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The removal of linear alkylbenzene sulphonate (LAS) in constructed wetlands

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THE REMOVAL OF LINEAR ALKYLBENZENE SULPHONATE (LAS) IN CONSTRUCTED WETLANDS

A thesis submitted to the University of Wales by

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ABSTRACT

This study investigated anionic surfactant LAS (Linear Alkylbenzene Sulphonate) removal processes in wetlands used for domestic wastewater treatment. The surfactant was monitored via a sensitive HPLC method or using radiolabelled techniques under controlled laboratory conditions, enabling a distinction to be made between primary biodegradation and mineralization. Hydrochemical parameters and enzyme activity (phosphatase, sulphatase and β-glucosidase) were also assessed in several investigations.

A field survey of three operational wetlands suggested that sub-optimal conditions occurred for LAS degradation with 55% removal observed. Climatological events influenced degradation with a springtime peak in LAS removal identified. Further laboratory investigation revealed possible controlling factors as temperature, pH and oxygen levels. However, effluent LAS concentration discharging from the wetland was still low (min. 0.02mg L^{-1}) and comparable to other sewage treatment studies.

Biodegradation by biofilm microbial processes was identified as being the major LAS removal mechanism. Pre-exposure to LAS enhanced initial mineralization probably via adaptation of the microbial community resulting in faster kinetics and a shorter lag phase. Biofilm presence also slightly increased LAS adsorption. Plants were also identified as significantly enhancing LAS removal mainly via oxygen root release and supporting a more active and/or different microbial biofilm populations in comparison to that on the gravel. However, plant uptake, species and exudation of organic matter were insignificant.

LAS at environmentally realistic concentrations did not impair natural wetland processes monitored, such as phosphate sorption, enzyme activity, plant growth, microbial activity and greenhouse gas emissions. Overall, the results of these studies support the hypothesis that LAS can be readily degraded in wetlands with similar removal processes involved as in more conventional sewage treatment processes. Hence this study suggests that constructed wetlands are a viable, cost-effective alternative for wastewater treatment for small communities in the UK and worldwide in facilitating LAS removal.

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List of Abbreviations

ABS	Alkyl Benzene Sulphonate
AE	Alcohol Ethoxylates
APE	Alkyl Phenol Ethoxylates
BOD_5	5-day Biological Oxygen Demand
CMC	Critical Micelle Concentration
CTAB	Cetyl Trimethylammonium Bromide
DOBS	Dodecyl Benzene Sulphonate
DOC	Dissolved Organic Carbon
HF	Horizontal Flow wetland
HPLC	High Performance Liquid Chromatography
HRT	Hydraulic Residence Time
k	Rate constant
LAS	Linear Alkylbenzene Sulphonate
LECA	Light Expanded Clay Aggregates
MBAS	Methylene Blue Active Substances
MUF	Methylumbelliferyl
NH_4^+	Ammonium
NO_3^-	Nitrate
NP	Nonyl Phenol
PO_4	Inorganic phosphate ($H_2PO_4^-$, HPO_4^{2-}, PO_4^{3-})
SEM	Scanning Electron Microscopy
SF	Surface Flow wetland
SO_4^{2-}	Sulphate
SPCs	Sulpho Phenyl Carboxylates
SPE	Solid Phase Extraction
SSF	Subsurface Flow wetland
$t_{1/2}$	Half-life
T	Temperature
TLC	Thin Layer Chromatography
TSS	Total Suspended Solids
VF	Vertical Flow wetland
WHO	World Health Organisation

CHAPTER 1: Introduction and Literature Review

1.1 INTRODUCTION

Surfactants are common components in household detergents encountered in the environment due to their disposal and subsequent presence in sewage. Research on their fate in sewage treatment plants is comprehensive with substantial focus on Linear Alkylbenzene Sulphonate (LAS), a major anionic surfactant. However, although the potential application of wetlands for wastewater treatment has been recognized worldwide in recent years, limited information is available on surfactants removal in these ecosystems. The majority of research on wetland treatment is on e.g. BOD, nutrient, coliform and metal removal efficiency. Hence the main aim of this thesis is to investigate the fate and impacts of LAS in wetlands used for wastewater treatment. The main issues to be addressed are quantification of LAS removal, identification of factors affecting removal and potential detrimental impacts of LAS on critical wetland processes.

1.2 SURFACTANTS

Surfactants are surface-active compounds that consist of both polar and non-polar parts (Swisher 1987). Typically surfactants exhibit both hydrophilic (having an affinity for water) and hydrophobic (having an aversion to water) characteristics at the opposite ends of a long alkyl chain (Castles *et al.* 1989). These compounds are widely used in detergents due to their unique surface-active properties that lowers the surface tension of water enabling dirt to be released from both fabrics and surfaces (Swisher 1987). At low concentrations surfactants are soluble in water. However, at higher concentrations surfactants associate to form small aggregates known as micelles (Figure 1.1). In water, the hydrophobic end of the surfactant forms the center of the micelle and the hydrophilic end the outside (Swisher 1987). The concentration at which micelles begin to form is known as the Critical Micelle Concentration (CMC) (Swisher 1987). At concentrations above the CMC surfactants can solubilise otherwise insoluble organic material by incorporating it into the interior of the micelles (Figure 1.2) consequently limiting bioavailability (Zhang *et al.* 1999).

However, at environmental concentrations the CMC is usually not reached, except perhaps in industrial discharges from detergent manufacturing industry.

Figure 1.1: Example of micelle structure (Shaw 1992).

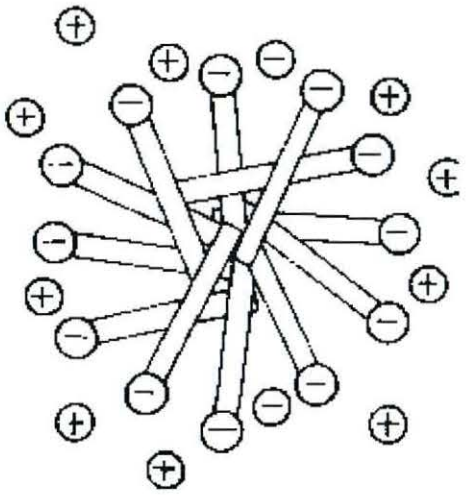
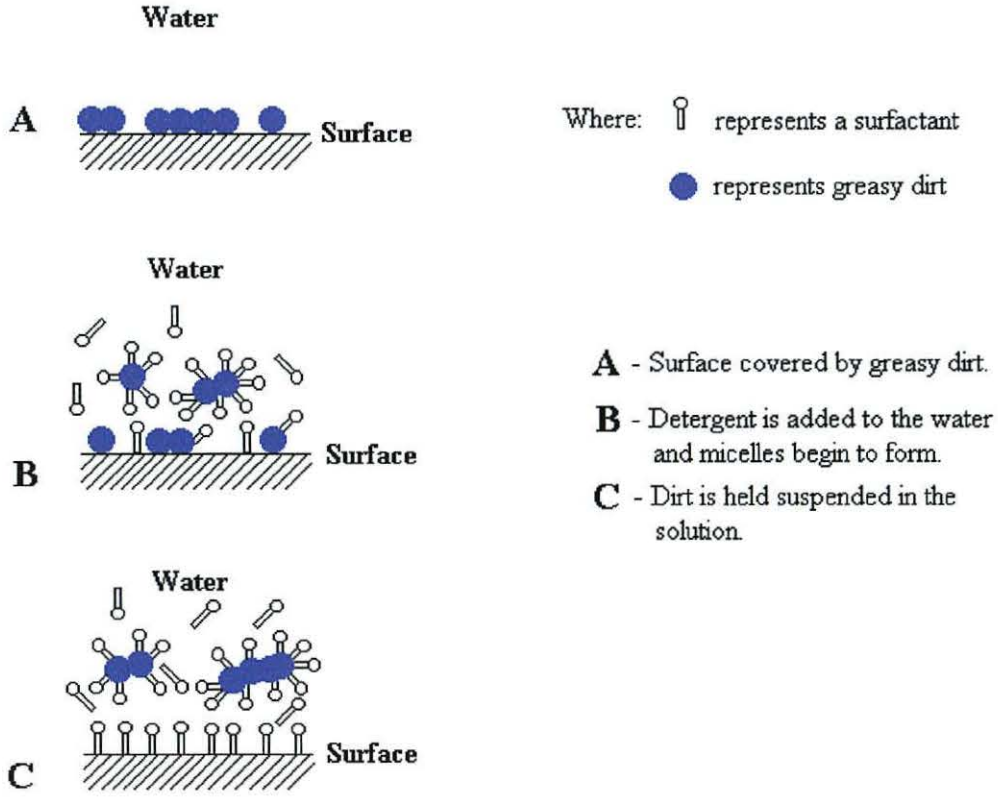


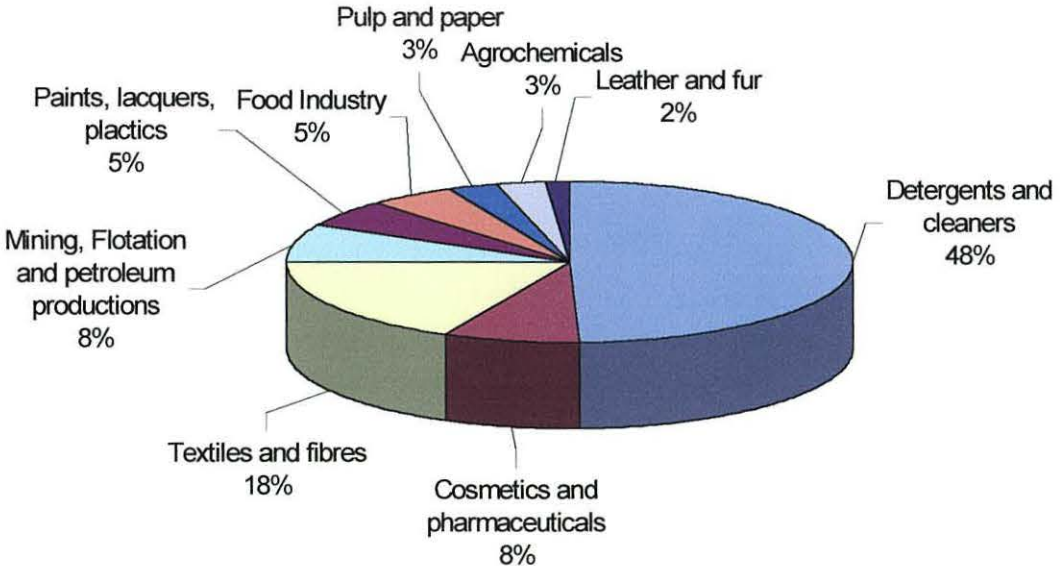
Figure 1.2: Removal of greasy dirt from a solid surface by surfactants in detergents (as amended from Shaw 1992)



Surfactants are generally classed as anionic, cationic, amphoteric or non-ionic according to the surface-active part of the molecule (Swisher 1987). In aqueous solutions anionic surfactants dissociate to form negatively charged surfactant ions (e.g. $-SO_3^-$), cationic surfactants give a positively charged surfactant ion (e.g. $-R_3N^+$), amphoteric surfactants have a positive and negative charge and non-ionic surfactants contain hydrophilic groups that do not ionize appreciably (Swisher 1987).

Figure 1.3 demonstrates that the main application of surfactants are in detergents and cleaners, with modern detergents containing approx. 10-35% surfactant (Swisher 1987, Callely *et al.* 1977). A broad spectrum of other industries also use surfactants, e.g. cosmetics and toiletries, textiles, agriculture, plastics, paints and photographic industries (Steber & Berger 1995, Scott & Jones 2000).

Figure 1.3: Surfactants application (as amended from Karsa 1987)



Recently surfactants have been considered for their potential use in environmental remediation via dissolution, desorption and biodegradation of pollutants from soil (van der Merren & Verstraete 1996). The concept of applying surfactants for such purposes originated from the petroleum industry that has successfully used surfactants for enhanced oil recovery (Allred & Brown 1996). Anionic surfactants have

commonly been used for in-situ desorption of heavy metals (Kornecki *et al.* 1997), polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) (van der Merren & Verstraete 1996). However, high surfactant concentrations are required with success of remediation dependent upon the mobility of the surfactant. Other beneficial effects observed due to the presence of surfactants include the enhanced dispersal, growth and mobility of specific bacteria in soil, improved anaerobic digestion process and so on (van der Merren & Verstraete 1996).

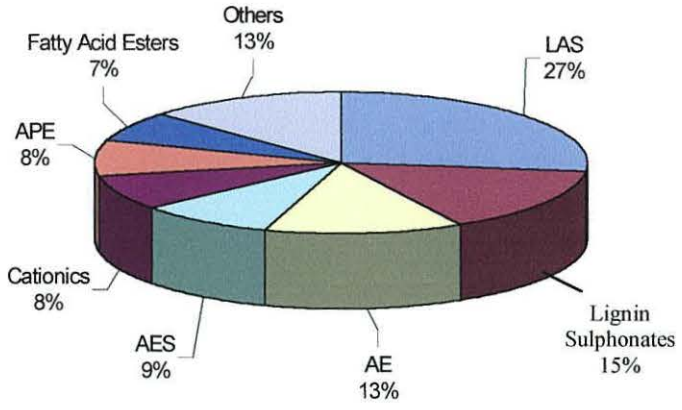
1.3 LAS (LINEAR ALKYLBENZENE SULPHONATE)

LAS is a major anionic surfactant used in detergents worldwide due to its effectiveness, cost/performance ratio, versatility and environmental safety record (de Wolfe & Feijtel 1998). The surfactant was introduced in the 1960s as a replacement for slowly degradable Alkyl Benzene Sulphonate (ABS). Foaming problems in sewage treatment plants, rivers and lakes mainly due to ABS are well documented (Jensen 1999) as shown in figure 1.4.

Figure 1.4: Illustration of foaming problems with surfactants in the 1960s, River Lee (courtesy of Unilever Research).

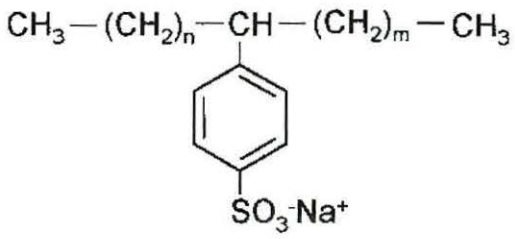
LAS can be synthesized from linear olefins or paraffins obtained from petroleum fractions which are then reacted with benzene in the presence of a catalyst. Sulphonation of the ring then occurs with an introduction of a sulphonate group usually in the para position (Swisher 1987). LAS has an annual worldwide consumption estimated at 2 x 10^6 t per year (Jensen 1999) and has a per capita consumption in Western Europe and US of between 1-2kg yr^{-1} (Cavalli *et al.* 1993). Figure 1.5 demonstrates that LAS accounts for a large proportion of surfactants manufactured. The human and environmental safety of LAS has been extensively researched in several environments, e.g. terrestrial (Jensen 1999), aquatic (Castles *et al.* 1989, Inaba & Amano 1988) and marine (Kikuchi *et al.* 1986), with computer modeling used to predict its fate, e.g. in river water the GREAT-ER project (Holt *et al.* 1998).

Figure 1.5: Production of different types of surfactants (as amended from Karsa 1987)



The common commercial formulation generally consists of a mixture of alkyl homologues of mainly C_{10}-C_{14}, mean $C_{11.8}$, with attached phenyl group at positions between 2-6 (Matthijs & De Henau 1987, de Wolfe & Feijtel 1998) as shown in figure 1.6. Concentration of detergent products in washing solutions range between 1000-3000mg L^{-1} (0.1-0.3%) with LAS comprising 5-10% part of the washing agents (Litz *et al.* 1987). LAS has a reported CMC of approximately 520mg L^{-1} (Zhang *et al.* 1999).

Figure 1.6: Structure of LAS (Matthijs & De Henau 1987).



Where n and m = 10 to 14

1.3.1 LAS REMOVAL PROCESSES

1.3.1.1 Biodegradation

Biodegradation is an essential mechanism for irreversible LAS removal. It refers to the breakdown of an organic substance by microorganisms and for LAS is cited in terms of primary degradation or mineralization (Brown 1995). The former refers to degradation required to change the structure of the compound so that the basic physical and chemical properties are lost, whereas mineralization refers to the complete or 'ultimate' conversion of a compound to carbon dioxide (CO_2), water and other inorganic compounds (Swisher 1987, Larson *et al.* 1993). Biodegradation is influenced not only by the presence, size and function of the LAS-utilizing microbial community but also the environmental and physical conditions that affect community activity and LAS bioavailability (Knaebel *et al.* 1994). LAS is generally regarded as a highly biodegradable surfactant under aerobic conditions (Scott & Jones 2000), whereas under anaerobic conditions remains debatable (Jensen 1999).

(i) Aerobic Biodegradation

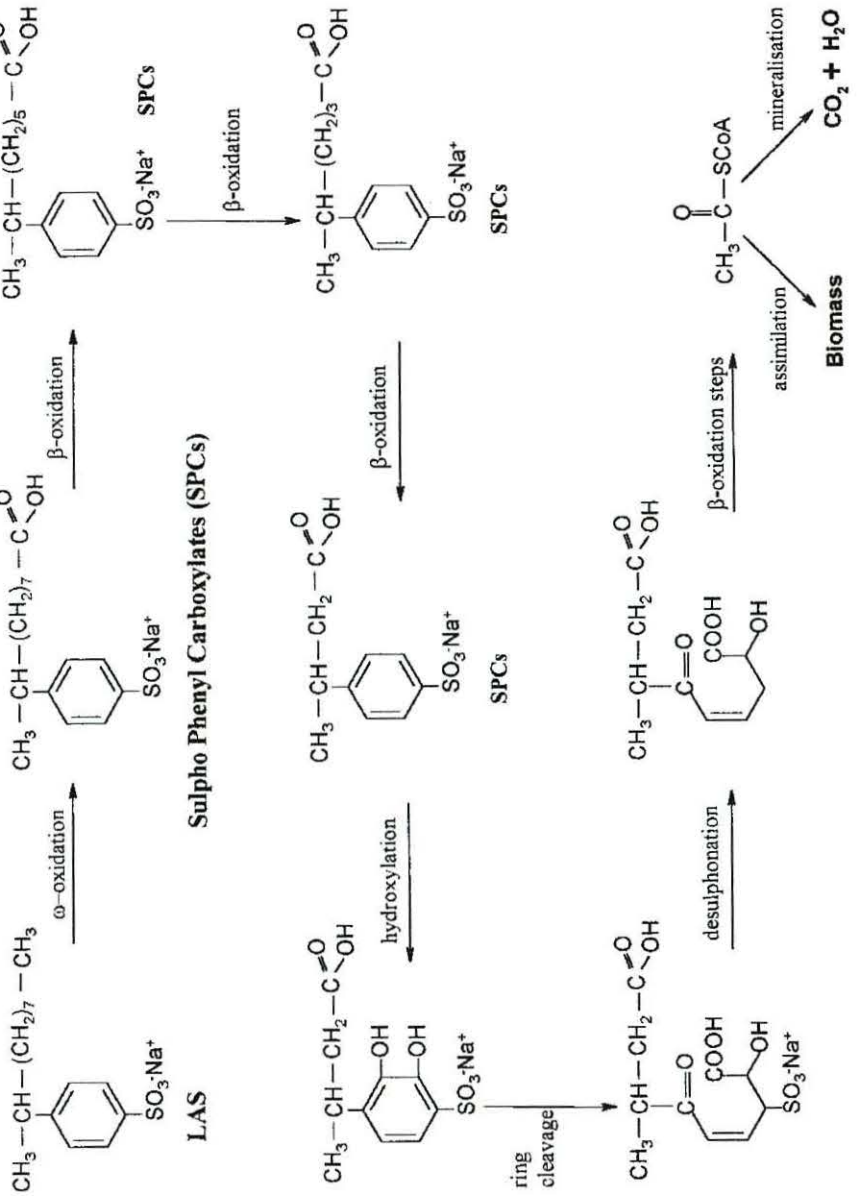
LAS is readily biodegradable under aerobic conditions (>90%) (Matthijs & De Henau 1987) and its mechanism is well documented as shown in figure 1.7. Swisher (1987) reports that the initial attack on LAS occurs with oxidation of the terminal methyl group firstly to an alcohol, then aldehyde and finally to a carboxyl group by ω-oxidation forming intermediates known as Sulpho Phenyl Carboxylates (SPCs). The alkyl chain is then rapidly degraded via β-oxidation involving successive shortening

of the alkyl chain by removing C_2-units at a time through a sequence of enzymatically catalysed reactions until 4-7 carbon atoms remain (Swisher 1987). After primary biodegradation has occurred much slower oxidation of the benzene ring component of the compound is then initiated. Cleavage of the aromatic ring involves the introduction of hydroxy groups followed by desulfonation of the ring degradation products, yielding sulfate (Steber & Berger 1995). Further catabolism by general metabolic routes of the compound results in the eventual production of CO_2 requiring a consortium of bacteria due to the limited metabolic capacities of individual microorganisms and the complexity of LAS structure (Jiménez *et al.* 1991, van Ginkel 1996).

(ii) Anaerobic Biodegradation

Conventionally LAS degradation under anaerobic conditions is perceived to occur very slowly or not at all due to initial ω-oxidation requiring molecular oxygen (Jensen 1999, Steber & Berger 1995, McEnvoy & Giger 1986, Wagener & Schink 1987). However, some researchers argue that alternative anaerobic degradation pathways exist. Field data support the argument for anaerobic degradation as no observed accumulation of LAS in anaerobic environments, e.g. river sediments (Waters & Feijtel 1995). Possible explanations include unrealistic test conditions, alternative degradation pathways and/or pervious oxygenation exposure affecting anaerobic degradation. The strict anaerobic tests conducted is not representative of anaerobic conditions in the environment, e.g. real environmental conditions may have some molecular oxygen present through diffusion. Laboratory studies on the degradation of LAS in anoxic conditions show that LAS will degrade. Alternative anaerobic degradation pathways exist, e.g. reduction of the sulphonate group to a sulfhydryl (-SH) group resulting in hydrolytic ring cleavage (Federle & Schwab 1992). In addition Denger & Cook (1999) argue that not all desulphonation reactions require oxygen and found that desulphonation of the C-SO_3^- bond of LAS can occur by anaerobic bacteria growing under anoxic conditions. Evidence of LAS degradation under anoxic conditions suggests that once initial biodegradation has occurred continuation even in anaerobic conditions will occur.

