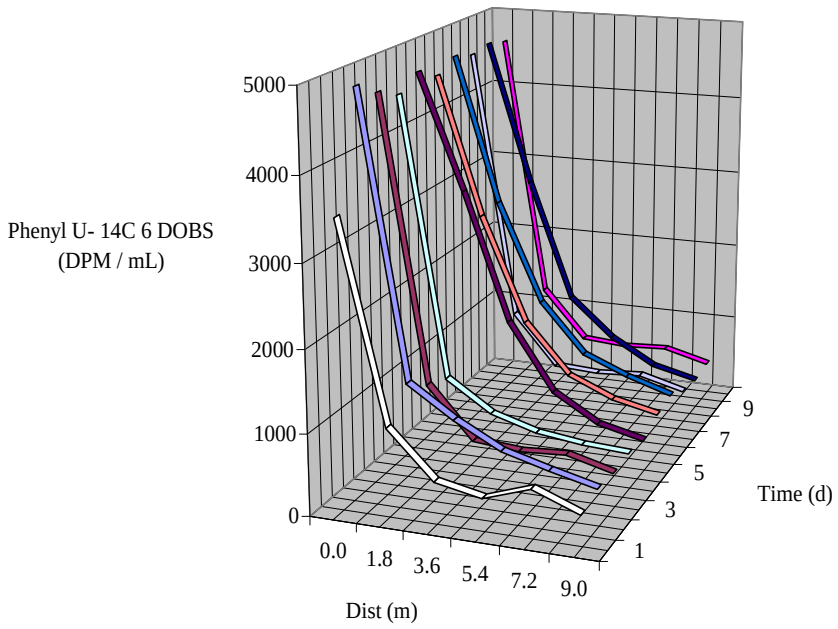


Fig 4.5. ^{14}C Phenyl 6-DOBS degradation curve of the continuous flow river model with attached biomass.

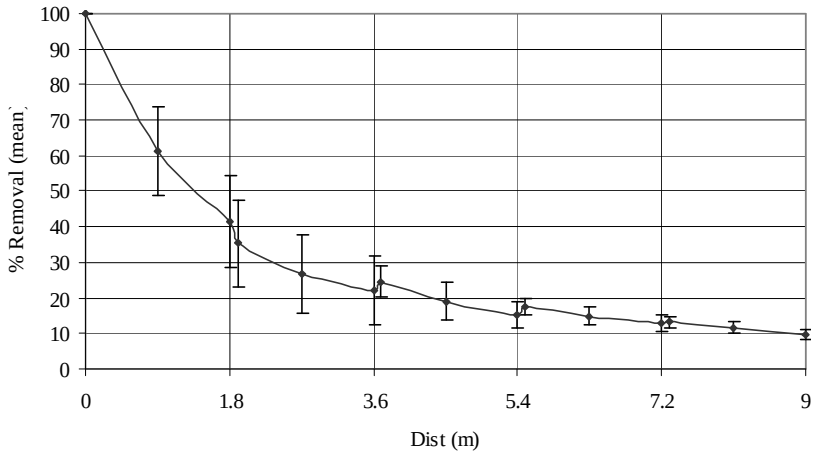


Fig 4.6. Plot of loss of ^{14}C Phenyl 6 DOBS (mean measured values) against distance (derived from data in Fig 4.5) in the test system operated under steady state conditions, (Error bars = ± standard deviation).

Calculated rate constants (k) for Phenyl 6-DOBS = 0.13 h^{-1} and half life $t_{½}$ = 5.11 h^{-1}

Values of the rate constant calculated with axial flow and distance were very similar to aniline, although less variable.

Comment [S39]: I've taken this out because I don't think it belongs here. I think it comes under the LC-MS results

4.6.2 Water quality analysis

The temperature and pH remained stable in all channels throughout the duration of the study. The mean temperature during the experiment was 20^{o}C +/- 0.3 ^{o}C . The pH during the test had a mean of 8.09 with a standard deviation of 0.12 (Appendix 3). A slight increase was observed from the inlet along the course of the channels to the outlet of the last channel. This could have been due to the increasing algal formation as the channel progressed, with increasing presence of algae photo-synthesising and utilising the dissolved carbon dioxide, in effect, reducing the acidity of the water and increasing the pH. The calculations for the determination of percent free ammonia used these mean pH and temperature measurements.

Chemical Oxygen Demand (COD)

Comment [S40]: I would send this to the appendix

Changes in COD with distance downstream of injection during the acclimation period, showed a general decrease in COD with distance downstream (Fig 4.7). The artificial river model was rapidly removing COD from the test media with steady state conditions being established after 22 days. The period day 22 to day 104 was used as the calculation period (the period over which data was used in calculating rate constants). The mean concentration measured in the test medium during the calculation period was 88.4 mg/L (standard deviation = 9.34). The mean percent biodegradation during this period was 71% with a degradation rate constant (k) = 0.071 h^{-1} , and half life $t_{½}$ = 9.9 h^{-1}, cv = 13%, calculated using the axial flow speed and distance.

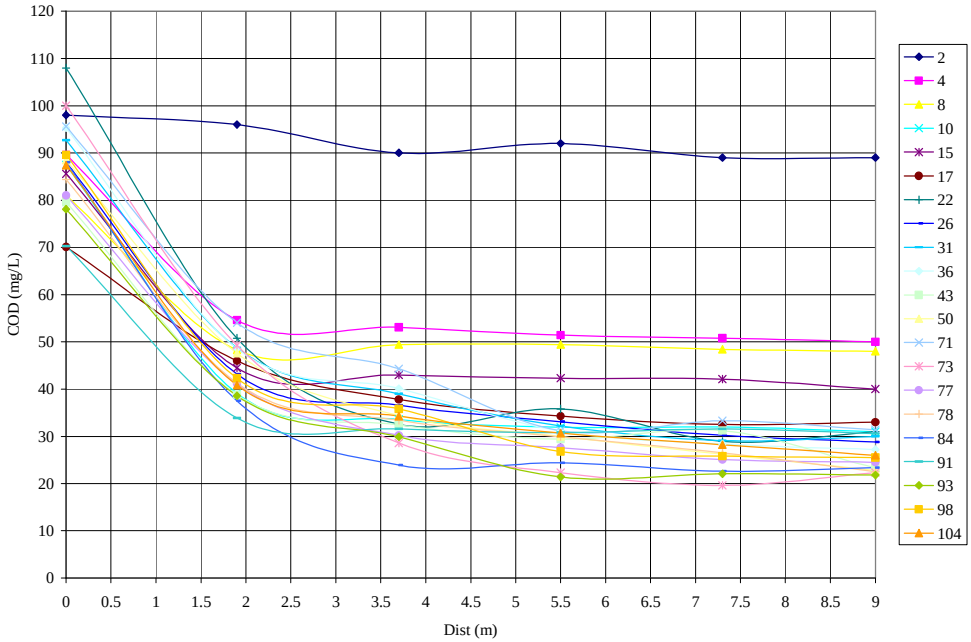


Fig 4.7. Plot of selected COD measurements in the artificial river model, during the acclimation period and calculation period.

The ratio of the half lives of LAS to COD, $t_{1/2}$ (LAS/COD) was = 0.51,

This confirms that the removal of LAS was more rapid than the key water quality determinant COD.

Ammonical Nitrogen analysis

Ammonical Nitrogen measurements were made in the artificial river model, during the acclimation period and calculation period (Fig 4.8).

Mean values for the calculation period were derived from this data and the mean concentration of free ammonia plotted against distance (Fig 4.9). The mean concentration of ammonium measured in the input to the first channel during the calculation period was 12.2 mg/L, equivalent to 9.5mg N / L, stand deviation = 1.1.

Comment [S41]: Is this correct?

Concentrations of NH_4N at 9m, after the establishment of steady state, were < 0.1mg/L , meaning that removal during this period was > 99% . This corresponds to a degradation rate constant k (h^{-1}) = 0.12, half life $t_{½}$ (h^{-1}) = 5.7.

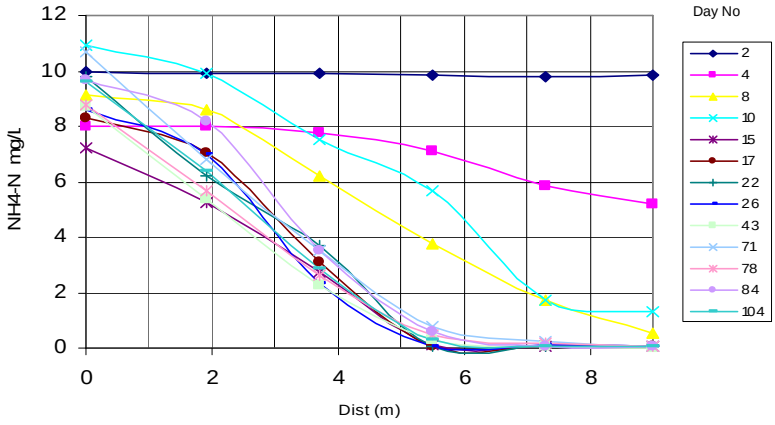


Fig 4.8. Plot of selected NH_4N measurements in the artificial river model, during the acclimation period and calculation period.

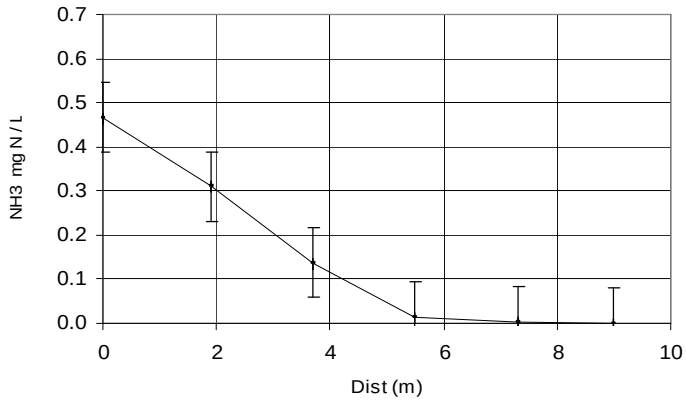


Fig 4.9. Plot of mean NH_3 measurements in the artificial river model against distance. Error bars represent ± one standard deviation.

The fraction of total ammonical nitrogen which was free NH_3 was 3.8%, a fraction that was consistent during the study due to the stable pH and temperatures observed in the cascade system. (Appendix 3). The levels fell below the toxicity threshold of free ammonia (European EIFAC= 0.025mg/L toxicity levels to freshwater fish) by the 5.5m distance mark, equivalent to HRT = 15.9 hrs in the system. The reported half life for the NH_4 in the system corresponds well with reported field data by McAvoy *et al.,* (2003) who reported 5.4 h^{-1} .

Plots of measured inorganic nitrogen species, nitrite (Fig 4.9.1) and nitrate (Fig 4.9.2) also confirmed that the system had achieved steady state conditions. The data shows the expected downstream changes in concentrations of ammonium, nitrite and nitrate, with decreasing ammonium and nitrite levels and increasing nitrate concentrations, reflecting the micro-biological processes associated with nitrifying bacteria.
By day 22 all of the nitrite was rapidly being converted to nitrate.
A mass balance could not be calculated for the inorganic nitrogen cycle because N was entering the test system from the settled sewage / river water test media.
Some mineralization to nitrogen will have taken place and some losses of NH_3-N due to volatilisation (at pH 8 this is likely) but these losses were not quantified.

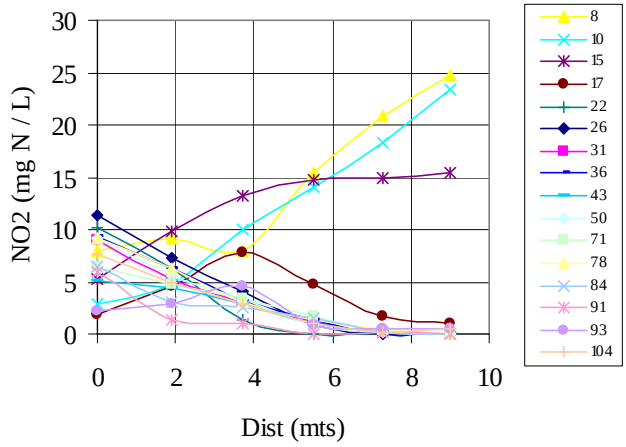


Fig 4.9.1 Plot of selected NO_2N measurements in the artificial river model, during the acclimation period and calculation period.

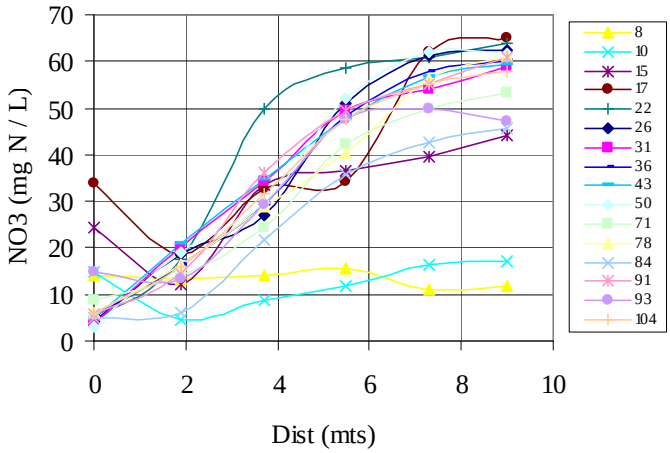


Fig 4.9.2 Plot of selected NO_3N measurements in the artificial river model, during the acclimation period and calculation period.

Analysis of test media

Analysis of the test medium (river water / sewage influent) for linear alkyl benzene sulphonate (LAS) was carried out within this phase of the study by liquid chromatography / mass spectrometry (LC/MS). An alternative method of measuring LAS concentration using derivatisation and gas chromatography / mass spectrometry (GC/MS) to analyse individual LAS isomer concentrations was also investigated. This was specifically to measure the amount of 6 Ph-C_{12} in the samples. Although not sensitive enough for measuring environmental sample concentration, a profile of the LAS standard used in the work was generated.

LC/MS Analysis

The concentration of LAS in each sample is shown in Table 4.1. The method showed good mean recoveries of total LAS, from spiked samples at 5 mg/L of 99.1 ± 2.5 % (n=5), see Table 4.2. Typical chromatograms of the LAS in the media (river water/sewage influent) and a standard are shown in the single ion monitoring (SIM) data. The C_{12} homologues of a standard and a sample collected from the test medium prior to the calibration of the radiolabelled material, show a similar profile by HPLC (Fig 4.9.3).

GC/MS Analysis

As shown in the total ion chromatogram (TIC) of 15mg total LAS after derivatization (Fig 4.9.4), the different LAS homologues are separated and each homologue is split into separate isomers with only 2-Ph-C_{12} and 6/7-Ph C_{13} co-eluting. The co-eluting

peaks could be resolved by extracting the individual ions for each homologue, i.e. m/z 346, 360, 374,and 388 which represent the derivatization products of LAS for C_{10}, C_{11}, C_{12} and C_{13}, respectively. The standards were also analysed using a single ion monitoring (SIM) method. The SIM chromatogram for LAS C_{12} where m/z = 374 was performed, and from this data the relative abundances for each isomer (Table 4.3) were calculated. The method was found to have a detection limit which was equivalent to 5 mg of LAS when dried.

This relatively high detection limit meant that large sample volumes (approximately 5L) would be required to detect LAS in the environmental samples, which in this study were at concentration levels of approximately 1mg/L.

For the LC/MS procedure, 20 mL of each sample was extracted and reserved at a final volume of 20 mL in methanol (equivalent to 20 µg LAS). Even if these extracts were pooled and dried there would be an insufficient amount of LAS present to be detected by this method. However using a combination of results obtained (i.e. the fact there is an average 23.7 % of the 6 phenyl C_{12} isomer in the LAS standard and given the similarity in profile of the LAS in the test media and standard as analysed by HPLC, this allows a reasonable estimate of the content of this isomer in the media to be made. The GC/MS derivatisation method requires further method development as the calibration curve produced, even in standards, was not quantitative or linear. For calculation purposes it was assumed that 20% of the LAS C_{12} would be attributable to the 6-phenyl C_{12} isomer.

Table 4.1. Concentrations of native LAS homologues in test media prior to the addition of radiolabelled material

Sample	Average LAS Concentration (mg/L) (Individual Replicates, n=2)				
	C_{10}	C_{11}	C_{12}	C_{13}	Total
Settled Sewage / River Water 06/05/05	0.196 (0.195 , 0.197) **18.5%**	0.445 (0.438 , 0.453) **42.0%**	0.290 (0.290 , 0.291) **27.4%**	0.131 (0.133, 0.130) **12.4%**	1.06 (1.06 , 1.07)
Settled Sewage / River Water 09/05/05	0.238 (0.235 , 0.241) **22.5%**	0.574 (0.574 , 573) **52.4%**	0.393 (0.391 , 0.394) **37.1%**	0.191 (0.187 , 0.194) **18%**	1.40 (1.39 , 1.40)
Settled Sewage / River Water 11/05/05	0.230 (0.229 , 0.231) **21.7%**	0.561 (0.570 , 0.552) **52.9%**	0.380 (0.386 , 0.374) **35.8%**	0.182 (0.189 , 0.174) **17.2%**	1.35 (1.38 , 1.33)
Settled Sewage / River Water 13/05/05	0.205 (0.198 , 0.211) **19.3%**	0.480 (0.462 , 0.497) **45.3%**	0.323 (0.311 , 0.336) **30.5%**	0.152 (0.145 , 0.159 **14.3%**	1.16 (1.12 , 1.20)
Settled Sewage / River Water 16/05/05	0.233 (0.230 , 0.237) **22.0%**	0.521 (0.507 , 0.535) **49.2%**	0.330 (0.319 , 0.340) **31.1%**	0.150 (0.145 , 0.154) **14.2%**	1.23 (1.20 , 1.27)
Settled Sewage / River Water 18/05/05	0.271 (0.276 , 0.266) **25.6%**	0.623 (0.636 , 0.610) **58.8%**	0.393 (0.399 , 0.387) **37.1%**	0.177 (0.182 , 0.172) **16.7%**	1.46 (1.49 , 1.44)

* Figures in bold show the concentrations as percentages of the total

Table 4.2. Spike recoveries at 5mg/L from settled sewage / river water

Sample	Recovery (%)				
	C_{10}	C_{11}	C_{12}	C_{13}	Average
Settled Sewage / River Water 05/05/05	104	104	101	93.9	101
Settled Sewage / River Water 06/05/05	106	104	102	97.6	102
Settled Sewage / River Water 09/05/05	102	101	102	98.5	101
Settled Sewage / River Water 11/05/05	99.4	96.9	95.6	92.3	95.8
Settled Sewage / River Water 13/05/05	99.8	99.6	97.3	92.2	97.2
Settled Sewage / River Water 16/05/05	100	101	97.8	93.6	98.1
Average Spike Recovery	102	101	99.2	94.7	99.1
Standard Deviation	2.5	2.6	2.7	2.7	2.5

Table 4.3. % Isomer distribution of LAS C_{12} at various concentrations in the standard material

Isomer	6Ph-C12	5Ph-C12	4Ph-C12	3Ph-C12	2Ph-C12
0 mg LAS*	0	0	0	0	0
1 mg LAS*	16.7	33.4	17.2	12.6	20.1
5 mg LAS*	26.0	31.0	17.6	12.1	13.3
10 mg LAS	23.9	24.0	17.3	15.2	19.6
15 mg LAS	23.8	23.0	17.4	15.6	20.3
20 mg LAS	23.4	22.3	17.4	16.0	20.8
Average	23.7	23.1	17.4	15.6	20.2
Standard Dev.	0.26	0.85	0.08	0.38	0.59

- Data not used for average and standard deviation

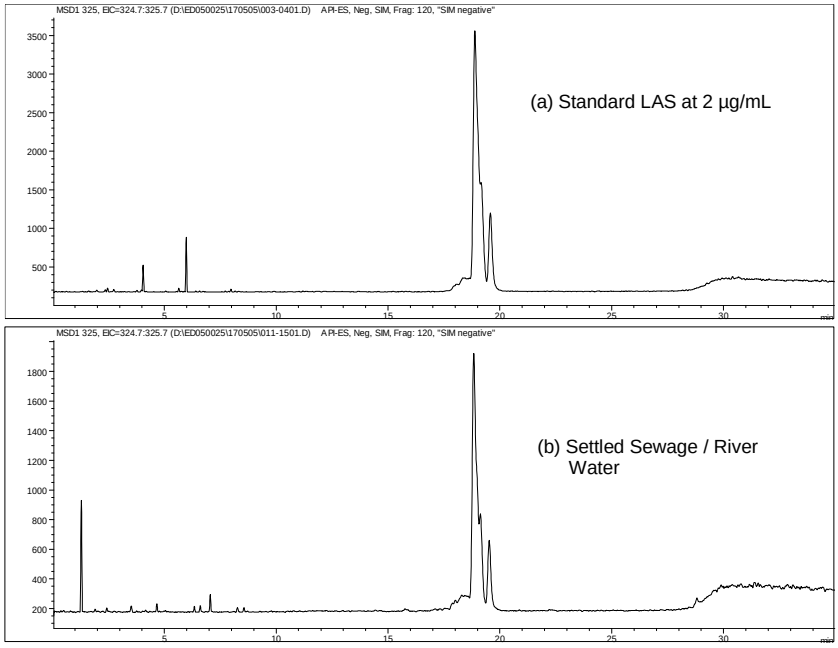


Fig 4.9.3. Comparison between HPLC-MS chromatograms of LAS C_{12} homologue in a standard and in a sample collected from the test media.

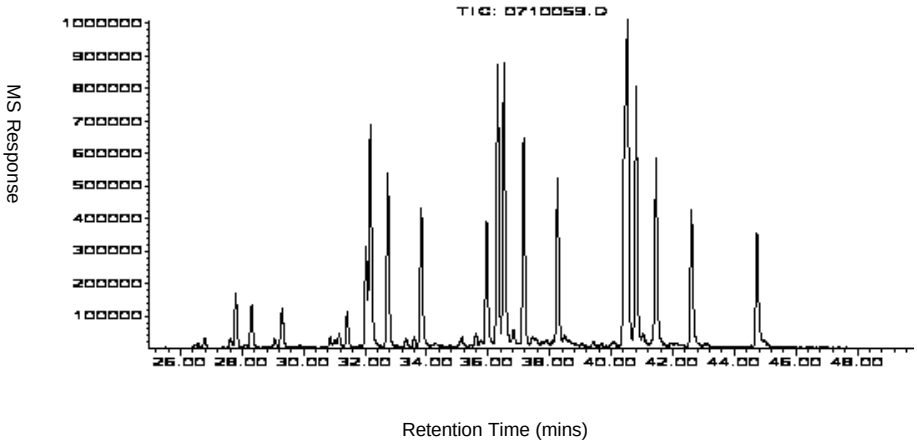
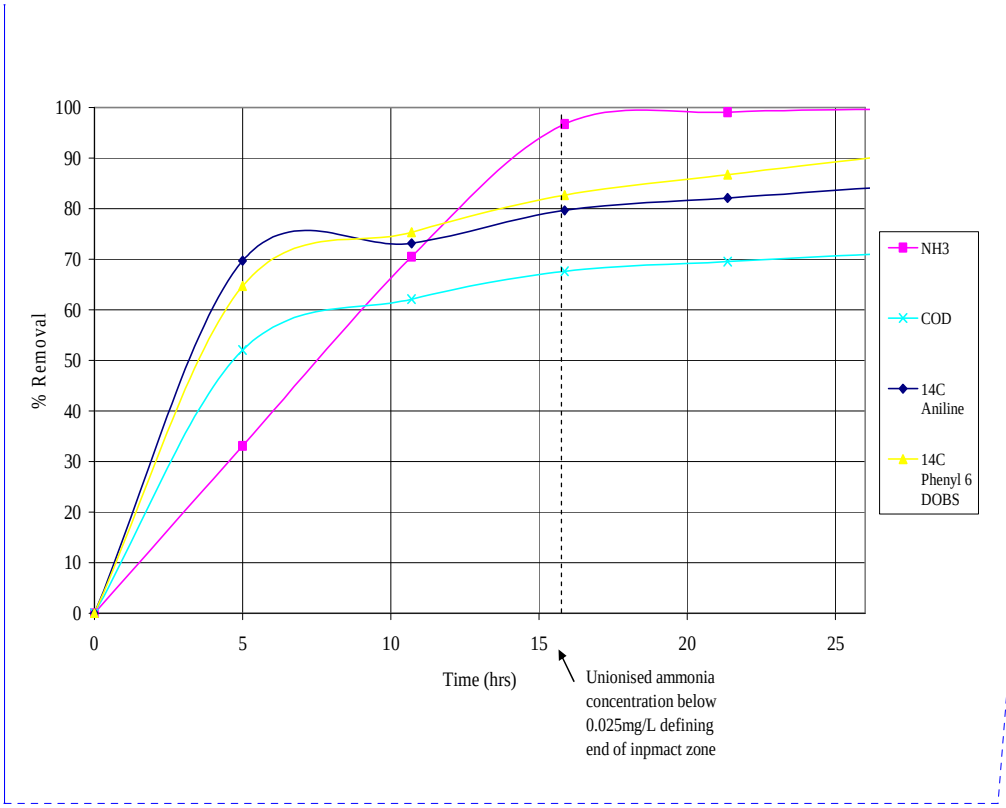


Fig 4.9.4. Total ion chromatogram (TIC) of 15mg total LAS after derivatisation.