Figure 1.7: LAS biodegradation pathway (Steber & Berger 1995)



1.3.1.2 Adsorption

Adsorption is well documented as an important LAS removal mechanism, due to surface-active properties (Swisher 1987). In terms of sewage treatment, LAS adsorption is significant with approximately 30% adsorbed onto suspended particles entering during primary treatment (Matthijs & De Henau 1985) with the other approximate 70% degraded by microorganisms. However, desorption can return LAS to the aquatic phase. Matthijs & De Henau (1985) in a study on river sediments found the total LAS adsorption of 57% but desorption of 26%. This suggested that sediments act as a sink for LAS (Matthijs & De Henau 1985).

1.3.1.3 Chemical structure effects

Compound structures of the alkyl homologues and isomers of the LAS commercial mixture can have profound effect on LAS biodegradation and adsorption (Swisher 1987). In the late 70s Swisher *et al.* (1978) and Wichbold (1975) independently reported that the longer chain alkyl homologues were degraded more rapidly than short chain homologues (as cited in Swisher 1987). This has since been confirmed by several authors for C_6 to C_{16} homologues (Swisher 1987, Terzic *et al.* 1992). Similarly longer chain homologues have higher adsorption tendencies due to the greater hydrophobicity (McEnvoy & Giger 1986, Jensen 1999). Faster degradation and greater adsorption of isomers with the phenyl attached near the end of the chain than those with more central attachment is reported (Swisher 1987, Hand & Williams 1987). These structural effects are related to the compound hydrophobic characteristics and have become known as the distance principle.

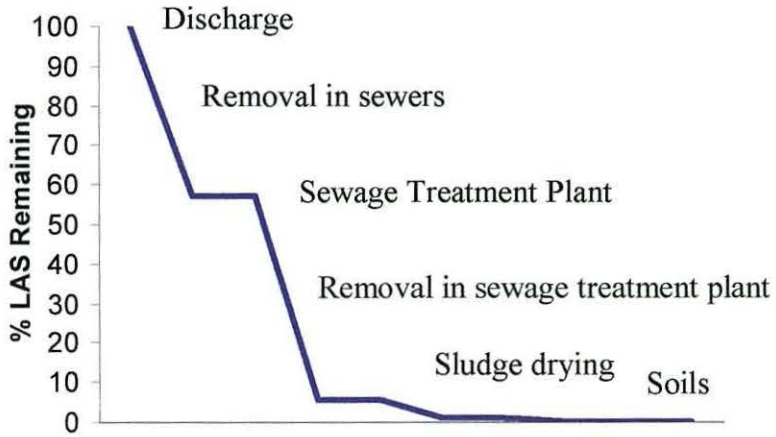
1.3.2 Fate of LAS in the Environment

On account of disposal of detergents into the sewage system LAS can be present in wastewater entering a sewage treatment plant at significant concentrations. Biodegradation is initiated prior to reaching sewage treatment plants with evidence of substantial removal in the sewer system, e.g. >50% (Moreno *et al.* 1990, Berna *et al.* 1993, Holt *et al.* 1998). Levels of 3.25-15.1mg L^{-1} LAS in raw sewage are published (Holt *et al.* 1998, Schröder *et al.* 1999, Waters & Feijtel 1995) with evidence of the high removal (≥95%) during traditional treatment (Jensen 1999, Castles *et al.* 1989, Matthijs & De Henau 1987, Leal *et al.* 1994) and >90% with lagoon treatment

(Moreno *et al.* 1994). Complete degradation is not often achieved resulting in discharges at <1mg L^{-1} (Moreno *et al* 1990) with reported levels of e.g. 240µg/l (Holt *et al.* 1998) and 0.01mg L^{-1} (Waters & Feijtel 1995). However, although the average LAS measured in raw wastewater is low it is possible in industrial waters from surfactant manufacturing to exceed 300mg L^{-1} (Wagner & Schink 1987).

LAS adsorbed and not degraded in sewage sludge can enter the soil environment via application of sludge to soils as a fertilizer (Jensen 1999). However, once applied to the soil sufficient aerobic conditions will result in the biodegradation of the surfactant, with a half-life of 1-3 weeks, preventing bioaccumulation depending on concentration and adaptation of the microbial community (Jensen 1999). Moreno *et al.* (1990) produced a fate cycle of LAS in the environment that can be used as a good indication of the behaviour of the surfactant after initial discharge and is shown in figure 1.8.

Figure 1.8: The fate cycle of LAS in the environment (Moreno *et al.* 1990).



1.3.3 Detection

Several analytical methods have been developed for the detection of LAS with the most widely used being the colorimetric methylene blue method (Matthijs & De Henau 1987). The methylene blue cation and anionic surfactants react to form a complex that is readily extracted into chloroform forming a characteristic blue colour that is easily quantified by absorbance (Swisher 1987). However, this method is not specific to LAS as it also detects other anionic materials with the measurements

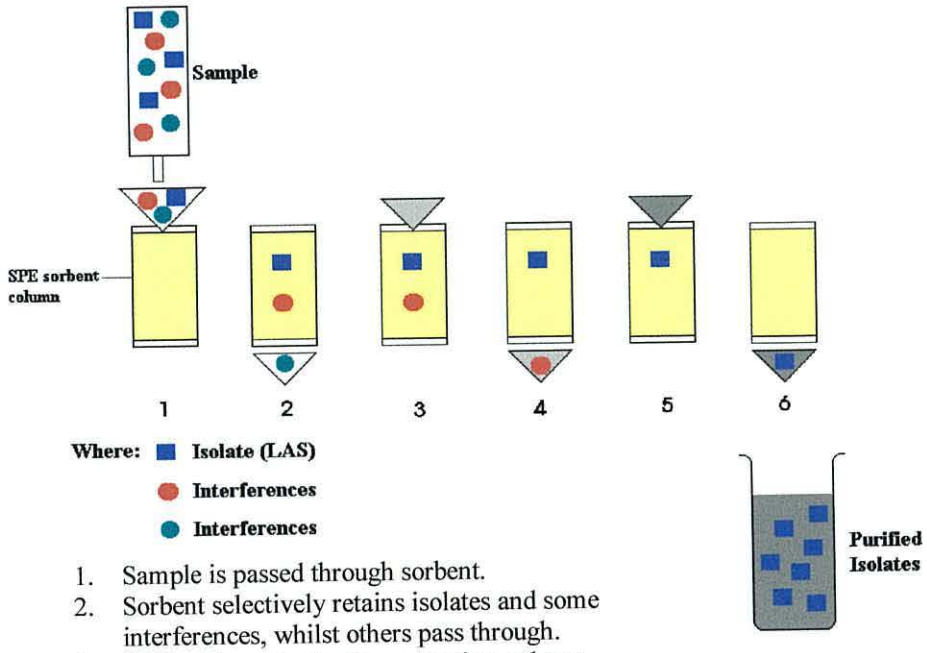
consequently being referred to as Methylene Blue Active Substances (MBAS), with many interferences especially in environmental samples, e.g. humic substances (Matthijs & De Henau 1987).

Alternative methods for specific determination of LAS have recently been developed including potentiometric methods, gas chromatography, mass spectrometry and Nuclear Magnetic Resonance (NMR). However, one of the most promising methods to date is High Performance Liquid Chromatography (HPLC). This method is specific to LAS, highly sensitive and can identify individual LAS homologues. In order to isolate, concentrate and determine trace levels of LAS in environmental samples solid phase extraction (SPE) procedures are required prior to HPLC analysis (Kikuchi *et al.* 1986, Matthijs & De Henau 1987, Castles *et al.*, 1989). SPE is used in order to separate LAS from the broad range of other components, including other anionic surfactants with the principles involved illustrated in figure 1.9.

1.3.4 Intermediates

Intermediates Sulpho Phenyl Carboxylates (SPCs) are formed during the first step of biodegradation of LAS (Berna *et al.* 1993) as shown in figure 1.7. The chain length of the SPCs are shortened by 2 carbon atoms through β-oxidation steps until ring cleavage by certain bacteria occurs (Gonzalez-Mazo *et al.* 1997). These intermediates are very polar compounds and hence are only found in water (Berna *et al.* 1993). However, concern has recently been voiced about the accumulation of these relatively stable intermediates in the environment (van der Meeren & Verstraete 1996). These intermediates may have different effects and toxicity in comparison to the original parent compound and can exhibit 120-240% higher LC_{50} values (Kimerle & Swisher 1977). However, complex analytical methods are required for SPC detection, e.g. LC-MS (Gonzales-Mazo *et al.* 1997).

Figure 1.9: Solid Phase Extraction (SPE)



1. Sample is passed through sorbent.
2. Sorbent selectively retains isolates and some interferences, whilst others pass through.
3. Sorbent is washed with appropriate solvent.
4. Isolates remain on the sorbent bed whereas interferences are eluted.
5. Concentrated isolates are eluted from sorbent with appropriate solvent.

1.4 WETLANDS

In the last twenty years wetlands have shown great potential in wastewater treatment. Their ability to transform and/or inactivate a variety of nutrients, metals and chemical substances into harmless forms is well documented. The research has developed from monitoring biogeochemical processes of natural wetlands to the design and application of thousands of constructed wetlands worldwide. Both natural and constructed wetlands are sources, sinks and transformers of nutrients with the dominant process dependent upon several factors, e.g. vegetation presence and species, climatological influences and hydrology.

1.4.1 Natural Wetlands

There are several definitions of natural wetlands in the literature which take into account various conditions, locations, functions and size. Hence there is no one single correct definition. However, there are several main distinguishing features of natural wetlands the most important of which are the presence of standing water, the unique wetland soils and the vegetation which adapt to, or are tolerant of, the saturated soil conditions (Mitsch & Gosselink 1993). In terms of formal definitions according to the U.S. 1977 Clean Water Act (Section 404):

'The term "wetlands" means those areas that are inundated or saturated by surface or ground water at a frequency and duration sufficient to support, and that under normal circumstances do support, a prevalence of vegetation typically adapted for life in saturated soil conditions. Wetlands generally include swamps, marshes, bogs and similar areas' (as quoted in Kent 1994).

From a global perspective, natural wetlands make up approximately 6% of the land surface of the world and are found in every climate from the tropics to tundra (Mitsch & Gosselink 1993). They are transitional between terrestrial and aquatic systems and are recognized as diverse and productive ecosystems. Many natural wetlands have been indirectly treating wastewater worldwide for centuries (Kent 1994, Hammer & Bastain 1989) with, for example, riparian wetlands recognized as nutrient sinks. Wetlands were recognized for their storage and transformations of nutrients and pollutants in wastewater from upland regions (Hammer & Bastain 1989). More recently biogeochemical processes, e.g. greenhouse gas emissions (Freeman *et al.*

1997), enzyme activity (Freeman *et al.* 1995, Kang & Freeman 2000) and nutrient retention (Kent 1994) have been monitored in natural wetlands indicating their water quality amelioration potential.

This potential for wetlands as a form of natural passive biological treatment has only relatively recently, in the last 50 years, been recognized. Further investigation resulted in development and application of constructed wetlands as effective and feasible systems for wastewater treatment (Kadlec & Knight 1996). In some countries, e.g. Finland, wastewater treatment plants are designed to incorporate natural wetlands. There are several advantages of using these natural systems, including their established diverse population of bacteria that live and grow in the ecosystem. However, signing of the Ramsar Convention (1971) has ensured the protection and preservation of valuable natural wetlands from exploitation and coupled with other problems with geographical locations constructed wetlands are much more widely applied.

1.4.2 Constructed Wetlands

Constructed wetlands are designed to mimic the biogeochemical characteristics and functions of a natural wetland under more controlled and manipulative conditions. Hammer & Bastain (1989) define constructed wetlands as a 'designed and man-made complex of saturated substrates, emergent and submergent vegetation, animal life, and water that simulates natural wetlands for human use and benefits'. Applications include treatment of sewage (Kadlec 1999), landfill leachate (Kowalik *et al.* 1996), industrial effluent, mining effluent (Dunbabin & Bowmer 1992) and agricultural wastes (Kern & Idler 1999).

On account of the natural nutrient cycling processes occurring in constructed wetlands, the application for sewage treatment has been popular with approximately 500-1000 systems in operation in Europe reported in 1995 (Haberl *et al.* 1995), usually in a secondary or tertiary capacity. The effectiveness of the wetland is based on various complex physical, chemical and biological processes occurring in parallel between the substrate, plants and microorganisms. Physical processes involve settling of suspended particulate matter and filtration, chemical processes include adsorption and precipitation, and biological processes include oxidation or reduction reactions

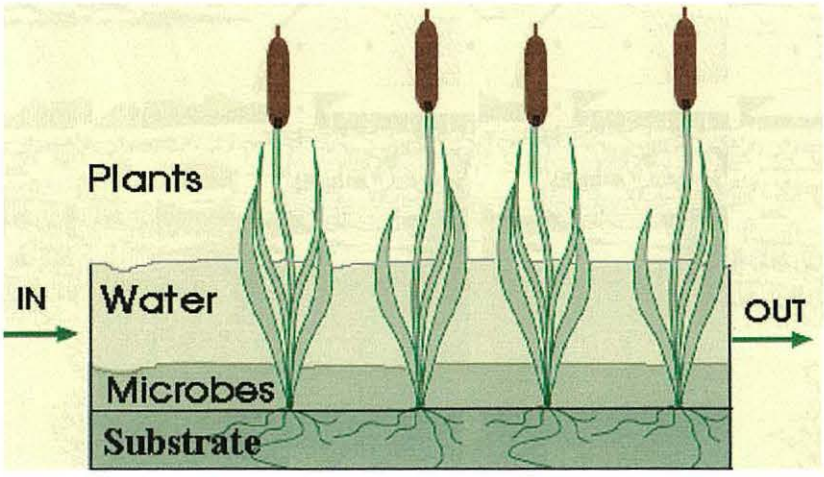
(Gopal 1999). Surveys on the removal of various pollutants in constructed wetlands have been conducted globally, e.g. 5-day Biochemical Oxygen Demand (BOD_5) (Haberl *et al.* 1995), phosphate (Brix *et al.* 2001), nitrate (Hammer & Knight 1994) and metals (Dunbabin & Bowner 1992).

In the UK the Water Resources Act (1991) states that it is an offence to 'cause or knowingly permit' pollutants to enter controlled waters without permission. The permission is usually granted by the Environment Agency (EA) and normally specifies conditions in relation to the volume and concentration of substances in the effluent. Hence water authorities are responsible for treatment of sewage effluent to a high standard. Wetlands can not only provide effective treatment of wastewater but are also cost-effective. Additional secondary benefits such as ease of construction and maintenance, less energy intensive, renewable and aesthetically pleasing contribute to making constructed wetlands an attractive addition, or alternative, to conventional wastewater treatment technologies even though they can be land intensive.

1.4.3 Wetland Components

In wetlands there are several components involved in the various processes that occur within the system. The basic components are water, substrate, plants and microorganisms as shown in figure 1.10.

Figure 1.10: The basic components of a wetland system.



(a) Water

Hydrology is the most important design factor for all constructed wetlands with even small changes having a large influence on nutrient availability, organic matter accumulation, oxygen availability and primary productivity (Kadlec & Knight 1996, Mitsch & Gosselink 2000). The hydraulic residence time (i.e. average time that water remains in the wetland) affects treatment efficiency and is the dominant consideration in wetland design. Other important hydrological factors include weather and climate conditions, hydraulic loading and evapotranspiration (Kent 1994).

(b) Substrate

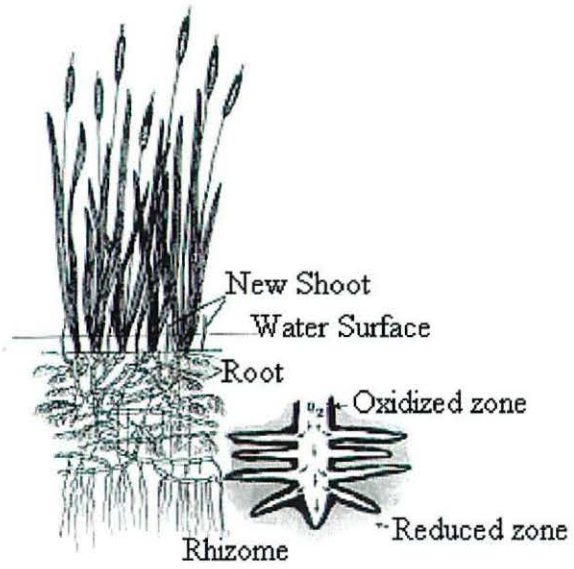
In constructed wetlands the most common substrates used include soil, gravel and sand. Gravel is often applied for greater hydraulic conductivity and percolation with better access to the oxygenated conditions at the plant roots and rhizosphere (Dunbabin & Bowmer 1992). The substrate not only physically supports the plants but also acts as a filter, stores pollutants and provides attachment sites for microbial and chemical transformations to occur (Hammer & Bastain 1989). In addition the anaerobic conditions, resulting from the presence of standing water, in some wetlands is characterised by a narrow oxidised zone at the substrate surface allowing aerobic processes to occur (Mitsch & Gosselink 1993).

(c)Plants

Vegetation is the most visible component of any wetland and is often included in the definition of natural wetlands. The three main macrophytes are normally planted in constructed wetlands, which are reeds (*Phragmites* spp.), cattails (*Typha* spp.) or bullrushes (*Scripus* spp.) with species selection commonly dependent on wastewater contaminant, climate, wetland design and local species (Kent 1994). *Phragmites australis* is most common species used in constructed wetlands worldwide, particularly in Europe (Haberl *et al.* 1995, Tanner 1996), hence the common term of 'reed beds'. Important plant functions in a wetland include uptake and storage of nutrients and pollutants, attachment site for microbial growth within the water column and transport of atmospheric oxygen into the root zone (Brix 1997, Armstrong & Armstrong 1988, Hammer & Bastain 1989). Oxygenation of the root and rhizosphere, as shown in figure 1.11, results in promotion of aerobic microbial processes, e.g. nitrification (Reddy *et al.* 1989) and oxidation of organic matter (May *et al.* 1990). In

addition other plant roles include filtration and increasing retention time resulting in greater interaction for microorganisms and pollutants (Brix 1997, Findlater *et al.* 1990).

Figure 1.11: Transport of oxygen to root zone by wetland plants (Hammer & Bastain 1989).



(d) Microorganisms

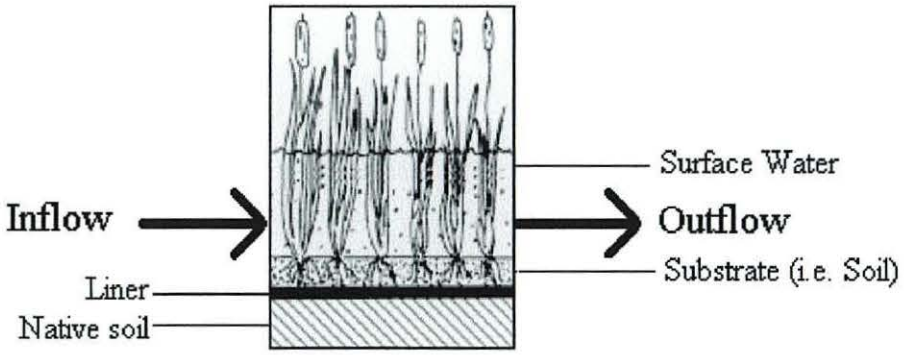
Microbes play a key role in wetland function in terms of pollutant transformations and nutrient cycling to gain energy for growth (Hammer & Bastain 1989). The unique conditions within the root-zone and rhizosphere create an environment suitable for a large diversity of both aerobic and anaerobic bacterial communities which can treat wastewater containing a variety of pollutants (Findlater *et al.* 1990).

1.4.4 Types of Constructed Wetlands

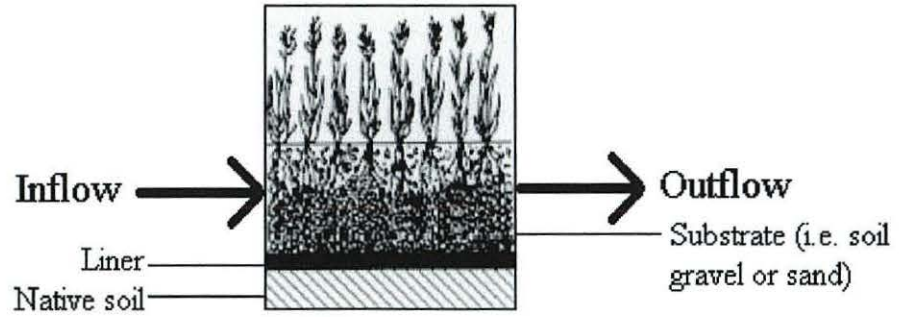
There are two basic types of constructed wetlands; surface flow (SF) and subsurface flow (SSF) (Mitsch & Gosselink 1993). Figures 1.12 a and b illustrate the differences between these constructed wetlands. The design employed will depend upon wastewater characteristics, location, cost and maintenance considerations.

Figure 1.12: Types of constructed wetlands.

(a) Surface Flow Wetland (Mitsch & Gosselink 1993).



(b) Subsurface Flow Wetland (Mitsch & Gosselink 1993).



In a surface flow constructed wetland the water flows over the substrate surface where the near-surface layer is aerobic and deeper waters and substrate are anaerobic (Kadlec & Knight 1996). This type of constructed wetland is often also referred to as free water surface wetland and is more commonly used in the US (Mitsch & Gosselink 1993).

In a subsurface flow constructed wetland the water level is designed to remain below the surface of the porous substrate, usually gravel (Kadlec & Knight 1996). Wastewater comes into contact with aerobic microbes living on the plant roots and on substrate and is more commonly employed in Europe (Mitch & Gosselink 1993). The operational expectancy of these wetlands are generally shorter due to clogging problems and are also much more expensive to build and maintain. However, they are

generally more efficient. Thus overall surface flow wetlands are more economically feasible on basis of performance and cost.