Summary of results

The mean percentage removal of the key water quality parameters which characterise the impact zone and subsequent "self-purification" are summarised, and compared with the removal of the reference and test materials, whilst the system was judged to be operating under steady state conditions (Fig 4.9.5).

The end of the impact zone was defined as the point when the NH_3 concentration fell below 0.025 mg/L, which is the PNEC based on of free ammonia toxicity levels to freshwater fish (EIFAC). It is clear at this point (ca. 16 hours), under these conditions, more than 80% removal of both LAS and aniline had occurred.



Comment [S42]: This is big enough for the figure, but the font size is too small making it difficult to read. It's the key final figure, so it's worth getting it to look nice

Fig 4.9.5. Mean % removal of key parameters – calculated during system operating under steady state conditions

Applying the impact zone concept model, the PEC for the chemical of interest was calculated, and compared with the PNEC, at the end of the IZ (i.e. the point in the system where the toxicity of free ammonia falls below the ammonia PNEC). Assuming that the degradation rate observed for the C_{12} isomer is representative of all the LAS homologues, and only slightly different decay rates were observed , then this would suggest that with the total LAS concentration measured entering the test system (1.26 mg/L), would be reduced to 0.12 mg/L at the end of the impact zone.

Test Medium

Water quality variables in the test medium are shown in Appendix 3 for samples checked between Feb 05 and May 05. The coefficient of variation (cv) for most water quality variables suggests that the water quality was varying typically by about 10% over the course of the study. This is considered to be acceptable but must be borne in mind when interpreting the results.

4.7 Conclusions

The artificial river model proved an appropriate test system to model a point source pollution episode simulating direct discharge conditions. Following work in a similar system (Boeije *et al.*, 2000), glass beads were used to encourage the development of biofilm on the channel bed. The system clearly reproduced the expected relationship between the key water quality parameters resulting from an emission of untreated waste water, including high oxygen demand, associated dissolved oxygen sag, followed by recovery and high ammonium concentrations which decrease with distance downstream.

Observations were made of the fate of two radiolabelled synthetic organic materials (C_{12} linear alkyl benzene sulphonate and aniline hydrochloride).

Rapid changes in ^{14}C counts were observed with flow-time for both of these substances, representing complete mineralisation. In these experiments the impact zone was assumed to be the point at which the concentration of the unionised ammonia fell below the predicted no effect concentration for salmonids, (0.025 mg/L). In relation to this, LAS had been significantly removed (89%).

The calculated decay rates $t_{0.5}$ = 5 h corroborated well with reported field data.

More recent studies carried out in some rivers specifically to measure in-stream removal kinetics of LAS, showed $t_{0.5}$ in the 1-3 h range (Takada *et al.*, 1992; Schröder, 1995; Fox *et al.*, 2000). It also supports the studies by McAvoy *et al*. (2003) and Dyer *et al*. (2003), which reported the relative in-stream removal of LAS as higher than the removal of BOD.

The mean concentration of total LAS entering the artificial model system measured in the test media test media was 1.26 mg/L. At the point in the system identified as being the end of the impact zone the total LAS levels would be approximately = 0.12 mg/L. Downstream of the impact zone, a conventional ecological risk assessment can then be performed, in which the concentration of the chemical of interest is compared with the predicted no-effect concentration derived for laboratory toxicity test data (Table 4.4).

Table 4.4. Reported toxicity data of LAS (Schöberl, 1997).

EC50 (Daphnia)	8.8 mg/L
EC50 (fish)	4.8 mg/L
NOEC (Daphnia)	0.3 mg/L
NOEC (fish)	0.16 mg/L

NOEC: no observed effect concentration.

The profile of the C_{12} 6-DOBS in this system thus indicates that LAS levels would be expected to be below LAS PNEC levels by the time self purification was complete. This also confirms similar field observations conducted under tropical conditions reported by McAvoy *et al.*,(2003) and Whelan *et al.,* (2007).

The method used here did not confirm metabolites but did measure complete mineralization (conversion to CO_2). In the case of LAS the formation of persistent biodegradation intermediates can be excluded as demonstrated by high tier tests (Gerike *et al.*, 1986; Moreno *et al.*, 1991; Cavalli *et al.*,1996).

Biodegradation intermediates, i.e. the sulpho phenyl carboxylates are not persistent and their toxicities is several orders of magnitude lower than that of the parent molecule (Kimerle *et al.*,1977).

Aniline Hydrochloride is similarly classified to LAS as readily biodegradable, and again achieved >80% removal at the point defined 'end of the impact zone'.

Comment [S43]: I suggest moving this sentence to the final discussion chapter.

The cascade system was designed to provide a confirmatory methodology for assessing the ecological risks of chemicals under direct discharge conditions, without the need for expensive field campaigns. Although successful, further work is required both in the field and in the laboratory to ascertain the extent to which the patterns observed for LAS can be generalised to other readily biodegradable and inherently degradable substances.

CHAPTER 5

General Discussion

General Discussion

Environmental risk assessments for “down the drain” chemicals often apply simple models of exposure and effect, based on experience gained in systems in which waste water is treated before emission into the environment. These models are often inappropriate for evaluating ecological risks associated with chemicals emitted under direct discharge conditions. This is especially the case in the tropics where environmental conditions are often very different.

The screening tests explored in these studies have made a step towards obtaining more relevant data for risk assessment of ingredients in this environmental compartment and thus provide a better understanding of the fate and effects of detergent ingredients in an untreated discharge scenario and on the subsequent self-purification process.

In the case of the batch system explored in this thesis for determination of biodegradation rates (Chapter 2), the test system proved suitable to compare the relative biodegradation rate of a specific chemical to the other organic components in wastewater under defined conditions. The key finding in these experiments was that the primary biodegradation of the compounds chosen, LAS, and AE, is more rapid in an aerobic environment than the general organic loading present from the sewage. Some improvements could be made to the system used, for example the test system had to be aerated as no control over dissolved oxygen levels could be applied or monitored. It would be expected that oxygen would be present in the environment but possibly at reduced levels (1-4mg/L) due to the high organic loadings particularly below the wastewater outfall. The ability to control dO_2 in batch systems and its

effects on removal rates could be explored further. It was noted that for new chemicals or chemicals not consistently present in wastewater, sufficient test chemical (radiolabelled and unlabelled) should be added to approximate the expected concentration in wastewater diluted into surface water during an episodic release or following commercialisation of a new chemical.

The inhibition studies described in Chapter 3 demonstrated alternative short term toxicity tests which would be more applicable to ingredients which are released in to rivers in the presence of untreated sewage.

Both of the respiration and nitrification inhibition tests were designed to look at the potential inhibitory effects of detergent ingredients on micro-organisms that perform the key processes which influence self purification in the impact zone. The data provided alternative and more relevant PNEC's for these ingredients in this scenario. The tests confirmed that detergent ingredients can show selective inhibition towards heterotrophic and autotrophic micro-organisms. All of the detergent ingredients tested required concentrations above their predicted environmental concentrations to inhibit these processes. This was also observed in the case of ingredients known to be used in formulations for their anti bacterial properties, e.g. Triclosan.

Comment [S44]: No reference to Chapter 3!

The artificial river model described in Chapter 4 was a simulation test designed to provide more realistic estimates of removal rates. It is still only a model in terms of simulating the environment (as all standardised screening tests are) as it can not replicate all the processes which occur and which could be considered from monitoring studies in rivers.

However, it did allow the measurement of the rates of key parameters under defined conditions and the comparison of these rates of removal to each other.

Simulation tests of this nature can use specific analytical methods or, as in this case, the utilisation of radio-labelled test substances. These tests then can provide more extensive kinetic data of the parent disappearance or supply a comprehensive assessment of the full fate of a chemical. The model drew from the ISO 14952 standard, with reference to agreed criteria based on established scientific principles, but it remains critical that all the conditions are not fully standardised. Flexibility is required to maximise the realism for each specific chemical tested within its emission scenario. The cost and effort associated with more realistic simulation tests make it impossible to make them routine. Nevertheless for chemicals used at high volume or potential environmentally problematic chemicals these tests should supersede the less realistic tests.

The data from these screening tests provide perspective on biodegradation kinetics in key compartments relevant for chemicals discharged in wastewater. In addition, measured first order biodegradation rates can be used in a variety of exposure models to assess loading to the environment. Importantly in this experiment in comparison to the batch system investigated in Chapter 2, steady state conditions are allowed to develop, which provides more confidence in comparing the key parameters.

The continually changing conditions in a static system in terms of available nutrients and water quality have the potential to have an influence on the observed decay rates. A key finding in the experiments with the artificial river was the observed influence of the biofilm that and its important role in the self purification process.

The experiment again confirmed that beyond the impact zone LAS had been extensively removed and that current the approach of assuming no biodegradation of LAS and readily biodegradable materials in the risk assessment in an untreated discharge scenario is overly stringent. It has also demonstrated that a 'true' untreated discharge scenario can be modeled in the laboratory.

Risk Assessment

The European Union has established specific technical guidance (EU-TGD) and a formal model (EUSES) for environmental risk assessment, and the SEAC Pelican system referred to in this thesis adopts the same risk assessment principles upon which EUSES is based.

In the current version of EUSES, the first-order biodegradation rates derived from the batch system and the artificial river model biodegradation tests are clearly relevant for chemicals whose primary mode of entry into the environment is via wastewater, as opposed to others where atmospheric deposition or soil run-off might be more relevant. An appropriate biodegradation rate for surface water (*k biowater)* in such cases, for input into EUSES might be logically determined as follows:

kbiowater = keff-water x Feff + kww-water x Fww

Comment [S45]: This isn't quite clear! Some notes right at the end.

Where :

keff-water is the first-order biodegradation rate in the effluent in surface water.

kww-water is the first-order biodegradation rate in the untreated wastewater in surface water (derived from the screening tests discussed).

Feff is the fraction of wastewater released as treated effluent and *Fww* is the fraction of wastewater released without treatment.

This allows for a weighted average of the decay rates depending on the fraction that receive treatment or remain untreated prior to discharge (as discussed in many developing regions no treatment will be received at all). Knowledge of these decay rates could also be applied to exposure models and compared with decay rates of the key water quality parameters described in the impact zone concept to confirm if they would be expected to still be present or removed beyond the impact zone.

Although the tests explored worked well, further refinement of various parameters should be explored. This should include in the case of the artificial river model, the effects of hydraulic retention time and the effects of alternative environmental matrices such as sediment rather than artificial substrate, as the structure of the micro-organism communities (i.e. the number of species and their ecological preferences) may differ.

In all of these screening tests the effects of temperature would also be relevant as in many regions where this is a typical scenario, varying temperatures are found between temperate and tropical regions.

In the case of both the batch screening tests and the artificial river model, further work in these systems should also include broadening the range of chemicals tested and in particular including those which are classified as ‘inherently’ biodegradable, to examine if there is some potential for their removal prior to self purification after an untreated discharge. Through investigation of model chemicals (e.g. LAS for ready biodegradable chemicals), a read across to all chemicals of a particular classification could potentially then be made and an override removal factored in to the risk assessment and exposure models.

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APPENDIX 1

Biodegradation of linear alkylbenzenesulphonate and alcohol ethoxylates in river water simulating untreated discharge conditions (Raw data)

Batch die away raw data study 1

Time	Time	Conc (mg/l)			% remaining of initial conc					
(hrs)	(days)	COD	NH_4	MBAS	COD	NH_4	MBAS	dO_2	pH	Temp
0	0	246	23.2	9.95	100	100	100	9.98	7.91	19.7
2	0	234	22	9.15	95	94.8	92	9.98	7.97	19.7
19	0.8	162	18.2	5.15	65.9	78.4	51.8	9.89	8.01	20.2
22	0.9	150	17.9	4.58	61.0	77.2	46.0	10	7.99	20
43	1.8	132	17.2	1.85	53.7	74.1	18.6	10	8.04	19.9
46	1.9	128	16.4	1.69	52.0	73.0	17.0	9.9	8	20.1
48	2							9.9	8.09	20.1
90	3.8	111	14.9	1.5	45.1	64.2	15.1	9.91	8.14	20.4
94	3.9							9.6	8.12	20
114	4.8	103	12.3	1.45	41.9	53.0	15.1	9.8	8.08	19.6

Batch die away raw data study 2

Time (hrs)	Time (days)	dO_2 (mg/l)	dO_2 %sat	Temp (^oC)	pH	Concn (mg/l)			%remaining of initial conc.		
						COD	MBAS	NH4	COD	MBAS	NH4
0	0.0	9.1	100	19.8	8.26	187	7.8	13.6	100	100.0	100
5	0.2	9.38	102	19.9	8.21	170	5.8	12.2	90.9	74.7	89.7
8	0.3	9.06	100	19.9	8.01	147	4.9	11.0	78.6	62.5	80.9
24	1.0	9.02	99	19.8	8.18	118	3.7	9.4	63.1	47.1	69.3
26	1.1	8.71	97	20	8.22	109	3.1	9.3	58.3	40.1	68.4
32	1.3	8.72	97	20	8.24	111	3.3	9.1	59.4	41.8	66.9
48	2.0	8.9	98	20	8.37	83	2.4	8.0	44.4	30.4	57.1
51	2.1	8.9	98	20	8.43	78	2.2	7.8	41.7	27.7	57.1
55	2.3	8.97	99	19.7	8.46	79	2.0	7.5	42.2	25.3	54.8
72	3.0	8.75	97	20.1	8.4	71	1.5	6.7	38.0	19.7	48.9
76	3.2	8.9	98	20	8.47	67	1.5		35.8	18.6	
79	3.3	8.72	97	20	8.41	65	1.13	6.6	34.8	18.6	48.5
96	4.0	9.02	98	20	8.33	61	1.1	6.3	32.6	13.8	46.3
120	5.0	9.l	100	19.8	8.31	64	1.2	6.4	34.2	14.0	47.3

Concentrations of C_{10}-C_{13} LAS (mg/L), analysis by LC ESI MS (figures in bold are quoted at method detection limit)

Day	Time (hr)	$C_{10\ LAS}$	$C_{11\ LAS}$	$C_{12\ LAS}$	$C_{13\ LAS}$	Total
0.0	0	0.464	0.942	0.499	0.324	2.229
0.2	5	0.479	0.849	0.414	0.320	2.062
0.3	8	0.467	0.781	0.400	0.274	1.922
1.0	24	0.379	0.651	0.279	0.163	1.472
1.2	28	0.368	0.556	0.202	0.133	1.259
1.3	32	0.307	0.404	0.143	0.088	0.942
2.0	48	0.131	0.083	0.033	0.033	0.280
2.1	51	0.080	0.037	0.033	0.033	0.183
2.3	55	0.051	0.033	0.033	0.033	0.150
3.0	72	0.033	0.033	0.033	0.033	0.132
3.2	76	0.033	0.033	0.033	0.033	0.132
3.3	79	0.033	0.033	0.033	0.033	0.132

C_{12} AE Analysis raw data (Figures in bold are quoted at method detection limit)

C_{12} (mg/L)	Time (hrs)			
	0	5	8	24
EO_0	0.0970	0.0300	0.0067	**0.0040**
EO_1	0.1506	0.1079	0.0613	**0.0040**
EO_2	0.1183	0.1189	0.0832	**0.0040**
EO_3	0.1177	0.1011	0.0800	**0.0040**
EO_4	0.0935	0.0763	0.0574	**0.0040**
EO_5	0.0591	0.0440	0.0492	**0.0040**
EO_6	0.0526	0.0481	0.0368	**0.0040**
EO_7	0.0433	0.0415	0.0320	**0.0040**
EO_8	0.0360	0.0286	0.0225	**0.0040**
EO_9	0.0294	0.0226	0.0203	**0.0040**
EO_{10}	0.0214	0.0194	0.0158	**0.0040**
EO_{11}	0.0210	0.0194	0.0143	**0.0040**
EO_{12}	0.0141	0.0109	0.0097	**0.0040**
EO_{13}	0.0111	0.0060	0.0052	**0.0040**
EO_{14}	0.0096	0.0082	0.0076	**0.0040**
EO_{15}	0.0070	0.0067	0.0070	**0.0040**
EO_{16}	**0.0040**	**0.0040**	**0.0040**	**0.0040**
EO_{17}	**0.0040**	**0.0040**	**0.0040**	**0.0040**
EO_{18}	**0.0040**	**0.0040**	**0.0040**	**0.0040**
EO_{19}	**0.0040**	**0.0040**	**0.0040**	**0.0040**
EO_{20}	**0.0040**	**0.0040**	**0.0040**	**0.0040**

C_{13} AE Analysis raw data (Figures in bold are quoted at method detection limit)

	Time (hrs)			
C_{13} (mg/L)	0	5	8	24
EO_0	0.1176	0.0723	0.0596	0.0071
EO_1	0.0364	0.0256	0.0198	0.0052
EO_2	0.0282	0.0171	0.0198	**0.0040**
EO_3	0.0280	0.0169	0.0155	0.0043
EO_4	0.0249	0.0209	0.0118	**0.0040**
EO_5	0.0245	0.0162	0.0165	**0.0040**
EO_6	0.0280	0.0169	0.0156	**0.0040**
EO_7	0.0235	0.0157	0.0102	**0.0040**
EO_8	0.0193	0.0189	0.0142	**0.0040**
EO_9	0.0250	0.0139	0.0127	**0.0040**
EO_{10}	0.0190	0.0160	0.0150	**0.0040**
EO_{11}	0.0201	0.0170	0.0144	**0.0040**
EO_{12}	0.0114	0.0136	0.0117	**0.0040**
EO_{13}	0.0109	0.0088	0.0060	**0.0040**
EO_{14}	0.0051	0.0049	0.0055	**0.0040**
EO_{15}	0.0066	**0.0040**	0.0056	**0.0040**
EO_{16}	**0.0040**	**0.0040**	**0.0040**	**0.0040**
EO_{17}	**0.0040**	**0.0040**	**0.0040**	**0.0040**
EO_{18}	**0.0040**	**0.0040**	**0.0040**	**0.0040**
EO_{19}	**0.0040**	**0.0040**	**0.0040**	**0.0040**
EO_{20}	**0.0040**	**0.0040**	**0.0040**	**0.0040**

C_{14} AE Analysis raw data (Figures in bold are quoted at method detection limit)

C_{14} (mg/L)	Time (hrs) 0	5	8	24
EO_0	0.0907	0.0342	0.0087	**0.0040**
EO_1	0.0408	0.0257	0.0199	**0.0040**
EO_2	0.0421	0.0360	0.0207	**0.0040**
EO_3	0.0224	0.0176	0.0105	**0.0040**
EO_4	0.0360	0.0220	0.0148	0.0054
EO_5	0.0347	0.0223	0.0173	**0.0040**
EO_6	0.0202	0.0177	0.0152	**0.0040**
EO_7	0.0225	0.0244	0.0142	**0.0040**
EO_8	0.0302	0.0243	0.0175	**0.0040**
EO_9	0.0232	0.0229	0.0169	**0.0040**
EO_{10}	0.0236	0.0184	0.0158	**0.0040**
EO_{11}	0.0120	0.0151	0.0142	**0.0040**
EO_{12}	0.0126	0.0079	0.0059	**0.0040**
EO_{13}	0.0067	0.0076	0.0076	**0.0040**
EO_{14}	0.0075	0.0077	0.0069	**0.0040**
EO_{15}	0.0042	0.0053	0.0040	**0.0040**
EO_{16}	0.0041	0.0044	0.0046	**0.0040**
EO_{17}	**0.0040**	**0.0040**	**0.0040**	**0.0040**
EO_{18}	**0.0040**	**0.0040**	**0.0040**	**0.0040**
EO_{19}	**0.0040**	**0.0040**	**0.0040**	**0.0040**
EO_{20}	**0.0040**	**0.0040**	**0.0040**	**0.0040**

C_{15} AE Analysis raw data (Figures in bold are quoted at method detection limit, no C_{15} AE > EO_{15} was detected)

	Time (hrs)			
C_{15} (mg/L)	0	5	8	24
EO_0	0.0215	0.0148	0.0120	**0.0040**
EO_1	0.0109	**0.0040**	**0.0040**	**0.0040**
EO_2	0.0161	0.0049	**0.0040**	**0.0040**
EO_3	0.0122	0.0077	0.0104	**0.0040**
EO_4	0.0149	0.0095	0.0101	**0.0040**
EO_5	0.0160	0.0114	0.0132	**0.0040**
EO_6	0.0172	0.0141	0.0110	**0.0040**
EO_7	0.0140	0.0102	0.0107	**0.0040**
EO_8	0.0159	0.0172	0.0128	**0.0040**
EO_9	0.0107	0.0095	0.0141	**0.0040**
EO_{10}	0.0133	0.0096	0.0092	**0.0040**
EO_{11}	0.0134	0.0059	0.0113	**0.0040**
EO_{12}	0.0094	0.0051	0.0049	**0.0040**
EO_{13}	0.0071	0.0042	0.0051	**0.0040**
EO_{14}	0.0071	**0.0040**	0.0054	**0.0040**
EO_{15}	0.0054	**0.0040**	**0.0040**	**0.0040**

C_{16} AE Analysis

	Time (hrs)			
C_{16} (mg/L)	0	5	8	24
EO_0	0.0510	0.0219	0.0146	**0.0040**
EO_1	0.0127	0.0139	0.0165	**0.0040**
EO_2	0.0111	0.0119	**0.0040**	**0.0040**
EO_3	0.0098	0.0083	**0.0040**	**0.0040**
EO_4	0.0068	**0.0040**	**0.0040**	**0.0040**
EO_5	0.0052	**0.0040**	**0.0040**	**0.0040**

(C_{16} > EO_5 not detectable).

C_{18} AE Analysis

C_{16} (mg/L)	Time (hrs) 0	5	8	24
EO_0	0.1526	0.1157	0.1254	0.0195
EO_1	**0.0040**	**0.0040**	**0.0040**	**0.0040**

APPENDIX 2

Investigation of short term toxicity tests for micro-organisms in the aquatic environment under direct discharge conditions (Raw Data)

Nitrifying sludge activity determination

Nitrfying sludge activity

Flask No	**1**	**2**	**3**	**4**	**5**	**6**
Medium (mL)	25	25	25	25	25	25
Activated Sludge (mL)	125	125	125	125	125	125
Reference Inhibitor ATU (mL)	0	2.5	0	2.5	0	2.5
Diluted Water (mL)	100	97.5	100	97.5	100	97.5
Total Volume (mL)	250	250	250	250	250	250
Concn of activated Sludge (mg/L)	125	125	250	250	500	500
$N0_2$-N	0.99	0.12	1.31	0.09	2.59	0.08
$N0_3$-N	1.61	0.22	3.28	0.47	6.51	0.95
Total oxidised N	2.60	0.35	4.59	0.56	9.10	1.03

	1	2
Wt. of paper	0.37	0.35
Volume	50	50
Wt. of paper + solids	0.57	0.57
Solids	0.2	0.22

			Mean
MLSS (mg/L)	4092	4492	4292

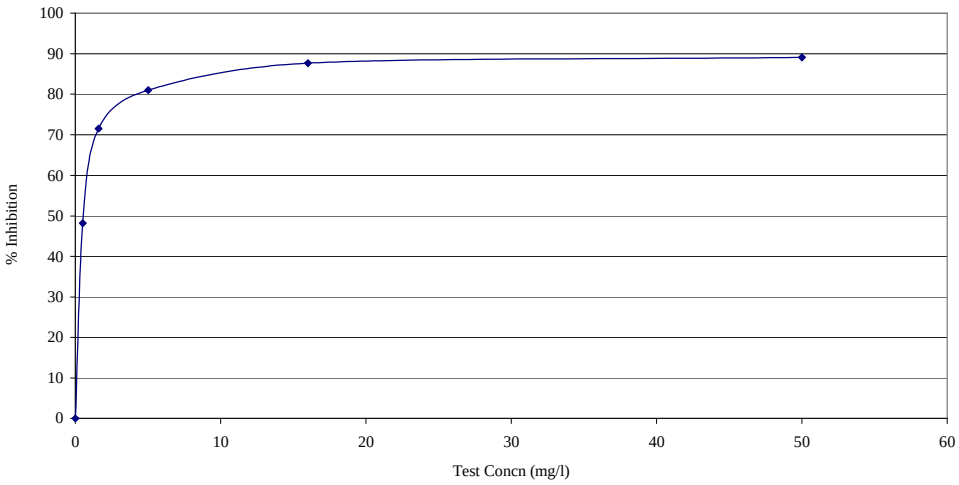
SS required (mg/L)	125	250	500
Inoculum vol required in test vessel.	7	15	29

SS (mg/L)	125	250	500
mg of N (g.h)	4.5	4.0	4.0

(3,5 DICHLOROPHENOL)

Nitrification Inhibition (raw data)

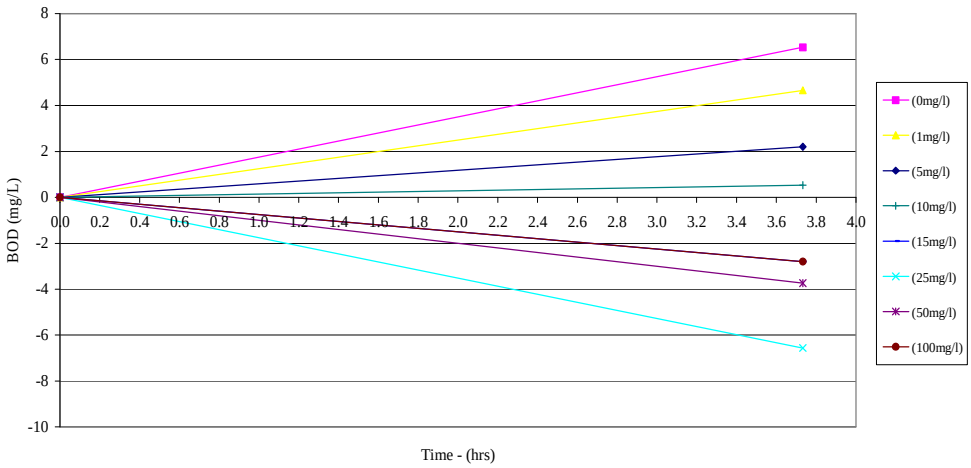
Flask No	**1**	**2**	**3**	**4**	**5**	**6**	**7 (ref)**
Concentration of test substance (mg/L)	0	0.5	1.6	5	16	50	2.5
Oxidised N (mg/L)							
$N0_2$-N	0.585	0.139	0.027	0.014	0.05	0.089	0.002
$N0_3$-N	1.38	1.02	0.741	0.596	0.448	0.385	0.29
Total	1.965	1.159	0.768	0.61	0.498	0.474	0.292
mg of N (g.h) control	2.18						
% Inhibition	0	48.2	71.5	81.0	87.7	89.1	



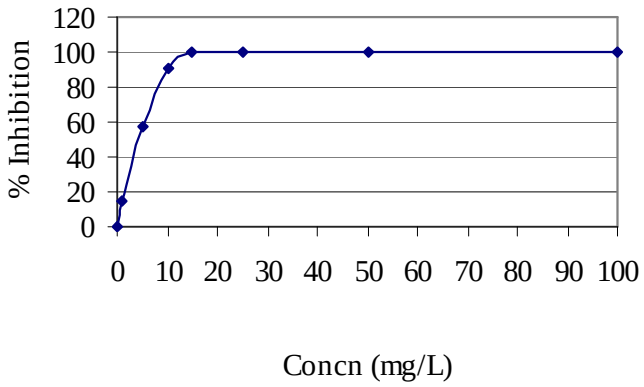
Plot of percentage inhibition versus test material concentration.