Constructed wetlands may also be described in terms of the hydraulic system, i.e. horizontal (HF) or vertical flow (VF). The vast majority of wetlands constructed employ a horizontal flow design where the wastewater flows through the bed, maintaining a water depth of 30-50cm (Kadlec & Knight 1996). These systems promote denitrification and processes for TSS and BOD removal (Cooper 1999) and are by far the most common used in Europe (Haberl *et al.* 1995). However, in recent years, vertical flow wetlands have been constructed whereby intermittent flow is used so that the wastewater can percolate through the bed medium before being discharged at the bottom (Kadlec & Knight 1996). Although the VF wetlands are praised for their nitrification removal performance (Cooper 1999), problems of clogging with high TSS loadings can occur. On account of the different oxygenation conditions horizontal flow systems are commonly used for tertiary treatment whereas vertical flow for primary of secondary treatment. However, due to the collective advantages of both types of wetlands, treatment involving combined systems have been reported (Cooper 1999).

1.4.5 BIOGEOCHEMISTRY IN WETLANDS

Biogeochemistry is a study of the surface of the Earth and includes reactions in the atmosphere, oceans, crustal minerals and living organisms (Schlesinger 1991). Hence biogeochemistry involves the cycling of elements via several transformation reactions. Natural wetlands exhibit unique biogeochemistry in comparison to terrestrial or aquatic systems. Constructed wetlands are designed in order to mimic and maximize these reactions for pollution control.

1.4.5.1 Nutrient Cycling in Wetlands

Nutrients are essential components for which living tissue requires for energy. In wetlands the transformation, consumption and re-utilization of nutrient processes are referred to as cycling. The major nutrient cycles in wetlands frequently researched are the carbon, nitrogen, phosphorus and sulphur cycles. These cycles are discussed below in general and in relation to LAS.

(a) Carbon cycling

Figure 1.13 shows the main processes involved in the carbon cycle. Photosynthesis and aerobic respiration mainly dominate aerobic carbon transformations in wetlands with organic carbon used as an energy source and released as CO_2 under aerobic conditions (Mitsch & Gosselink 2000):

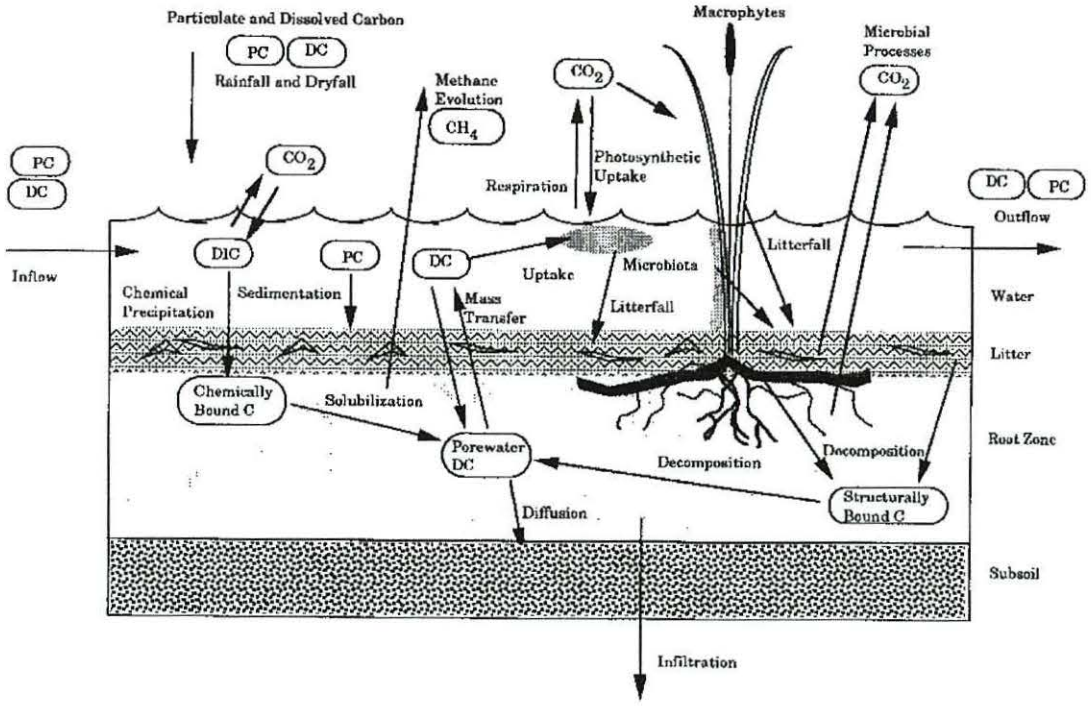
$$6CO_2 + 12H_2O + \text{light} \rightarrow C_6H_{12}O_6 + 6O_2 + 6H_2O$$
$$C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 12e^- + 6H_2O + \text{Energy}$$

Under anaerobic conditions the major carbon processes are fermentation and methanogenesis (Mitsch & Gosselink 2000). Fermentation of organic matter forms various low molecular weight acids and alcohols, and CO_2. Methanogens use the CO_2, or a low molecular weight organic compound, as an electron acceptor for production of gaseous CH_4 at extremely reduced conditions (-250 to -350 mV) (Mitsch & Gosselink 2000):

$$CH_3COO^- + 4H_2 \rightarrow 2CH_4 + 2H_2O$$

LAS is mainly composed of alkyl groups with CO_2 a major breakdown product after mineralization of the surfactant has occurred (see figure 1.7). Hence the surfactant would interact with the carbon cycle of the wetland. The amount of CO_2 released would be expected to vary in the wetland depending upon the aerobic or anaerobic conditions with the former promoting rapid LAS degradation as discussed in section 1.3.1. As the surfactant is degraded DOC would be expected to decrease (Zhang *et al.* 1999) resulting in possible faster C cycling in the wetland. However, this would depend upon LAS concentration and factors such as temperature, oxygen availability and microbial communities.

Figure 1.13: The carbon cycle (Kadlec & Knight 1996).



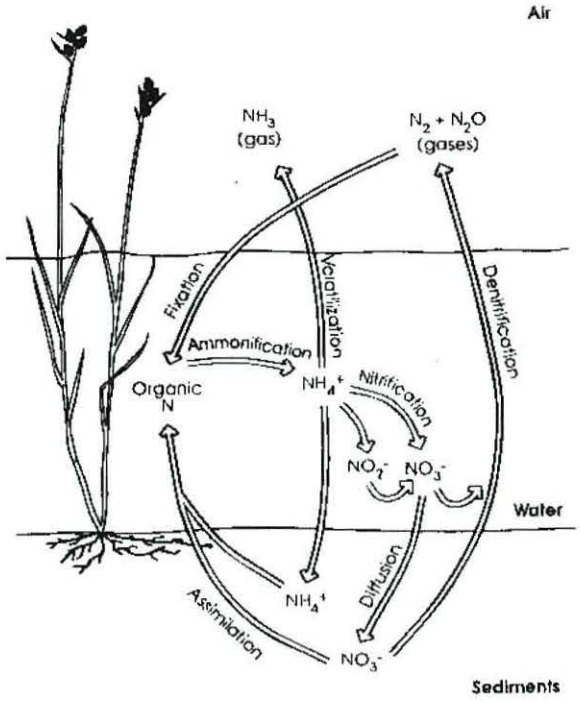
(b) Nitrogen Cycling

Removal of nitrogen (N) compounds is a primary concern in wastewater treatment due to their role in eutrophication, toxicity to aquatic species and effect on oxygen content of receiving waters (Kadlec & Knight 1996). Oxidation states of nitrogen forms present include NH_4^+, NO_2^-, NO_3^-, N_2O and N_2 with transformations mediated by soil organisms (Kadlec & Knight 1996). N-compounds wetland transformation processes are mainly nitrification and denitrification, with ammonification and assimilation also important (Kent 1994) and are shown in figure 1.14. The balance between nitrification and denitrification rates can be incorporated into wetland design with subsurface flow wetlands more effective than surface flow wetlands for nitrogen removal. Published example removal rates of N are 18-86% (Greenway & Woolley 1999) 36% (Haberl *et al* 1995) and 64% (House *et al.* 1994). However, overall removal will depend upon nitrogen source, temperature, mass loading rate and dissolves oxygen (Kadlec & Knight 1996, Kadlec & Reddy 2001).

Although LAS is not directly involved in the N cycle, indirect effects may occur due to the presence of the surfactant. At high concentrations LAS may be toxic to microbes involved in N cycling. The effect of LAS on nitrification has been studied in alternative wastewater treatment processes with little or no detrimental effect

observed at relatively high concentrations of LAS reported (Painter & Zabel 1989). Hence similar insensitive effects on N cycling in wetlands may also prevail. LAS toxicity to wetland plants may also occur which could affect the amount of N uptake. Hence possible build up of N compounds could occur due to the presence of LAS resulting in detrimental effects for N cycling.

Figure 1.14: The nitrogen cycle (Kadlec & Knight 1996)



Nitrification

Nitrification involves the conversion of ammonia to nitrate and occurs at a redox potential greater than +300mV (Reddy *et al.* 1986). Nitrification is an aerobic process with 4.3mg of oxygen required for each milligram of ammonia nitrified (Reddy *et al.* 1989).

$$NH_4^+ + 2O_2 \rightarrow NO_3^- + 2H^+ + H_2O$$

Hence this process is reported to occur in the oxidized rhizosphere of plants where adequate oxygen is often available to facilitate ammonium conversion to nitrate (Mitsch & Gosselink 2000). In temperature climates, soil nitrifier populations are reported highest in spring and lowest during hot summers and winter with nitrification slow below 5°C (Hammer & Knight 1994) and above 40°C (Paul & Clark 1989), with

an optimal temperature between 30-35°C. pH can also affect nitrification with optimum pH reported at 6.6-8.0 (Paul & Clarke 1989) and a marked decline at pH <6.0 (Hammer & Knight 1994).

Denitirifcation

Denitrification is a reduction process (i.e. anaerobic) resulting in the production of N_2, N_2O or NO (Kadlec & Knight 1996), at redox potentials under +300mV (Mitsch & Gosselink 2000), with NO_3^- acting as a terminal electron acceptor. This process is the major mechanism of nitrogen loss from wetlands.

$$C_6H_{12}O_6 + 4NO_3^- \rightarrow 6CO_2 + 6H_2O + 2N_2$$

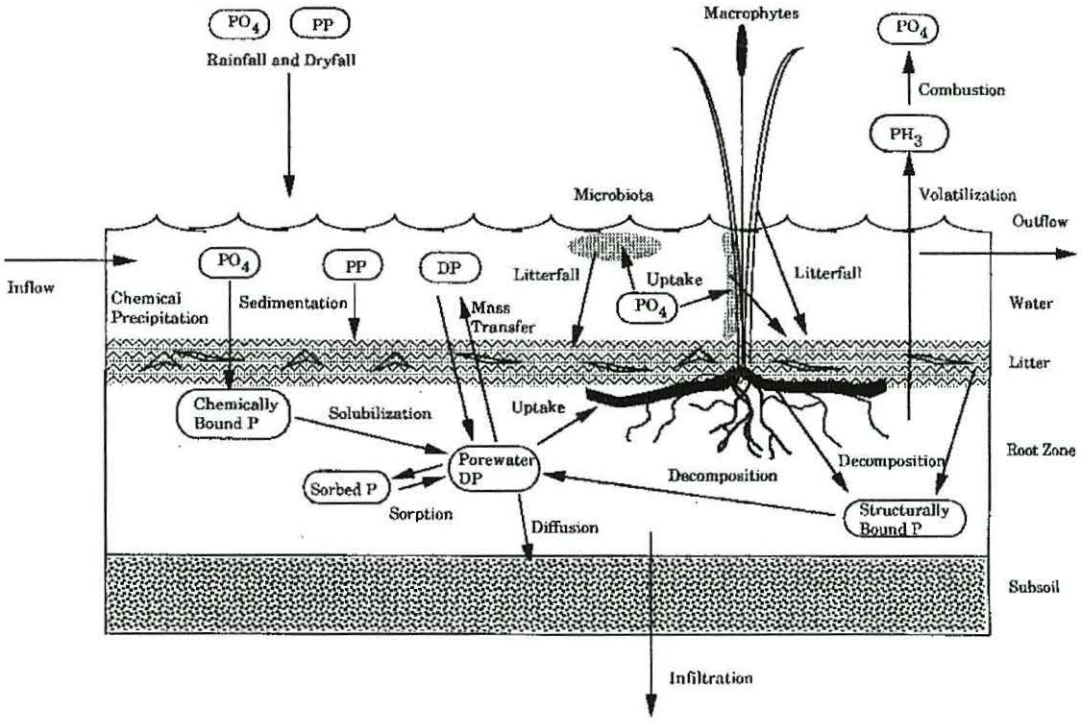
Rate of denitirification is dependent on, e.g. nitrate availability, denitrifying bacteria, temperature, pH and organic carbon content (Corbitt & Bowen 1994). Optimum temperature for denitirification at 25-65°C (Hammer & Knight 1994) and pH of 7-8 (Hammer & Knight 1994) are reported, with rate of denitirification slow below pH 5 (Paul & Clarke 1989). Denitrification is also reduced if the carbon supply is low (Hammer & Knight 1994).

(c) Phosphorus Cycling

Phosphorus is an essential nutrient required for plant growth. Natural sources of phosphorus into constructed wetland include atmospheric deposition (Kadlec & Knight 1996), whereas anthroprogenic sources include detergents whereby tripolyphosphate builders in detergents are used (Scott & Jones 2000). Key phosphorus (P) removal mechanisms in wetlands include adsorption, plant uptake, precipitation and fixation by algae and bacteria (Corbitt & Bowen 1994, House *et al.* 1994, Kadlec & Knight 1996) and are shown in figure 1.15. Principal organic forms encountered in wetlands are PO_4^{3-}, HPO_4^{2-}, $H_2PO_4^-$ and occurs in the sedimentary rather than gaseous cycle (Mitsch & Gosselink 2000). Unlike most other nutrients, P is not directly altered by redox potential but is indirectly by its association with several elements that are affected (Mitsch & Gosselink 2000). Variable P removal rates of e.g. <13, 63 and 86% are reported (Greenway & Wolley 1999, Haberl *et al.* 1995, House *et al.* 1994).

As stated in section 1.3.1 adsorption is also an important LAS removal mechanism. In a wetland, due to the negative charge of both LAS and PO_4, interacting processes and possible competition for adsorption sites may occur. Possible toxicity to plants could also affect P uptake in the wetland. Hence, LAS may have important consequences for the P cycle.

Figure 1.15: The phosphorus cycle (Kadlec & Knight 1996).



(d) Sulphur

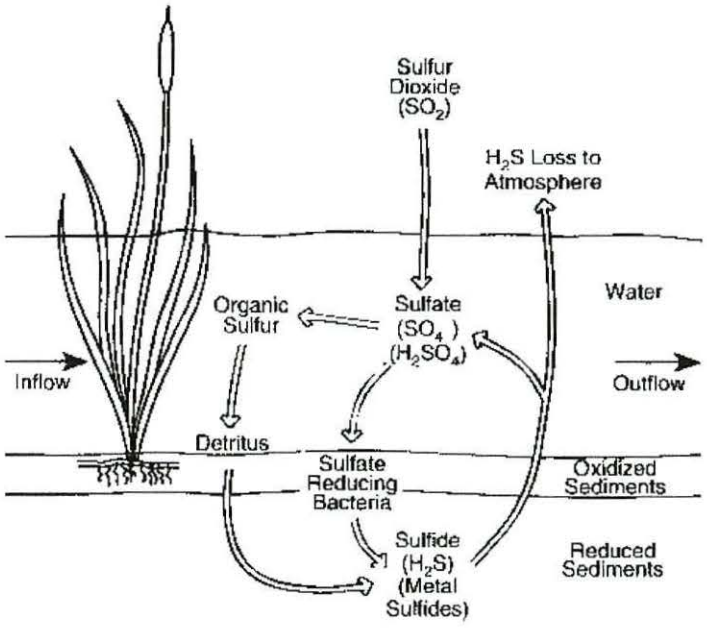
Wetlands can act as sinks of sulphur (S) via precipitation of insoluble sulphides, internal production and release of hydrogen sulphide (H_2S) gas (Kadlec & Knight 1996). S is an essential nutrient present in wetlands as two main forms, i.e. sulphate (SO_4), in aerobic waters, and H_2S, in anaerobic waters (Kadlec & Knight 1996). In the redox scale, S compounds are next major electron acceptors after NO_3, Fe and Mn with reduction occurring at -75 to -150mV (Mitsch & Gosselink 2000). In this range SO_4 can act as a terminal electron acceptor (Mitsch & Gosselink 2000):

$$4H_2 + SO_4^{2-} \rightarrow H_2S + 2H_2O + 2OH^-$$

Research into S cycling in constructed wetlands is mainly focused on acid mine drainage (AMD) application whereby high quantity of removal is observed at high input concentrations (Dunbabin & Bowmer 1992). The main processes are exhibited in figure 1.16.

LAS is also composed of a sulphonate group which forms sulphate during the mineralization process (Swisher 1987). Hence, depending upon the concentration of SO_4 released, the surfactant may have an important effect on S cycling in the wetland which may result in greater production of H_2S gas.

Figure 1.16: The Sulphur cycle (Kadlec & Knight 1996)



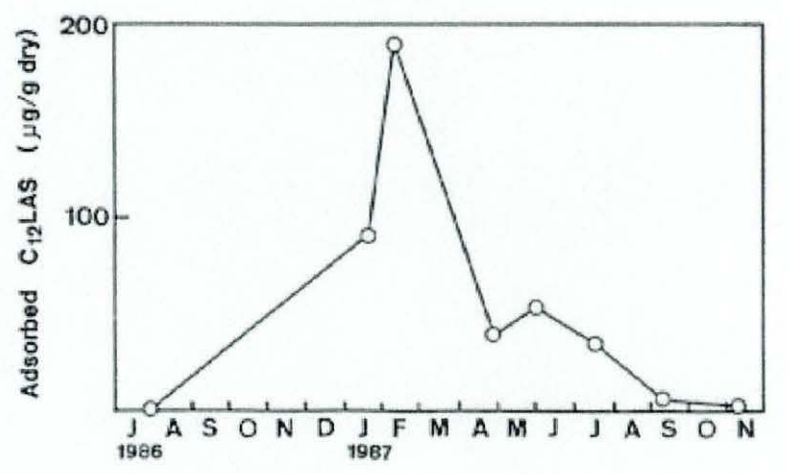
However, sulphate-reducing bacteria can compete with methanogens for energy sources. Thermodynamically, SO_4 reduction is more favourable than methanogenesis resulting in low CH_4 production with high SO_4 concentration (Mitsch & Gosselink 2000). However, when SO_4 availability is low, methanogenesis would be the dominant mechanism. Proposed explanations include competition for substrates, inhibitory effects of sulfate or sulfide on methane bacteria and redox potential is not low enough (Kadlec & Knight 1996). Hence LAS may possibly result in higher levels of SO_4 resulting in inhibiting methanogenesis in the wetland.

1.5 LAS REMOVAL IN WETLAND SYSTEMS

As discussed the majority of research on constructed wetlands for wastewater treatment focuses on removal of BOD, N, P and metals. However, although surfactants are major components of urban wastewater, as highlighted in section 1.2, they have largely been ignored. Hence the fate and removal of LAS in wetlands is poorly understood with limited research available.

In terms of the published work available, Inaba *et al.* (1988) assessed the quality of purification of LAS in a large-scale natural wetland system in Japan. The authors reported that the amount and isomer content of LAS in the influent did not vary much throughout the year but that seasonal changes in both was found in the effluent. This seasonal variation was due to biodegradation by bacteria and/or adsorption on sediment particles. LAS adsorption was higher in the winter than summer, as shown in figure 1.17, suggesting greater biodegradation at warmer temperatures (Inaba *et al.* 1988). The authors further investigated this temperature dependence and concluded that LAS biodegradation was temperature controlled with an "ON-OFF" switch operating at 6 to 9°C (Inaba *et al.* 1988).

Figure 1.17: Seasonal changes in concentration of C_{12}LAS adsorbed on the sediment particles from surface to 5cm depth in a natural wetland (Inaba *et al.* 1988).



In a further study Inaba (1992) quantitatively assessed the removal of LAS in the wetland. In the summer approximately 95% of the influent LAS was removed but

only 50% in the winter. The author suggested biodegradation of LAS already adsorbed on sediment particles as well as that in the inflow occurs in the summer.

However, the published work is limited in terms of the amount of data available, influencing factors investigated and the low concentrations of surfactant present. It is essential to assess LAS removal at higher concentrations and possible subsequent effects on the wetland. Relatively high concentrations of surfactants may enter wetlands via wastewater from the surfactant manufacturing industry, incidents such as the Foot and Mouth crisis where large quantities of disinfectants and detergents were used or where raw sewage is directly discharged into surface waters without treatment. In addition the research did not focus on other important parameters in investigating the fate of LAS in wetlands. Potential detrimental or beneficial impacts to microbes and removal of other pollutants in the system also need to be addressed.

1.6 RESEARCH OBJECTIVES

The high potential for LAS removal in various environmental compartments, illustrated by various publications discussed above, raises the question as to why it is important to monitor this surfactant in wetlands. The vast volume and diversity of domestic and industrial application and disposal of LAS has extended the interest and concern to monitor the surfactant when released into the environment (Knaebel *et al.* 1994). Since the foaming problems of the 1960s various legislative regulations govern that surfactants released into the environment must exhibit high biodegradation capacities in the environment (see Chapter 3). The lack of knowledge and publications on the fate of LAS and other surfactants in wetlands, bar work from Japan (section 1.5), coupled with the increase in application of wetlands for sewage treatment indicates that research in this area is required.

Hence the main aim of this thesis was to investigate the fate and impacts of LAS removal processes in wetlands used for wastewater treatment. The specific aims of this research was to investigate:

1. LAS concentrations inflowing into constructed wetlands and, after treatment, discharged and resulting removal efficiencies.
2. Relationships between LAS removal and;
   (a) wetland characteristics, e.g. hydraulic residence time, vegetation, bed size, age and hydraulic loading rate.
   (b) environmental factors, e.g. temperature/climate, pH, and season.
   (c) surfactant properties, e.g. concentration, isomers and alkyl chain length.
3. Possible modification of the influencing factors to improve LAS removal.
4. Whether LAS has any secondary detrimental effect on natural wetland processes, such as nutrient cycling, microbial activity and plant growth.

To fulfill these aims this Thesis has been sectioned into 5 experimental chapters. Chapter 2 investigates LAS removal and concentrations in operational constructed wetlands in the UK and worldwide, whereas the third chapter investigates LAS removal processes and corresponding kinetics in the laboratory. Chapter 4 investigates the effect of plant mechanisms on LAS degradation and chapter 5 is concerned with factors that may influence LAS removal in wetlands. The final

experimental chapter investigates the possible detrimental effect of LAS on natural wetland processes with chapter 7 summarizing the research presented in previous chapters and the main conclusions drawn.

CHAPTER 2: Survey of LAS Removal in Constructed Wetlands in the UK and Worldwide

This chapter presents monitoring data of LAS concentrations and removal efficiencies in several wetlands used for domestic wastewater treatment in the UK and worldwide. The data are presented in three parts; 12-month field study of LAS in three UK constructed wetlands, continuation for a further 18 month period of one wetland for long term data and comparison of these sites with samples collected from wetlands on a latitudinal gradient worldwide. Physicochemical data, water chemistry and enzyme activities are also presented.

2.1 LAS REMOVAL IN UK CONSTRUCTED WETLANDS

2.1.1 Introduction

Since its introduction in the 1960s LAS has become a major surfactant present in detergents and cleaning agents. With an estimated annual worldwide consumption at 2 x 10^6 t per year (de Wolfe & Feijtel 1998) LAS represents >40% of all surfactants used (Scott & Jones 2000). LAS can be present at significant concentrations in the environment depending on, e.g. source, location and treatment facilities. Vast monitoring data accumulated worldwide indicate LAS levels in raw sewage of <10mg L^{-1} as summarised table 2.1. Low concentrations in rivers have also been published, e.g. <2.1-2.9µg/l in the River Leidsche Rijn, Netherlands (Feijtel *et al.* 1995) and 0.04mg L^{-1} (0.01-0.09 mg L^{-1}) in Germany (Matthijs & De Henau 1987).

Table 2.1: LAS concentrations measured in sewage treatment plants.

Country	Inflow (mg L^{-1})	Outflow (mg L^{-1})	Reference
UK	1.1-5.58		Holt *et al.* (1998)
	15.1	0.010	Waters & Feijtel (1995)
USA	3.8		De Henau *et al.* (1986)
Germany	8.0	0.067	Waters & Feijtel (1995)
	4.0	0.070	Matthijs & De Henau (1987)
		0.007-0.016	Schröder *et al.* (1999)
Netherlands	3.1-7.2	<0.008	Feijtel *et al.* (1995)
	4.0	0.009	Waters & Feijtel (1995)
Spain	11.2-21.0	0.150-0.230	Berna *et al.* (1989)
	9.6	0.140	Waters & Feijtel (1995)
	5.0	0.160	Leal *et al.* (1994)
Italy	4.6	0.068	Waters & Feijtel (1995)

However, despite the increase in application of constructed wetlands in sewage treatment the fate of LAS has largely been ignored. In the UK the introduction of constructed wetlands has greatly expanded in recent years with >350 systems in operation (Nuttall *et al.* 1997). These systems are also widely used in Europe (>500-1000, Haberl *et al.* 1995), e.g. Sweden (Whittgren & Mæhlum 1997), Norway

(Mæhlum & Stalnacke 1999), and worldwide, e.g. India (Billore *et al.* 1999), Australia (Greenway & Woolley 1999) and US (Kadlec & Knight 1996).

In this section, a 12-month field survey of LAS concentrations and water chemistry was conducted in three constructed wetlands receiving domestic wastewater in the UK that varied in design, size and flow rates. The main aims are:

1. To determine nominal influent and effluent LAS concentrations in UK constructed wetlands
2. To assess effects of seasonal variations on LAS removal
3. To determine the distribution and removal of individual alkyl homologues
4. To seek possible relationships of LAS removal with hydrochemistry and microbial activities
5. To assess hydrochemical data in relation to general wetland performance

Some of the findings detailed in this chapter have been presented in Thomas *et al.* (2003) as given in appendix B.

2.1.2 METHODS

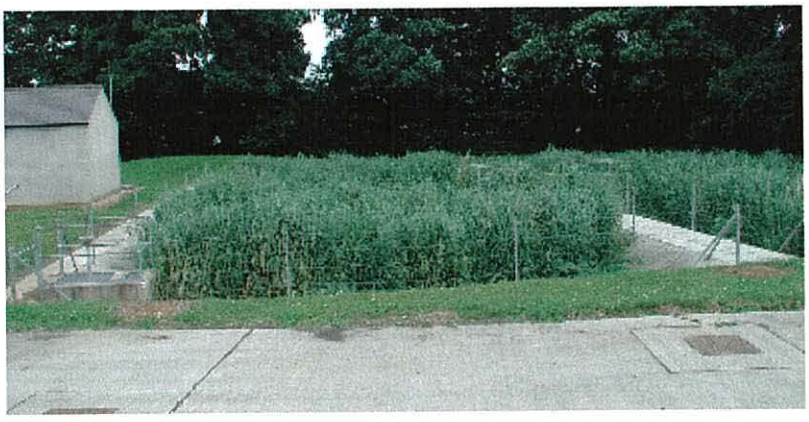
2.1.2.1 Sampling sites

Three constructed wetlands used in sewage treatment plants in the UK for tertiary treatment were monitored (Table 2.2). All wetlands were subsurface flow constructed wetlands planted with *Phragmites australis* in gravel media. The first site at Brynsiencyn, Anglesey (National Grid Ref. SH491666) had the largest total surface area (500m^2), served the largest population (2000) and received the highest loading (665 m^3 d^{-1}). This treatment plant had a Rotary Biological Contractor (RBC) as a secondary treatment process. The second largest (172m^2) wetland served a small community (200) at Clutton, Wrexham (National Grid Ref. SH460544) and had been in operation for the longest period. The third study site treated wastewater at Rosset, Chester (National Grid Ref. SH370565) receiving wastewater from a small hospital and a few nearby households (150) and is the most recently constructed. A photograph of the Brynsiencyn constructed wetland is shown in Figure 2.1 as an example.

Table 2.2: Characteristics of the wetland sampling sites.

Site	Area (m^2)	Population	Loading (m^3 d^{-1})	Construction Year
Brynsiencyn	500	2000	665	1998
Clutton	172	200	25	1996
Rosset	130	150	100	1999

Figure 2.1: Photograph of the Brynsiencyn constructed wetland.



2.1.2.2 Samples

From each constructed wetland inflow and outflow water samples were taken, using methanol washed bottles, on a monthly basis from January to December 2000 (except for Clutton that commenced in March). These were preserved on site with 3-5% formaldehyde (37% v/v) and refrigerated (<4°C) whilst awaiting analyses for LAS content. Water samples were also taken and filtered (0.2μm CA) for water chemistry analyses. Five replicate gravel substrate (top 10cm) grab samples were collected and incubated at 4°C.

2.1.2.3 Laboratory analysis

LAS Analytical Procedure

The method adopted was based on that developed by Matthijs & De Henau (1987) but modified slightly to improve the selectivity. Solid Phase Extraction (SPE) was used to isolate and concentrate the LAS in the aqueous samples before HPLC analyses. Each sample was initially passed through a preconditioned C18 (1g/6ml) SPE column (10ml methanol followed by 10ml distilled water) and then eluted with methanol onto a preconditioned SAX (500mg/3ml) anion-exchange SPE column (3ml hexane followed by 10ml methanol). LAS was then eluted into a glass vial with 3ml of CH_3OH:HCl solution (80:20) and evaporated to dryness at 75°C under a gentle stream of nitrogen. The samples were stored in the dry state at <4°C whilst awaiting analyses. In order to minimise contamination all glassware were washed in methanol before use and appropriate glassware then conditioned for 24 hours prior to use with LAS solution to reduce loss of surfactant to the glass surface. Figure 2.2 shows the scheme for the analytical procedure.

HPLC Analyses

Separation of LAS homologues was achieved by reversed phase separation using the DX-300 HPLC system, with a 10μm, 30cm x 3.9mm i.d., μ-Bondclone C18 analytical column. The mobile phase (22:78 water:methanol), containing sodium perchlorate buffer (0.0875M), at a flow rate of 2 ml min^{-1} was used and a Perkin Elmer LS-4 fluorescence detector (Excitation wavelength = 232nm; emission wavelength = 290nm; slit width = 10nm). For calibration Nansa® HS 80/S was used containing C_{10}-

C_{13} LAS homologues (with distribution of alkyl chains of C_{10} 15.8%; C_{11} 41.5%; C_{12} 30.1%; C_{13} 12.5%).

Method Development

The analytical method was validated via reproducibility and recovery analysis. Reproducibility was assessed by repetitive analysis of standard solutions and environmental samples. Recovery of spiked samples was measured by adding known concentrations of LAS to the aqueous samples prior to processing of the sample.

Anions

Ion Chromatography (DX-120) was used to determine anion concentration (nitrate, phosphate and sulphate) of the filtered samples with an IonPac AS4A column, with 1.7 mM $NaHCO_3$/1.8 mM Na_2CO_3 eluent (1ml/min).

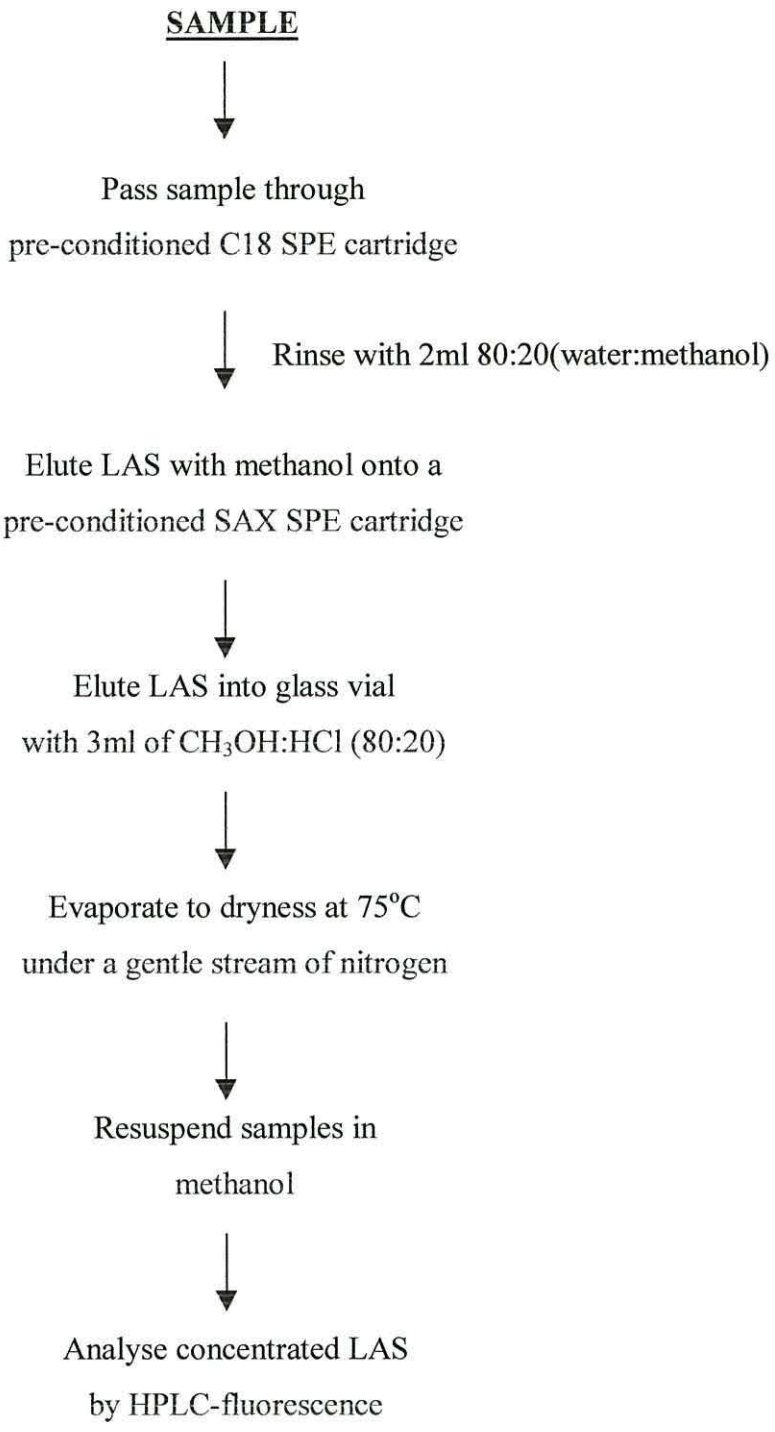
DOC

Dissolved Organic Carbon (DOC) content was analysed using the SHIMADZU TOC-5000 with zero grade air carrier gas (150ml/min).

Phenolics

Phenolic contents were assayed using Folin-Ciocalteau phenol reagent (Box 1983). The method involved successively adding 0.15ml of Na_2CO_3 (200 g L^{-1}) and 50µl of Folin-Ciocalteau reagent to 1 ml of sample. Mixture was gently shaken and assayed for 90 minutes at room temperature and then measured with a spectrophotometer at 750nm against a reagent blank. Calibration was achieved using phenol standards of 0-2 mg L^{-1}.

Figure 2.2: Scheme for the LAS analytical procedure.



Enzyme assays

Activities of three hydrolytic enzymes (β-glucosidase, sulphatase and phosphatase) were determined in 5 replicate gravel samples from each wetland using fluorogenic methylumbelliferyl (MUF) substrates for the last six months of the study (Freeman *et*

al. 1995). 2ml cellosolve (ethylene glycol monoethyl ether) was used to pre-dissolve all MUF substrates freshly prepared for each assay as substrates have minimal solubility in pure water. Cellosolve does not affect enzyme activity (Hoppe 1983).

MUF-substrate (7ml) was added to 1g of gravel samples, homogenised and after incubation at field temperature for 1 hour the reaction was terminated via centrifugation (10,000 rpm for 5 minutes). The supernatant (0.5ml) was transferred to 2.5ml of deionised water and fluorescence determined with a Perkin-Elmer LS50 fluorometer at 450nm emission and 330nm excitation (slit-width 2.5cm). Calibration curves were constructed using 0-100μM MUF-free acid solution and assayed as above to correct quench interference of phenolics (Freeman *et al.* 1995).

To initially determine the appropriate substrate concentration for optimum enzyme activity to be measured, assays of gravel from each wetland at a range of MUF-substrate concentrations (0-800μmol L^{-1}) were conducted. Dry mass of each sample was determined (furnace at 104°C for 24hrs) to calculate the rate of enzyme activity as nmol MUF g^{-1} hr^{-1}.

2.1.2.4 Statistical analysis of results

Relationships between LAS concentrations, physico-chemical factors and enzyme activities for each site, with data which conformed to the normal distribution, were determined via correlation analysis (Pearson) using Minitab™ version 13.1 (Minitab Inc. 2000). Nonparametric data were assessed using Spearman rank correlation distribution. Between-site comparisons were assessed with one-way ANOVA tests for the data that conformed to the normal distribution and had homogenous variance.

2.1.3 RESULTS

a. LAS

The annual mean LAS inflow concentration from the three sites was 1.1mg L^{-1} and 0.43mg L^{-1} in the outflow. Figure 2.3 shows the mean annual LAS concentrations measured at each sampling site. Highest LAS concentration was at the Rosset site (3.2mg L^{-1}, March) and lowest at Brynsiencyn (0.03mg L^{-1}, December). A minimum of 0.02mg L^{-1} was measured in the outflow (Brynsiencyn, December). The sites exhibited similar mean LAS removal capacities of 57, 55 and 54% respectively at Clutton, Brynsiencyn and Rosset. The wetland in operation longest, i.e. Clutton, exhibited the highest maximum LAS removal recorded at 84% (June), whereas 21% removal at Rosset (October) the lowest.

Figure 2.3: Annual mean LAS concentrations at the sampling sites.

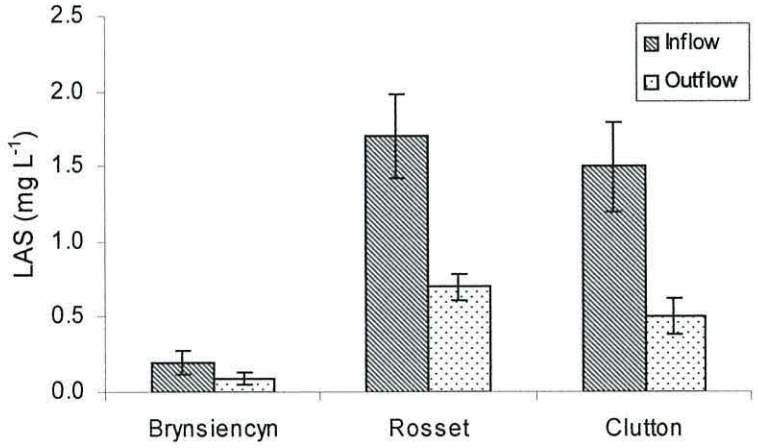
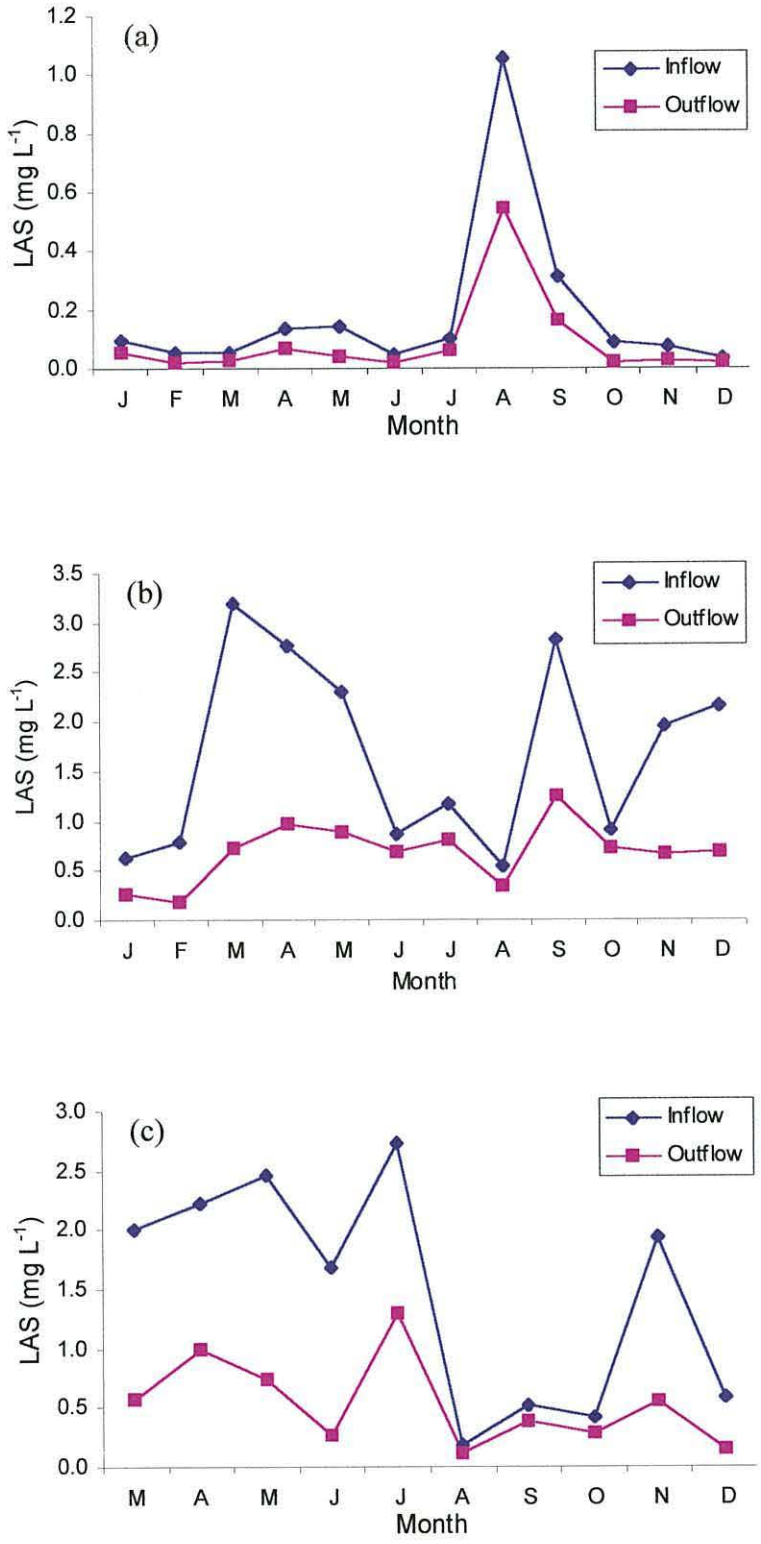


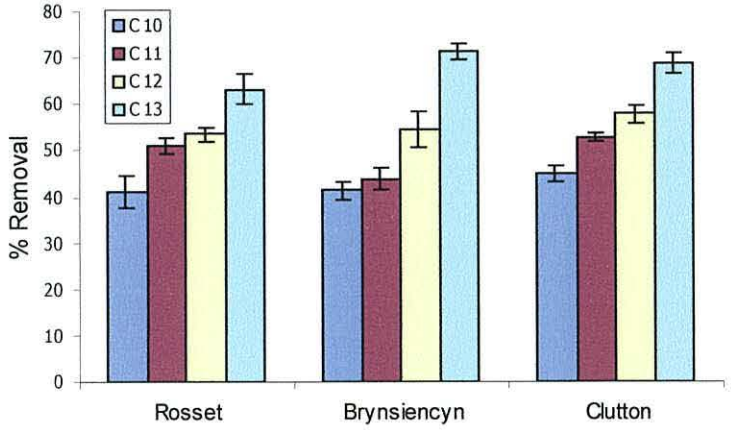
Figure 2.4 shows the annual variation on a monthly basis of LAS concentrations measured at the sampling sites. A sharp peak in LAS inflow concentration was observed in August at Brynsiencyn whereas, in contrast, a sharp decline is observed at the other two sites. Low concentrations in the inflow in October at these two sites were also exhibited.