Respiration Inhibition (raw data)

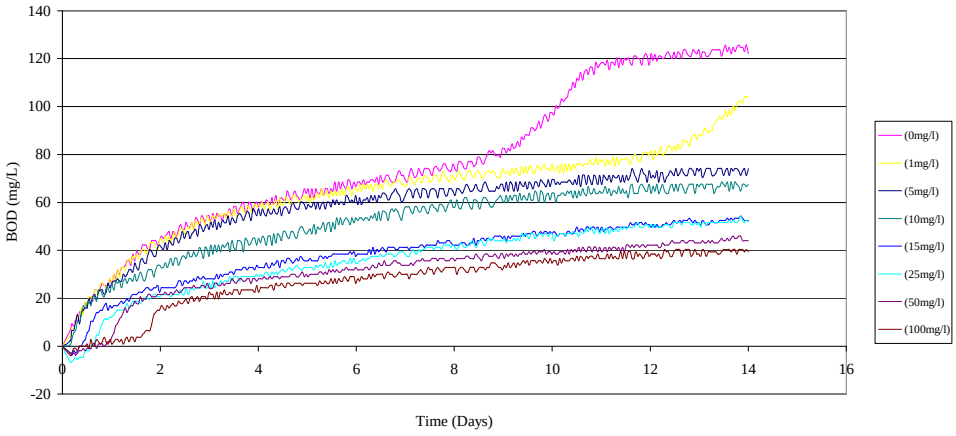
Test Material Vessel No	3,5 DCP	Test concn mg/L	BOD (avg) 4hr	% Inhib 4hr
1,2,3	Control	0	6.5	0
4,5,6	Test	1	5.6	14.3
7,8,9	" "	5	2.8	57.1
10,11,12	" "	10	0.6	90.3
13,14,15	" "	15	-2.8	100.0
16,17,18	" "	25	-6.6	100.0
19,20,21	" "	50	-3.7	100.0
22,23,24	" "	100	-2.8	100.0



3,5 Dichlorophenol – 4hr BOD plot



Plot of percentage inhibition versus test material concentration

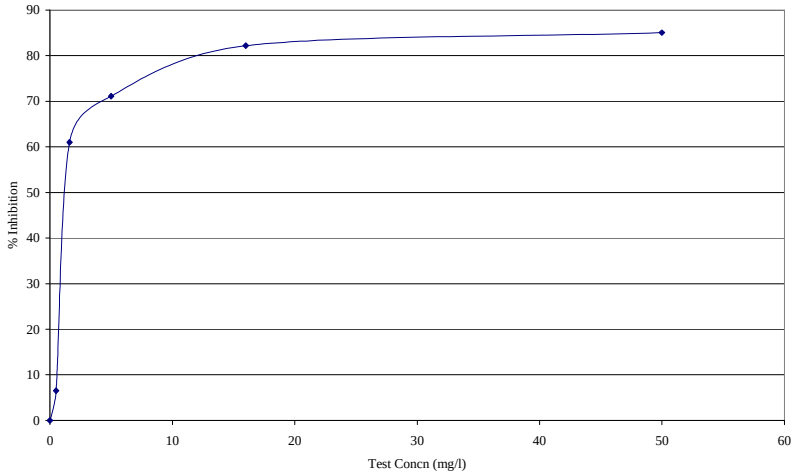


3,5 Dichlorophenol – 14day BOD plot

(IRGASAN DP300)

Nitrification Inhibition (raw data)

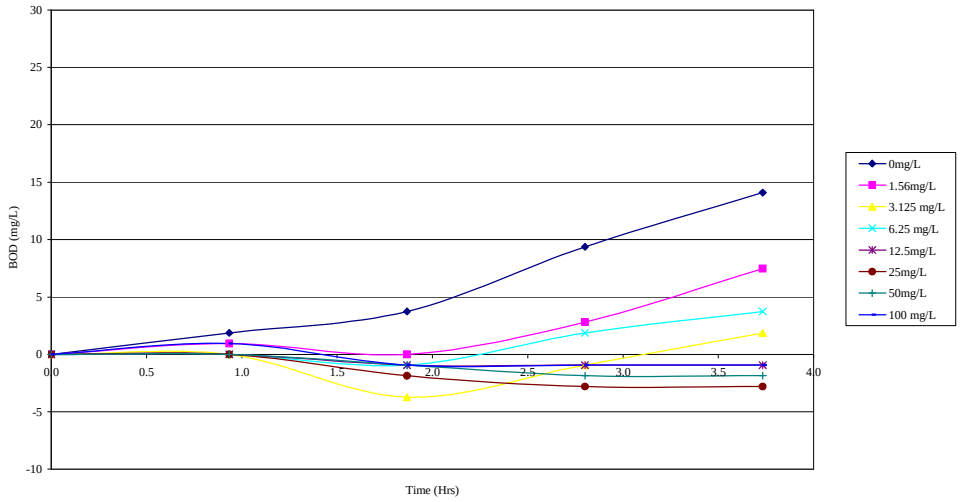
Flask No	**1**	**2**	**3**	**4**	**5**	**6**	**7 (ref)**
Concentration of Test substance (mg/L)	0	0.5	1.6	5	16	50	2.5
Oxidised N (mg/L)							
$N0_2$-N	0.45	0.36	0.33	0.29	0.10	0.05	0.00
$N0_3$-N	3.29	3.16	1.39	1.09	0.91	0.87	0.42
Total	3.74	3.52	1.72	1.38	1.01	0.92	0.42
mg of N (g.h) control	5.74						
% Inhibition	0.00	6.52	60.97	71.10	82.17	85.04	



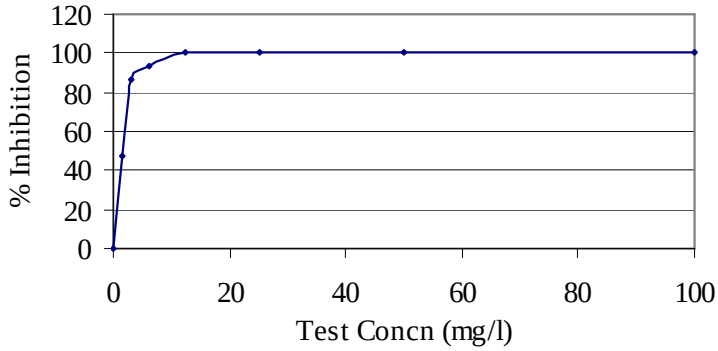
Plot of percentage inhibition versus test material concentration.

Respiration Inhibition (raw data)

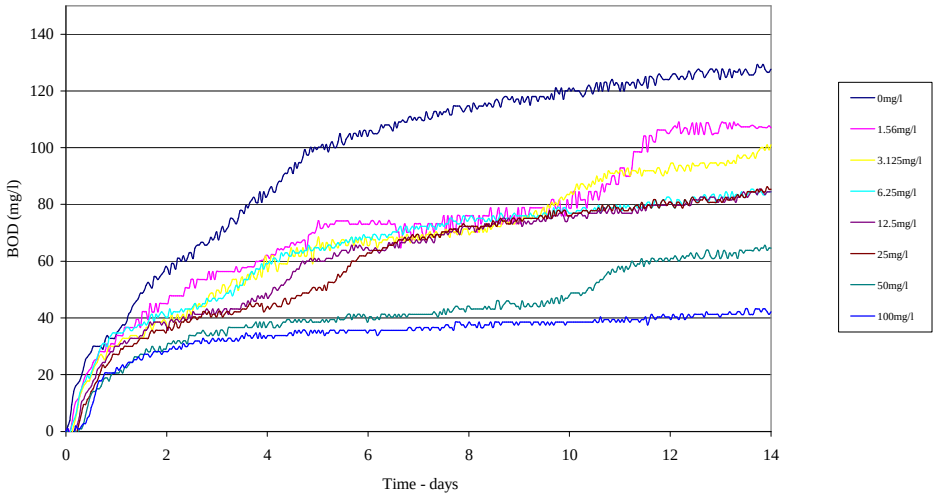
Test Material : Irgasan DP300 Vessel No		Test concn mg/L	BOD (avg) 4hr	% Inhib 4hr
1,2,3	Control	0	14.1	0.0
4,5,6	Test	1.56	7.5	47.0
7,8,9	" "	3.12	1.9	86.8
10,11,12	" "	6.25	0.9	93.4
13,14,15	" "	12.5	-0.9	100.0
16,17,18	" "	25	-2.8	100.0
19,20,21	" "	50	-2.8	100.0
22,23,24	" "	100	-2.8	100.0



Irgasan DP300 – 4hr BOD plot



Plot of percentage inhibition versus test material concentration

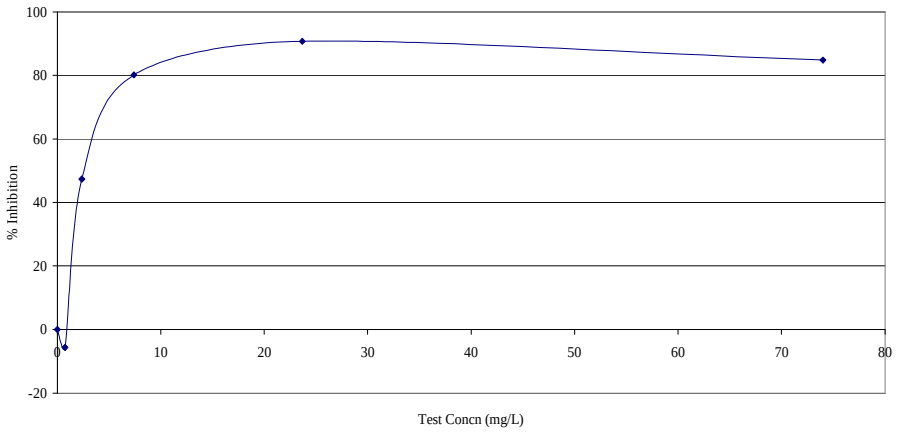


Irgasan DP300 – 14day BOD plot

(CATIGENE LM80)

Nitrification Inhibition (raw data)

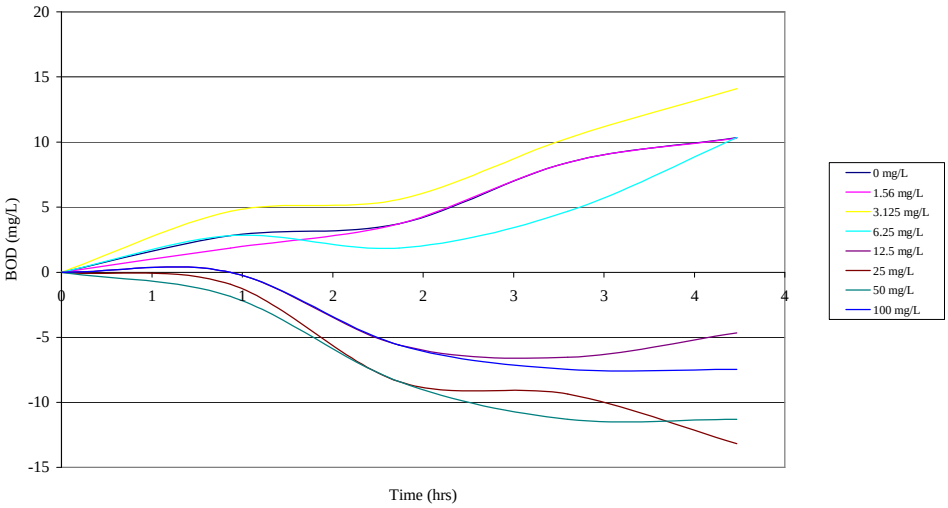
Flask No	**1**	**2**	**3**	**4**	**5**	**6**	**7 (ref)**
Concentration of test substance (mg/L)	0	0.7	2.4	7.4	23.7	74.0	2.5
Oxidised N (mg/L)							
$N0_2$-N	0.328	0.28	0.142	0.054	0.04	0.1	0.001
$N0_3$-N	1.21	1.32	0.916	0.672	0.579	0.579	0.524
Total	1.538	1.595	1.058	0.726	0.619	0.679	0.525
mg of N (g.h) control	2.026						
% Inhibition	0	-5.6	47.4	80.2	90.7	84.8	



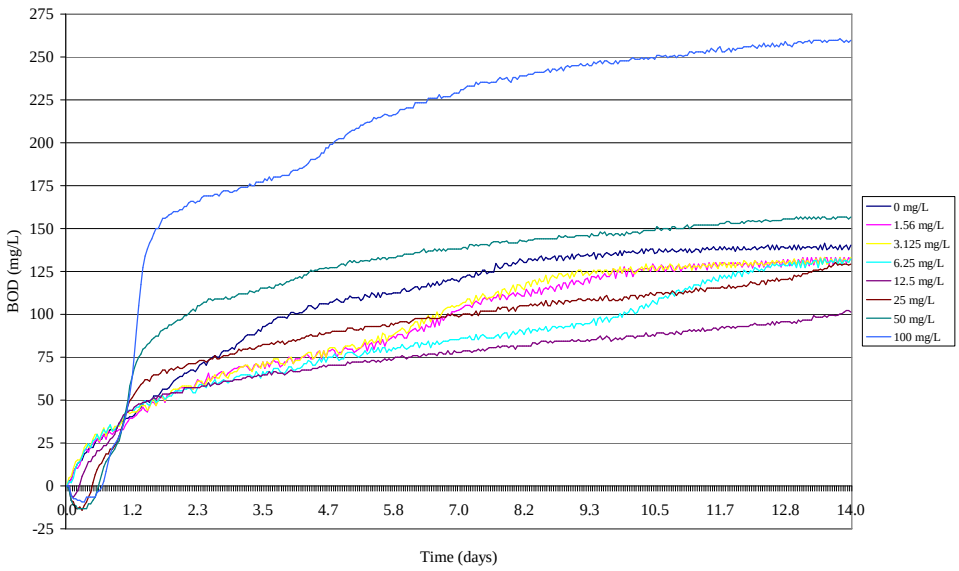
Plot of percentage inhibition versus test material concentration.

Respiration Inhibition (raw data)

Test Material: Catigene LM80 Vessel No		Test Concn mg/L	BOD (avg) 3hr	% Inhib 3hr
1,2	Control	0	13.2	0.0
3,4	Test	1.24	11.3	14.2
5,6	" "	2.48	10.5	20.0
7,8	" "	4.96	10.3	21.5
9,10	" "	9.92	-4.7	100.0
11,12	" "	19.8	-13.2	100.0
13,14	" "	39.7	-11.3	100.0
15,16	" "	79.4	-7.5	100.0



Catigene LM80 - 4hr BOD plot

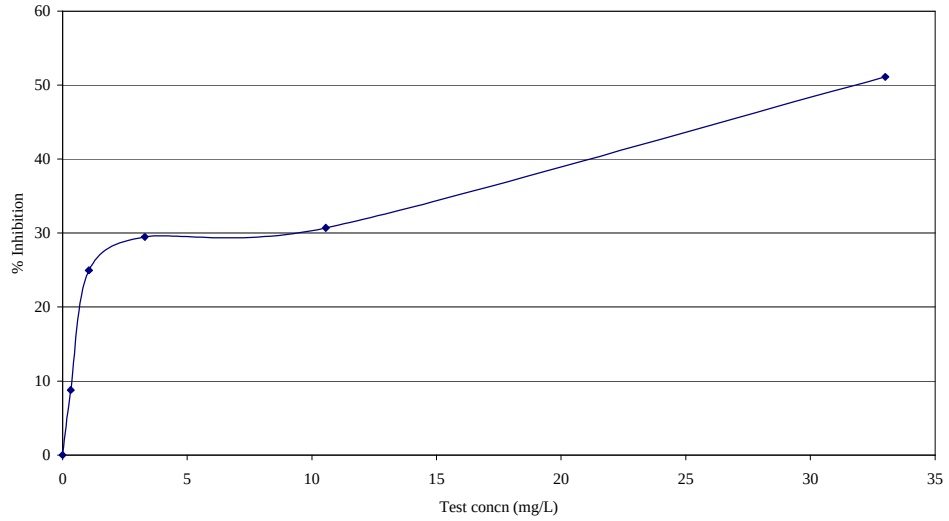


Catigene LM80 - 14day BOD plot

(TINOFIX CL)

Nitrification Inhibition (raw data)

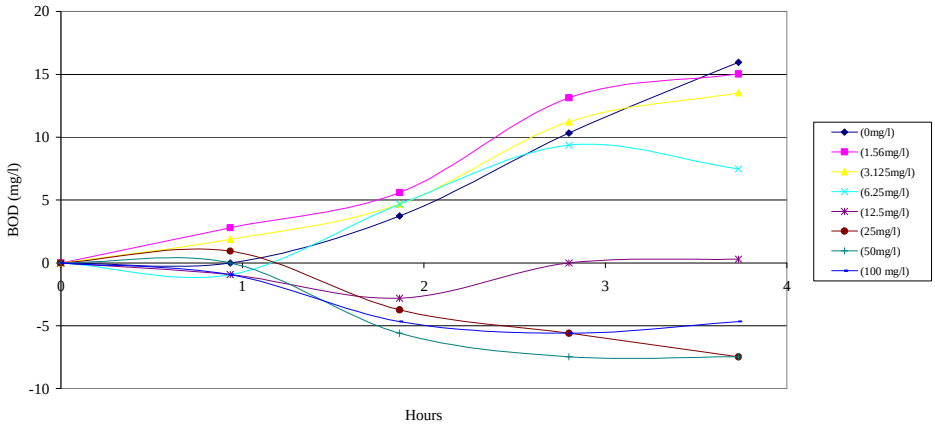
Flask No	**1**	**2**	**3**	**4**	**5**	**6**	**7 (ref)**
Concentration of Test substance (mg/L)	0	0.33	1.056	3.3	10.56	33	2.5
Oxidised N (mg/L)							
$N0_2$-N	0.469	0.483	0.449	0.499	0.433	0.263	0.008
$N0_3$-N	1.5	1.37	1.19	1.08	1.13	1.03	0.638
Total	1.969	1.853	1.639	1.579	1.563	1.293	0.646
mg of N (g.h)	2.65						
% Inhibition	0	8.8	24.9	29.5	30.7	51.1	



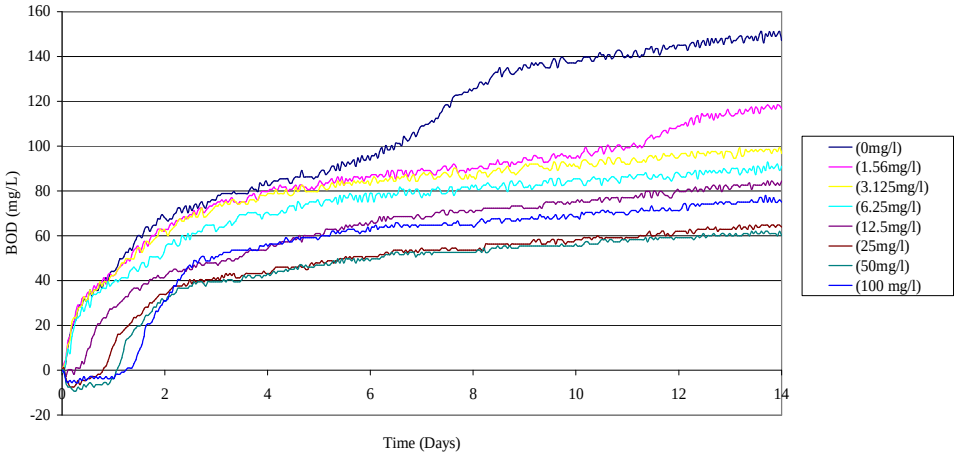
Plot of percentage inhibition versus test material concentration.

Respiration Inhibition (raw data)

Test Material Vessel No	Tinofix CL	Test Concn mg/L	BOD (avg) 4hr	% Inhib 4hr
1,2,3	Control	0	16.0	0.0
4,5,6	Test	1.56	15.0	5.8
7,8,9	" "	3.12	13.5	15.2
10,11,12	" "	6.25	11.3	29.2
13,14,15	" "	12.5	7.5	53.2
16,17,18	" "	25	0.3	98.1
19,20,21	" "	50	-7.5	100.0
22,23,24	" "	100	-4.7	100.0



Tinofix CL – 4hr BOD plot



Tinofix CL – 14day BOD plot

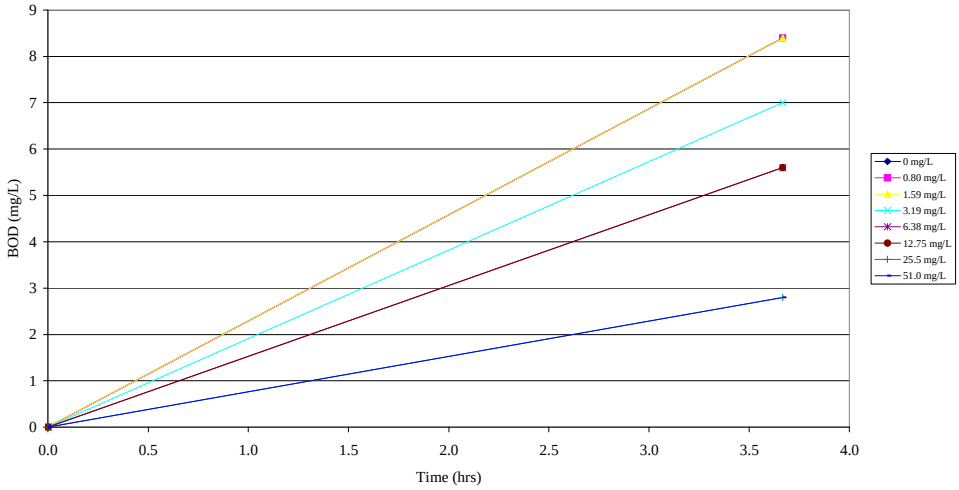
(LINEAR ALKYLBENZENE SULPHONATE)

Nitrification Inhibition (raw data)

Flask No	**1**	**2**	**3**	**4**	**5**	**6**	**7 (ref)**
Concentration of Test substance (mg/L)	0	0.5	1.6	5.1	16.4	51.1	0
Oxidised N (mg/L)							
$N0_2$-N	0.555	0.69	0.59	0.589	0.526	0.946	0.013
$N0_3$-N	0.786	1.14	0.915	1.07	0.952	0.556	0.101
Total	1.341	1.83	1.505	1.659	1.478	1.502	0.114
mg of N (g.h) control	2.45						
% Inhibition	0	-39.9	-13.4	-25.9	-11.2	-13.1	

Respiration Inhibition (raw data)

Test Material LAS Vessel No		Test Concn mg/l	BOD (avg) 4hr	% Inhib 4hr
1,2	Control	0.00	8.4	0.0
3,4	Test	0.80	8.4	0.0
5,6	" "	1.59	8.4	0.0
7,8	" "	3.19	7	16.7
9,10	" "	6.38	5.6	33.3
11,12	" "	12.75	5.6	33.3
13,14	" "	25.50	2.8	66.7
15,16	" "	51.00	2.8	66.7



Linear alkylbenzene sulphonate – 4hr BOD plot

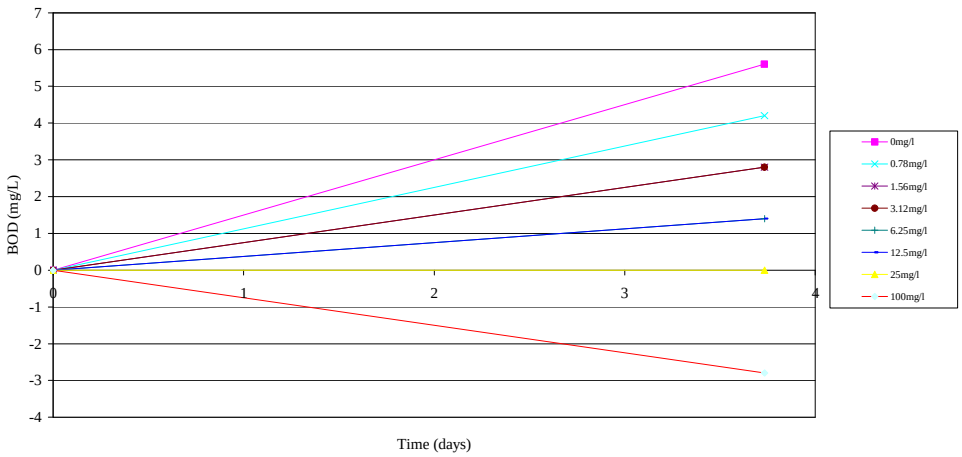
(LUTENSOL TO20)

Nitrification Inhibition (raw data)

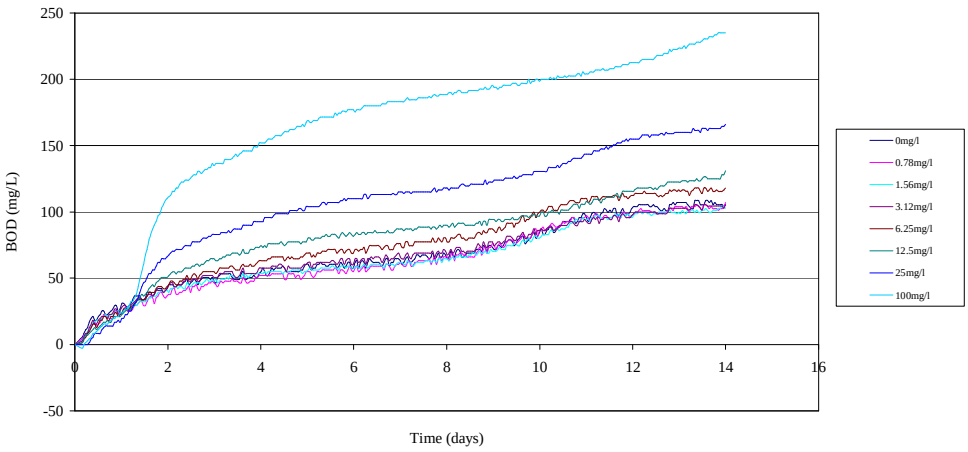
Flask No	**1**	**2**	**3**	**4**	**5**	**6**	**7 (ref)**
Concentration of Test substance (mg/L)	0	1	3.2	10	32	100	0
Oxidised N (mg//L)							
$N0_2$-N	0.53	0.502	0.531	0.508	0.52	0.421	0.008
$N0_3$-N	1.27	1.23	1.4	1.23	1.15	1.12	0.658
Total	1.8	1.732	1.931	1.738	1.67	1.541	0.666
mg of N (g.h) control	2.268						
% Inhibition	0	6.0	-11.6	5.5	11.5	22.8	

Respiration Inhibition (raw data)

Test Material $C_{13} - C_{15}$ EO_{20}		Test Concn mg/l	BOD (avg) 4hr	% Inhib 4hr
Vessel No				
1,2,	Control	0.0	5.6	0
3,4	Test	0.78	4.2	25.0
5,6	" "	1.56	2.8	50.0
7,8	" "	3.13	2.8	50.0
9,10	" "	6.25	1.4	75.0
11,12	" "	12.5	1.4	75.0
13,14	" "	25	-2.8	100.0
15,16	" "	50	0.7	87.5
17,18	" "	100	-2.8	100.0



Lutensol TO20 – 4hr BOD plot

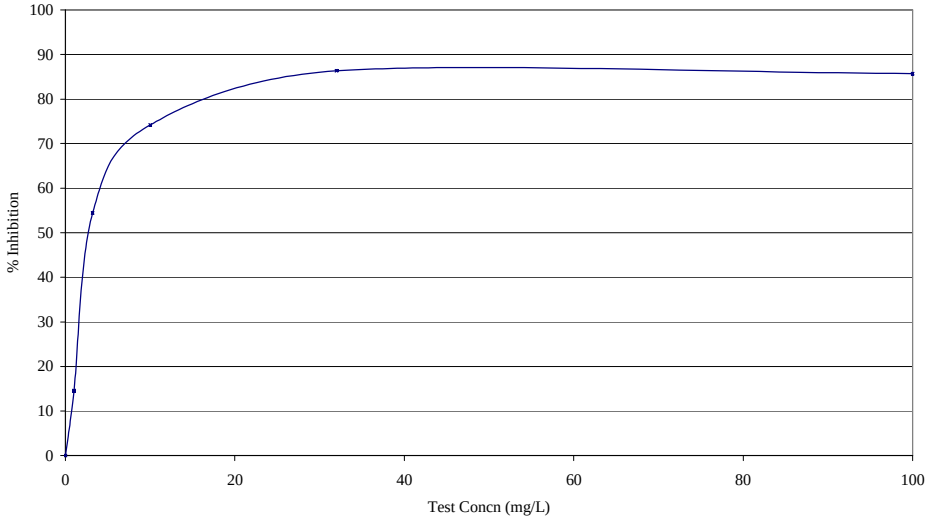


Lutensol TO20 – 14day BOD plot

(ANILINE)

Nitrification Inhibition (raw data)

Flask No	**1**	**2**	**3**	**4**	**5**	**6**	**7 (ref)**
Concentration of Test substance (mg/L)	0	1	3.2	10	32	100	2.5
Oxidised N (mg/L)							
N02-N	0.382	0.244	0.071	0.015	0.007	0.014	0.007
N03-N	1.3	1.28	1.02	0.862	0.738	0.738	0.59
Total	1.682	1.524	1.091	0.877	0.745	0.752	0.597
mg of N (g.h)	2.2						
% inhibition	0	14.6	54.5	74.2	86.4	85.7	

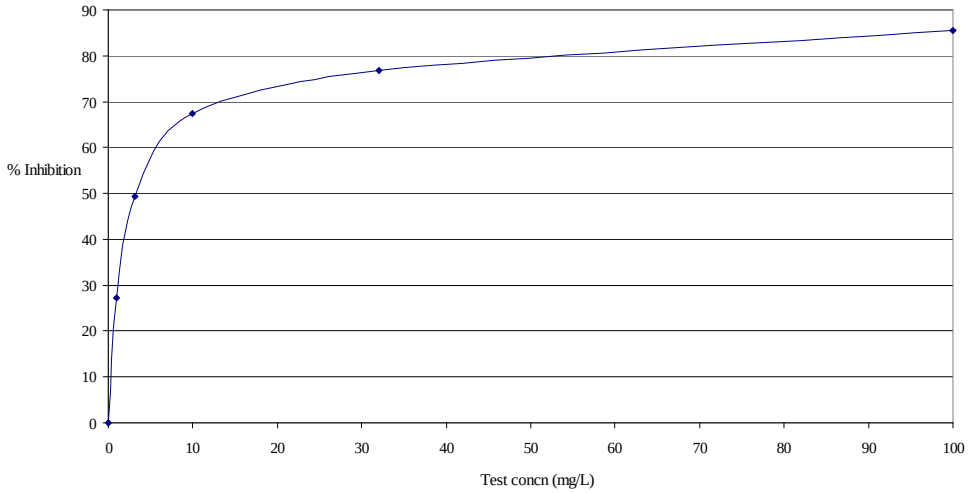


Plot of percentage inhibition versus test material concentration

(PHENOL)

Nitrification Inhibition (raw data)

Flask No	**1**	**2**	**3**	**4**	**5**	**6**	**7 (ref)**
Concentration of Test substance (mg/L)	0	1	3.2	10	32	100	2.5
Oxidised N (mg/l)							
$N0_2$-N	0.286	0.035	0.007	0.009	0.01	0.005	0.005
$N0_3$-N	1.12	1.06	0.845	0.645	0.54	0.45	0.29
Total	1.406	1.095	0.852	0.654	0.55	0.455	0.295
mg of N (g.h)	2.22						
% inhibition	0.0	28.0	49.9	67.7	77.0	85.6	



Plot of percentage inhibition versus test material concentration.

APPENDIX 3

Biodegradation of an anionic surfactant in a continuous flow simulation of untreated discharge conditions (Raw Data)

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14C Aniline LSC – Raw data

	EDE050025	14C ANILINE									
	10/03/2005										
	Sample Code	14C TOC	Mean dpm	14C TOC - Control (dpm)	conc as Aniline(ug/L)	14C DOC	Mean dpm	14C DOC - Control (dpm)	conc as Aniline(ug/L)	14C TOC - 14C DOC	% Biodeg
	C1	35.31				78.48					
	(Test Media)	32.57				81.64					
Dist (m)		30.09	32.66			81.34	80.69				
0	AU	2364.07			11.77						
(inlet)		2355.98			0.000012						
		2368.32	2362.99	2330.33	198027031						
0.9	BU	1644.87			7.88	1107.27			4.94		
		1546.70			0.000008	1040.55			0.000005		
		1530.08	1593.88	1561.23	198027031	1029.32	1053.05	978.36	198027031	1352	58.02
1.8	CU	1526.93			7.34	928.88			4.17		
		1436.27			0.000007	902.76			0.000004		
		1437.26	1486.82	1454.16	198027031	888.60	906.75	826.06	198027031	1504	64.55
1.9	DU	1413.05			6.80	823.67			3.79		
		1385.62			0.000007	837.04			0.000004		
		1340.92	1379.86	1347.21	198027031	827.85	831.52	750.83	198027031	1580	67.78
2.7	EU	1105.03			5.55	666.62			2.91		
		1150.34			0.000006	655.01			0.000003		
		1136.90	1130.96	1098.30	198027031	650.91	657.51	576.83	198027031	1754	75.25
3.6	FU	1041.92			5.22	616.57			2.68		
		1082.06			0.000005	607.64			0.000003		
		1077.03	1067.00	1034.35	198027031	607.75	610.65	529.97	198027031	1800	77.26
3.7	GU	773.83			4.23	656.32			2.76		
		704.11			0.000004	606.59			0.000003		
		1125.05	867.66	837.01	198027031	621.19	628.03	547.35	198027031	1783	76.51
4.5	HU	978.12			4.87	588.42			2.52		
		1004.33			0.000005	583.98			0.000003		
		1010.90	998.00	965.35	198027031	565.93	579.44	498.76	198027031	1832	78.60
5.4	IU	888.41			4.23	505.70			2.15		
		840.11			0.000004	485.04			0.000002		
		885.25	871.26	838.60	198027031	527.17	505.97	425.28	198027031	1905	81.75
5.5	JU	888.46			4.24	576.76			2.41		
		857.97			0.000004	577.48			0.000002		
		872.07	872.83	840.18	198027031	519.37	557.87	477.18	198027031	1853	79.52
6.3	KU	716.35			3.53	488.63			2.04		
		731.15			0.000004	510.96			0.000002		
		747.17	731.56	698.90	198027031	452.11	483.90	403.21	198027031	1927	82.70
7.2	LU	644.35			2.32	401.57			1.60		
		595.80			0.000003	398.72			0.000002		
		591.85	610.67	578.01	198027031	394.29	398.19	317.51	198027031	2013	86.38
7.3	MU	612.85			2.74	394.80			1.52		
		554.20			0.000003	379.47			0.000002		
		559.36	575.67	543.01	198027031	371.70	381.99	301.30	198027031	2029	87.07
8.1	NU	482.61			2.16	352.76			1.30		
		454.41			0.000002	343.24			0.000001		
		444.15	460.39	427.73	198027031	318.24	338.08	257.39	198027031	2073	88.95
9	OU	272.08			1.22	230.47			0.71		
		272.41			0.000001	223.29			0.000001		
		278.94	274.48	241.82	198027031	211.58	221.78	141.09	198027031	2189	93.95

14C Aniline LSC – Raw data

	11/03/2005										
	Sample Code	14C TOC	Mean	14C TOC - Control	conc as Aniline (ug/l)	14C DOC	Mean	14C DOC - Control	conc as Aniline (ug/L)	14C TOC - 14C DOC	% Biodeg
Test Media	C1	35.34				50.48					
		31.65				45.92					
		34.80	33.93			52.52	49.64				
0	AU	2247..41			11.15						
(inlet)		2253.54			0.000011						
		2225.26	2242.40	2208.47	198027031						
0.9	BU	1560.18			7.85	1290.36			6.16		
		1717.75			0.000008	1287.02			0.000006		
		1485.34	1587.76	1553.83	198027031	1234.00	1270.46	1220.82	198027031	366	44.72
1.8	CU	1419.81			6.54	1030.45			5.75		
		1248.32			0.000007	1165.20			0.000006		
		1315.99	1328.04	1294.11	198027031	1371.52	1189.06	1139.42	198027031	1063	48.41
1.9	DU	1280.22			6.04	1018.32			4.68		
		1179.09			0.000006	918.04			0.000005		
		1230.49	1229.93	1196.00	198027031	994.06	976.81	927.17	198027031	1281	58.02
2.7	EU	1011.69			5.35	670.95			3.04		
		1175.44			0.000005	654.18			0.000003		
		1095.06	1094.06	1060.13	198027031	627.34	650.82	601.18	198027031	1607	72.78
3.6	FU	1053.90			5.03	577.19			2.63		
		1057.35			0.000005	586.70			0.000003		
		978.35	1029.87	995.94	198027031	585.00	582.96	533.32	198027031	1675	75.85
3.7	GU	990.64			5.06	575.86			3.20		
		968.79			0.000005	869.48			0.000003		
		1148.79	1036.07	1002.14	198027031	603.51	682.95	633.31	198027031	1575	71.32
4.5	HU	1058.85			4.84	504.76			2.31		
		1009.08			0.000005	527.82			0.000002		
		908.58	992.17	958.24	198027031	489.26	507.28	457.64	198027031	1751	79.28
5.4	IU	930.03			4.47	465.27			2.09		
		894.65			0.000004	462.40			0.000002		
		931.90	918.86	884.93	198027031	464.12	463.93	414.29	198027031	1794	81.24
5.5	JU	941.63			4.39	435.54			2.21		
		855.07			0.000004	487.76			0.000002		
		910.56	902.42	868.49	198027031	476.56	486.62	436.98	198027031	1771	80.21
6.3	KU	827.69			4.07	445.84			2.01		
		820.85			0.000004	449.74			0.000002		
		874.06	840.87	806.94	198027031	448.85	448.14	398.50	198027031	1810	81.96
7.2	LU	796.68			3.86	407.98			1.80		
		782.93			0.000004	405.16			0.000002		
		813.88	797.83	763.90	198027031	406.75	406.63	356.99	198027031	1851	83.84
7.3	MU	801.20			3.76	393.82			1.80		
		771.49			0.000004	420.75			0.000002		
		765.44	779.38	745.45	198027031	402.56	405.71	356.07	198027031	1852	83.88
8.1	NU	802.73			3.78	383.22			1.67		
		773.07			0.000004	376.15			0.000002		
		773.29	783.03	749.10	198027031	382.61	380.66	331.02	198027031	1877	85.01
9	OU	761.08			3.50	346.53			1.51		
		730.88			0.000004	340.04			0.000002		
		691.37	727.78	693.85	198027031	360.37	348.98	299.34	198027031	1909	86.45

14C Aniline LSC – Raw data

	12/03/2005										
	Sample Code	14C TOC	Mean	14C TOC - Control	conc as Aniline (ug/L	14C DOC	Mean	14C DOC - Control		14CTOC - 14C DOC	% Biodeg
Test Media	C1	34.34				57.38					
		39.29				53.40					
		42.80	38.81			46.38	52.59				
0	AU	2261.36			11.24						
(inlet)		2285.87			0.000011						
		2243.75	2263.66	2224.85	138027031						
0.9	BU	1415.00			6.78	1228.95			5.39		
		1328.60			0.000007	1201.88			0.000006		
		1399.81	1381.14	1342.33	138027031	1285.40	1238.74	1186.16	138027031	1039	46.63
1.8	CU	1194.81			6.18	1119.37			5.40		
		1287.10			0.000006	1157.58			0.000005		
		1304.59	1262.17	1223.36	138027031	1088.30	1121.75	1063.16	138027031	1156	51.34
1.9	DU	1120.14			5.51	923.26			4.31		
		1159.29			0.000006	903.80			0.000004		
		1111.44	1130.29	1091.48	138027031	893.36	906.81	854.22	138027031	1371	61.61
2.7	EU	1044.00			5.11	787.23			3.70		
		1049.00			0.000005	772.39			0.000004		
		1061.75	1051.58	1012.77	138027031	794.77	785.00	732.41	138027031	1432	67.08
3.6	FU	1041.42			4.30	794.11			3.73		
		1036.13			0.000005	800.38			0.000004		
		951.59	1003.71	970.90	138027031	777.21	790.77	738.18	138027031	1487	66.82
3.7	GU	1012.26			4.31	764.25			3.75		
		1004.22			0.000005	873.33			0.000004		
		1015.22	1010.57	971.76	138027031	750.56	796.05	743.46	138027031	1481	66.58
4.5	HU	938.56			4.29	710.95			3.27		
		750.94			0.000004	687.95			0.000003		
		874.62	888.04	843.23	138027031	638.89	679.26	646.68	138027031	1578	70.33
5.4	IU	914.80			4.40	638.49			2.97		
		905.25			0.000004	647.89			0.000003		
		909.07	909.71	870.90	138027031	637.69	641.36	588.77	138027031	1636	73.54
5.5	JU	883.06			3.87	642.70			2.96		
		828.46			0.000004	639.70			0.000003		
		903.15	804.89	766.08	138027031	636.19	639.53	586.94	138027031	1638	73.62
6.3	KU	887.60			4.02	618.23			2.88		
		909.63			0.000004	625.39			0.000003		
		706.29	834.51	795.70	138027031	623.44	622.35	569.77	138027031	1655	74.39
7.2	LU	840.96			3.37	567.01			2.53		
		570.06			0.000003	527.88			0.000003		
		706.29	705.77	666.96	138027031	568.36	554.42	501.83	138027031	1723	77.44
7.3	MU	561.01			3.57	553.68			2.54		
		825.86			0.000004	553.59			0.000003		
		850.45	745.77	706.96	138027031	560.81	556.03	503.44	138027031	1721	77.37
8.1	NU	575.69			3.01	522.11			2.40		
		539.06			0.000003	533.40			0.000002		
		789.43	634.73	595.92	138027031	528.61	528.04	475.45	138027031	1749	78.63
9	OU	777.17			3.72	473.91			2.15		
		773.35			0.000004	481.54			0.000002		
		775.32	775.48	736.67	138027031	480.63	478.69	426.11	138027031	1799	80.85

14C Aniline LSC – Raw data

	13/03/2005										
	Sample Code	14C TOC	Mean	14C TOC - Control	conc as Aniline (ug/L	14C DOC	Mean	14C DOC - Control		14CTOC - 14C DOC	% Biodeg
Test Media	C1	35.14				39.67					
		37.75				40.38					
		34.02	35.64			47.38	42.88				
0	AU	1869.65			3.53						
(inlet)		1876.24			0.000010						
		2023.39	1923.29	1887.66	138027031						
0.3	BU	1544.03			7.64	1467.00			7.25		
		1580.41			0.000008	1666.74			0.000007		
		1519.16	1547.87	1512.23	138027031	1302.02	1478.59	1435.71	138027031	452	23.34
1.8	CU	1377.62			6.47	348.30			4.70		
		1298.22			0.000006	342.66			0.000005		
		1274.78	1316.87	1281.24	138027031	1023.84	373.60	330.72	138027031	357	50.69
1.9	DU	1333.12			5.89	304.04			3.90		
		1127.68			0.000006	778.33			0.000004		
		1144.76	1201.85	1166.22	138027031	764.61	615.86	772.38	138027031	1115	53.05
2.7	EU	1023.43			4.85	670.45			3.19		
		971.82			0.000005	665.32			0.000003		
		987.23	936.20	900.56	138027031	686.31	674.23	631.35	138027031	1256	66.55
3.6	FU	988.90			4.76	628.10			2.98		
		974.48			0.000005	633.66			0.000003		
		970.76	978.05	942.41	138027031	635.45	632.40	589.53	138027031	1238	68.77
3.7	GU	985.00			4.79	630.76			3.24		
		985.00			0.000005	769.17			0.000003		
		985.00	985.00	949.36	138027031	650.81	683.58	640.70	138027031	1247	66.06
4.5	HU	921.49			4.02	613.14			2.78		
		930.67			0.000004	574.65			0.000003		
		941.50	931.22	895.58	138027031	594.28	594.02	551.15	138027031	1337	70.80
5.4	IU	872.35			4.20	556.24			2.59		
		854.02			0.000004	553.88			0.000003		
		872.93	866.43	830.80	138027031	559.88	556.67	513.79	138027031	1374	72.78
5.5	JU	963.13			3.65	522.49			2.49		
		960.18			0.000004	540.48			0.000002		
		951.88	958.40	922.76	138027031	542.94	535.30	492.43	138027031	1395	73.91
6.3	KU	976.05			4.17	539.07			2.48		
		931.01			0.000004	525.16			0.000002		
		975.17	960.74	925.11	138027031	537.71	533.98	491.10	138027031	1397	73.98
7.2	LU	920.84			3.86	473.33			2.13		
		923.12			0.000004	476.14			0.000002		
		958.51	900.82	865.19	138027031	482.17	477.21	434.34	138027031	1453	76.99
7.3	MU	985.84			3.77	473.33			2.18		
		902.41			0.000004	473.19			0.000002		
		958.65	982.30	946.66	138027031	475.12	474.08	431.20	138027031	1456	77.16
8.1	NU	988.81			3.81	467.36			2.17		
		995.28			0.000004	475.00			0.000002		
		983.47	989.19	953.55	138027031	476.38	472.91	430.04	138027031	1458	77.22
9	OU	943.52			3.57	432.69			1.98		
		941.84			0.000004	429.73			0.000002		
		943.76	943.04	907.40	138027031	440.18	434.20	391.32	138027031	1436	79.27

14C Aniline LSC – Raw data

	14/03/2005										
	Sample Code	14C TOC	Mean	14C TOC - Control	conc as Aniline	14C DOC	Mean	14C DOC - Control	Aniline conc	14CTOC - 14C DOC	% Biodeg
	C1	40.40				40.35					
		37.92				41.78					
		35.52	37.95			37.66	39.93				
0	AU	1848.41			3.38						
(inlet)		1937.66			0.000003						
		1890.00	1892.02	1858.09	138027031		#REF!	#REF!			
0.9	BU	1638.03			8.07	868.58			4.07		
		1643.63			0.000008				0.000004		
		1611.08	1632.91	1598.98	138027031	844.27	856.43	806.79	138027031	1051	56.58
1.8	CU	1150.15			5.57	953.59			4.62		
		1125.86			0.000006				0.000005		
		1136.87	1137.63	1103.70	138027031	975.23	964.41	914.77	138027031	943	50.77
1.9	DU	978.14			4.42	625.98			2.75		
		797.14			0.000004	565.90			0.000003		
		951.13	908.80	874.87	138027031	626.01	594.13	544.49	138027031	1314	70.70
2.7	EU	920.48			4.56	590.47			2.70		
		978.48			0.000005	574.47			0.000003		
		910.53	936.50	902.57	138027031	586.43	583.79	534.15	138027031	1324	71.25
3.6	FU	854.25			4.44	504.71			2.47		
		933.63			0.000004	543.18			0.000002		
		887.44	913.77	879.84	138027031	564.62	539.50	489.86	138027031	1368	73.64
3.7	GU	1015.01			4.75	673.00			2.89		
					0.000005	569.23			0.000003		
		933.70	974.36	940.43	138027031		621.12	571.48	138027031	1287	69.24
4.5	HU	950.13			4.64	521.06			2.44		
		938.10			0.000005				0.000002		
		967.44	951.89	917.96	138027031	546.08	533.57	483.93	138027031	1374	73.96
5.4	IU	833.04			4.07	520.04			2.47		
		819.70			0.000004	536.19			0.000002		
		859.93	839.56	805.63	138027031	560.42	538.88	489.24	138027031	1369	73.67
5.5	JU	843.56			4.08	468.76			2.13		
		862.21			0.000004	472.05			0.000002		
		820.30	842.02	808.09	138027031	473.74	471.52	421.88	138027031	1436	77.30
6.3	KU	860.84			4.18	561.18			2.37		
		844.28			0.000004	512.41			0.000002		
		879.65	861.59	827.66	138027031	482.82	518.80	469.16	138027031	1389	74.75
7.2	LU	826.45			4.14	447.35			2.03		
		874.60			0.000004	455.71			0.000002		
		859.40	853.48	819.55	138027031	443.74	450.93	401.29	138027031	1457	78.40
7.3	MU	849.93			4.19	487.75			2.25		
		886.70			0.000004	439.80			0.000002		
		851.55	862.73	828.80	138027031	436.46	434.67	385.03	138027031	1413	76.05
8.1	NU	859.57			4.23	541.11			2.35		
		871.60			0.000004	529.11			0.000002		
		883.55	871.57	837.64	138027031	473.17	514.46	464.82	138027031	1393	74.98
9	OU	807.02			3.79	435.42			2.00		
		700.57			0.000004	453.03			0.000002		
		848.31	785.30	751.37	138027031	450.60	446.35	396.71	138027031	1461	78.65