Figure 2.4: Annual LAS trends at (a) Brynsiencyn, (b) Rosset and (c) Clutton.



The sensitive HPLC procedure adopted not only enabled total LAS concentrations to be measured but also individual LAS alkyl chain homologues. Four LAS homologues were identified and quantitatively measured with chain lengths of C_{10}-C_{13}. Variation in homologue distribution was evident with an average alkyl chain length of 11.5 in the inflow for both Rosset and Clutton sites and 11.3 in the outflow. At Brynsiencyn an average chain length of 11.7 (inflow) and 11.5 (outflow) was measured. Mean annual percentage removal distribution of the LAS alkyl homologues show that the longer alkyl chain homologues are removed to a greater extent than the shorter chain homologues in the order of $C_{13}>C_{12}>C_{11}>C_{10}$ (see figure 2.5)

Figure 2.5: LAS alkyl chain homologue distribution.



Reproducibility

The results (table 2.3) show a maximum relative standard deviation (SD) of *c.* 4% for total LAS measured in the samples. The SD for the alkyl homologues were higher as expected at lower environmental concentrations.

Table 2.3: Reproducibility of LAS analysis (Where Σ_{LAS} = total LAS).

Sample	**Relative standard deviation (%) (n=6)**				
	C10	**C11**	**C12**	**C13**	**Σ_{LAS}**
Standard	5.2	2.1	2.8	2.4	2.0
Influent Sewage	4.5	5.1	5.2	3.6	3.8
Outflow Sewage	5.1	5.4	6.3	7.2	4.2

Recovery

Results of recovery analysis for spiked LAS samples for total LAS and alkyl homologues are shown in table 2.4. The minimum mean recovery of total LAS was 87% and only small differences were observed between the individual alkyl homologues.

Table 2.4: Recovery of spiked LAS samples (Where Σ_{LAS} = total LAS).

Sample	**Mean Recovery (%) (n=6)**				
	C10	**C11**	**C12**	**C13**	**Σ_{LAS}**
Influent Sewage	91	86	105	83	93
Outflow Sewage	86	81	92	82	87

b. Water chemistry

Anions

Although nitrate concentration was highest in the inflow at Brynsiencyn (19.2-96.0mg L^{-1}), this site exhibited the lowest mean removal (59%) (table 2.5). Outflow nitrate concentrations were lowest at the Rosset site (1.1mg L^{-1}) and demonstrated the highest annual removal (84%). The annual trend in nitrate is shown in figure 2.6.

The lowest phosphate concentration in the inflow was measured at the Brynsiencyn site (3.7mg L^{-1}; October), whereas Clutton exhibited the highest at 54.3mg L^{-1} (November) and mean at 40.2mg L^{-1} (table 2.5). The annual phosphate variation is presented in figures 2.7.

The highest sulphate concentration in the inflow was detected at the Clutton site with a maximum of 82.2mg L^{-1} (mean 58.6mg L^{-1}). Lowest sulphate measured was 17.6mg L^{-1} at Brynsiencyn (November) as shown in table 2.5. Seasonal variation was most distinctive at Rosset and Brynsiencyn, where removal was highest during August (figure 2.8).

Figure 2.6: Nitrate concentrations for (a) Brynsiencyn, (b) Rosset and (c) Clutton.

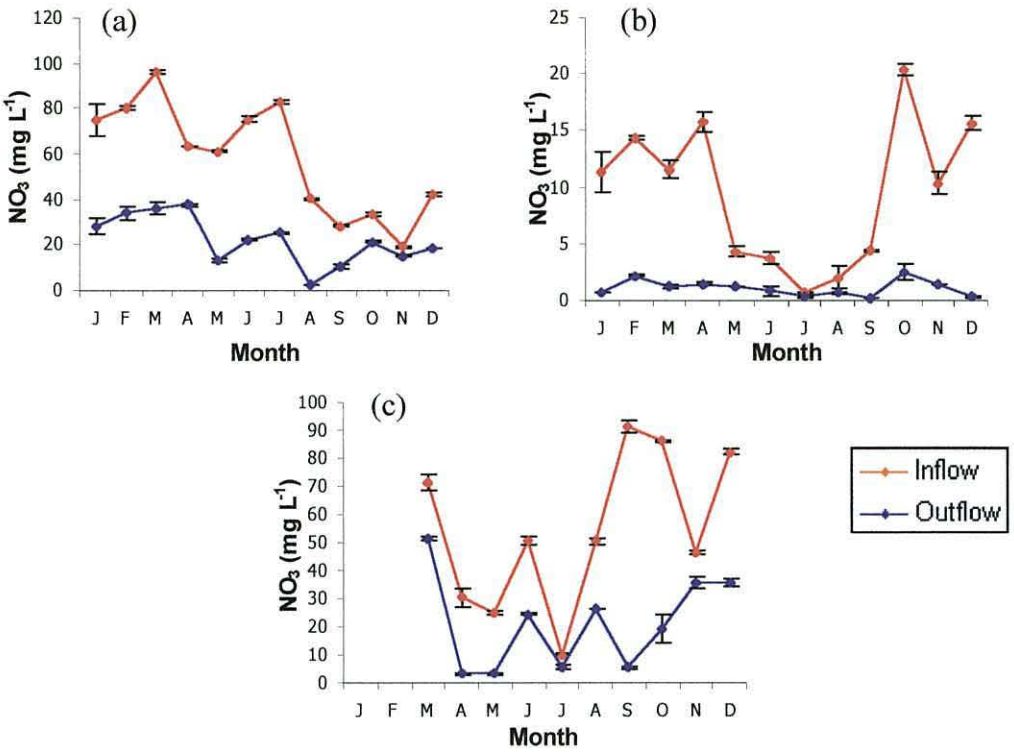


Figure 2.7: Phosphate concentrations for (a) Brynsiencyn, (b) Rosset and (c) Clutton.

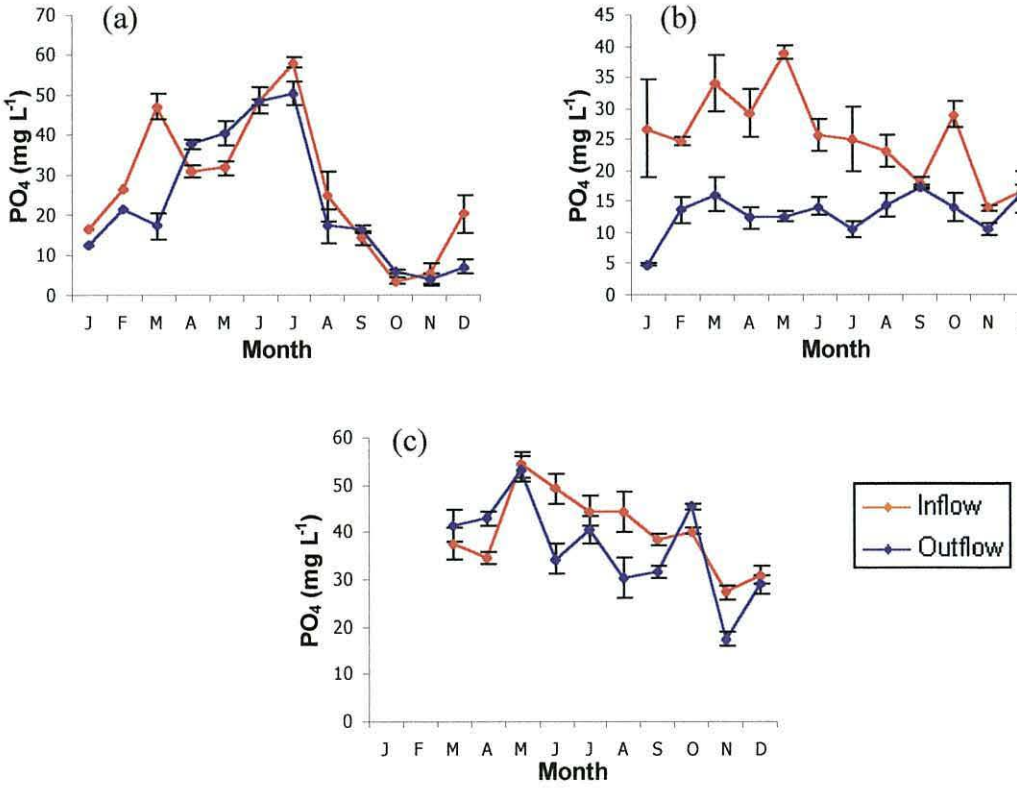
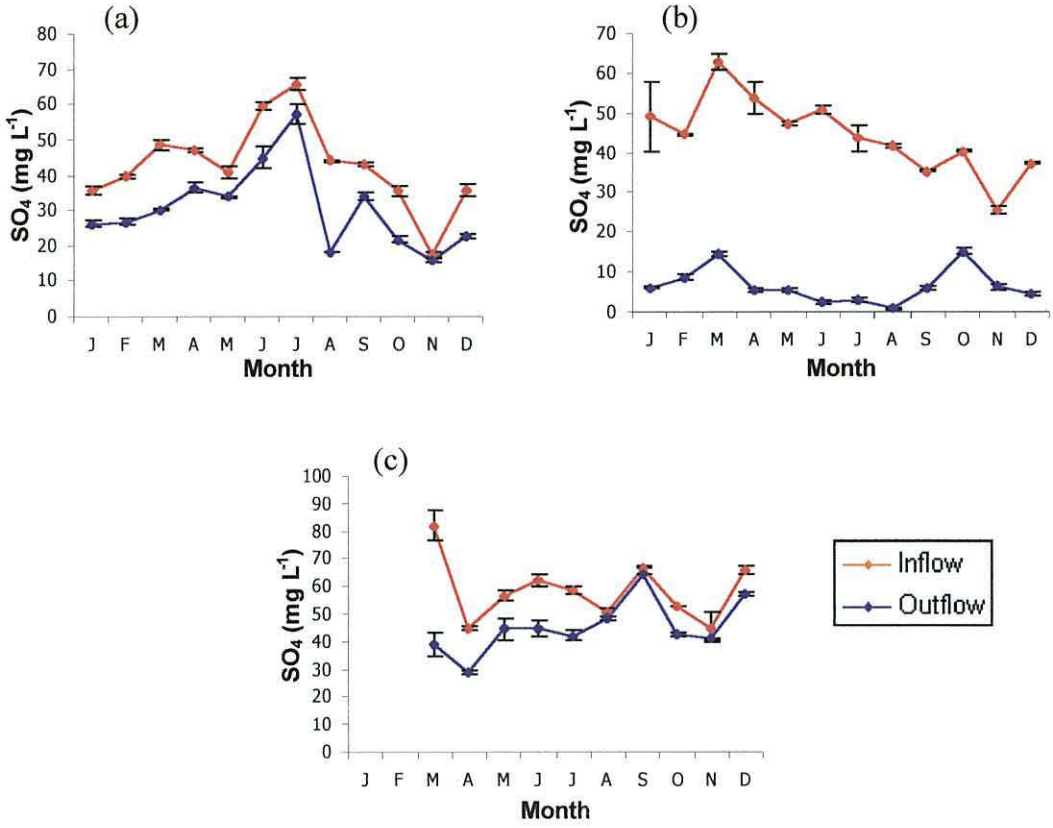


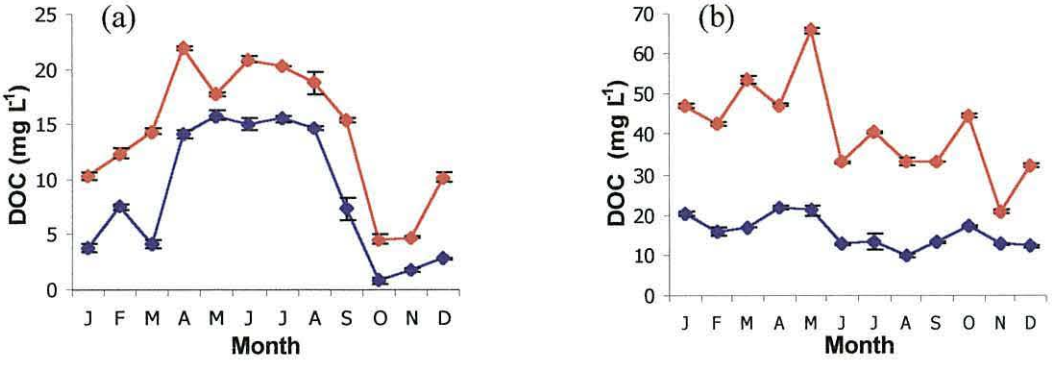
Figure 2.8: Sulphate concentrations for (a) Brynsiencyn, (b) Rosset and (c) Clutton.



DOC and Phenolics

Brynsiencyn exhibited the lowest inflow (4.6mg L^{-1}) and outflow (0.9mg L^{-1}) DOC with variations at all sites shown in figure 2.9. Highest overall removal was observed at Rosset (61.1%) and lowest at Clutton (36.4%) (Table 2.5). The annual phenolic concentrations are shown in figure 2.10. In the inflow 1.9-5.2mg L^{-1} (Clutton), 2.1-4.6mg L^{-1} (Rosset) and 0.4-2.0mg L^{-1} (Brynsiencyn) were measured and means of 1.6, 2.0 and 0.8mg L^{-1} respectively in the outflow (table 2.5).

Figure 2.9: DOC concentrations for (a) Brynsiencyn, (b) Rosset and (c) Clutton.



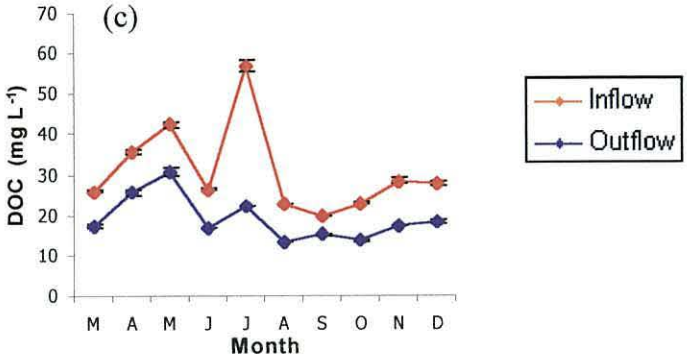
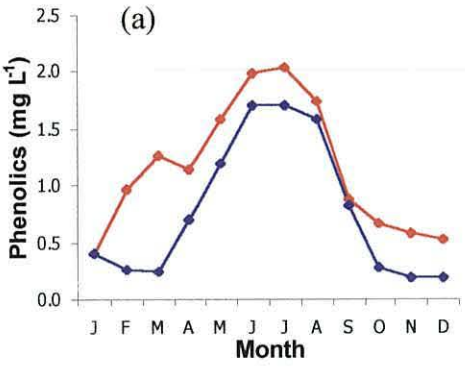
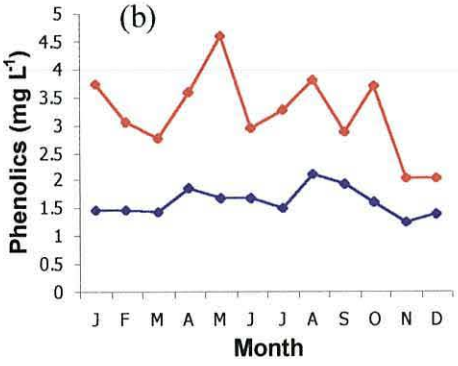


Figure 2.10: Phenolics concentrations for (a) Brynsiencyn, (b) Rosset and (c) Clutton.





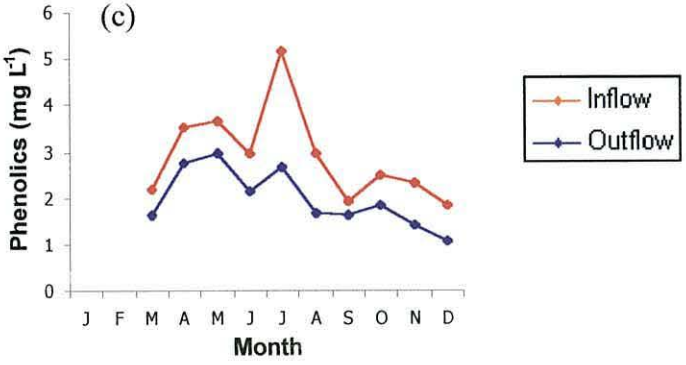


Table 2.5: Mean inflow and outflow water chemistry (where % = % removal)

	Rosset			Brynsiencyn			Clutton		
	In	Out	%	In	Out	%	In	Out	%
NO_3	9.6	1.1	83.6	58.1	22.2	59.0	54.5	21.1	60.0
PO_4	25.5	13.1	43.8	27.4	23.3	9.8	40.2	36.7	8.6
SO_4	44.4	6.6	84.7	42.8	30.7	28.3	58.7	45.4	21.5
DOC	41.2	15.6	61.1	14.3	8.6	46.8	31.0	19.1	36.4
Phenolics	3.2	1.6	47.6	1.2	0.8	37.6	2.9	2.0	30.9

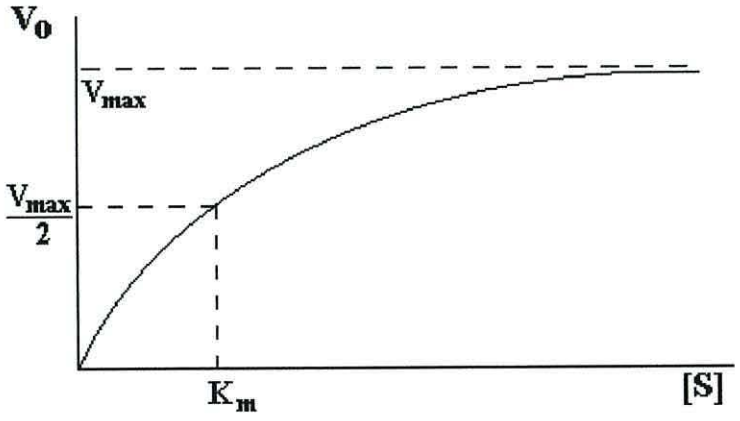
c. Enzyme activity

The kinetic data for enzyme activity measured over a range of MUF-substrate concentrations are plotted as reaction velocity (v) versus substrate concentration ([S]) in Figure 2.11. Kinetic parameters K_m, the Michaelis constant which is the half saturation concentration, and V_{max}, the maximum velocity, were determined for each enzyme. These parameters are defined by the Michaelis-Menten equation as;

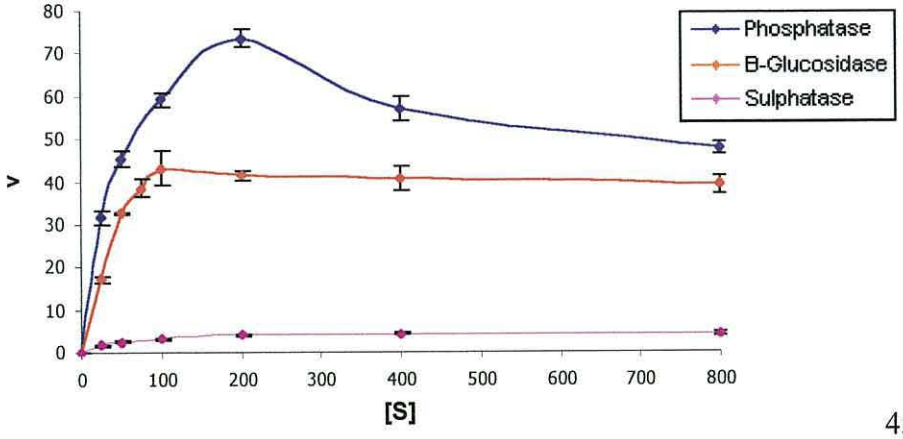
$$v = \frac{V_{max}[S]}{K_m + [S]}$$

Figure 2.11: Enzyme kinetics (a) example, (b) Brynsiencyn, (c) Rosset, (d) Clutton.

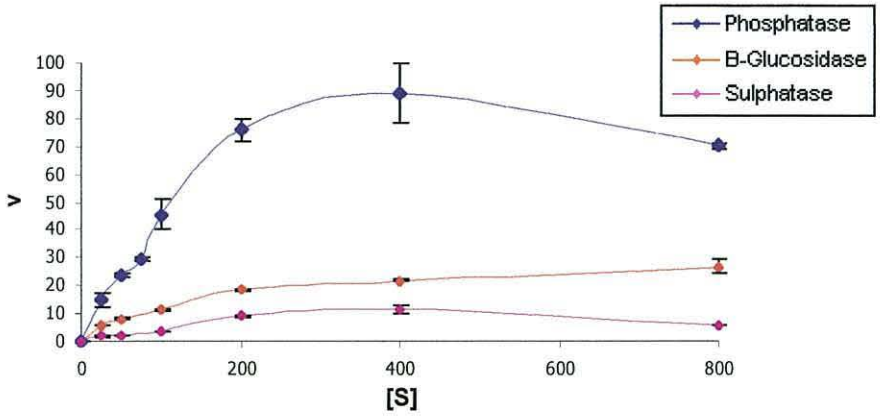
(a)



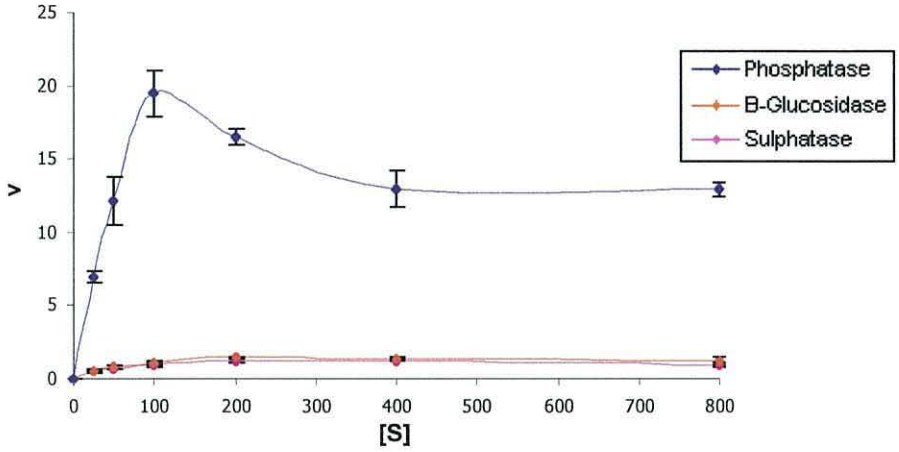
(b)



(c)



(d)



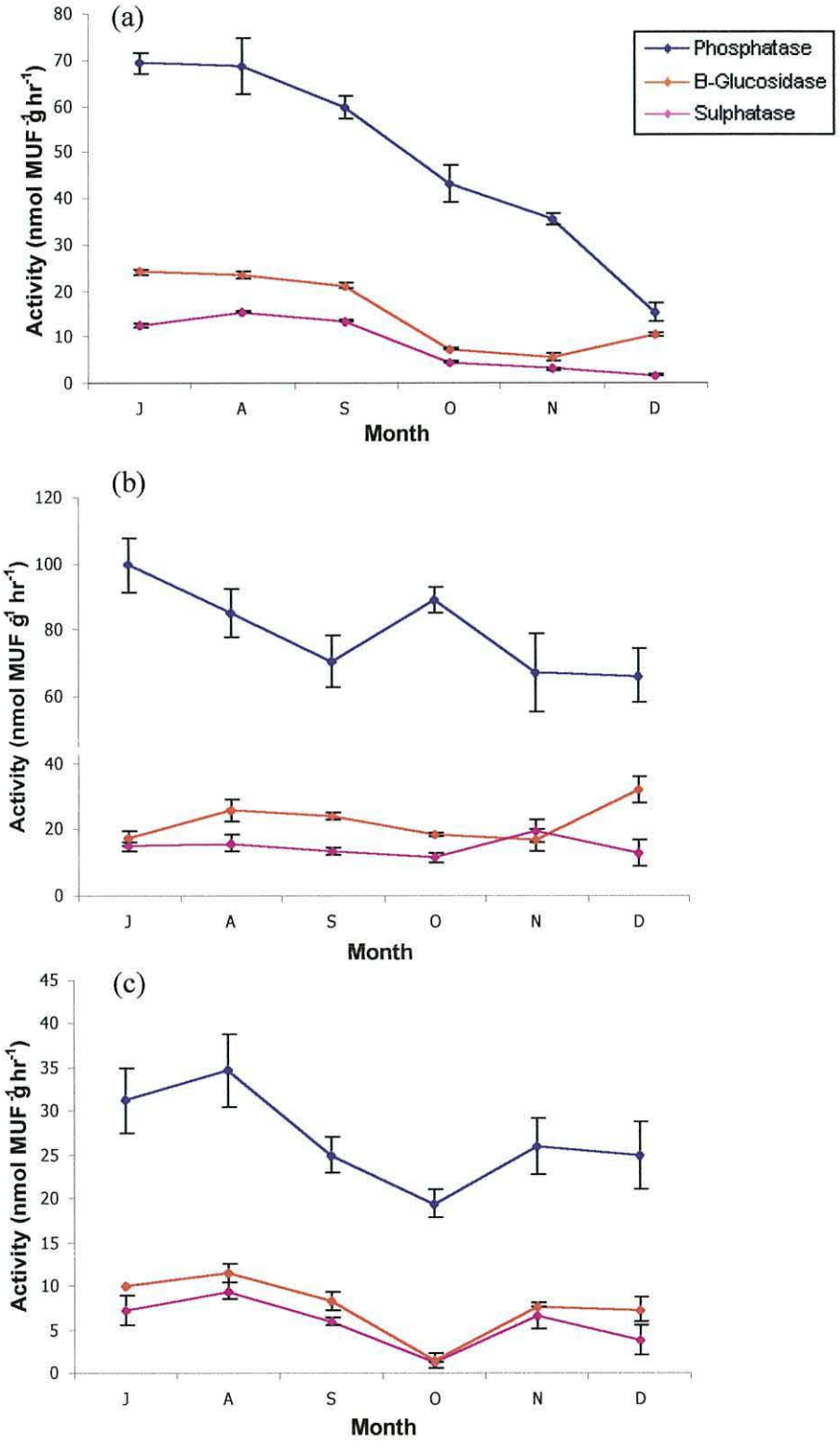
However, it is not possible to accurately determine the kinetic parameters from the curved non-linear Michaelis-Menten plot. Several solutions have been traditionally used to overcome this with the original equation re-arranged and the data plotted in several ways to give a linear graphical representation (Price & Stevens 1982). The three main ways to interpret that data are commonly used are Lineweaver-Burk, Eadie-Hoftsee and Hanes equations (Price & Stevens 1982) and were applied to this study using the EnzPack software (Version 3.1a, BIOSOFT). The calculated constants, and correlation coefficient (r), for each enzyme activity measured are summarised in table 2.6. The highest V_{max} value was calculated for phosphatase followed by β-glucosidase and sulphatase.

Table 2.6: V_{max}, K_m and r for Lineweaver-Burk, Eadie-Hoftsee and Hanes plots for Brynsiencyn, Clutton and Rosset constructed wetlands (K_m= μM L^{-1}, V_{max}=nmol MUF g^{-1} hr^{-1}).

Site	Enzyme	Lineweaver-Burk			Eadie-Hoftsee			Hanes		
		K_m	V_{max}	r	K_m	V_{max}	r	K_m	V_{max}	r
Bryn.	β-gluco	74.6	71.5	0.97	41.1	55.1	0.75	29.4	49.4	0.98
	Pho	44.3	87.0	1.00	46.1	88.6	0.99	50.3	91.5	1.00
	Sul	40.7	4.7	1.00	41.9	4.71	0.99	36.4	4.61	0.99
Clutton	β-gluco	51.0	1.59	0.99	45.1	1.54	0.89	17.4	1.34	1.00
	Pho	32.8	17.4	0.85	12.2	15.2	0.32	10.3	12.9	1.00
	Sul	31.1	1.29	0.96	33.3	1.33	0.94	38.0	1.38	1.00
Rosset	β-gluco	83.1	24.1	0.98	105	27.4	0.94	146	31.3	1.00
	Pho	158	106	0.99	134	99.6	0.78	87.4	83.6	0.97
	Sul	103	8.38	0.91	74.5	8.6	0.40	38.3	7.05	0.93

Figure 2.12 demonstrates the trend in enzyme activity from July to December for the three sites. Highest activity was exhibited by phosphatase at each site (26.9-79.7nmol MUF g^{-1} hr^{-1}) and lowest for sulphatase (5.7-14.7 nmol MUF g^{-1} hr^{-1}). The Rosset site demonstrated the highest overall activity. Greatest sulphate and phosphate removal was also exhibited at this site. Temporal trends were most distinctive at Brynsiencyn with a general decrease in enzyme activity over time. Activity peaked in August at the Clutton site and a dip observed in October.

Figure 2.12: Trend in enzyme activity for (a) Brynsiencyn, (b) Rosset and (c) Clutton.



d. Environmental Factors

Air temperature and rainfall data were attained at the end of the sampling period via the UK Met. Office (Pers. Com.). Mean overall air temperature of 10.5(±0.8)°C was recorded. Brynsiencyn received the highest monthly mean rainfall (99.6mm), followed by Rosset (78.5mm) and Clutton (77.5mm).

e. Statistical analysis of results

(i) Correlation coefficients

Brynsiencyn

Several inter-correlations were established for the Brynsiencyn site with relation to the hydrochemistry. The significant correlations only are outlined in table 2.7. Anion, DOC and phenolics inflow and outflow concentrations were significantly correlated ($p<0.001$-0.050) and inversely with rainfall ($p<0.010$) which may suggest dilution influences. In addition, interestingly, outflow nitrate was the only identified correlation (inverse) with outflow LAS ($r=-0.618$, $p<0.05$). Statistical analysis were also interpreted in terms of percentage removal which revealed that LAS removal was inversely correlated with PO_4 removal ($r=-0.578$, $p<0.05$). NO_3 removal was negatively correlated with local rainfall ($r=-0.727$, $p<0.01$) and both DOC removal ($r=-0.573$, $p<0.05$) and phenolics ($r=-0.628$, $p<0.05$) with air temperature. Correlation coefficients between the three enzymes were high and highly significant ($p<0.01$) and also with air temperature ($p<0.01$). However, negative correlation was found for enzyme activity and percentage removal for DOC ($p<0.05$) and also phenolics ($p<0.05$). In addition, correlation (inverse) between β-glucosidase and local rainfall ($r=-0.891$, $p<0.05$) was identified.

Table 2.7a: Significant correlations between inflow water chemistry parameters, Brynsiencyn site (r = Pearson correlation coefficient, p = probability).

		Rain	Inflow SO_4	Inflow PO_4	Inflow DOC
Inflow SO_4	r	-0.724			
	p	0.008			
Inflow PO_4	r	-0.816	0.876		
	p	0.001	0.000		
Inflow NO_3	r	-0.780	0.645	0.795	
	p	0.003	0.023	0.002	
Inflow	r	-0.826	0.810	0.764	
DOC	p	0.001	0.001	0.004	
Inflow	r	-0.672	0.810	0.811	0.806
Phenolics	p	0.017	0.001	0.001	0.002

Table 2.7b: Significant correlations between outflow water chemistry parameters, Brynsiencyn site (r = Pearson correlation coefficient, p = probability).

		Rain	Outflow SO_4	Outflow PO_4	Outflow DOC	Outflow LAS
Outflow	r	-0.705	0.884			
PO_4	p	0.010	0.000			
Outflow	r					-0.618
NO_3	p					0.032
Outflow	r	-0.826	0.642	0.657		
DOC	p	0.001	0.024	0.020		
Outflow	r	-0.672	0.635	0.601	0.885	
Phenolics	p	0.017	0.026	0.039	0.000	

Clutton

Table 2.8 below shows the correlation coefficient for various parameters calculated at the Clutton site. LAS was highly significantly correlated with DOC and phenolic concentration but, interestingly, inversely correlated to inflow NO_3 (see table 2.8). In terms of percentage removal PO_4 was inversely correlated with local rainfall ($r=-0.733$, $p<0.05$), but phenolics and DOC removal exhibiting a positive strong relationship ($r=0.808$, $p<0.005$). Activities of β-glucosidase, phosphatase and

sulphatase were highly significantly correlated ($p<0.01$). Phosphatase activity was also correlated with PO_4 removal ($p=0.002$).

Table 2.8a: Significant correlations between inflow water chemistry parameters, Clutton site (r = Pearson correlation coefficient, p = probability).

		Inflow NO_3	**Inflow LAS**	**Inflow DOC**
Inflow	r	-0.787		
LAS	p	0.007		
Inflow DOC	r	-0.838	0.776	
	p	0.002	0.008	
Inflow	r	-0.888	0.652	0.897
Phenolics	p	0.001	0.041	0.000

Table 2.8b: Significant correlations between outflow water chemistry parameters, Clutton site (r = Pearson correlation coefficient, p = probability).

		Outflow PO_4	**Outflow NO_3**	**Outflow LAS**	**Outflow DOC**
Outflow	r			0.682	
DOC	p			0.030	
Outflow	r	0.646	-0.740	0.727	0.706
Phenolics	p	0.043	0.014	0.017	0.022

Rosset

At the Rosset site few correlations were established and hence a table is not presented. Inflow sulphate was found to be positively correlated with phosphate ($p<0.01$) but inversely with rainfall ($r=-0.743$, $p<0.01$) suggesting dilution influences. The relationship between DOC and phenolics was again strong with inflow ($p<0.01$) and outflow ($p<0.001$) positive correlations. In terms of removal, DOC was inversely correlated with rainfall ($r=-0.628$, $p<0.05$). Whereas PO_4 and phenolics removal were positively correlated ($r=0.929$, $p<0.001$). No relationship between the activities of the enzymes was observed at this site. However, a significant correlation was established between phosphatase activity and inflow PO_4 concentration ($p<0.05$) and PO_4 removal ($p<0.01$). Interestingly a negative correlation was observed between percentage LAS (and individual alkyl homologues) removal and phosphatase activity ($p<0.05$).

(ii) Between-site statistical comparisons

Between-site comparisons of LAS concentrations using one-way ANOVA tests revealed extremely significant differences between sites for inflow ($F=12.51$, $p<0.001$) and outflow ($F=13.88$, $p<0.001$). However, post hoc Tukey test revealed that the difference was not significant for the Clutton and Rosset sites. In addition no significant differences for percentage LAS removal were observed ($p>0.05$).

One-way ANOVA tests on water chemistry parameters (anions, DOC and phenolics) identified significant differences for the inflow and outflow concentrations for the 3 sites ($p<0.010$). Significant differences were also revealed for percentage removal rates of NO_3 ($F=6.018$, $p<0.001$), PO_4 ($F=5.775$, $p>0.01$), SO_4 ($F=8.489$, $p<0.001$), DOC ($F=6.792$, $p<0.01$) but not phenolics ($p>0.05$). However, post hoc Tukey tests revealed that the differences were not significant between the Brynsiencyn and Clutton sites. In terms of enzyme activity, significant differences between sites for β-glucosidase ($F=8.21$, $p<0.01$), phosphatase ($F=18.92$, $p<0.001$) and sulphatase ($F=7.29$, $p<0.01$) were also calculated. However, post hoc Tukey tests showed that these were only significant between the Rosset and Clutton sites for all enzymes and also Brynsiencyn and Rosset sites for phosphatase.

2.1.4 DISCUSSION

a. LAS

The HPLC analytical procedure adopted successfully separated LAS from the other components and interferences in sewage wastewater. Measured reproducibility and mean recovery supports the validation and accuracy of the method and are comparable to other publications, e.g. reproducibility relative SD of 3-4% (Matthijs & De Henau 1987, Kikuchi *et al.* 1986) and recovery of 98±5 and 85±6% respectively for influent and effluent (Castles *et al.* 1989) and mean of *c.*95% (Matthijs & De Henau 1987, Prats *et al.* 1993).

Holt *et al.* (1998) reported an average LAS concentration of 3.25mg L^{-1} in UK raw sewage (range 1.10-5.58mg L^{-1}). Comparable to the lower range (1.1mg L^{-1}) mean LAS was measured in this study as expected for tertiary treatment. LAS concentration depended on the source and extent of biodegradation in previous treatment processes, e.g. the sharp decrease in the inflow LAS concentration observed at Rosset and Clutton in August. At Brynsiencyn the peak in inflow in July/August is almost certainly due to increased tourism in the area and a possible overload of the secondary treatment process. The considerably higher mean inflow concentration at Rosset may be explained by the hospital wastewater source expected to have a higher consumption of detergents and cleaning agents than the average household. Unexpectedly the LAS inflow concentration is higher at Clutton than Brynsiencyn even though the former serves a 10-fold smaller population. However, this may be due to a lower dilution from other sources. Smaller works can normally receive a higher concentration of LAS since it is not diluted by industrial effluents (Painter & Zabel 1989). In addition the retention time in the sewer prior to reaching the sewage treatment plant is normally lower in smaller treatments works, allowing less time for biodegradation to occur (Painter & Zabel 1989). For example, Painter & Zabel (1989) reported 20.8mg L^{-1} MBAS measured inflow in a small domestic sewage but only 7.4mg L^{-1} MBAS in a nearby larger treatment plant.

A typical annual mean LAS removal of *c.*55% was calculated at each site with no significant ($p>0.05$) difference between sites. Similarly Inaba (1992) reported that 60% of inflow LAS was annually removed, and a LAS biodegradation potential of

0.15g m^{-2} day^{-1}, in a natural wetland receiving gray water investigated in Japan. The author also reported seasonal temperature effects with 95% of the influent LAS removed in the summer, but only 50% in the winter due to inhibition of bacterial activity (<7°C). In this study seasonal variation was suggested with greatest LAS removal observed at all three sites in the spring. This springtime peak in LAS removal coincides with the growth season of the *Phragmites australis* in the constructed wetlands. Several factors may be suggested including enhanced oxygen rhizosphere release, greater surface area for attached microbial growth and DOC root release (Brix 1997) (see chapter 4). Takada *et al.* (1992) reported greater LAS depletion in a river study in summer suggesting higher microbial degradation activity and warmer water temperatures resulting in enhanced removal. This was highlighted by the average LAS chain length which was lower in summer (11.3±0.2, n=10) than winter (11.6±0.1, n=10). However, Nishihara *et al.* (1997) found no significant seasonal variation in LAS biodegradation in 3 different ponds.

Considerable removal was also observed during the colder winter months in this study. Evidence of water purification at lower temperatures is limited in the literature and hence the efficiency of constructed wetlands in pollutant removal during colder months is questionable (Mæhlum *et al.* 1995). However, the performance of constructed wetlands under cold-climate conditions is evident in other countries, e.g. Sweden (min. –3°C) (Whitgren & Mæhlum 1997) and Norway (min. –5°C) (Mæhlum & Stålnackle 1999). LAS removal efficiencies of 77% at Rosset (February), 73% at Clutton (December) and 64% at Brynsiencyn (February) were measured in this study suggesting that winter temperature conditions did not detrimentally affect treatment performance for the surfactant.

However, periods of high rainfall affected LAS removal via dilution, especially in October at Rosset and Clutton. The impact of rain on average may be relatively small, but individual events can have large effects of water quality (Kadlec 1999). In this study no attempt was made to quantify the effects of rainfall on LAS concentrations which could have affected the percentage removal rates. However, the excessive rainfall observed was unusual, as September to November 2000 in the UK were the wettest in over twenty years, with October being the wettest in almost a century (UK

Met Office, Pers. com.). Brynsiencyn on the other hand was not as badly affected as this system is much larger and is designed for stormwater flows. Stark *et al.* (1994) investigated storm events on small and large constructed wetlands for AMD treatment and found a significant drop to near zero in treatment efficiency during a substantial rain event in the smaller wetland. However, no significant reduction in performance was observed at the larger wetland. In addition, Spieles & Mitsch (2000) reported much higher NO_3 concentration in the outflow than inflow during extreme rainfall events (400% higher).

In terms of the LAS chain length distribution a mean of 11.5 (n=12) for both Rosset and Clutton and 11.7 (n=12) at Brynsiencyn was measured in the inflow. A lower average alkyl chain length, and resulting lower molecular weight, was measured in the outflow by a factor of 0.2 at each site indicating the greater removal of longer chain alkyl homologues. This decrease has been found by other authors and has been attributed to the differences in the degree to which the homologues are adsorbed onto suspended particles and different biodegradation rates (Painter & Zabel 1989, Moreno *et al.* 1994).

In this study the average percentage removal of the longer chain homologues was found to be greater than the shorter alkyl chain homologues in the order of $C_{13}>C_{12}>C_{11}>C_{10}$ and a similar decreasing order has been observed in another wetland (Del Bubba *et al.* 2000, Appendix A). This is in accord with the distance principle. The distance principle states that the position of the phenyl group and length of the alkyl chain influence the rate of biodegradation of LAS (Swisher 1987). It states that the longer chain alkyl homologues will have faster degradation than shorter chain homologues (Swisher 1987). The increased biodegradation rate and adsorption are related to the increased hydrophobicity due to the longer alkyl chain (Swisher 1987). Faster rate of degradation of the longer chain homologues has been confirmed for all LAS chain lengths from C_6 to C_{16} (Swisher 1987, Terzic *et al.* 1992). The faster biodegradation and greater adsorption tendencies of the longer chain alkyl homologues have important ecotoxicological implications as lower molecular weight LAS homologues have lower aquatic toxicity values (Swisher 1987). Greater removal of the longer chain alkyl homologues in various test systems, other sewage treatment systems and different aquatic environments has been reported (Prats *et al.* 1993,

Swisher 1987, Terzic *et al.* 1992). Hence the findings of this study confirm that LAS is removed in constructed wetlands by similar processes to that in other more conventional sewage treatment systems.

b. Water Chemistry

Nitrate

Nitrate removal in wetlands is mainly governed by denitrification and is assisted by macrophytes through supply of organic carbon via plant root and litter release (Weisner *et al.* 1994). Wetland design, aeration, plant uptake, available carbon and pH (House *et al.* 1994) influence treatment of nitrate. In this study nitrate was found to negatively correlate with LAS at two of the wetlands monitored perhaps suggesting N-limitation on LAS removal efficiency. However, the inverse correlation between LAS and nitrate observed in the inflow for the Clutton site may suggest that previous treatment stages may be an influencing factor. LAS did not appear to affect nitrate removal with the Rosset site exhibiting both the highest inflow LAS concentrations and nitrate removal. No inhibition of nitrification at 20mg L^{-1} LAS in other forms of wastewater treatment is reported (Baillod & Boyle 1968 as cited in Painter & Zabel 1989). However, at higher LAS concentrations (>20mg L^{-1}) inhibition has been observed (Janicke *et al.* 1973 as reported in Painter & Zabel 1989). Section 6.5 further investigates the effect of LAS on the N cycle.

In terms of general wetland performance, similar N concentrations to that reported in domestic sewage (20-70mg L^{-1}, Horan 1990) were observed in the inflow. Hammer & Knight (1994) associated a decrease in nitrogen removal efficiency with increasing hydraulic loading rate. Similarly, this study shows that Brynsiencyn received the highest loading rate and exhibited the lowest nitrate removal. However, this site received high inflow nitrate with runoff water from agricultural fields a possible source. Greater net N removal has been demonstrated during warmer seasons (Frankenback & Meyer 1999) with temperatures of approx. 10°C partially inhibiting nitrate reduction (Kadlec & Reddy 2001). However, no seasonal trends were recognised in this study and the data agree with Hammer & Knight (1994) in that N removal is variable with a broad spectrum of removal data published.

Phosphate

Phosphate release from sewage treatment plants is of concern due to excess P resulting in enhanced eutrophication in lakes and rivers. In constructed wetlands P removal mechanisms include sedimentation, precipitation, plant uptake and adsorption, with the latter accounting for the greatest removal (Kadlec & Reddy 2001). The inverse correlation associated between percentage PO_4 and LAS removal at Brynsiencyn is interesting and possibly suggests competition for available adsorption sites. Both PO_4 and LAS have negative charges and hence may be governed by similar chemical adsorption processes. LAS may compete for adsorption sites directly or indirectly via the production of SO_4^{2-} during desulphonation. Hence wetland treatment of wastewater containing high PO_4 concentrations may be detrimental for LAS removal efficiency. The importance and quantification of LAS adsorption in the field is further investigated in section 2.2 and its effect on phosphate adsorption in Chapter 6.