14C Aniline LSC – Raw data

		15/03/2005									
	Sample Code	14C TOC	Mean	14C TOC - Control	aniline conc conc	14C DOC	Mean	14C DOC - Control	aniline conc	14CTOC - 14C DOC	% Biodeg
	C1	34.17				48.14					
		31.52				45.28					
		36.24	33.98			44.80	46.07				
0	AU	2027.31			10.08				#DIV/0!		
(inlet)		2045.84			0.000010				#DIV/0!		
		2018.46	2030.54	1996.56	198027031		#DIV/0!	#DIV/0!	#DIV/0!		
0.3	BU	1040.86			6.68	931.46			4.60		
		1277.11			0.000007	921.91			0.000005		
		1750.88	1356.28	1322.31	198027031	1016.74	956.70	910.63	198027031	1086	54.39
1.8	CU				5.50	862.16			4.19		
		970.11			0.000006	896.06			0.000004		
		1276.29	1123.20	1089.22	198027031	870.99	876.40	830.33	198027031	1166	58.41
1.9	DU	577.74			3.64	583.00			2.64		
		1022.96			0.000004	581.03			0.000003		
		665.25	755.32	721.34	198027031	544.57	569.53	523.46	198027031	1473	73.78
2.7	EU	899.35			3.37	520.90			2.37		
		769.09			0.000004	506.37			0.000002		
		793.17	820.74	786.76	198027031	518.26	515.18	463.10	198027031	1527	76.50
3.6	FU	894.45			4.24	531.09			2.34		
		857.81			0.000004	502.38			0.000002		
		868.09	873.45	839.47	198027031	493.95	509.14	463.07	198027031	1533	76.81
3.7	GU	1032.77			4.71	709.27			3.09		
		890.05			0.000005	681.59			0.000003		
		374.80	965.87	931.90	198027031	582.06	657.64	611.57	198027031	1385	69.37
4.5	HU	860.32			4.15	481.31			2.49		
		835.72			0.000004	664.08			0.000002		
		870.30	855.45	821.47	198027031	472.97	539.45	493.38	198027031	1503	75.29
5.4	IU	838.93			3.21	435.47			2.00		
		465.00			0.000003	440.04			0.000002		
		706.19	670.04	636.06	198027031	450.31	441.94	395.87	198027031	1601	80.17
5.5	JU	808.47			3.87	432.85			1.95		
		822.61			0.000004	437.22			0.000002		
		768.28	799.79	765.81	198027031	426.94	432.34	386.26	198027031	1610	80.65
6.3	KU	843.83			4.12	428.58			#VALUE!		
		844.41			0.000004	446.31			#VALUE!		
		854.53	849.59	815.61	198027031	437.73	437.54	391.47	#VALUE!	1605	80.39
7.2	LU	836.89			3.37	421.66			1.91		
		799.07			0.000004	420.30			0.000002		
		825.81	820.59	786.61	198027031	428.78	423.58	377.51	198027031	1619	81.09
7.3	MU	780.23			3.81	438.38			1.89		
		780.87			0.000004	412.23			0.000002		
		803.08	788.06	754.08	198027031	403.31	420.17	374.10	198027031	1622	81.26
8.1	NU	805.81			3.31	441.02			1.90		
		813.06			0.000004	413.63			0.000002		
		806.02	808.30	774.32	198027031	403.43	421.36	375.29	198027031	1621	81.20
9	OU	724.49			3.21	422.50			1.91		
		818.30			0.000003	422.43			0.000002		
		467.33	670.04	636.06	198027031	425.69	423.54	377.47	198027031	1619	81.09

14C Aniline LSC – Raw data

	16/03/2005										
	Sample Code	14C TOC	Mean	14C TOC - Control	aniline conc	14C DOC	Mean	14C DOC - Control	aniline conc	14CTOC - 14C DOC	% Biodeg
	C1	88.32				44.45					
		81.03				37.46					
		83.37	84.44			46.31	42.74				
0	AU	2228.08			10.85						
(inlet)		2235.85			0.000011						
		2232.54	2232.16	2147.72	138027031						
0.9	BU	1382.40			6.70	376.21			4.77		
		1442.55			0.000007	382.11			0.000005		
		1408.64	1411.20	1326.76	138027031	1006.63	388.32	345.58	138027031	1202	55.37
1.8	CU	1701.97			8.02	802.18			3.46		
		909.18			0.000006	712.45			0.000003		
		1216.39	1275.85	1191.41	138027031	669.30	727.98	685.24	138027031	1462	68.03
1.9	DU	1025.06			4.77	561.52			2.54		
		1033.57			0.000005	532.15			0.000003		
		1023.77	1029.47	945.03	138027031	544.68	546.12	503.38	138027031	1644	76.56
2.7	EU	979.43			4.65	526.13			2.34		
		1011.90			0.000005	507.17			0.000002		
		1026.80	1006.04	921.60	138027031	483.45	505.58	462.84	138027031	1685	78.45
3.6	FU	966.24			4.51	459.70			2.11		
		1016.69			0.000005	480.33			0.000002		
		947.99	976.97	892.53	138027031	442.33	460.79	418.05	138027031	1730	80.54
3.7	GU	979.43			4.51	525.39			2.36		
		954.26			0.000005	496.68			0.000002		
		1001.03	978.24	893.80	138027031	508.62	510.23	467.49	138027031	1680	78.23
4.5	HU	942.01			4.26	471.15			2.07		
		940.31			0.000004	453.08			0.000002		
		904.15	928.82	844.38	138027031	433.46	452.56	409.82	138027031	1738	80.92
5.4	IU	870.81			3.90	402.92			1.77		
		840.20			0.000004	392.08			0.000002		
		857.63	856.21	771.77	138027031	383.34	392.78	350.04	138027031	1738	80.70
5.5	JU	873.65			3.87	479.22			1.93		
		827.93			0.000004	396.11			0.000002		
		848.93	850.17	765.73	138027031	402.14	425.82	383.08	138027031	1765	82.16
6.3	KU	798.73			3.60	350.27			1.59		
		808.38			0.000004	357.51			0.000002		
		783.17	796.76	712.32	138027031	365.36	357.91	315.17	138027031	1833	85.33
7.2	LU	740.04			3.38	334.69			1.53		
		732.03			0.000003	348.11			0.000002		
		791.73	754.60	670.16	138027031	356.47	346.42	303.68	138027031	1844	85.86
7.3	MU	758.23			3.40	332.16			1.42		
		744.29			0.000003	314.36			0.000001		
		770.60	757.71	673.27	138027031	324.89	323.80	281.06	138027031	1867	86.91
8.1	NU	760.32			3.24	309.07			1.39		
		695.70			0.000003	321.17			0.000001		
		724.54	726.85	642.41	138027031	325.31	318.52	275.78	138027031	1872	87.16
9	OU	398.83			2.39	313.41			1.36		
		531.34			0.000002	303.84			0.000001		
		740.41	557.19	472.75	138027031	317.23	311.51	268.77	138027031	1879	87.43

14C Aniline LSC – Raw data

	17/03/2005										
	Sample Code	14C TOC	Mean	14C TOC - Control	aniline conc	14C DOC	Mean	14C DOC - Control	aniline conc	14CTOC - 14C DOC	% Biodeg
	C1	44.84				59.94					
		43.10				56.76					
		44.08	44.01			72.63	63.11				
0	AU	2002.46			3.20	1702.59			#VALUE!		
(inlet)		1876.43			0.000003	1681.93			#VALUE!		
		1838.55	1905.81	1821.37	138027031	1705.51	1696.68	1653.94	#VALUE!		
0.3	BU	1770.58			8.56	737.06			3.74		
		1801.10			0.000003	802.41			0.000004		
		1768.14	1779.94	1695.50	138027031	811.02	783.50	740.76	138027031	1081	58.33
1.8	CU	1652.14			6.08	596.76			2.83		
		1105.10			0.000006	625.33			0.000003		
		1107.43	1288.24	1203.80	138027031	589.26	603.78	561.04	138027031	1260	63.20
1.9	DU	1011.53			5.75	533.63			2.47		
		1083.98			0.000006	529.25			0.000002		
		1575.31	1223.83	1139.39	138027031	531.50	531.46	488.72	138027031	1333	73.17
2.7	EU	971.61			3.86	555.26			2.55		
		603.50			0.000004	527.47			0.000003		
		973.89	849.67	765.23	138027031	563.31	548.68	505.94	138027031	1315	72.22
3.6	FU	1018.60			4.84	513.94			2.42		
		1044.43			0.000005	535.36			0.000002		
		1063.76	1042.28	957.84	138027031	515.83	521.71	478.97	138027031	1342	73.70
3.7	GU	951.17			4.44	629.14			2.66		
		953.86			0.000004	534.89			0.000003		
		985.09	963.37	878.93	138027031	541.52	568.52	525.78	138027031	1296	71.13
4.5	HU	900.16			4.33	507.15			2.33		
		934.83			0.000005	439.56			0.000002		
		1283.66	1061.55	977.11	138027031	505.83	504.18	461.44	138027031	1360	74.67
5.4	IU	874.82			4.54	455.41			2.10		
		874.21			0.000005	460.95			0.000002		
		1001.60	983.54	899.10	138027031	458.88	458.41	415.67	138027031	1406	77.18
5.5	JU	908.73			4.28	452.47			2.07		
		918.81			0.000004	466.71			0.000002		
		966.15	931.23	846.79	138027031	435.88	451.69	408.95	138027031	1412	77.55
6.3	KU	908.93			3.73	476.28			2.16		
		875.17			0.000004	457.56			0.000002		
		886.90	823.67	739.23	138027031	476.64	470.16	427.42	138027031	1394	76.53
7.2	LU	939.52			4.22	476.28			2.16		
		948.93			0.000004	457.56			0.000002		
		873.55	920.67	836.23	138027031	476.64	470.16	427.42	138027031	1394	76.53
7.3	MU	879.78			4.12	440.79			2.11		
		884.22			0.000004	457.39			0.000002		
		935.67	899.89	815.45	138027031	480.89	459.69	416.95	138027031	1404	77.11
8.1	NU	893.95			4.07	450.73			2.03		
		893.08			0.000004	436.39			0.000002		
		923.59	883.54	805.10	138027031	446.72	444.61	402.07	138027031	1419	77.92
9	OU	699.74			4.19	445.12			2.04		
		1005.61			0.000004	450.34			0.000002		
		1038.35	914.57	830.13	138027031	446.86	447.44	404.70	138027031	1417	77.78

14C Aniline LSC – Raw data

	21/03/2005										
	Sample Code	14C TOC	Mean	14C TOC - Control	aniline conc	14C DOC	Mean	14C DOC - Control	aniline conc	14CTOC - 14C DOC	% Biodeg
	C1	36.68				43.55					
		34.30				57.97					
		35.71	35.56			51.77	51.10				
0	AU	2015.13			3.19				#DIV/0!		
(inlet)		1847.20			0.000003				#DIV/0!		
		1850.00	1904.11	1819.67	198027031		#DIV/0!	#DIV/0!	#DIV/0!		
0.9	BU	1149.75			5.18	598.93			2.75		
		1144.26			0.000005	596.19			0.000003		
		1039.11	1111.04	1026.60	198027031	565.36	586.83	544.09	198027031	1276	70.10
1.8	CU	1110.20			5.24	436.08			2.01		
		1139.48			0.000005	460.82			0.000002		
		1116.55	1122.08	1037.64	198027031	423.09	440.00	397.26	198027031	1422	78.17
1.9	DU	972.85			4.55	423.86			1.90		
		980.12			0.000005	403.71			0.000002		
		1003.69	985.55	901.11	198027031	420.95	416.17	375.43	198027031	1444	79.37
2.7	EU	973.51			4.53	382.61			1.68		
		947.66			0.000005	363.17			0.000002		
		1025.08	982.08	897.64	198027031	379.81	375.20	332.46	198027031	1487	81.73
3.6	FU	983.51			4.47	353.19			1.48		
		954.28			0.000004	327.86			0.000001		
		972.56	970.12	885.68	198027031	325.92	335.66	292.92	198027031	1527	83.90
3.7	GU	917.62			4.39	316.89			1.42		
		969.14			0.000004	346.97			0.000001		
		1358.03	953.38	868.94	198027031	308.02	323.96	281.22	198027031	1538	84.55
4.5	HU	897.01			4.17	336.74			1.44		
		893.67			0.000004	327.89			0.000001		
		936.40	911.03	826.59	198027031	321.24	328.62	285.88	198027031	1534	84.29
5.4	IU	878.60			3.83	285.47			1.21		
		808.07			0.000004	277.34			0.000001		
		839.31	842.19	757.75	198027031	285.86	283.03	240.35	198027031	1579	86.79
5.5	JU	883.94			3.95	293.92			1.28		
		853.30			0.000004	288.61			0.000001		
		864.86	867.57	783.13	198027031	287.82	295.45	252.71	198027031	1567	86.11
6.3	KU	844.37			4.01	295.76			1.22		
		945.72			0.000004	273.32			0.000001		
		848.15	879.41	794.97	198027031	285.55	284.88	242.14	198027031	1578	86.69
7.2	LU	803.75			3.58	282.44			1.18		
		753.01			0.000004	279.35			0.000001		
		821.23	792.66	708.22	198027031	268.97	276.92	234.18	198027031	1585	87.13
7.3	MU	798.51			3.55	272.85			1.15		
		779.70			0.000004	277.83			0.000001		
		782.46	786.89	702.45	198027031	263.11	271.26	228.52	198027031	1591	87.44
8.1	NU	806.73			3.45	267.78			1.15		
		737.08			0.000003	274.86			0.000001		
		761.29	768.37	683.93	198027031	268.41	270.35	227.61	198027031	1592	87.49
9	OU	765.64			3.42	230.05			1.04		
		756.25			0.000003	259.75			0.000001		
		765.48	762.46	678.02	198027031	256.77	248.86	206.12	198027031	1614	88.67

14C Aniline LSC – Raw data

	22/03/2005											
	Sample Code	14C TOC	Mean	14C TOC - Control	aniline conc	14C DOC	Mean	14C DOC - Control	aniline conc	14CTOC - 14C DOC	% Biodeg	14CTOC - 14C DOC
	C1	40.60				39.80						
		40.22				41.31						
		40.77	40.53			41.83	41.18					
0	AU	1862.68			3.20				#DIV/0!			
(inlet)		1935.13			0.000003				#DIV/0!			
		1862.57	1906.81	1822.37	138027031		#DIV/0!	#DIV/0!	#DIV/0!			#DIV/0!
0.9	BU	1458.27			6.90	359.43			4.45			
		1430.06			0.000007	314.32			0.000004			
		1461.89	1450.07	1365.63	138027031	838.64	324.13	881.33	138027031	341	51.64	484.24
1.8	CU	1475.41			6.33	605.75			2.84			
		1145.43			0.000007	605.42			0.000003			
		1751.36	1457.62	1373.18	138027031	603.41	604.86	562.12	138027031	1260	69.15	811.06
1.9	DU	1003.85			4.63	473.58			2.12			
		1001.15			0.000005	461.95			0.000002			
		996.71	1000.57	916.13	138027031	453.17	462.90	420.16	138027031	1402	76.34	435.37
2.7	EU	333.12			4.15	395.42			1.83			
		330.25			0.000004	411.02			0.000002			
		846.72	505.36	820.92	138027031	410.32	405.79	363.05	138027031	1459	80.06	457.88
3.6	FU	873.67			4.21	379.72			1.76			
		922.06			0.000004	353.14			0.000002			
		960.08	918.60	834.16	138027031	441.06	391.31	348.57	138027031	1474	80.87	485.60
3.7	GU	871.58			3.97	475.96			1.99			
					0.000004	397.62			0.000002			
			871.58	787.14	138027031		436.79	394.05	138027031	1428	78.38	393.09
4.5	HU	830.61			3.14	346.85			1.52			
		900.17			0.000003	334.24			0.000002			
		987.00	705.93	621.49	138027031	352.68	344.59	301.85	138027031	1521	83.44	319.64
5.4	IU	1021.64			3.20	289.76			1.23			
		803.73			0.000003	285.98			0.000001			
		930.78	918.72	834.28	138027031	283.98	286.57	243.83	138027031	1579	86.62	390.44
5.5	JU	795.04			3.58	298.71			1.32			
		805.34			0.000004	293.37			0.000001			
		778.43	792.94	708.50	138027031	314.46	304.18	261.44	138027031	1561	85.65	447.06
6.3	KU	323.49			2.74	289.99			1.26			
		784.73			0.000003	292.67			0.000001			
		774.21	627.48	543.04	138027031	295.64	292.77	250.03	138027031	1572	86.28	293.01
7.2	LU	729.04			2.57	276.87			1.18			
		734.10			0.000003	271.80			0.000001			
		314.82	592.65	508.21	138027031	280.27	276.31	233.57	138027031	1589	87.18	274.64
7.3	MU	729.50			2.56	278.90			1.20			
		308.27			0.000003	285.00			0.000001			
		736.43	592.07	507.63	138027031	279.56	281.15	238.41	138027031	1584	86.32	269.21
8.1	NU	722.38			2.50	274.70			1.23			
		305.66			0.000002	288.21			0.000001			
		708.54	578.86	494.42	138027031	293.18	285.36	242.62	138027031	1580	86.63	251.80
9	OU	630.09			3.10	263.82			1.17			
		716.63			0.000003	280.69			0.000001			
		691.10	679.27	614.83	138027031	271.88	274.13	231.39	138027031	1531	87.30	383.44

14C Phenyl 6 DOBS LSC – Raw data

	07/05/2005										
	Sample Code	14C TOC	Mean dpm	14C TOC - Control (dpm)	conc as 6-DOBS	14C DOC	Mean dpm	14C DOC - Control (dpm)	conc as 6-DOBS	% Biodeg	14C TOC - 14C DOC
	C1	35.74				32.03					
	(Test Media)	31.19				32.65					
Dist (m)		33.71	33.55			32.37	32.55				
0	AU	3416.00			28.69	3738.25			#VALUE!		
(inlet)		3434.43			0.000023	3727.22			#VALUE!		
		3730.00	3526.81	3493.26	121771773	3531.10	3665.52	3632.97	#VALUE!		
0.9	BU	1734.00			13.57	1416.74			11.01		
		1643.00			0.000014	1400.01			0.000011		
		1674.00	1685.67	1652.12	121771773	1303.07	1373.27	1340.72	121771773	61.62	311.40
1.8	CU	1524.82			12.10	1156.96			9.03		
		1507.08			0.000012	1088.43			0.000009		
		1483.09	1507.00	1473.45	121771773	1143.51	1131.63	1099.08	121771773	68.54	374.37
1.9	DU	1109.06			9.43	770.30			5.76		
		1197.66			0.000009	675.57			0.000006		
		1237.34	1181.35	1147.81	121771773	756.63	734.17	701.62	121771773	79.32	446.19
2.7	EU	1051.14			8.21	580.80			4.63		
		1013.55			0.000008	592.90			0.000005		
		1033.84	1032.84	999.30	121771773	613.96	595.89	563.34	121771773	83.87	435.96
3.6	FU	976.95			7.92	587.00			4.43		
		966.51			0.000008	566.81			0.000004		
		1043.80	997.75	964.21	121771773	560.41	571.41	538.86	121771773	84.57	425.35
3.7	GU	1476.00			10.96	955.64			7.40		
		1259.33			0.000011	911.72			0.000007		
			1367.70	1334.15	121771773		933.68	901.13	121771773	74.20	433.02
4.5	HU	1084.36			8.51	616.09			4.69		
		1078.49			0.000009	603.26			0.000005		
		1047.9	1070.25	1036.70	121771773	586.60	603.98	571.43	121771773	83.64	465.27
5.4	IU	962.62			7.68	467.91			3.51		
		1007.79			0.000008	452.02			0.000004		
		934.34	968.45	934.90	121771773	459.18	459.70	427.15	121771773	87.77	507.75
5.5	JU	1008.81			8.51	568.23			4.18		
		1083.67			0.000009	515.15			0.000004		
		1116.46	1069.65	1036.10	121771773	540.13	541.17	508.62	121771773	85.44	527.48
6.3	KU	1086.66			8.65	614.10			4.68		
		1088.24			0.000009	618.62			0.000005		
		1087.32	1087.41	1053.86	121771773	573.85	602.19	569.64	121771773	83.69	484.22
7.2	LU	1125.92			9.09	637.72			5.11		
		1128.20			0.000009	643.87			0.000005		
		1166.75	1140.29	1106.74	121771773	678.03	655.21	622.66	121771773	82.18	484.09
7.3	MU	1172.08			9.33	594.90			4.58		
		1148.46			0.000009	604.13			0.000005		
		1189.56	1170.03	1136.49	121771773	573.28	590.77	558.22	121771773	84.02	578.27
8.1	NU	1126.48			8.92	526.07			3.91		
		1113.43			0.000009	505.14			0.000004		
		1118.67	1119.53	1085.98	121771773	496.44	509.22	476.67	121771773	86.35	609.31
9	OU	1056.20			8.47	438.41			3.32		
		1059.47			0.000008	429.14			0.000003		
		1078.13	1064.60	1031.05	121771773	442.36	436.64	404.09	121771773	88.43	626.97

14C Phenyl 6 DOBS LSC – Raw data

	08/05/2005									
	Sample Code	14C TOC	Mean	14C TOC - Control	conc as 6-DOBS	14C DOC	Mean	14C DOC - Control	conc as 6-DOBS	% Biodeg
Test Media	C1	31.20				34.62				
		32.59				30.68				
		32.63	32.14			30.04	31.78			
0	AU	4330.85			33.88				#DIV/0!	
(inlet)		4306.36			0.000040				#DIV/0!	
		4767.08	4888.10	4855.96	121771773		#DIV/0!	#DIV/0!	#DIV/0!	
0.9	BU	1879.30			15.26	1638.35			13.33	
		1923.26			0.000015	1625.54			0.000013	
		1870.31	1890.96	1858.82	121771773	1701.33	1655.07	1623.29	121771773	66.57
1.8	CU	1718.63			13.44	1522.76			11.74	
		1642.22			0.000013	1429.93			0.000012	
		1647.03	1669.29	1637.15	121771773	1432.74	1461.81	1430.03	121771773	70.55
1.9	DU	1682.58			13.45	1221.68			10.34	
		1638.22			0.000013	1291.14			0.000010	
		1683.84	1670.21	1638.07	121771773	1359.43	1290.82	1259.04	121771773	74.07
2.7	EU	1469.00			11.80	1152.93			8.93	
		1426.74			0.000012	1120.86			0.000009	
		1510.16	1468.63	1436.49	121771773	1082.31	1118.72	1086.94	121771773	77.62
3.6	FU	1411.42			11.48	1103.68			8.69	
		1436.52			0.000011	1090.94			0.000009	
		1440.89	1429.61	1397.47	121771773	1076.52	1090.38	1058.60	121771773	78.20
3.7	GU	1793.75			14.23	1441.48			11.34	
		1855.18			0.000014	1427.53			0.000012	
		1645.40	1764.78	1732.64	121771773	1587.63	1485.55	1453.77	121771773	70.06
4.5	HU	1491.2			9.30	922.86			7.05	
		1346.52			0.000009	865.99			0.000007	
		1333.4	1164.11	1131.97	121771773	882.90	890.58	858.80	121771773	82.31
5.4	IU	1161.88			9.30	792.28			6.26	
		1179.00			0.000009	785.34			0.000006	
		1151.46	1164.11	1131.97	121771773	805.49	794.37	762.59	121771773	84.30
5.5	JU	1313.49			10.55	885.67			7.11	
		1275.99			0.000011	911.72			0.000007	
		1360.63	1316.70	1284.56	121771773	893.93	897.11	865.33	121771773	82.18
6.3	KU	1238.50			9.61	734.91			5.53	
		1199.91			0.000010	706.70			0.000006	
		1167.64	1202.02	1169.88	121771773	673.71	705.11	673.33	121771773	86.13
7.2	LU	1155.02			9.18	663.65			5.02	
		1145.53			0.000009	625.45			0.000005	
		1149.21	1149.92	1117.78	121771773	638.74	642.61	610.83	121771773	87.42
7.3	MU	1212.83			10.09	648.35			5.50	
		1264.00			0.000010	716.41			0.000006	
		1305.22	1260.68	1228.54	121771773	740.96	701.91	670.13	121771773	86.20
8.1	NU	1214.37			9.37	580.83			4.51	
		1163.76			0.000009	580.08			0.000005	
		1140.24	1172.79	1140.65	121771773	582.29	581.07	549.29	121771773	88.69
9	OU	1092.57			8.70	506.40			3.89	
		1094.96			0.000009	520.12			0.000004	
		1087.27	1091.60	1059.46	121771773	489.18	505.23	473.45	121771773	90.25