In relation to wetland efficiency, phosphate removal was highly variable with, often, discharged effluent concentrations exceeding the influent, as observed elsewhere (Greenway & Woolley 1999, Davison *et al.* 2001). Cooper & Green (1995) reported that no significant phosphate removal had been noted in constructed wetlands in the UK. PO_4 removal capacity generally declined with time. This is frequently reported by other researchers (Tanner *et al.* 1998, Davison *et al.* 2001) with greatest P removal observed in the first year of operation (Mann & Bavor 1993). The decline has been associated with the adsorption capacities of the substrate media. The capacity of gravel for P removal is generally low (Gray *et al.* 2000), e.g. <50 mg kg^{-1} (Breen 1990). Artificial substrates have also been investigated and exhibited greater adsorption capacities, such as calcite, crushed marble (Brix *et al.* 2001a), industrial waste products (Mann 1990), and shale (Drizo *et al.* 1997) (see chapter 6). Hence the low phosphate removal observed may be attributed to the low adsorption capacity of the gravel substrate used as planting medium at the sites investigated. The decline observed suggests increasing occupation of P-adsorption sites with time resulting in a reduction in removal and is illustrated as,

Rosset (1999) > Brynsiencyn (1998) > Clutton (1996)

Temperature is reported to have a minimal effect on PO_4 sorption (Kadlec & Reddy 2001, Kadlec & Knight 1996). In this study no relationship with air temperature was established. Interestingly an inverse relationship with rainfall was established perhaps indicating that dilution effects are important and/or as the hydraulic retention time decreases the contact time for PO_4 adsorption to occur also declines, resulting in a detrimental effect on treatment performance.

Sulphate

Mineralization of LAS can act as a source of S in the wetland due to the release of SO_4 (Swisher 1987). However, due to the low LAS concentrations observed in the wetlands and the other sources of SO_4 in the wastewater it was not possible to distinguish any effect due to the surfactant in this field study.

In terms of general wetland performance seasonal variation was most distinctive in sulphate removal for Rosset and Brynsiencyn sites with highest removal observed during August. In general, the growing season should show a tendency to deplete nutrients (Kadlec & Knight 1995). Stober *et al.* (1997) reported removal of SO_4, PO_4 and NO_3 to be significantly greater in early spring than in the winter period and suggested the improvement efficiency probably occurred by increased surface water temperature, vegetative uptake and greater detention times.

DOC and Phenolics

LAS concentration as a percentage of DOC was low at <5% for the Rosset and Clutton sites, but lower at <2% for Brynsiencyn. Berna *et al.* (1993) reported the LAS contribution to DOC to be very low (<1%). This suggests that although DOC is a parameter that is indicative of LAS mineralization (Zhang *et al.* 1999), with a decrease observed as LAS is broken down, it does not give reliable information in wetlands due to the natural and anthroprogenic compounds that cannot be distinguished from the breakdown product. Similarly, although during LAS mineralization a phenolic product is observed (see figure 1.7) it is not possible to distinguish this from other sources. Radiochemical methods would enable

identification and measurement of these compounds but was not applicable in this field study.

In terms of general wetland performance, DOC has been extensively studied in relation to, for example, heterotrophic bacterial growth (Mann & Wetzel 1995), CO_2 production (Bianchi *et al.* 1996), and extracellular enzyme activity (Kang *et al.* 1998). The inverse correlation between percentage DOC removal with enzyme activity in this study may suggest stimulation by high DOC concentrations. However, the inverse correlation ($p<0.05$) also observed between DOC removal efficiency and air temperature may suggest that the enzyme activity is also related to temperature. A similar relationship between DOC removal and temperature is reported elsewhere (Quanrud *et al.* 2001). Elevated temperatures and associated biochemical activities and increased evapotranspiration may raise DOC levels in the effluents resulting in lower removal efficiency (Quanrud *et al.* 2001). Leaching of DOC into water flowing through a wetland as plants, algae and bacteria grow, die and decay can also occur. Pinney *et al.* (2000) attributed low DOC removal during warmer summer temperatures to the elevated contributions of plant-derived DOC.

Phenolic compounds have also been monitored in wetlands, e.g. in relation to enzyme activity (Kang & Freeman 2000) with phenolic inhibition of enzyme activities reported (Wetzel 1992, Wetzel 1993). In this study an inverse correlation between percentage phenolics removal with enzyme activity was observed at the Brynsiencyn site. However, in contrast, a positive correlation was observed with phosphatase activity at the Rosset site. Shackle *et al.* (2000a) also reported a positive correlation between phenolics and enzyme activity. Hence, the data from this study are inconclusive.

The greater capacity at the Rosset site for DOC and phenolics removal may be associated with the operational age of the wetland. This wetland is the youngest that may suggest that the maximum capacity of the other two mature wetlands have been reached. This may result in these wetlands acting as a source rather than a sink for DOC and phenolics. However, further monitoring is required to establish whether the maximum capacity has been reached.

c. Enzyme Activity

Soil enzymes are involved in a variety of essential biogeochemical transformations (Burns 1978). In constructed wetlands LAS may affect enzyme activities. The production of SO_4 via LAS mineralization could affect sulphatase activity. Competition between LAS and PO_4 for adsorption sites may result in the release of PO_4 that could affect phosphatase activity. The various carbon-based products of LAS mineralization could also affect β-glucosidase activity. The relationship between LAS and soil enzyme activity has been investigated elsewhere with higher surfactant concentrations (<100-fold) than observed in this study required for a direct inhibitory effect to be observed (Elsgaard *et al.* 2001a, Elsgaard *et al.* 2001b).

Various methods have been developed to quantify enzyme activity. However, due to the relatively low activity expected with gravel (due to high dry mass and lower surface area per gram ratio than soil) a sensitive method using methylumbelliferyl (MUF) compounds was adopted based on the measurement of 4-MUF, a fluorescent product liberated on hydrolysis of enzyme substrate (Darrah & Harris 1986). MUF-substrates have been employed in various studies since its application in the 70s with soil (Pancholy & Lynd 1971) and more recently in peatlands (Freeman *et al.* 1995, Kang & Freeman 2000) and constructed wetlands (Kang *et al.* 1998, Shackle *et al.* 2000a). MUF substrates have also been used in soil enzyme studies investigating the effect of LAS (Elsgaard *et al.* 2001a, Elsgaard *et al.* 2001b). In this study assays were conducted under conditions as representative as possible of the environment in which the samples were taken, i.e. incubation under field temperature conditions and no buffer applied to reaction mixture.

Enzyme activities have been related to several biogeochemical processes in wetlands, e.g. trace gas emission (Freeman *et al.* 1997), nutrient cycling (Kang *et al.* 1998) and particulate organic matter decomposition (Sinsabaugh *et al.* 1992). Commonly β-glucosidase, sulphatase and phosphatase have been monitored in C, S and P cycles respectively, i.e. the main biogeochemical cycles in wetlands (Speir & Ross 1978). In this study, the enzymes mainly exhibited maximum rates of reaction at 200-400μM substrate concentration, except for β-glucosidase at 100μM for Brynsiencyn and Clutton sites. Similar ranges have been reported in wetland soils (Kang 1999) and a saturation concentration of 150μM for β-glucosidase (Freeman *et al.* 1995). The

noticeable decrease in activity at higher substrate concentration in some cases suggests that inhibition is occurring possibly due to end product inhibition. Freeman *et al.* (1995) observed this for β-glucosidase and phosphatase in peatland soils.

In terms of enzyme kinetics, literature suggests that the 3 linear transformations are equally applicable for estimation of K_m value of enzymes in soils (Pettit *et al.* 1977). The highest V_{max} value was calculated for phosphatase followed by β-glucosidase and sulphatase at each site. This order for V_{max} has been reported in various ecosystems, e.g. peatlands (Freeman *et al.* 1995) and fresh stream waters (Chappell & Goulder 1992). V_{max} will vary with the total concentration of enzyme present, whereas K_m is independent of enzyme concentration (Price & Stevens 1982). Tabatabai & Bremner (1971) suggested that enzymes with similar K_m values from different sources indicated similar type or origin, or that association of enzymes affected K_m with soil constituents. However, the authors also suggested that the K_m for phosphatase and sulphatase activity was affected by shaking of the soil-substrate mixture during incubation.

In terms of mean annual activity, the sites were ranked in the order of: Rosset>Brynsiencyn>Clutton. The level of enzyme activity over the sampling period was highest at Rosset by approximately 1.5 and 2.5-3.0 orders of magnitude in comparison to Brynsiencyn and Clutton respectively. Due to the lack of data of enzyme activities in constructed wetlands, especially gravel-based, comparable activities are scarce. However, in soil-based wetlands, Shackle *et al.* (2000a) reported maximum activities of approx. 2μmol g^{-1} min^{-1} for phosphatase, 1.5μmol g^{-1} min^{-1} for β-glucosidase and 0.5μmol g^{-1} min^{-1} for sulphatase in comparison to Kang *et al.* (1998) with 0.4, 0.2 and 0.02μmol g^{-1} min^{-1} respectively. A dip in enzyme activity was observed at Clutton in October coinciding with the month of high rainfall. Waterlogging has been reported to restrict enzyme activity in natural wetlands (Kang & Freeman 1999, Pulford & Tabatabai 1988). However, in contrast a peak was observed in phosphatase activity at Rosset for this month.

Activities of the enzymes were significantly correlated with each other ($p<0.01$) for the Brynsiencyn and Clutton sites supporting previous evidence, e.g. sulphatase and

phosphatase (Speir 1977). Despite the known importance of temperature as a controlling regulator of enzyme activity, only at one site (Brynsiencyn) was a significant correlation established with air temperature ($p<0.05$). Several researchers (Kang & Freeman 1998, Kang & Freeman 1999, Shackle 2000) have reported an increase in enzyme activity with increasing temperature. Lack of correlation with temperature and other physico-chemical parameters may suggest that interactions with other factors are paramount, e.g. vegetation exudates (Shackle *et al.* 2000b), substrate type (McClaugherty & Linkuns 1990), hydraulic loading and flow rate.

Despite published evidence that activity of a specific enzyme and the target substrate is related, few correlations were found in this study. Phosphatase activity was correlated with percentage PO_4 removal at both Rosset and Clutton sites, and an additional positive correlation with inflow PO_4 concentration also at the former site. However, an inverse relationship has been reported for phosphatase activity and availability of PO_4 elsewhere (Nannipieri *et al.* 1978, Spier & Ross, 1978). Nutrient supply along with temperature and pH has been reported as controlling factors of enzyme activity in upland soils (Sinsabough *et al.* 1991) with microorganisms controlling their enzyme production in response to nutrient availability (Chròst 1991).

2.1.5 CONCLUSION

1. Mean inflow LAS concentration of 1.1mg L^{-1} and effluent concentration of 0.43mg L^{-1} LAS was measured.
2. LAS mean annual removal of *c.*55% was observed at all three sites with no significant ($p>0.05$) difference in treatment efficiency between sites.
3. Seasonal variation in LAS removal was suggested with greatest removal efficiency observed in the spring.
4. Greater removal of the longer chain LAS alkyl homologues was evident ($C_{13}>C_{12}>C_{11}>C_{10}$) suggesting that similar removal mechanisms as that reported in more conventional sewage treatment processes also prevail in wetlands.

5. Overall the Rosset wetland exhibited greatest treatment efficiency in terms of nutrient cycling. However, generally inconsistent phosphate removal was observed at all three sites.

2.2 LONG TERM STUDY OF LAS REMOVAL IN CONSTRUCTED WETLANDS

2.2.1 INTRODUCTION

The majority of published LAS monitoring studies focus on LAS removal on a short-term basis. LAS removal has been published in sewage treatment processes over periods of e.g. hours (Leal *et al.* 1994), days (Holt *et al.* 1995), months (Inaba *et al.* 1988, Di Corcia *et al.* 1999) and, infrequently, years (e.g. almost 2 years, Moreno *et al.* 1994). However, due to the lack of knowledge on removal in constructed wetlands, it is important to monitor for longer periods in order to establish any trends with time, e.g. decline or improvement.

In this extensive survey longer term LAS trends were monitored by the continuation of sampling at the Brynsiencyn site for a further 18-month period until May 2002. Substrate samples from the wetland were also collected and analysed for LAS content to measure change in amount of LAS adsorbed. The main aim of this section is to establish any long-term trends and/or seasonal variations in LAS, nutrients and enzyme activity. Moreno et al. (1994) reported high LAS removal (<97%) in a lagoon treatment plant over almost 2 years suggesting no decline in treatment efficiency over time. Hence it is hypothesised that;

1. LAS removal efficiency will not decline over time
2. Seasonal variations in treatment efficiency will prevail
3. Treatment efficiency of nutrients will be related to enzyme activity and possibly environmental factors (e.g. temperature, rainfall, etc.)

2.2.2 METHODS

2.2.2.1 Sampling and laboratory analysis

Monthly sampling of the inflow and outflow for LAS and water chemistry (section 2.2.3) were conducted from January 2000-May 2002. Additional samples were also taken in the latter 18 months for pH (in situ using portable pH electrodes, Phillip Harris International, UK) and Total Suspended Solids (TSS) analysis. TSS was measured by passing a known volume of water through a pre-washed and weighed GF/C glass microfibre Whatman® filter (9cm), then oven dried (104°C) and re-weighed. Replicate gravel samples were taken for enzyme analysis (Section 2.2.3) and LAS analysis (see below). However, due to the foot and mouth outbreak in the UK in January 2001 and the location of the treatment plant, sampling was suspended due to legal restrictions until June 2001. Thereafter monthly sampling continued.

2.2.2.2 LAS concentration/extraction procedure for gravel substrate samples

Gravel substrate samples were taken from the top 10cm of the wetland and frozen (-15°C) for storage in the last 12 months of the study. For analysis, gravel samples were dried and placed in weighed, pre-extracted with methanol, cellulose acetate extraction thimbles. Samples were extracted with 200ml methanol under sohlex extraction for 4-6 hours. The methanol extract was then dried down using a rotary evaporator and adjusted to 100ml with 95:5 water:methanol solution. This was then treated as an aqueous sample and passed through the prepared C_{18} and subsequent SAX SPE cartridges (section 2.2.3).

Recovery

Spiked samples were prepared by pipetting a known concentration of LAS into an extraction thimble containing an aliquot of gravel and drying in the oven before sohlex extraction. The analytical procedure in section 2.2.3 above was then followed.

2.2.2.3 Statistical analysis of results

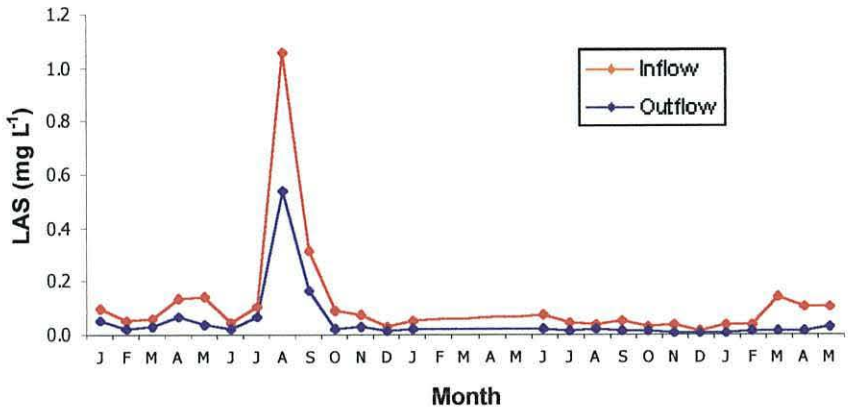
Correlation relationships were assessed using Pearson correlation coefficients for normally distributed data or Spearman rank correlation coefficients for other data (Minitab Inc. 2000). One-way ANOVA tests, for the data that conformed to the normal distribution and had homogenous variance, were applied to hydrochemical percentage removal results and to compare enzyme activities.

2.2.3 RESULTS

a. LAS

Measured LAS concentration (mean 0.12mg L^{-1}) was highest in the inflow at 1.06mg L^{-1} (August, 2001) and lowest at 0.02mg L^{-1} (December, 2001). In the outflow (range 0.004-0.54mg L^{-1}) a mean of 0.05mg L^{-1} was measured with maximum exhibited in August (2001) and lowest in November (2001). Figure 2.13a shows the measured LAS concentrations. Removal distribution of the alkyl chain homologues was highly significant ($p<0.001$), i.e. $C_{13}(78\%)>C_{12}(64\%)>C_{11}(52\%)>C_{10}(44\%)$.

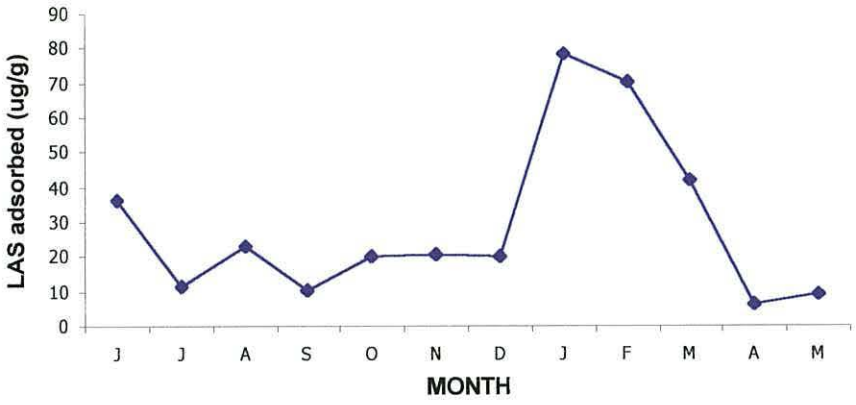
Figure 2.13a: Measured LAS concentrations.



b. LAS concentration/extraction procedure for gravel substrate samples

The mean amount of LAS adsorbed on the gravel samples collected was 30μg/g (range 7-79μg/g) at 0-10cm depth from the surface. Seasonal change was clearly observed with greater adsorption in the winter (Figure 2.13b). Peak measured LAS concentration adsorbed was measured in January (79μg/g) and February (71μg/g) 2002. The mean distribution of the homologues adsorbed onto the gravel were of the opposite trend found in water in order of; $C_{13}>C_{12}>C_{11}>C_{10}$ with a mean chain length of 11.6. Reproducibility as percentage relative standard deviation for the gravel samples (C_{10}=6.4%, C_{11}=7.6%, C_{12}=6.6%, C_{13}=5.7%, Total LAS=5.1%). The minimum mean recovery of total LAS was measured at 87%. In the range tested recovery of LAS was not affected by the concentration added.

Figure 2.13b: Seasonal change in LAS adsorbed on Brynsiencyn wetland gravel.



c. Water Chemistry

Anions

High mean nitrate concentration was observed in the inflow at 66.8mg L^{-1} and was reduced by an overall mean of 62%. Highest removal was observed at 97% (May 2002) and lowest at 20% (November 2000). Figure 2.14a shows the overall trend in nitrate concentrations.

Phosphate removal continued to be variable with an overall mean of only 16%, but with a high of 75% in February (2002). However the range of phosphate concentrations measured in the inflow varied widely (3.7-58.2mg L^{-1}) and was often higher in the outflow (2.7-50.4mg L^{-1}) as shown in figure 2.14b.

Inflow sulphate (mean 46.2mg L^{-1}) was detected at a peak of 83.6mg L^{-1} in November 2001 and outflow (mean 33.5mg L^{-1}) at a minimum of 15.7mg L^{-1} for November 2000. Overall sulphate removal was relatively low at an average of 26%. Measured sulphate concentrations are exhibited in figure 2.14c.

DOC and Phenolics

Continuation of the similar trend of DOC (figure 2.14d) and phenolics (figure 2.14e) with time both in measured concentrations and removal efficiencies was observed. DOC (mean inflow 16.9mg L^{-1}, outflow 11.0mg L^{-1}) removal was greatest during the autumn/early winter period (Oct-Jan) for both years monitored with a maximum of 81% observed in October 2000. Low DOC removal also exhibited temporal trends in

the spring/early summer with the overall minimum of 7.8% exhibited in April 2002. Similar phenolics removal (6-72%) with time was also exhibited.

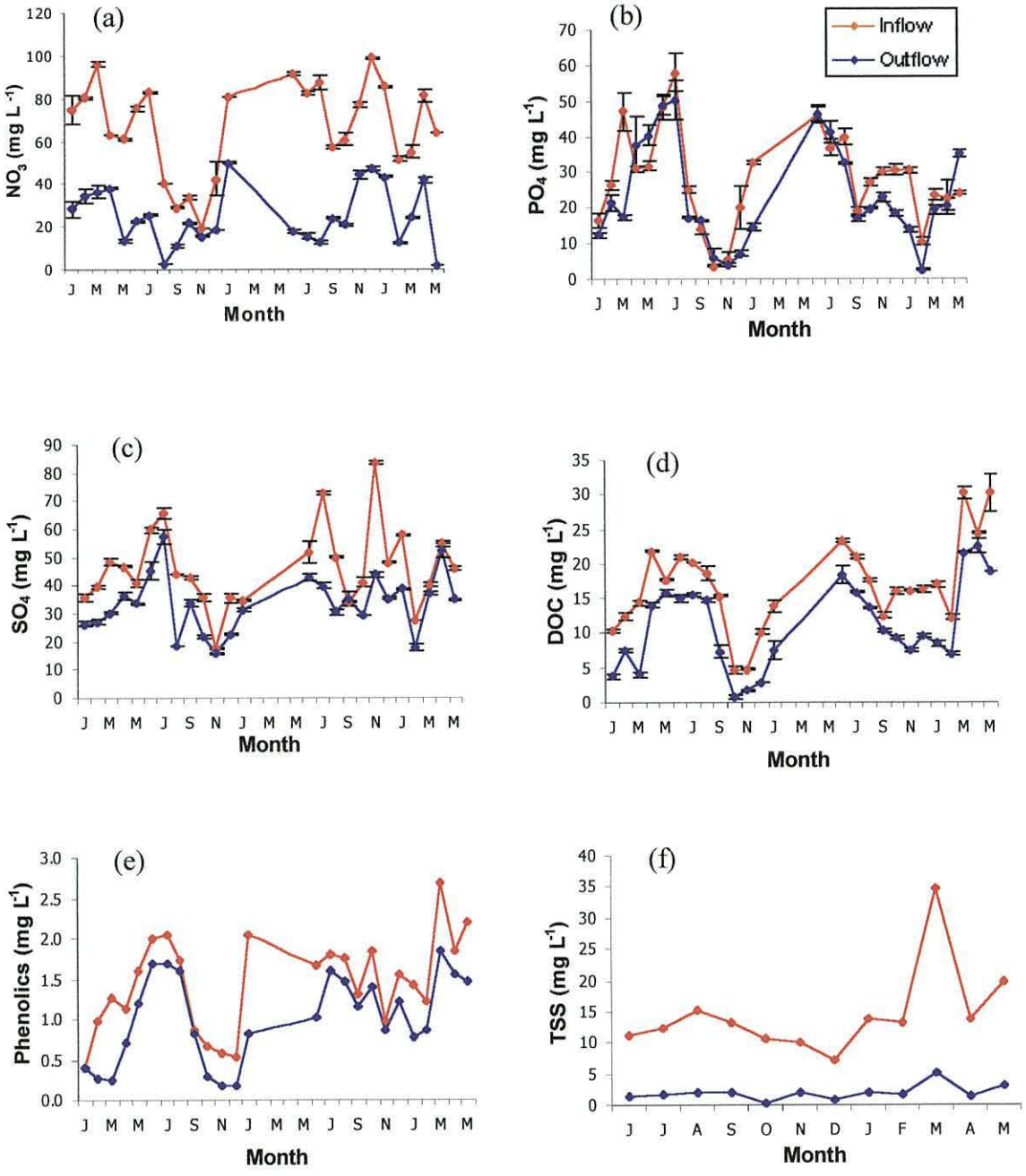
TSS

TSS concentrations both in the inflow and outflow were stable throughout the year with the exception of peaks observed in March 2002. High TSS removal (figure 2.14f) was observed with a mean of 87% (range 80-96%). However, overall, low TSS concentrations were measured in the inflow (mean 14.7mg L^{-1}) and outflow (mean 2.0mg L^{-1}).

pH

A mean pH of 6.6(±0.2) was observed in the inflow and was found to be slightly more neutral at pH 6.8(±0.2) in the outflow.