14C Phenyl 6 DOBS LSC – Raw data

	09/05/2005									
	Sample Code	14C TOC	Mean	14C TOC - Control	conc as 6-DOBS	14C DOC	Mean	14C DOC - Control		% Biodeg
Test Media	C1	32.08				39.63				
		31.65				38.04				
		31.30	31.68			38.55	38.74			
0	AU	4647.15			39.18				#DIV/0!	
(inlet)		4754.10			0.000039				#DIV/0!	
		4721.62	4707.62	4675.95	119347166		#DIV/0!	#DIV/0!	119347166	
0.9	BU	2454.42			20.19	2279.53			18.92	
		2433.48			0.000020	2259.52			0.000019	
		2437.11	2441.67	2409.99	119347166	2350.56	2296.54	2257.80	119347166	51.71
1.8	CU	1586.01			13.59	1236.35			10.15	
		1636.42			0.000014	1293.14			0.000010	
		1739.69	1654.04	1622.36	119347166	1215.91	1250.47	1211.73	119347166	74.09
1.9	DU	1586.20			12.80	948.68			8.09	
		1457.39			0.000013	925.37			0.000008	
		1633.67	1559.09	1527.41	119347166	1137.38	1003.81	365.07	119347166	79.36
2.7	EU	1280.11			10.12	693.19			5.69	
		1244.86			0.000010	756.20			0.000006	
		1192.79	1239.25	1207.58	119347166	705.31	718.23	679.49	119347166	85.47
3.6	FU	1204.07			9.83	606.75			4.93	
		1206.54			0.000010	632.73			0.000005	
		1203.81	1204.81	1173.13	119347166	643.24	627.57	588.83	119347166	87.41
3.7	GU	1787.29			13.76	1153.26			8.41	
		1709.65			0.000014	981.38			0.000008	
		1523.76	1673.57	1641.89	119347166	991.40	1042.01	1003.27	119347166	78.54
4.5	HU	1338.40			10.87	700.69			5.41	
		1330.00			0.000011	672.51			0.000005	
		1318.67	1329.02	1297.35	119347166	680.31	684.50	645.76	119347166	86.19
5.4	IU	1250.99			9.79	586.31			4.57	
		1167.89			0.000010	582.81			0.000005	
		1179.86	1199.58	1167.90	119347166	584.45	584.52	545.78	119347166	88.33
5.5	JU	1448.17			11.58	737.58			6.45	
		1407.25			0.000012	830.18			0.000006	
		1384.05	1413.16	1381.48	119347166	796.80	808.19	769.45	119347166	83.54
6.3	KU	1357.04			11.20	744.74			5.73	
		1409.26			0.000011	702.85			0.000006	
		1340.06	1368.79	1337.11	119347166	719.76	722.45	683.71	119347166	85.38
7.2	LU	1304.41			10.56	613.07			4.85	
		1274.90			0.000011	637.86			0.000005	
		1297.11	1292.14	1260.46	119347166	602.72	617.88	579.14	119347166	87.61
7.3	MU	1403.49			11.29	674.37			5.37	
		1352.78			0.000011	687.77			0.000005	
		1380.39	1378.89	1347.21	119347166	677.82	679.99	641.25	119347166	86.29
8.1	NU	1368.77			11.05	592.79			4.42	
		1370.80			0.000011	538.63			0.000004	
		1312.57	1350.71	1319.04	119347166	566.00	565.81	527.07	119347166	88.73
9	OU	1246.53			10.33	462.79			3.63	
		1362.11			0.000010	475.65			0.000004	
		1185.23	1264.62	1232.95	119347166	477.70	472.05	433.31	119347166	90.73

14C Phenyl 6 DOBS LSC – Raw data

	10/05/2005									
	Sample Code	14C TOC	Mean	14C TOC - Control	conc as 6-DOBS	14C DOC	Mean	14C DOC - Control		% Biodeg
Test Media	C1	29.16				33.67				
		37.09				30.48				
		29.78	32.01			37.98	36.04			
0	AU	4564.40			38.03				#DIV/0!	
(inlet)		4551.24			0.000038				#DIV/0!	
		4536.14	4570.59	4538.58	119347166		#DIV/0!	#DIV/0!	119347166	
0.9	BU	2531.95			20.66	2485.00			20.37	
		2497.90			0.000021	2395.02			0.000020	
		2461.79	2497.21	2465.20	119347166	2522.52	2467.51	2431.47	119347166	46.43
1.8	CU	1331.51			11.50	1130.83			9.13	
		1331.98			0.000012	1183.09			0.000009	
		1431.14	1404.88	1372.87	119347166	1082.96	1132.29	1096.25	119347166	75.85
1.9	DU	1664.57			14.03	1388.24			11.08	
		1748.75			0.000014	1330.87			0.000011	
		1706.43	1706.58	1674.57	119347166	1355.32	1358.14	1322.10	119347166	70.87
2.7	EU	1221.24			10.38	797.98			6.37	
		1316.03			0.000010	796.63			0.000006	
		1273.55	1270.27	1238.26	119347166	793.00	795.87	759.83	119347166	83.26
3.6	FU	1260.51			9.77	800.28			6.09	
		1132.07			0.000010	753.26			0.000006	
		1161.73	1198.10	1166.09	119347166	734.93	762.82	726.78	119347166	83.99
3.7	GU	1366.95			11.71	853.51			6.68	
		1335.60			0.000012				0.000007	
		1585.88	1429.48	1397.47	119347166	813.05	833.28	797.24	119347166	82.43
4.5	HU	1272.93			10.30	737.80			5.92	
		1245.47			0.000010	730.81			0.000006	
		1263.74	1260.71	1228.70	119347166	759.45	742.69	706.64	119347166	84.43
5.4	IU	884.97			8.44	582.09			4.68	
		1125.59			0.000008	602.44			0.000005	
		1106.84	1039.13	1007.12	119347166	598.50	594.34	558.30	119347166	87.70
5.5	JU	1237.74			10.18	685.23			5.69	
		1198.05			0.000010	639.14			0.000006	
		1303.70	1246.50	1214.49	119347166	761.21	715.19	679.15	119347166	85.04
6.3	KU	992.79			9.09	602.29			4.79	
		1137.20			0.000009	615.25			0.000005	
		1221.13	1117.04	1085.03	119347166	604.16	607.23	571.19	119347166	87.41
7.2	LU	1092.64			9.21	511.01			4.10	
		1159.62			0.000009	524.28			0.000004	
		1142.52	1131.59	1099.58	119347166	539.32	524.87	488.83	119347166	89.23
7.3	MU	1244.15			10.32	589.67			4.60	
		1256.86			0.000010	578.92			0.000005	
		1289.60	1263.54	1231.53	119347166	587.53	585.37	549.33	119347166	87.90
8.1	NU	1279.27			10.02	589.38			4.60	
		1241.46			0.000010	578.92			0.000005	
		1164.31	1228.35	1196.34	119347166	587.53	585.28	549.23	119347166	87.90
9	OU	1224.43			9.89	485.00			3.83	
		1257.04			0.000010	435.53			0.000004	
		1154.30	1211.92	1179.91	119347166	437.87	452.80	456.76	119347166	89.94

14C Phenyl 6 DOBS LSC – Raw data

	11/05/2005									
	Sample Code	14C TOC	Mean	14C TOC - Control	conc as 6-DOBS	14C DOC	Mean	14C DOC - Control	6-DOBS conc	% Biodeg
Test Media	C1	29.83				30.36				
		29.32				32.51				
		33.36	31.04			33.79	32.22			
0	AU	5516.22			39.43					
(inlet)		4266.49			0.000039					
		4431.32	4738.01	4705.87	1.19E+08		#DIV/0!	#DIV/0!		
0.9	BU	4420.39			35.86	3832.95			34.71	
		4284.01			0.000036	4667.33			0.000035	
		4231.08	4311.83	4279.69	1.19E+08	4023.24	4174.51	4142.73	119347165.7	11.97
1.8	CU	3597.74			29.15	3400.00			27.47	
		3489.58			0.000029	3339.11			0.000027	
		3445.66	3510.33	3478.85	1.19E+08	3132.23	3310.45	3278.67	119347165.7	30.33
1.9	DU	2963.36			23.39	2868.90			24.13	
		2826.24			0.000024	2768.90			0.000024	
		2831.34	2835.85	2863.71	1.19E+08	3037.37	2911.72	2879.34	119347165.7	38.80
2.7	EU	2638.64			22.08	2095.04			18.32	
		2603.58			0.000022	2234.81			0.000018	
		2700.90	2667.71	2635.57	1.19E+08	2325.76	2218.54	2186.76	119347165.7	53.53
3.6	FU	2396.35			19.70	1847.44			14.22	
		2372.51			0.000020	1630.04			0.000014	
		2379.50	2382.79	2350.65	1.19E+08	1710.82	1729.43	1697.65	119347165.7	63.92
3.7	GU				18.97	1211.57			9.36	
		2443.52			0.000019	1228.13			0.000010	
		2147.81	2295.67	2263.53	1.19E+08	1222.41	1220.70	1188.92	119347165.7	74.74
4.5	HU	2117.72			16.25	1158.36			9.36	
		1827.79			0.000016	1141.10			0.000009	
		1970.59	1972.03	1939.89	1.19E+08	1144.79	1148.28	1116.50	119347165.7	76.27
5.4	IU	1684.00			14.67	889.25			7.26	
		1872.80			0.000015	902.64			0.000007	
		1791.28	1782.69	1750.55	1.19E+08	903.67	898.52	866.74	119347165.7	81.58
5.5	JU	1829.85			14.70	823.01			6.69	
		1806.28			0.000015	836.57			0.000007	
		1724.58	1786.90	1754.76	1.19E+08	831.47	830.35	798.57	119347165.7	83.03
6.3	KU	1654.65			13.64	703.90			4.46	
		1631.89			0.000014	692.92			0.000004	
		1631.38	1653.51	1627.37	1.19E+08	293.37	563.60	531.82	119347165.7	88.70
7.2	LU	1519.81			11.68	571.79			4.44	
		1346.32			0.000012	551.54			0.000004	
		1412.80	1426.31	1394.17	1.19E+08	562.00	561.78	530.00	119347165.7	88.74
7.3	MU	1512.18			12.14	529.83			4.29	
		1378.22			0.000012	541.48			0.000004	
		1553.35	1481.25	1449.11	1.19E+08	560.39	543.92	512.14	119347165.7	89.12
8.1	NU	1428.92			11.46	493.57			3.85	
		1422.67			0.000011	481.37			0.000004	
		1347.60	1399.73	1367.59	1.19E+08	493.67	491.54	459.76	119347165.7	90.23
9	OU	1330.54			10.69	428.01			3.33	
		1351.1			0.000011	428.95			0.000003	
		1242.15	1307.93	1275.79	1.19E+08	431.53	429.50	397.72	119347165.7	91.55

14C Phenyl 6 DOBS LSC – Raw data

		12/05/2005								
	Sample Code	14C TOC	Mean	14C TOC - Control	6-DOBS conc	14C DOC	Mean	14C DOC - Control	6-DOBS conc	% Biodeg
Test Media	C1	34.61				25.38				
		32.33				23.57				
		32.65	33.20			21.69	23.55			
0	AU	4565.53			38.18				#DIV/0!	
(inlet)		4589.87			0.000038				#DIV/0!	
		4615.04	4590.15	4556.95	119347166		#DIV/0!	#DIV/0!	#DIV/0!	
0.9	BU	4563.05			38.15	4361.69			36.29	
		4475.04			0.000038	4265.73			0.000036	
		4719.08	4585.72	4552.53	119347166	4437.03	4354.82	4331.27	119347166	4.95
1.8	CU	3530.41			29.08	2789.00			23.67	
		3519.26			0.000029	2980.00			0.000024	
		3461.86	3503.84	3470.65	119347166	2776.22	2848.41	2824.86	119347166	38.01
1.9	DU	3242.23			26.69	2676.40			21.24	
		3207.12			0.000027	2488.48			0.000021	
		3206.51	3218.62	3185.42	119347166	2511.23	2558.70	2535.16	119347166	44.37
2.7	EU	2802.67			22.33	2055.61			16.34	
		2808.00			0.000023	1927.43			0.000016	
		2693.37	2770.01	2736.82	119347166	1938.25	1973.76	1950.22	119347166	57.20
3.6	FU	2533.81			21.21	1533.00			12.66	
		2538.58			0.000021	1486.63			0.000013	
		2621.99	2564.79	2531.60	119347166	1584.42	1534.68	1511.14	119347166	66.84
3.7	GU				13.11	1424.52			11.44	
		2284.33			0.000019	1352.26			0.000011	
		2343.46	2313.90	2280.70	119347166		1388.39	1364.84	119347166	70.05
4.5	HU	2273.46			18.14				9.36	
		2238.46			0.000018	1213.05			0.000010	
		2215.45	2242.46	2209.26	121771773	1212.04	1212.55	1189.00	119347166	73.91
5.4	IU	1993.38			16.17	894.33			7.45	
		1939.21			0.000016	929.82			0.000007	
		1955.87	1962.82	1929.62	119347166	914.03	912.73	889.18	119347166	80.49
5.5	JU	1688.84			13.50	983.37			7.80	
		1617.56			0.000014	927.54			0.000008	
		1626.33	1644.44	1611.25	119347166	951.17	954.03	930.48	119347166	79.58
6.3	KU	1687.32			14.06	862.68			6.37	
		1712.76			0.000014	870.22			0.000007	
		1732.52	1710.87	1677.67	119347166	833.08	855.33	831.78	119347166	81.75
7.2	LU	1772.73			14.52	665.38			5.43	
		1750.97			0.000015	683.33			0.000005	
		1775.69	1766.46	1733.27	119347166	666.26	671.66	648.11	119347166	85.78
7.3	MU	1788.07			14.46	654.77			5.16	
		1731.27			0.000014	631.63			0.000005	
		1756.75	1758.70	1725.50	119347166	632.14	639.51	615.97	119347166	86.48
8.1	NU	1909.89			15.61	612.56			4.95	
		1919.22			0.000016	593.42			0.000005	
		1861.02	1896.71	1863.51	119347166	617.45	614.71	591.16	119347166	87.03
9	OU	1957.69			16.22	542.22			4.38	
		1945.86			0.000016	531.34			0.000004	
		2002.38	1968.64	1935.45	119347166	565.66	546.41	522.86	119347166	88.53

14C Phenyl 6 DOBS LSC – Raw data

	13/05/2005									
	Sample Code	14C TOC	Mean	14C TOC - Control	aniline conc	14C DOC	Mean	14C DOC - Control	aniline conc	% Biodeg
Test Media	C1	20.51				44.45				
		23.27				37.46				
		20.51	23.43			46.31	42.74			
0	AU	4764.60			39.35					
(inlet)		4722.89			0.000033					
		4673.03	4720.17	4696.74	1.19E+08		#DIV/0!	#DIV/0!		
0.9	BU	3603.37			29.77	3237.96			28.57	
		3687.34			0.000030	3410.06			0.000029	
		3436.87	3575.86	3552.43	1.19E+08	3648.63	3452.22	3409.48	1.19E+08	27.41
1.8	CU	3907.00			31.63	2933.28			23.92	
		3816.00			0.000032	2900.20			0.000024	
		3670.76	3797.92	3774.49	1.19E+08	2859.77	2897.75	2855.01	1.19E+08	39.21
1.9	DU	3441.59			28.58	2543.31			20.37	
		3446.21			0.000029	2520.83			0.000021	
		3414.81	3434.20	3410.77	1.19E+08	2567.01	2545.92	2503.18	1.19E+08	46.70
2.7	EU	3075.89			25.85	2117.32			17.69	
		3063.56			0.000026	2146.72			0.000018	
		3185.26	3108.24	3084.81	1.19E+08	2137.68	2133.91	2111.17	1.19E+08	55.05
3.6	FU	2778.87			22.86	1667.49			13.43	
		2802.60			0.000023	1657.57			0.000013	
		2674.28	2751.92	2728.49	1.19E+08	1610.99	1645.35	1602.61	1.19E+08	65.88
3.7	GU	2535.47			21.12	1442.62			11.52	
		2553.36			0.000021	1396.64			0.000012	
			2544.42	2520.99	1.19E+08	1414.89	1418.05	1375.31	1.19E+08	70.72
4.5	HU	2395.00			19.85	1286.81			10.42	
		2390.00			0.000020	1286.06			0.000010	
			2392.50	2369.07	1.19E+08	1285.37	1286.08	1243.34	1.19E+08	73.53
5.4	IU	2233.68			18.40	987.43			8.18	
		2191.45			0.000018	1024.00			0.000008	
		2233.63	2219.61	2196.18	1.19E+08	1046.00	1019.14	976.40	1.19E+08	79.21
5.5	JU	2319.63			18.56	1094.03			8.67	
		2237.04			0.000019	1072.46			0.000009	
		2159.28	2238.65	2215.22	1.19E+08	1066.92	1077.80	1035.06	1.19E+08	77.36
6.3	KU	2182.23			18.02	1001.94			7.90	
		2155.05			0.000018	988.82			0.000008	
		2184.36	2173.88	2150.45	1.19E+08	964.38	985.05	942.31	1.19E+08	79.94
7.2	LU	2116.12			17.72	800.25			6.26	
		2121.91			0.000018	784.04			0.000006	
		2176.43	2138.17	2114.74	1.19E+08	784.79	783.69	746.95	1.19E+08	84.10
7.3	MU	2073.95			17.14	735.09			5.73	
		2017.00			0.000017	694.22			0.000006	
		2116.07	2069.01	2045.58	1.19E+08	750.38	726.56	683.82	1.19E+08	85.44
8.1	NU	1983.28			16.49	690.55			5.56	
		2017.95			0.000016	730.88			0.000006	
		1967.24	1991.49	1968.06	1.19E+08	697.83	706.42	663.68	1.19E+08	85.87
9	OU	1976.25			16.29	585.89			4.77	
		1940.16			0.000016	636.21			0.000005	
		1986.90	1967.77	1944.34	1.19E+08	615.09	612.40	569.66	1.19E+08	87.87

14C Phenyl 6 DOBS LSC – Raw data

	14/05/2005									
	Sample Code	14C TOC	Mean	14C TOC - Control	aniline conc	14C DOC	Mean	14C DOC - Control	aniline conc	% Biodeg
Test Media	C1	24.07				22.70				
		26.24				23.51				
		26.51	25.61			25.70	23.37			
0	AU	4600.54			38.72				#DIV/0!	
(inlet)		4681.00			0.000033				#DIV/0!	
		4653.84	4645.13	4621.70	1.19E+08		#DIV/0!	#DIV/0!	#DIV/0!	
0.9	BU	2363.75			19.64	2242.11			18.89	
		2343.52			0.000020	2272.79			0.000013	
		2382.75	2367.34	2343.91	1.19E+08	2376.15	2297.02	2254.28	1.19E+08	51.22
1.8	CU	1386.40			12.32	1253.44			10.14	
		1511.75			0.000012	1290.05			0.000010	
		1582.09	1493.41	1469.98	1.19E+08	1216.60	1253.36	1210.62	1.19E+08	73.81
1.9	DU	1401.81			11.19	321.39			7.83	
		1241.70			0.000011	312.18			0.000008	
		1432.16	1358.56	1335.13	1.19E+08	1039.75	377.77	335.03	1.19E+08	79.77
2.7	EU	1058.62			8.50	688.93			5.52	
		1054.45			0.000009	703.15			0.000006	
		1001.54	1038.20	1014.77	1.19E+08	712.10	701.39	658.65	1.19E+08	85.75
3.6	FU	955.53			7.94	602.62			4.75	
		979.65			0.000008	619.04			0.000005	
		978.26	971.15	947.72	1.19E+08	608.72	610.13	567.39	1.19E+08	87.72
3.7	GU	1593.71			12.13				7.38	
		1528.00			0.000012	390.35			0.000008	
		1286.47	1471.39	1447.96	1.19E+08	398.18	394.57	351.83	1.19E+08	79.41
4.5	HU	1038.64			8.86	678.66			5.19	
		1071.79			0.000009	656.63			0.000005	
		1072.86	1061.10	1057.67	1.19E+08	651.70	662.33	619.59	1.19E+08	86.59
5.4	IU	990.38			7.89	570.81			4.48	
		956.10			0.000008	581.26			0.000004	
		947.94	964.81	941.38	1.19E+08	580.36	577.48	534.74	1.19E+08	88.43
5.5	JU	1196.14			9.68	816.22			6.44	
		1201.84			0.000010	838.93			0.000006	
		1138.79	1178.92	1155.49	1.19E+08	778.82	811.32	768.58	1.19E+08	83.37
6.3	KU	1148.05			9.25	727.87			5.59	
		1128.11			0.000009	685.64			0.000006	
		1105.42	1127.19	1103.76	1.19E+08	715.85	709.79	667.05	1.19E+08	85.57
7.2	LU	1032.21			8.53	627.42			4.79	
		1022.36			0.000009	637.58			0.000005	
		1063.36	1041.71	1018.28	1.19E+08	578.60	614.53	571.79	1.19E+08	87.63
7.3	MU	1095.74			8.86	672.43			5.32	
		1057.38			0.000009	697.52			0.000005	
		1089.79	1081.17	1057.74	1.19E+08	664.77	678.24	635.50	1.19E+08	86.25
8.1	NU	1091.86			8.75	587.83			4.37	
		1060.67			0.000009	534.42			0.000004	
		1050.04	1067.52	1044.09	1.19E+08	570.60	564.28	521.54	1.19E+08	88.72
9	OU	943.66			8.11	464.74			3.55	
		1106.21			0.000008	485.66			0.000004	
		918.65	991.51	968.08	1.19E+08	443.09	466.50	423.76	1.19E+08	90.83

14C Phenyl 6 DOBS LSC – Raw data

	15/05/2005									
	Sample Code	14C TOC	Mean	14C TOC - Control	aniline conc	14C DOC	Mean	14C DOC - Control	aniline conc	% Biodeg
Test Media	C1	25.32				28.65				
		23.47				31.33				
		27.24	25.34			24.56	28.18			
0	AU	5444.66			33.13				#DIV/0!	
(inlet)		4209.20			0.000033				#DIV/0!	
		4425.23	4693.03	4663.60	1.19E+08		#DIV/0!	#DIV/0!	#DIV/0!	
0.9	BU	4386.26			35.63	3968.41			33.37	
		4248.70			0.000036				0.000033	
		4193.29	4276.08	4252.65	1.19E+08	4082.87	4025.64	3982.90	1.19E+08	14.71
1.8	CU	3405.90			27.73	2808.32			23.89	
		3304.52			0.000028	2815.94			0.000024	
		3288.11	3332.84	3309.41	1.19E+08	3058.03	2894.10	2851.36	1.19E+08	38.94
1.9	DU	2779.62			22.35	2101.10			18.05	
		2599.35			0.000022	2189.24			0.000018	
		2692.29	2690.42	2666.99	1.19E+08	2293.44	2196.59	2153.85	1.19E+08	53.87
2.7	EU	2358.65			19.56	1739.69			13.80	
		2327.84			0.000020	1624.11			0.000014	
		2387.87	2358.12	2334.69	1.19E+08	1647.14	1690.31	1647.57	1.19E+08	64.72
3.6	FU	2076.64			17.17	1338.32			11.05	
		2087.92			0.000017	1356.63			0.000011	
		2051.91	2072.16	2048.73	1.19E+08	1390.73	1361.89	1319.15	1.19E+08	71.75
3.7	GU	2930.64			21.02	1223.47			9.78	
		2134.47			0.000021	1234.41			0.000010	
		1867.02	2532.56	2503.13	1.19E+08	1173.39	1210.42	1167.68	1.19E+08	74.39
4.5	HU	1774.85			14.05	1101.68			8.95	
		1623.58			0.000014	1105.62			0.000009	
		1703.04	1700.49	1677.06	1.19E+08	1125.32	1111.07	1068.33	1.19E+08	77.12
5.4	IU	1447.30			12.64	877.35			6.37	
		1621.72			0.000013	866.34			0.000007	
		1526.05	1531.69	1508.26	1.19E+08	880.19	875.03	832.29	1.19E+08	82.16
5.5	JU	1481.59			12.08	815.53			6.56	
		1475.01			0.000012	836.33			0.000007	
		1438.31	1465.17	1441.74	1.19E+08	823.53	825.13	782.39	1.19E+08	83.25
6.3	KU	1386.14			11.26	678.84			5.33	
		1370.29			0.000011	666.88			0.000005	
		1346.56	1367.66	1344.23	1.19E+08	692.06	679.26	636.52	1.19E+08	86.37
7.2	LU	1231.41			9.57	548.66			4.18	
		1090.42			0.000010	562.46			0.000004	
		1174.14	1165.32	1141.89	1.19E+08	543.31	551.48	508.74	121771773	89.11
7.3	MU	1221.07			9.78	545.12			4.19	
		1131.51			0.000010	551.95			0.000004	
		1219.78	1190.79	1167.36	1.19E+08	532.44	543.17	500.43	1.19E+08	89.28
8.1	NU	1122.28			9.01	473.58			3.61	
		1151.30			0.000009	449.54			0.000004	
		1024.14	1099.24	1075.81	1.19E+08	499.40	474.17	431.43	1.19E+08	90.76
9	OU	1072.11			8.66	425.48			3.15	
		1101.46			0.000009	420.17			0.000003	
		997.28	1056.95	1033.52	1.19E+08	410.56	418.74	376.00	1.19E+08	91.95

14C Phenyl 6 DOBS LSC – Raw data

	16/05/2005									
	Sample Code	14C TOC	Mean	14C TOC - Control	aniline conc	14C DOC	Mean	14C DOC - Control	aniline conc	% Biodeg
Test Media	C1	23.53				22.15				
		23.53				19.68				
		25.99	24.35			25.17	22.33			
0	AU	4574.30			38.58				#DIV/0!	
(inlet)		4676.45			0.000039				#DIV/0!	
		4632.53	4627.76	4604.33	1.19E+08		#DIV/0!	#DIV/0!	#DIV/0!	
0.9	BU	2354.80			19.68	2234.98			18.88	
		2412.97			0.000020	2300.24			0.000019	
		2343.88	2372.55	2343.12	1.19E+08	2351.22	2295.48	2252.74	1.19E+08	51.07
1.8	CU	1374.88			12.19	1242.24			10.09	
		1467.88			0.000012	1296.05			0.000010	
		1530.80	1477.85	1454.42	1.19E+08	1200.95	1246.41	1203.67	1.19E+08	73.86
1.9	DU	1402.63			11.06	338.67			7.93	
		1194.57			0.000011	313.45			0.000008	
		1432.50	1343.25	1319.82	1.19E+08	1115.42	989.18	946.44	1.19E+08	79.44
2.7	EU	1046.20			8.19	705.92			5.59	
		988.52			0.000008	715.44			0.000006	
		969.55	1001.42	977.99	1.19E+08	709.12	710.16	667.42	1.19E+08	85.50
3.6	FU	964.58			7.87	608.10			4.78	
		987.93			0.000008	615.13			0.000005	
		935.86	962.79	939.36	1.19E+08	617.28	613.50	570.76	1.19E+08	87.60
3.7	GU	1535.06			12.50	1166.55			8.36	
		1435.52			0.000013	962.14			0.000008	
		1297.18	1515.29	1491.86	1.19E+08	994.15	1040.95	998.21	1.19E+08	78.32
4.5	HU	1080.71			8.55	693.15			5.34	
		1023.97			0.000009	661.87			0.000005	
		1028.44	1044.37	1020.94	1.19E+08	684.78	679.93	637.19	1.19E+08	86.16
5.4	IU	977.40			7.77	574.59			4.45	
		912.00			0.000008	583.40			0.000004	
		963.62	951.01	927.58	1.19E+08	563.06	573.68	530.94	1.19E+08	88.47
5.5	JU	1175.88			9.49	835.52			6.36	
		1165.94			0.000009	822.06			0.000006	
		1125.91	1155.91	1132.48	1.19E+08	749.05	802.21	759.47	1.19E+08	83.51
6.3	KU	1088.85			8.88	711.28			5.49	
		1119.62			0.000009	688.87			0.000005	
		1041.64	1083.37	1059.94	1.19E+08	694.89	698.35	655.61	1.19E+08	85.76
7.2	LU	1001.20			8.12	607.82			4.70	
		966.26			0.000008	627.42			0.000005	
		1010.40	992.62	969.19	1.19E+08	575.50	603.58	560.84	1.19E+08	87.82
7.3	MU	1058.82			8.79	662.95			5.19	
		1070.62			0.000009	657.30			0.000005	
		1087.34	1072.26	1048.83	1.19E+08	666.48	662.24	619.50	1.19E+08	86.55
8.1	NU	1080.57			8.45	569.44			4.33	
		1016.81			0.000008	548.12			0.000004	
		997.37	1031.58	1008.15	1.19E+08	559.36	558.97	516.23	1.19E+08	88.79
9	OU	935.10			7.98	454.36			3.47	
		1091.65			0.000008	477.82			0.000003	
		899.18	975.31	951.88	1.19E+08	439.22	457.13	414.39	1.19E+08	91.00

Water Quality Analysis – Temperature measurements from artificial river model

	Temp °C									
	Channel									
	1		2		3		4		5	
Time	Dist									
Days	(m)									
0	0	1.8	1.9	3.6	3.7	5.4	5.5	7.2	7.3	9
1	19.7	19.6	19.7	19.7	19.6	19.6	19.7	19.7	19.7	19.9
2	20.1	19.9	19.9	19.9	20.1	20.3	20.7	20.3	20.3	20.6
3	20.4	20.3	20	19.9	19.5	19.6	19.6	19.4	19.2	19.2
4	19.8	19.6	19.4	19.1	19	19	18.9	19.1	19.1	19
6	20	19.9	19.8	19.6	19.7	19.7	19.7	19.7	19.6	19.7
8	20.6	19.8	19.9	20	20	19.6	19.4	19.2	19.1	19.1
10	19.5	19.6	19.5	19.4	19.5	19.3	19.3	19.3	19.2	19.1
13	19.9	19.7	19.7	19.6	19.5	19.5	19.2	19.1	19	19
15	19.9	19.7	19.8	19.6	19.4	19.4	19.3	19.3	19.1	19
17	20.3	20	19.8	19.6	19.5	19.1	19	19	19	19
22	20.1	20	19.6	19.4	19.3	19.2	19.3	19	19	19
24	19.7	19.7	19.6	19.5	19.3	19.2	19.2	19.1	19	19
25	20	19.8	19.5	19.2	19.3	19.2	19.2	19.1	19	19
29	19.9	19.8	19.8	19.6	19.5	19.4	19.4	19.3	19.1	19.1
32	20.2	20.1	20.1	20	20	19.8	19.8	19.6	19.5	19.5
38	19.8	19.7	19.6	19.6	19.5	19.4	19.3	19.2	19.1	19.1
43	19.9	19.8	19.7	19.6	19.5	19.4	19.3	19.2	19.1	19.1
45	20.1	20	19.9	19.8	19.7	19.6	19.7	19.6	19.6	19.5
49	20	20	19.9	19.9	19.8	19.7	19.7	19.7	19.7	19.6
51	19.9	19.9	19.9	19.9	19.8	19.8	19.8	19.7	19.7	19.7
71	20.3	20.1	20	19.9	19.9	19.7	19.6	19.7	19.6	19.6
74	20.6	20.5	20.6	20.3	20.1	20	20.1	19.8	19.9	19.9
78	21	20.8	20.9	21	20.9	20.2	20	20.1	20	19.8
84	20.4	20.5	20.6	20.3	20.1	20.2	20	20	19.8	19.9
88	20.1	20	20.1	19.9	19.9	19.9	20	19.9	20	20
91	19.9	20	19.9	19.8	19.7	19.9	19.9	19.9	20	20.1
98	19.5	19.6	19.9	19.6	19.7	19.8	19.8	19.7	19.8	19.7
105	20	20.2	20.3	20.1	20	20.1	19.9	19.9	19.8	19.9
avg	19.94	19.9	19.8	19.8	19.7	19.6	19.6	19.5	19.4	19.4
sd	0.114	0.13	0.14	0.15	0.15	0.18	0.24	0.26	0.31	0.28

Water Quality Analysis – pH measurements from artificial river model

	pH									
	Channel									
	1		2		3		4		5	
Time	Dist									
Days	(m)									
0	0	1.8	1.9	3.6	3.7	5.4	5.5	7.2	7.3	9
1	7.88	8.19	8.26	8.36	8.37	8.41	8.41	8.42	8.42	8.47
2	8.16	8.21	8.2	8.27	8.14	8.19	8.22	8.3	8.26	8.21
3	7.6	7.71	8.06	8.26	7.99	8.16	8.38	8.29	8.31	8.27
4	7.61	8	8.01	8.31	8.23	8.36	8.4	8.36	8.31	8.42
6	7.72	7.89	8.01	8	8.16	8.06	8.15	8.16	8.22	8.37
8	7.81	7.9	7.92	7.99	8.11	8.1	8.14	8.2	8.21	8.24
10	7.73	8.06	8.19	8.19	8.23	8.26	8.3	8.26	8.31	8.34
13	8	8.01	8.05	8.09	8.11	8.13	8.15	8.2	8.22	8.26
15	7.66	7.94	7.8	7.95	8.03	8.06	8.2	8.29	8.44	8.45
17	7.7	7.98	8.03	8.11	8.05	8.08	8.09	8.11	8.1	8.13
22	7.92	7.9	7.99	8	8.06	8.09	8.12	8.16	8.12	8.15
24	7.83	7.87	7.9	7.93	7.94	7.95	7.96	7.98	8	8.01
25	7.89	7.9	7.95	7.99	8	8.05	8.09	8.13	8.15	8.15
29	7.81	7.83	7.9	7.91	7.95	8	8	8.03	8.11	8.17
32	7.98	8.01	8.09	8.13	8.15	8.18	8.21	8.25	8.25	8.31
38	8.05	8.07	8.09	8.11	8.13	8.15	8.2	8.22	8.21	8.23
43	7.83	7.97	8.03	8.1	8.1	8.14	8.19	8.21	8.23	8.26
45	8.01	8.06	8.1	8.11	8.13	8.22	8.2	8.25	8.27	8.26
49	7.96	7.9	8.04	8.06	8.1	8.16	8.2	8.22	8.25	8.25
51	8.16	8.2	8.2	8.24	8.14	8.19	8.22	8.22	8.23	8.21
71	7.87	7.9	8	7.92	7.96	7.94	8	8	8.03	8.06
74	7.66	7.7	7.7	7.71	7.69	7.74	7.79	7.83	7.85	7.89
78	7.8	7.71	7.73	7.79	7.81	7.8	7.83	7.84	7.89	7.88
84	7.59	7.62	7.65	7.71	7.73	7.7	7.66	7.69	7.71	7.73
88	7.96	7.98	8.01	8.03	7.99	8.02	7.98	7.96	7.93	7.91
91	8.03	8.05	8.01	7.97	7.95	7.96	7.98	8.01	8	8.01
98	7.88	7.86	7.91	7.95	7.99	8.01	8.03	8.01	8	8.06
105	8	8.04	8.09	8.12	8.17	8.2	8.22	8.24	8.22	8.21
avg	8	8.04	8.09	8.12	8.12	8.17	8.2	8.22	8.24	8.24
sd	0.12	0.11	0.07	0.07	0.02	0.03	0.01	0.02	0.02	0.02

Water Quality Analysis – Dissolved Oxygen measurements from artificial river model

	d02														
	Channel														
	1			2			3			4			5		
Time	Dist														
Days	(m)														
0	0	0.9	1.8	1.9	2.7	3.6	3.7	4.5	5.4	5.5	6.3	7.2	7.3	8.1	9
1	7.27	8	8.2	8.72	8.7	8.8	8.89	8.85	8.9	8.82	8.79	8.8	8.9	8.88	8.86
3	4.2	6.8	7	7.7	7.9	8	8.4	8.8	8.8	8.8	8.7	8.8	8.9	8.8	8.8
4	3.13	5.15	7.55	8	8.2	8.3	8.5	8.6	8.8	8.9	8.8	8.92	8.82	8.81	8.87
6	3.5	4.9	6.8	7.4	7.8	8.2	8.6	8.7	8.7	8.8	8.7	8.8	8.7	8.7	8.8
8	2.5	3.7	5.6	6.4	6.8	7.2	7.6	7.7	7.8	8	8.2	8.4	8.6	8.6	8.7
10	2.01	3.5	5.2	6.2	6.5	7.1	7.1	7.5	7.5	7.5	7.6	7.7	7.6	7.7	7.7
13	2.9	3.9	4.6	5.6	5.8	6.2	6.6	6.7	6.9	7.4	8	8.2	8.2	8.4	8.4
15	2.2	3.5	4.6	5.4	5.5	5.8	6.2	6.4	6.6	7	7.2	7.6	8	8.4	8.4
17	2.6	3.6	4.5	4.4	4.6	5	6.1	6	6	6	6.3	7.2	7.2	8	8.2
22	2	2.85	3.8	4.2	5.2	5.6	6	7	7.2	7.6	7.8	8	8	8.2	8.2
24	2.2	2.6	3.4	3.5	3.5	3.6	5	5.6	6	6.9	7.2	7.5	7.9	8	8
25	2.5	2.8	3.4	4	4.2	4.4	5.5	5.4	6	7.4	7.8	8	8.2	8.2	8
29	2.5	2.6	3	3.8	4.2	4.4	5.2	5.4	5.9	7	7.8	7.8	8	7.8	8
32	2.2	2.4	3	3.6	3.6	5	5.4	5.8	6.6	6.8	6.8	7	7.8	8	8
37	2.25	2.5	3.25	3.5	3.5	3.6	5	5.6	6.2	7.2	7.6	8	8.2	7.9	8.2
42	1.8	2.4	3.2	3.5	3.7	4	5.2	5.4	6	6.9	7.5	7.9	8	8	8.2
44	2.2	2.6	3.2	3.6	3.8	4.2	5.2	5.5	6.1	7	7.45	7.7	8	8	8
48	2	2.6	3.1	3.7	4	4.2	5.4	5.8	6.1	7.2	7.6	7.8	8	8.4	8.2
50	2.1	2.3	3	3.3	3.7	4.4	5.2	5.8	6.2	7	7.3	7.6	8	8	8.1
70	3.8	5.4	5.9	6.8	7	7.2	7.9	8.3	8.4	8.6	8.8	8.8	8.8	8.9	8.9
73	3.3	3.4	3.6	4	4.2	4.6	4.6	5.2	5.4	6.2	6.8	7.2	7.8	8	8.4
77	2.6	2.8	3.3	3.6	4	4.1	5	5.9	6.2	7.2	7.8	8	8.4	8.2	8.6
83	2.1	2.4	3	3.8	3.9	4.4	5	5.4	5.9	6.6	7.4	8	8.2	8.2	8.6
87	2.4	2.7	3.2	3.6	4.1	4.3	5.1	5.7	6.3	6.9	7.5	7.6	7.9	8.3	8.2
90	2.3	2.9	3.4	3.7	4.3	4.7	5.4	5.8	6	6.6	7.3	7.8	8	7.9	8.1
98	2.2	2.7	3.3	3.9	4.6	4.8	5.4	5.8	6.2	6.4	7.6	7.9	8.4	8.1	8.2
105	2.1	2.5	3.2	3.5	3.8	4.1	5.2	5.6	6.1	7	7.5	7.8	8	8	8.2
avg	2.07	2.48	3.15	3.52	3.74	4.08	5.2	5.62	6.12	7.06	7.49	7.8	8.04	8.06	8.14
sd	0.18	0.13	0.1	0.15	0.18	0.3	0.14	0.18	0.08	0.13	0.12	0.16	0.09	0.19	0.09

Water Quality Analysis – Chemical Oxygen Demand % removal

	COD % removal					
	Channel					
	1	2	3	4	5	Eff
	Dist (m)					
	0	1.9	3.7	5.5	7.3	9
	Time (hrs)					
Time	0.0	5.0	10.7	15.9	21.4	26.3
Days						
1		30.4	85.1	0.0	0.0	0.0
2	0	2.0	8.2	6.1	9.2	9.2
4	0	44.3	45.8	47.6	48.2	49.0
6	0					56.7
8	0	50.7	49.6	49.6	50.6	51.0
10	0	55.3	61.6	63.5	63.4	64.6
15	0	48.0	49.8	50.6	50.8	53.3
17	0	34.4	46.1	51.1	53.6	52.9
22	**0**	**53.0**	**69.8**	**66.9**	**73.2**	**71.3**
26	**0**	**60.0**	**66.1**	**69.4**	**72.0**	**73.3**
31	**0**	**54.9**	**63.9**	**70.2**	**73.1**	**72.2**
36	**0**	**55.0**	**62.9**	**71.2**	**73.7**	**74.8**
43	**0**	**51.3**	**59.3**	**63.1**	**60.8**	**70.7**
50	**0**	**46.2**	**56.8**	**62.3**	**67.1**	**71.4**
71	**0**	**43.4**	**53.7**	**67.4**	**65.2**	**67.2**
73	**0**	**50.7**	**71.4**	**77.7**	**80.4**	**77.7**
77	**0**	**49.3**	**62.7**	**65.9**	**69.0**	**69.8**
78	**0**	**51.1**	**60.4**	**64.6**	**68.6**	**73.3**
84	**0**	**57.4**	**67.2**	**72.3**	**74.3**	**73.4**
91	**0**	**51.8**	**55.0**	**56.2**	**55.0**	**56.5**
93	**0**	**50.6**	**61.7**	**72.6**	**71.7**	**72.1**
98	**0**	**52.8**	**60.0**	**70.1**	**71.2**	**71.5**
104	**0**	**53.3**	**60.8**	**64.9**	**67.7**	**70.3**
Day 22 - 104	Calculation period (steady state conditions)					
Average	0	52.0	62.1	67.6	69.5	71.0
sd	0	4.1	5.1	5.2	6.1	4.7

Descriptive statistics (Micro Excel) on decay rate / half-life data

calculation determined using axial flow speed and distance for Aniline

	k (h-1)	t1/2 (h-1)
Mean	0.112	6.362
Standard Error	0.007	0.357
Median	0.110	6.346
Mode	#N/A	#N/A
Standard Deviation	0.023	1.130
Sample Variance	0.001	1.276
Kurtosis	1.967	-0.343
Skewness	1.263	-0.438
Range	0.076	3.662
Minimum	0.088	4.236
Maximum	0.164	7.898
Sum	1.125	63.616
Count	10	10.000
Confidence Level(95.0%)	0.016	0.808

Comment [S46]: I don't think that this is useful. I would simply state the mean and SD

Descriptive statistics (Micro Excel) on decay rate / half-life data calculation determined using axial flow speed and distance for Phenyl 6-DOBS

	k (h-1)	t1/2 (h-1)
Mean	0.1357848	5.1182646
Standard Error	0.0024055	0.0916578
Median	0.1372773	5.0487662
Mode	0.1262972	5.487057
Standard Deviation	0.0076068	0.2898475
Sample Variance	5.786E-05	0.0840116
Kurtosis	-1.2613718	-1.3276624
Skewness	-0.1741884	0.3032432
Range	0.0211334	0.7920537
Minimum	0.1258228	4.7156922
Maximum	0.1469562	5.5077459
Sum	1.3578478	51.182646
Count	10	10
Confidence Level(95.0%)	0.0054416	0.2073444

Comment [S47]: As before, I don't think this is useful. Just mean and SD will do

COD decay rate and half-life calculation determined using axial flow speed and distance

Axial flow speed and distance				
Day No	k (h-1)	t1/2 (h-1)	k (d-1)	t1/2 (d-1)
22	41.938	9.518	1.747	0.397
26	37.530	10.636	1.564	0.443
31	37.907	10.530	1.579	0.439
36	42.128	9.475	1.755	0.395
43	41.280	9.670	1.720	0.403
50	45.570	8.759	1.899	0.365
71	37.409	10.670	1.559	0.445
73	50.420	7.917	2.101	0.330
77	40.178	9.935	1.674	0.414
78	44.421	8.986	1.851	0.374
84	44.507	8.969	1.854	0.374
91	27.948	14.283	1.164	0.595
93	42.876	9.310	1.787	0.388
98	42.224	9.454	1.759	0.394
104	40.737	9.799	1.697	0.408

NH4 decay rate and half-life calculation determined using

axial flow speed and distance

Axial flow speed and distance				
Day No	k (h-1)	t1/2 (h-1)	k (d-1)	t1/2 (d-1)
22	0.3271	2.119	7.849	0.09
26	0.3066	2.261	7.357	0.09
31	0.3286	2.109	7.885	0.09
36	0.3656	1.896	8.774	0.08
43	0.3948	1.755	9.476	0.07
50	0.3289	2.107	7.894	0.09
71	0.3024	2.292	7.257	0.10
78	0.3146	2.203	7.551	0.09
84	0.3058	2.266	7.340	0.09
91	0.5364	1.292	12.873	0.05
93	0.6744	1.028	16.186	0.04
104	0.3365	2.060	8.076	0.09

Descriptive statistics (Micro Excel) on NH4 decay rate / half-life data calculation

determined using axial flow speed and distance

	k (h-1)	t1/2 (h-1)	k (d-1)	t1/2 (d-1)
Mean	0.3768026	1.9488268	9.0432631	0.0812011
Standard Error	0.0328681	0.1165255	0.7888332	0.0048552
Median	0.3287305	2.1081103	7.8895323	0.0878379
Mode	#N/A	#N/A	#N/A	#N/A
Standard Deviation	0.1138583	0.4036563	2.7325985	0.016819
Sample Variance	0.0129637	0.1629384	7.4670946	0.0002829
Kurtosis	4.0402618	1.5923513	4.0402618	1.5923513
Skewness	2.1187017	-1.558374	2.1187017	-1.558374
Range	0.3720536	1.2642903	8.9292856	0.0526788
Minimum	0.3023798	1.0275292	7.2571162	0.0428137
Maximum	0.6744334	2.2918194	16.186402	0.0954925
Sum	4.5216316	23.385921	108.51916	0.9744134
Count	12	12	12	12
Confidence Level(95.0%)	0.0723421	0.256471	1.7362103	0.0106863

Water Quality Analysis – % Ammonium removal

	NH4	% Removal				
	Channel					
	1	2	3	4	5	Eff
	Dist (m)					
	0	1.9	3.7	5.5	7.3	9
	Time (hrs)					
Day	0.0	5.0	10.7	15.9	21.4	26.3
1						0.0
2	0	1.6	4.7	1.6	5.5	6.3
4	0	1.9	4.0	11.9	27.3	35.8
6						54.8
8	0	5.9	32.4	59.2	81.4	94.2
10	0	9.2	31.3	48.2	84.3	88.0
15	0	27.3	64.2	98.7	99.1	99.5
17	0	14.9	62.6	99.1	99.3	99.5
22	**0**	**36.4**	**62.5**	**99.4**	**99.6**	**99.6**
26	**0**	**31.7**	**72.9**	**99.0**	**99.1**	**99.5**
0	**0**	**32.8**	**67.4**	**95.8**	**98.9**	**99.6**
36	**0**	**39.2**	**71.1**	**95.5**	**99.3**	**99.8**
43	**0**	**38.0**	**74.1**	**96.4**	**99.1**	**100.0**
50	**0**	**26.1**	**65.7**	**97.1**	**99.5**	**99.6**
71	**0**	**36.2**	**66.8**	**92.7**	**97.5**	**99.4**
78	**0**	**35.6**	**69.7**	**94.3**	**97.9**	**99.5**
84	**0**	**15.3**	**63.7**	**94.1**	**99.1**	**99.5**
91	**0**	**27.1**	**79.3**	**99.8**	**99.8**	**99.8**
93	**0**	**38.6**	**80.5**	**99.8**	**99.9**	**99.9**
104	**0**	**33.8**	**70.2**	**96.8**	**99.1**	**99.7**
Avg	0.0	32.6	70.3	96.7	99.1	99.7
sd	0.0	6.9	5.7	2.4	0.7	0.2

Mean temperature measurements from each channel over complete duration

Temp C	Channel									
	1		2		3		4		5	
	Dist (m)									
	0	1.8	1.9	3.6	3.7	5.4	5.5	7.2	7.3	9
avg	19.94	19.9	19.8	19.8	19.7	19.6	19.6	19.5	19.4	19.4
sd	0.114	0.13	0.14	0.15	0.15	0.18	0.24	0.26	0.31	0.28

Comment [S48]: I would put this in the appendix

Mean pH measurements from each channel over complete duration

pH	Channel									
	1		2		3		4		5	
	Dist (m)									
	0	1.8	1.9	3.6	3.7	5.4	5.5	7.2	7.3	9
avg	8	8.04	8.09	8.12	8.12	8.17	8.2	8.22	8.24	8.24
sd	0.12	0.11	0.07	0.07	0.02	0.03	0.01	0.02	0.02	0.02

Descriptive statistics (Micro Excel) on decay rate / half-life data calculation determined using axial flow speed and distance for COD

	k (h-1)	t1/2 (h-1)
Mean	0.071	9.861
Standard Error	0.002	0.369
Median	0.073	9.518
Mode	#N/A	#N/A
Standard Deviation	0.009	1.429
Sample Variance	0.000	2.042
Kurtosis	3.205	6.782
Skewness	-0.979	2.185
Range	0.039	6.366
Minimum	0.049	7.917
Maximum	0.088	14.283
Sum	1.071	147.911
Count	15.000	15.000
Confidence Level(95.0%)	0.005	0.791

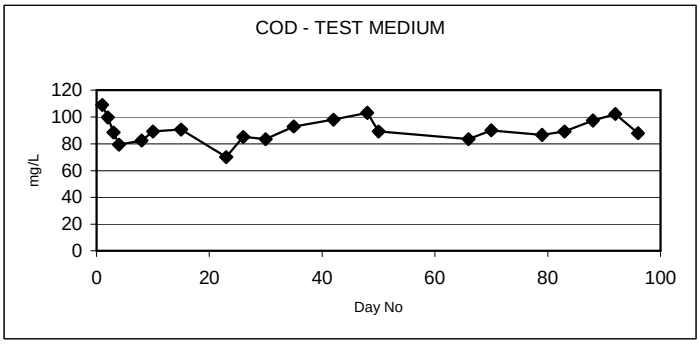
Comment [S49]: Again, I would send this to the appendix and just quote mean and SD

Values of water quality variables in showing the typical variability over the course of the tests.

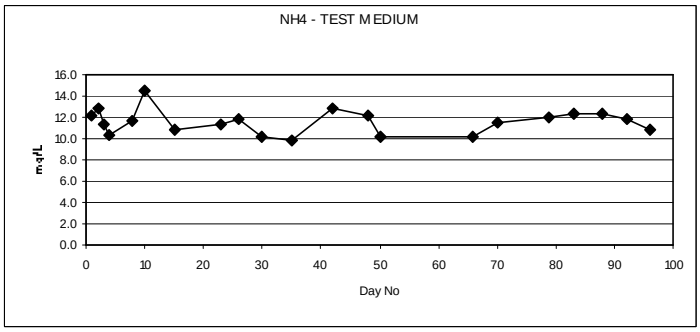
TEST MEDIUM - WATER QUALITY ANALYSIS

Date	Day No	COD (mg/l)	NH4 (mg/l)	N-NH4 (mg/l)	Total N (mg/l)	PO4 (mg/l)	PO4-P (mg/l)	Temp C	pH	dO (mg/l)	TDS (ppt)	EC (mS/cm)
01-Feb-05	1	109	12.1	9.4	23.6	6.6	2.19	20.4	7.82	5.62	0.59	1.19
02-Feb-05	2	99.6	12.8	9.97	20.5	6.61	2.16	19.7	7.69	5.02	0.6	1.21
03-Feb-05	3	88.4	11.4	9.17	22.4	6.1	2.01	19.6	7.81	4.24	0.59	1.19
04-Feb-05	4	79.3	10.3	7.99	28.2	6.27	2.05	19.8	7.57	5.61	0.56	1.14
08-Feb-05	8	82.4	11.7	9.13	26.9	7.12	2.13	19.8	7.66	5.72	0.59	1.19
10-Feb-05	10	89.2	14.5	11.3	28	7.33	2.39	20.1	7.45	4.98	0.6	1.2
15-Feb-05	15	90.6	10.8	7.97	26.3	4.51	1.62	20.6	7.87	5.07	0.59	1.09
23-Feb-05	23	70	11.4	8.85	24.2	5.88	1.86	19.8	7.85	5.23	0.53	1.12
26-Feb-05	26	85	11.8	8.92	26	6.01	1.98	19.2	7.99	5	0.58	1.11
02-Mar-05	30	83.3	10.2	7.89	23.6	6.2	2.13	19.6	7.87	4.2	0.58	1.15
08-Mar-05	35	93	9.8	7.22	32	7.51	2.55	19.8	8.01	5.8	0.58	1.12
15-Mar-05	42	98	12.9	10	22.2	6.8	1.98	19.9	8.05	4.9	0.59	1.1
21-Mar-05	48	103	12.2	9.7	25.1	6.2	2.13	20	7.92	5.1	0.6	1.17
23-Mar-05	50	89.2	10.15	8.64	21.4	6.44	2.21	20.1	8.04	4	0.59	1.19
8-Apr-05	66	83.3	10.2	7.89	23.6	6.2	2.13	19.6	7.87	4.2	0.58	1.15
12-Apr-05	70	90	11.5	9	25.3	6.39	2.09	19.8	7.8	4.2	0.58	1.15
22-Apr-05	79	86.6	12	9.6	24.4	6.66	2.14	20	7.76	3.9	0.59	1.14
27-Apr-05	83	89.1	12.3	9.4	24.9	6.72	2.26	19.9	7.67	3.7	0.58	1.16
03-May-05	88	97.3	12.4	9.1	22.3	5.98	1.9	20.2	7.89	4.4	0.59	1.13
11-May-05	92	102.3	11.9	9.23	25	5.11	1.54	20	7.94	4.8	0.57	1.18
17-May-05	96	87.8	10.8	7.9	24.8	6.49	2.18	19.9	7.81	4.1	0.59	1.17
	av	90.3	11.6	9.0	24.8	6.3	2.1	19.9	7.8	4.8	0.6	1.2
	sd	9.0	1.1	0.9	2.6	0.7	0.2	0.3	0.2	0.6	0.0	0.0
	cv %	9.9	9.8	10.5	10.5	10.6	10.9	1.5	2.0	13.6	2.7	3.0

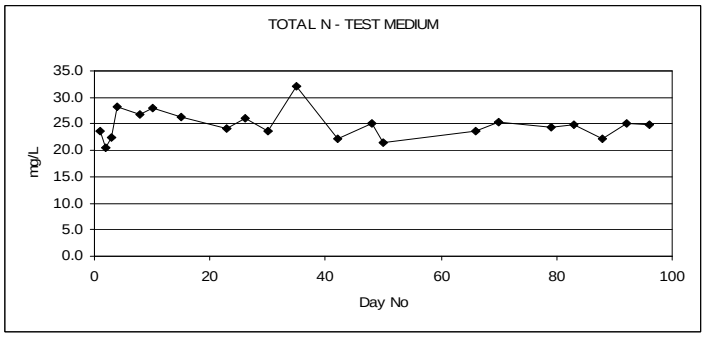
Plot of COD measurements in test medium vessel



Plot of NH4 measurements in test medium vessel



Plot of Total N measurements in test medium vessel



Plot of PO4 measurements in test medium vessel

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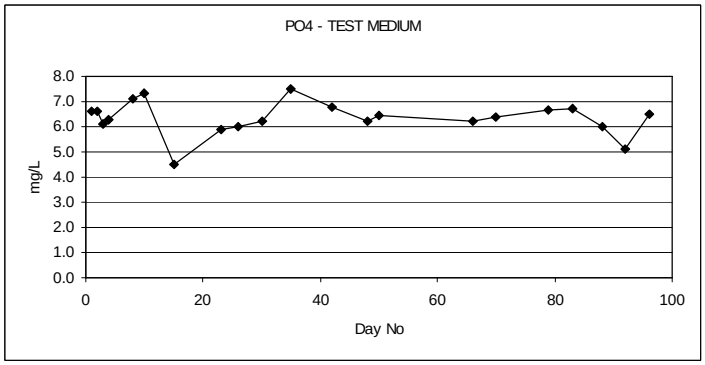

Summary of Aniline % biodegradation

% Biodeg											
		Day No									
Channel	Dist (m)	1	2	3	4	5	6	7	8	12	13
	0	0	0	0	0	0	0	0	0	0	0
1	0.9	58.02	44.72	46.69	23.94	56.58	54.39	55.97	59.33	70.10	51.64
	1.8	64.55	48.41	51.94	50.69	50.77	58.41	68.09	69.20	78.17	69.15
	1.9	67.78	58.02	61.61	59.05	70.70	73.78	76.56	73.17	79.37	76.94
2	2.7	75.25	72.78	67.08	66.55	71.25	76.50	78.45	72.22	81.73	80.08
	3.6	77.26	75.85	66.82	68.77	73.64	76.81	80.54	73.70	83.90	80.87
	3.7	76.51	71.32	66.58	66.06	69.24	69.37	78.23	71.13	84.55	78.38
3	4.5	78.60	79.28	70.93	70.80	73.96	75.29	80.92	74.67	84.29	83.44
	5.4	81.75	81.24	73.54	72.78	73.67	80.17	83.70	77.18	86.79	86.62
	5.5	79.52	80.21	73.62	73.91	77.30	80.65	82.16	77.55	86.11	85.65
4	6.3	82.70	81.96	74.39	73.98	74.75	80.39	85.33	76.53	86.69	86.28
	7.2	86.38	83.84	77.44	76.99	78.40	81.09	85.66	76.53	87.13	87.18
	7.3	87.07	83.88	77.37	77.16	76.05	81.26	86.91	77.11	87.44	86.92
5	8.1	88.95	85.01	78.63	77.22	74.98	81.20	87.16	77.92	87.49	86.69
	9	93.95	86.45	80.85	79.27	78.65	81.09	87.49	77.78	88.67	87.30

Summary of (Phenyl 6-DOBS) % biodegradation

		% Biodeg									
		Day No									
Channel	Dist (m)	1	2	3	4	5	6	7	8	9	10
	0	0	0	0	0	0	0	0	0	0	0
1	0.9	61.62	66.57	51.71	46.43	11.97	4.95	27.41	51.22	14.71	51.07
	1.8	68.54	70.55	74.09	75.85	30.33	38.01	39.21	73.81	38.94	73.86
	1.9	79.92	74.07	79.36	70.87	38.80	44.37	46.70	79.77	53.87	79.44
2	2.7	83.87	77.62	85.47	83.26	53.53	57.20	55.05	85.75	64.72	85.50
	3.6	84.57	78.20	87.41	83.99	63.92	66.84	65.88	87.72	71.75	87.60
	3.7	74.20	70.06	78.54	82.43	74.74	70.05	70.72	79.41	74.99	78.32
3	4.5	83.64	82.31	86.19	84.43	76.27	73.91	73.53	86.59	77.12	86.16
	5.4	87.77	84.30	88.33	87.70	81.58	80.49	79.21	88.43	82.18	88.47
	5.5	85.44	82.18	83.54	85.04	83.03	79.58	77.96	83.37	83.25	83.51
4	6.3	83.69	86.13	85.38	87.41	88.70	81.75	79.94	85.57	86.37	85.76
	7.2	82.18	87.42	87.61	89.23	88.74	85.78	84.10	87.63	89.11	87.82
	7.3	84.02	86.20	86.29	87.90	89.12	86.48	85.44	86.25	89.28	86.55
5	8.1	86.35	88.69	88.73	87.90	90.23	87.03	85.87	88.72	90.76	88.79
	9	88.43	90.25	90.73	89.94	91.55	88.53	87.87	90.83	91.95	91.00

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Nitrite measurements (cascade system)

NO2						
	Channel					
	1	2	3	4	5	Eff
Time	Dist					
Days	(m)					
	0.00	1.90	3.70	5.50	7.30	9.00
8	8.00	9.16	7.92	15.50	20.90	24.80
10	2.91	4.80	9.95	14.00	18.30	23.40
15	5.22	9.87	13.20	14.70	14.90	15.40
17	1.94	4.58	7.81	4.69	1.73	1.08
22	**10.20**	**6.29**	**1.29**	**0.03**	**0.03**	**0.02**
26	**11.30**	**7.21**	**4.01**	**0.86**	**0.04**	**0.02**
31	**8.92**	**5.32**	**3.03**	**1.12**	**0.20**	**0.00**
36	**9.31**	**6.33**	**3.23**	**1.43**	**0.07**	**0.01**
43	**5.03**	**4.42**	**2.87**	**0.99**	**0.10**	**0.04**
50	**9.16**	**5.54**	**3.32**	**0.89**	**0.12**	**0.00**
71	**6.45**	**4.78**	**3.46**	**1.76**	**0.32**	**0.04**
78	**9.22**	**6.23**	**2.99**	**1.34**	**0.43**	**0.05**
84	**6.37**	**3.00**	**2.62**	**1.48**	**0.24**	**0.00**
91	**5.92**	**1.35**	**1.10**	**0.07**	**0.10**	**0.00**
93	**2.24**	**2.96**	**4.58**	**0.91**	**0.56**	**0.46**
104	**7.65**	**4.86**	**2.95**	**0.98**	**0.20**	**0.58**
Avg	**7.65**	**4.86**	**2.95**	**0.99**	**0.20**	**0.10**
sd	**2.54**	**1.70**	**0.98**	**0.52**	**0.16**	**0.20**

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NO2	
	1
Time	
Days	
	0.00
8	8.00
10	2.91
15	5.22
17	1.94
22	**10.20**
26	**11.30**
31	**8.92**
36	**9.31**
43	**5.03**
50	**9.16**
71	**6.45**
78	**9.22**
84	**6.37**
91	**5.92**
93	**2.24**
104	**7.65**
Avg	**7.65**
sd	**2.54**

Nitrate measurements (cascade system)

N03						
	Channel					
	1	2	3	4	5	Eff
Time	Dist					
Days	(m)					
	0	1.9	3.7	5.5	7.3	9
8	**14.2**	**13.2**	**14.0**	**15.5**	**11.2**	**11.9**
10	**14.9**	**4.5**	**8.7**	**11.7**	**16.5**	**17.1**
15	**24.4**	**12.3**	**33.3**	**36.4**	**39.7**	**44.0**
17	**33.9**	**18.8**	**32.6**	**34.2**	**62.1**	**65.2**
22	**4.1**	**17.5**	**50.0**	**58.7**	**60.8**	**63.9**
26	**4.1**	**18.8**	**27.2**	**51.0**	**61.1**	**62.3**
31	**4.0**	**20.1**	**34.1**	**49.4**	**54.1**	**58.8**
36	**5.2**	**16.3**	**29.2**	**48.3**	**57.7**	**60.2**
43	**5.1**	**21.1**	**34.6**	**47.7**	**56.6**	**59.3**
50	**3.2**	**19.2**	**29.8**	**52.2**	**62.0**	**61.6**
71	**8.8**	**13.9**	**24.5**	**42.1**	**49.8**	**53.1**
78	**6.2**	**16.4**	**28.9**	**40.2**	**56.3**	**61.4**
84	**5.1**	**6.0**	**21.7**	**35.8**	**42.7**	**45.8**
91	**5.9**	**14.4**	**36.3**	**49.5**	**55.2**	**60.8**
93	**14.8**	**13.4**	**29.3**	**47.4**	**49.7**	**47.2**
104	**6.0**	**16.1**	**31.4**	**47.5**	**55.1**	**57.7**
Avg	**6.0**	**16.1**	**31.4**	**47.5**	**55.1**	**57.7**
sd	**3.1**	**4.0**	**7.2**	**5.9**	**5.5**	**5.9**
(Calculation period Day 22 - 104)						

Plot of Nitrogen cycle day 8 – 36

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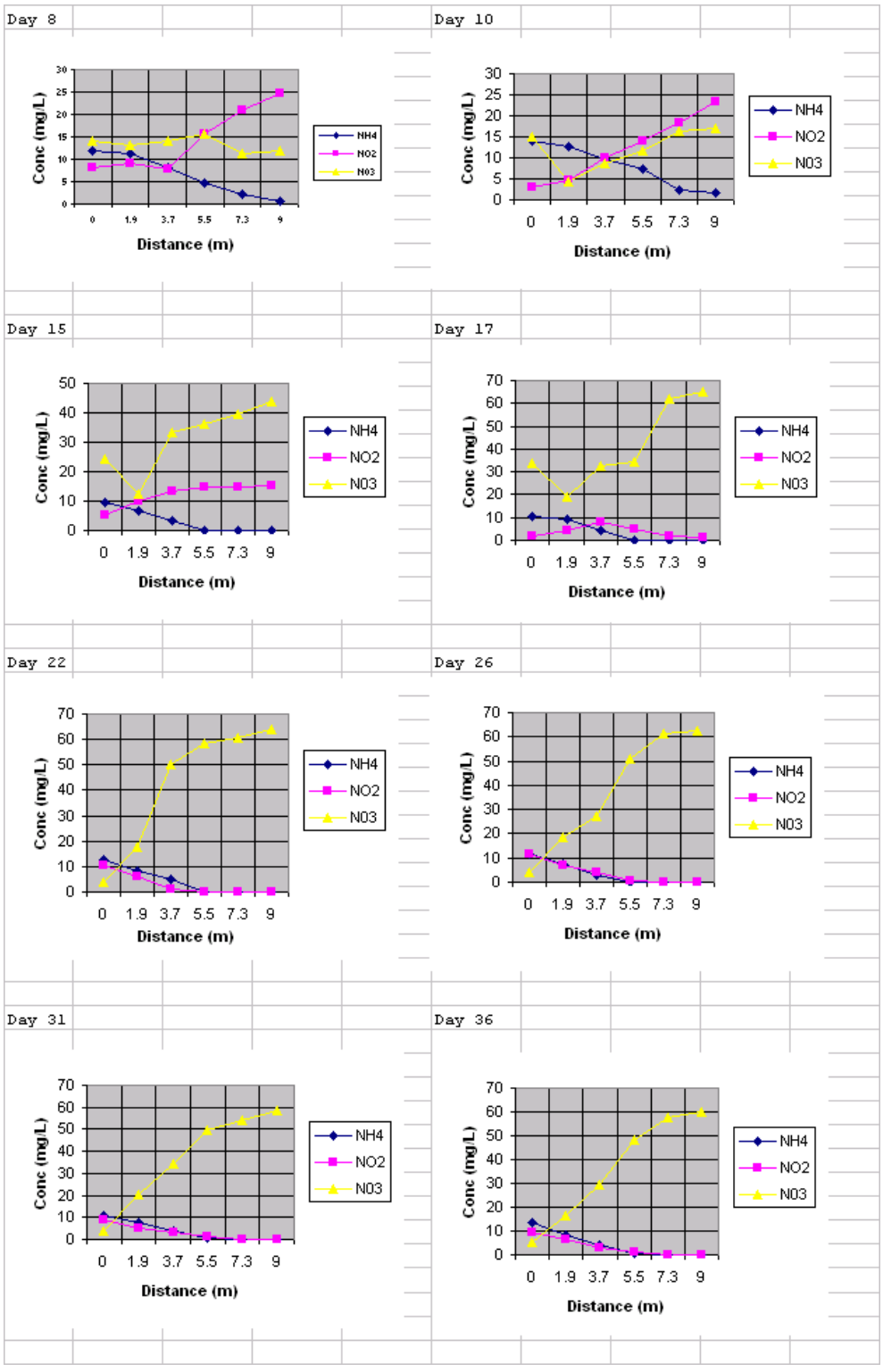
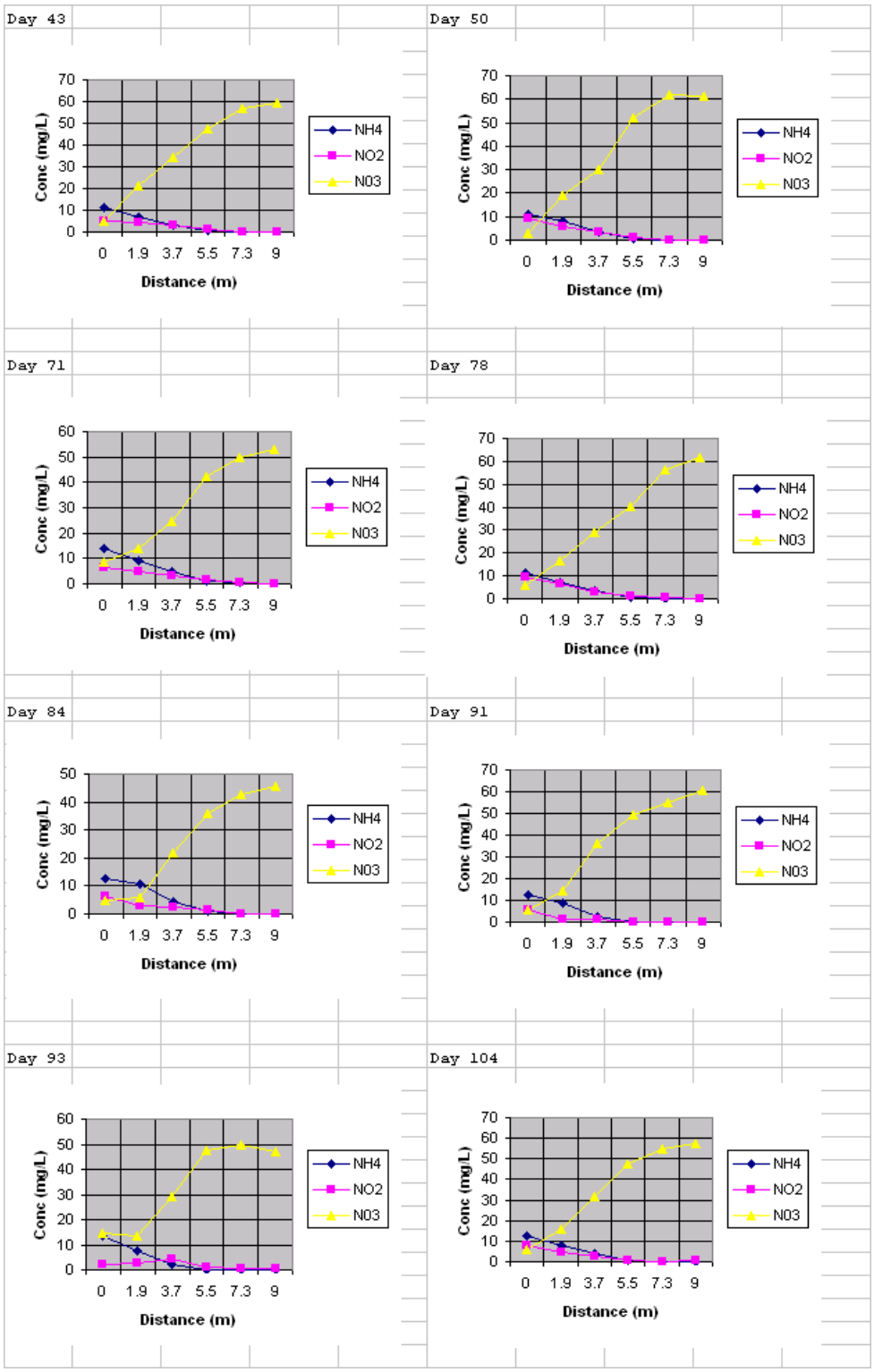
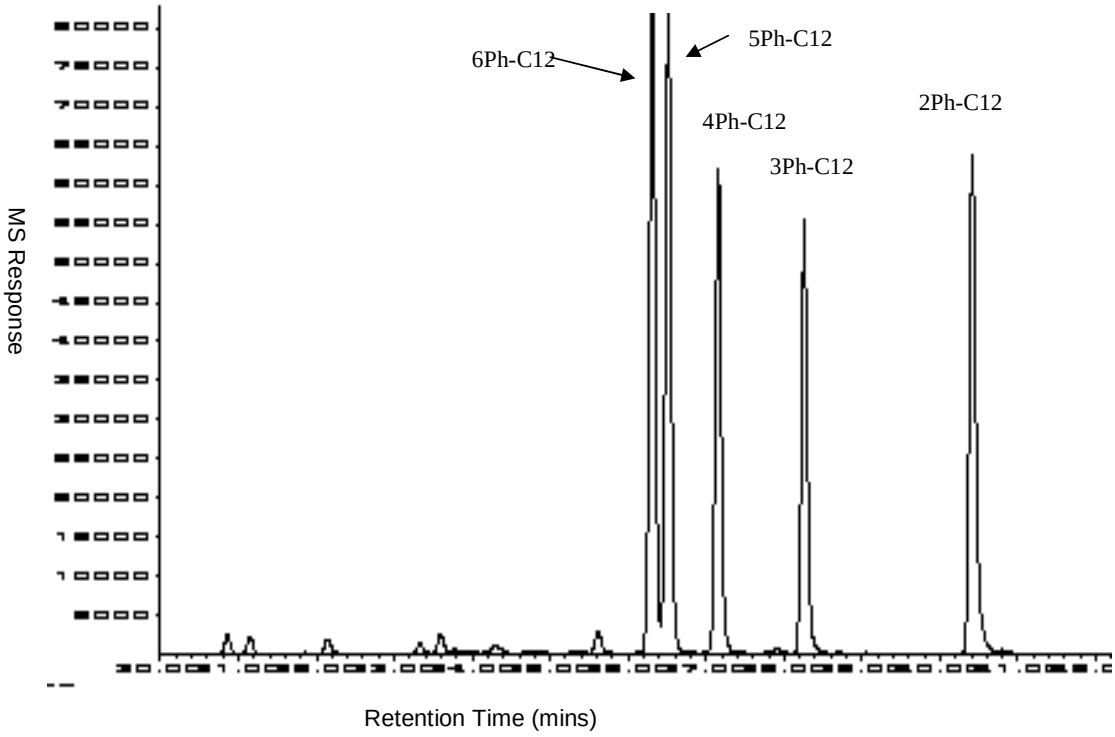


Plot of Nitrogen cycle day 43 – 104





Example of a single ion monitoring (SIM) chromatogram (m/z = 374) of 15mg total LAS after derivatisation