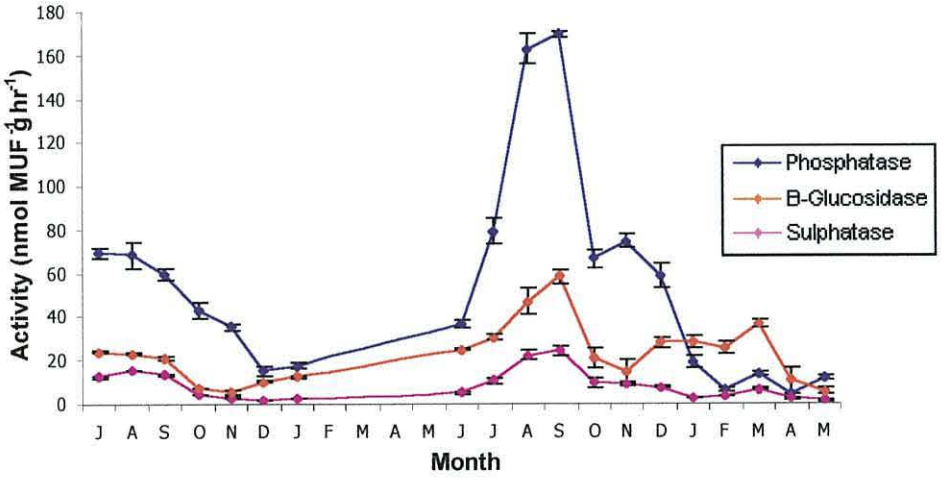
Figure 2.14: Measured concentrations at the Brynsiencyn wetland for (a) NO_3, (b) PO_4, (c) SO_4, (d) DOC, (e) Phenolics and (f) TSS.



d. Enzyme activity

Phosphatase activity was highest overall (53.4 nmol MUF g^{-1} hr^{-1}) followed by β-glucosidase (23.0 nmol MUF g^{-1} hr^{-1}) and sulphatase (8.4 nmol MUF g^{-1} hr^{-1}) (Figure 2.15). The activities of the enzymes exhibited seasonal variation with greater activities associated with the summer. Peak enzyme activity was observed for August and September 2001 for each enzyme. A drop of approximately 50% below the annual mean occurred during the winter in enzyme activity. Low activity was also exhibited for the spring (May) in comparison to the summer months.

Figure 2.15: Enzyme activities measured at Brynsiencyn wetland.



e. Environmental Factors

Mean air temperature of 10.7°C (min 3.2°C, max. 19°C) was measured. Rainfall was reported at a mean of 81.2mm (Met. Office, Pers. Com.).

f. Statistical Analysis

Significant results are presented in tables 2.9a and 2.9b for inflow and outflow data respectively.

Table 2.9a: Significant correlations between inflow water chemistry parameters, Brynsiencyn site (r = Pearson correlation coefficient, p = probability).

		Rain	Inflow SO_4	Inflow PO_4	Inflow DOC	Inflow Phenolics	Inflow pH
Inflow SO_4	r	-0.550					
	p	0.004					
Inflow PO_4	r	-0.701	0.645				
	p	0.000	0.000				
Inflow NO_3	r	-0.711	0.578	0.746			0.684
	p	0.000	0.002	0.000			0.014
Inflow DOC	r	-0.704	0.472	0.482			
	p	0.000	0.017	0.015			
Inflow Phenolics	r	-0.627		0.524	0.805		-0.663
	p	0.001		0.007	0.000		0.019
Inflow TSS	r				0.685	0.775	-0.842
	p				0.014	0.003	0.001
Inflow LAS	r						-0.647
	p						0.023

Table 2.9b: Significant correlations between outflow water chemistry parameters, Brynsiencyn site (r = Pearson correlation coefficient, p = probability).

		Outflow SO_4	Outflow PO_4	Outflow DOC	Outflow pH	Outflow NO_3
Outflow PO_4	r	0.703				
	p	0.000				
Outflow DOC	r	0.633	0.683			
	p	0.001	0.000			
Outflow Phenolics	r	0.556	0.581	0.840		
	p	0.004	0.002	0.000		
Outflow TSS	r				-0.793	
	p				0.002	
Outflow LAS	r					-0.401
	p					0.047
Rain	r	-0.589	-0.570	-0.6226		
	p	0.002	0.003	0.001		

Significant correlations were established between total LAS and individual alkyl homologues with TSS concentration. This may be related to the adsorptive behaviour of the surfactant on the solid particles. Table 2.9c summarises the correlation coefficients for LAS and TSS.

Table 2.9c: Significant correlations between TSS, LAS and LAS alkyl homologues, Brynsiencyn site (r = Pearson correlation coefficient, p = probability).

		Inflow LAS	**Inflow C_{10}**	**Inflow C_{11}**	**Inflow C_{12}**	**Inflow C_{13}**
Inflow	R	0.826	0.595	0.889	0.738	0.788
TSS	P	0.001	0.041	0.000	0.006	0.002
		Outflow LAS	**Outflow C_{10}**	**Outflow C_{11}**	**Outflow C_{12}**	**Outflow C_{13}**
Outflow	R	0.777	0.608	0.826	0.689	0.734
TSS	P	0.003	0.036	0.001	0.013	0.007

In addition, sulphatase and phosphatase positively correlated with air temperature ($p<0.01$). No correlations between percentage removals of the hydrochemical parameters and the activity of associated enzymes was significant except for, inversely, with phenolics ($p<0.05$) and β-glucosidase with DOC ($p<0.05$). Percentage removal of DOC and phenolics were closely correlated ($r= 0.531$, $p<0.006$).

One-way ANOVA tests revealed that percentage removal for NO_3 was significantly higher in comparison to PO_4 and SO_4 ($F=25.178$, $p<0.001$). In addition significant differences between enzyme activities ($F=12.253$, $p<0.001$), but not for β-glucosidase and sulphatase (Tukey test).

2.2.4 DISCUSSION

a. LAS

This long-term monitoring suggested that LAS removal possibly improved with time in the Brynsiencyn wetland investigated with mean removal of 69% in the latter 18-months compared to 55% reported in section 2.1. This is supported by the finding in section 2.1 that the constructed wetland in operation longest exhibited the greatest overall LAS removal. This may be explained by several possible reasons. Firstly,

during the initial 12 months more extreme weather conditions were observed with greater rainfall, especially October-December rainfall approx. three times higher in 2000 than in 2001. Secondly, adaptation of the microbial community to LAS has been reported to enhance LAS degradation in other studies (e.g. Branner *et al.* 1999, Jensen 1999, Scott & Jones 2000) and may also be an influencing factor here. Hence the hypothesis, that LAS removal efficiency will not decline over time, is supported by the data collected.

Seasonal trends prevailed with again greater removal coinciding with the spring plant growth period (67% in 2000, 82% in 2002) which was extensively discussed in section 2.1.4. Due to the Foot and Mouth epidemic, spring sampling for 2001 was suspended and hence data from two spring seasons (2000 and 2002) are the basis of this conclusion. Hence this may support the argument that plants enhance LAS removal as observed for other compounds in wetlands, e.g. nutrients (House *et al.* 1994, Heritage *et al.* 1995). The peak in LAS inflow concentration was not repeated in the summer of 2001 as occurred in 2000. However, it is difficult to conclude whether this is due to better performance by the secondary treatment stage or the decline in tourist influx in the area due to the Foot and Mouth epidemic outbreak that continued over the summer on Anglesey.

The mean LAS chain length in the inflow of 11.7 (n=25) was calculated which is equal to that for in section 2.1 for this wetland. However, in the outflow a mean chain length of 11.3 (n=25) was observed which is a factor of 0.2 lower than that measured in section 2.1 (11.5, n=12) suggesting greater removal of longer chain alkyl homologues in the later stages of the study (Painter & Zabel 1989). This is supported by the higher removal of the longer alkyl chain homologues as reported previously in section 2.1.3. Again this further supports the finding that the behaviour of LAS removal in constructed wetland systems is similar to that in other more conventional sewage treatment systems (section 2.1.4).

b. LAS concentration/extraction procedure for gravel substrate samples

Reproducibility data generated in this study was comparable to other researchers, e.g. relative SD of 2-18.2% and 3% (Holt *et al.* 1989, Kikuchi *et al.* 1986) with an increase in SD with decreasing concentration of LAS (Holt *et al.* 1989). Comparable

recoveries of >90% and *c.*85% (Holt *et al.* 1989, Matthijs & De Henau 1987) are published with Holt *et al.* (1989) reporting no significant improvements in extracting for longer periods from 4 to 16 hours. Matthijs & De Henau (1987) reported LAS recovery was not affected by concentration added but was inversely proportional to alkyl chain length.

The mean LAS adsorbed on the gravel varied throughout the year (7-79μg/g). Seasonal change was clearly observed with greater adsorption in the winter in comparison to the summer months. Inaba *et al.* (1988) also reported similar seasonal variations in the adsorption of the C_{12} LAS homologue in a wetland. The authors explained the data by the ability of bacteria to degrade LAS during the summer resulting in the adsorbed LAS decreasing. In winter, when activity of bacteria becomes lower, LAS was only reduced by adsorption. Adsorption near the outflow in the wetland winter was >400 μg/g, but almost zero in the summer (Inaba 1992).

Greater adsorption of the longer chain homologues was observed in the order of $C_{13}>C_{12}>C_{11}>C_{10}$. These results are consistent with published laboratory analysis on adsorption equilibrium studies of LAS by previous workers (e.g. Hand & Williams, 1987, Swisher 1987). The mean chain length of 11.6 is comparative to 11.7 reported by Holt *et al.* (1989). However, no significant difference was found between the concentration of the alkyl homologues adsorbed on the gravel even though a visual trend is observed.

c. Water Chemistry

Anions

As discussed in section 2.1.4 the various components in the sewage wastewater makes it difficult to identify relationships between LAS and the hydrochemical data in this field study. However, an inverse correlation between outflow LAS and nitrate was observed possibly suggesting N-limitation on LAS removal efficiency (see section 2.1.4b).

In terms of general wetland performance the inflow source greatly influenced the water chemistry fluctuations with a strong relationship between DOC and nutrients.

Extremely significant ($p<0.001$) differences between percentage removal of nutrients was observed with NO_3 removed greater than SO_4 and PO_4. The high levels of nitrate suggests that runoff from agricultural land is still a problem at this site with a mean of *c.*25mg L^{-1} discharged which is higher than the recommended levels of nitrate in public water supplies at <10mg L^{-1} (Gersberg *et al.* 1983). However, dilution of the effluent in river water would result in a much lower concentration downstream. Nitrate removal efficiency has been found to be greater in spring and summer months (Gersberg *et al.* 1983) as discussed in section 2.1.4 and observed in the latter 18-months of the study. Similarly to LAS, this enhanced treatment corresponds with the plant growth season. There is evidence that plant nutrient uptake is an important removal mechanism (Breen 1990). For example, nutrient uptake capacity of harvested plants measured at 200-2500 kg N ha^{-1} yr^{-1} and 30-150 kg P ha^{-1} yr^{-1} are reported (Gumbricht 1993, Brix 1994), with debate over harvesting affecting removal. However, there is debate about whether nutrient uptake is significant in an operational context (Gersberg *et al.* 1993, Green *et al.* 1997). Hence it is likely that microbial denitrification is the major process involved in nitrate removal in the wetland investigated.

Phosphate removal continued to be variable throughout the course of the study with an overall mean of only 16%. However, this is higher than the 9.8% reported at this site after 12 months which somewhat contradicts the findings in section 2.1, i.e. that a decline in phosphate removal with time was observed. In addition, as discussed in section 2.2.4a, LAS removal also improved during this time. This may suggest that the possible competition between LAS and PO_4 for adsorption sites may not occur at environmentally realistic LAS concentrations received by constructed wetlands and hence that LAS is not detrimental to PO_4 adsorption. However, outflow phosphate concentrations were higher than the inflow on 8 occasions (n=25) during the sampling period. This suggests that the wetland is on occasions acting as a source rather than a sink for phosphate. Several suggestions may explain this phenomenon. The gravel phosphate adsorption capacity may be exceeded, phosphate desorption has occurred and/or there is low microbial activity. Hence further investigation into phosphate removal capacity of gravel based wetlands is required and is discussed in greater detail in chapter 6.

Observations in the first 12 months of the study highlighted a seasonal trend in sulphate removal with highest removal occurring during August. Significant removal was also exhibited the following year in July 2001 (45%). This somewhat contradicts the suggestion that the SO_4 released during LAS mineralization would affect SO_4 measurements in the wetland as greatest LAS removal was observed in the spring (section 2.2.4a). However, as SO_4 data for the rest of the season were variable it is not possible to draw any firm conclusions.

DOC and Phenolics

LAS concentration as a percentage of DOC declined from that reported in section 2.1.4b (<2%) to <1% as reported by Berna *et al.* (1993). In relation to general wetland performance a similar trend in DOC and phenolics concentrations was observed in this study. However, although phenolic materials form part of the DOC concentration these two parameters do not necessarily vary together (Shackle 2000). Seasonal variations in DOC removal were observed with, in contrast to LAS and nutrient treatment efficiency, noticeably less removal during late spring/summer months by 23% (2000, n=12) and 11% (2001, n=7) in comparison to the rest of the year. This is also highlighted by the inverse correlation with air temperature. This is attributed to greater leaching by plants as discussed in section 2.1.4b.

TSS

It is proposed that LAS adsorption onto TSS is a significant removal mechanism in sewage treatment (Prats *et al.* 1997, Berna *et al.* 1989, McEnvoy & Giger 1986) and hence would be expected to occur during wetland treatment. Lower LAS concentration is observed in settled than raw sewage due to this phenomenon, e.g. reductions of 22% (Swanwick *et al.* 1969). Takada *et al.* (1992) found that dissolved LAS is enriched in short chain homologues (C_{11} and C_{12}), while suspended LAS is enriched in longer chain homologues (C_{12} and C_{13}) and is supported by similar findings by Moreno *et al.* (1994) and Holt *et al.* (1998). Although LAS adsorbed onto SS was not quantified in this study an interesting similar temporal trend, supported by highly significant correlation, was observed with aqueous LAS concentration in the last 12 months of the study. This may be attributed to the adsorptive behaviour of LAS onto solid particles.

In wetlands removal of TSS is primarily due to settling which is promoted by the vegetation and low water velocity (Kadlec & Knight 1996). In this study high removal of TSS for the 12 month period monitored with a mean of 87% (±10%) in accordance with that reported in similar systems, e.g. 86.1% (Marques *et al.* 2001), 95.6% (Merlin *et al.* 2002). Although no physico-chemical correlations were identified temperature seems to be an influencing factor in other studies with a general decrease in removal in winter months (Kadlec & Knight 1996) possibly due to a faster retention time associated with greater rainfall. However, other researchers have reported a decrease in removal in the summer (Kadlec & Knight 1996). Hence such trends are currently unclear. Reports of a decline in TSS removal with time due to clogging are cited (Tanner *et al.* 1998). However, no decline was visible throughout the duration of this study.

d. Enzyme activity

As discussed in section 2.1.4c it is not possible in this field study to investigate the effect of LAS on enzyme activity due to the other components in the wastewater. Hence enzyme activities in a general context to wetland processes are discussed in this section with the effect of LAS investigated in chapter 6.

Significant ($p<0.001$) differences were found between enzyme activities in the order of phosphatase>β-glucosidase>sulphatase. Increases in enzyme activity may occur due to stimulated growth of the microbial population directly leading to more enzyme activity, the required microbial substrate present at a premium and/or activation of enzymes previously immobilised in the soil (Shackle *et al.* 2000b). Overall there was no obvious relationship expressed between enzyme activity and nutrient concentration nor removal at this site ($p>0.05$). pH can be a controlling factor in enzyme activity regulation and this has been reported for phosphatase activity in natural wetland soils (Kang & Freeman 1999). However, again no statistical evidence in support was identified in this study.

Correlation with air temperature was found ($p<0.01$) for sulphatase and phosphatase with greater activity observed during the summer and drop of approximately 50% in the winter in comparison to the overall mean. An autumn decrease in enzyme activity

has been reported elsewhere (Kang & Freeman 1999, Shackle *et al.* 2000a). A drop in enzyme activity in the spring (May) was also observed as reported in other constructed (Shackle *et al.* 2000a) and natural (Kang 1999, Kang & Freeman 1999) wetlands. However, this is in contrast to observations of an increase in soil phosphatase activity in the spring by other authors (Ramírez-Martínez & McLaren 1966, Kiss *et al.* 1975 as quoted in Speir & Ross 1978). Kang (1999) associated the decline to competition between plant root systems and microorganisms for nutrients with a reduction in root exudation due to fast plant growth in the spring resulting in a decline in rhizosphere bacteria and associated enzyme activity.

2.2.5 CONCLUSION

1. Possible greater LAS removal by *c.*15% in the latter 18-month sampling in comparison to the first 12-months was suggested, giving an overall mean of 62%.
2. Strong correlation was identified between LAS and TSS concentrations suggesting adsorption onto solid particles in wastewater.
3. LAS adsorption showed seasonal variation with greater adsorption exhibited in the winter and overall greater adsorption of longer chain alkyl homologues.
4. Nutrient removal was of the order nitrate>sulphate>phosphate with no correlations established between enzyme activity and target substrate.

2.3 LATITUDINAL GRADIENT OF LAS REMOVAL IN WETLANDS

2.3.1 INTRODUCTION

Theoretically there are various chemical, physical and biological processes involved in wetland wastewater treatment which may affect LAS degradation and nutrient cycling. Climatic conditions can potentially affect several of these processes and have to date been considered infrequently on a global scale. Studies of wetland processes on a latitudinal gradient have focused on, for example, enzyme activity (Kang & Freeman 2000), methane emissions (Bartlett & Harris 1993), and primary productivity and decomposition (Brinson *et al.* 1981).

Climatic conditions can affect the hydrology, vegetation growth and microbial activity via water level fluctuations, precipitation and temperature. Constructed wetlands have been investigated individually in various climates, e.g. tropical conditions of India (Billore *et al.* 1999) and Thailand (Kantawanichkul *et al.* 1999), and cold-climate wetlands in Norway (Mæhlum & Stalnacke 1999) and Sweden (Whitgren & Mæhlum 1997). However, no collective comparison of surfactant removal has been published.

Evident temperature effects on LAS degradation in laboratory (e.g. Terzic *et al.* 1992) and field tests, e.g. rivers (Takada *et al.* 1992) and natural wetland (Inaba *et al.* 1988), are reported. Hence this study investigates LAS removal efficiency in wetlands treating domestic wastewater treatment on a global latitudinal gradient. Secondary data on nutrient removal and DOC levels were also collected. Effect of climatic conditions on wetland performance is assessed.

2.3.2 METHODS

2.3.2.1 Sampling sites

Characteristics of the six-wetland sampling sites sampled in different countries are described below. The wetlands sampled all received domestic wastewater and were incorporated as either secondary or tertiary treatment stages.

a. Nynäshamn, Sweden (58.5°N)

This large wetland (28 ha.) is situated in Nynäshamn, approx. 70km south of Stockholm, Sweden. The wetland was constructed in 1997 and has been in operation since spring 1998. The main vegetation is *Phragmites australis*, although *Typha latifolia*, *Elodea Canadensis* and *Schoenoplectus lacustris* are also present. The wastewater is treated with mechanical and chemical treatment before reaching the wetland.

b. Capel Coch, UK (53.2°N)

This site is a natural wetland that receives run off from a domestic sewage treatment works in Anglesey, UK during high flow conditions. The site (300m length) is dominated by *Juncus* and *Sphagnum* vegetation.

c. Girona, Spain(42.0 °N)

The large wetland treatment system (length 500m) was constructed in 1995 situated on the Costa Brava, Spain. The wetland receives variable flow with greatest rate occurring in the summer due to the large population increase in tourism. Dominant vegetation is *Phragmites spp.* with *Typha* and *Juncus* also present.

d. Seoul, Korea (37.0°N)

A large (14, 300m^2) soil-based wetland mainly planted with *Phragmites communis* situated in Seoul, Korea. The wetland has been operational receiving sewage wastewater since April 2001 on an approximate retention time design of 2 days.

e. Ujjain, India (23.1 °N)

A field-scale subsurface flow (SF) constructed wetland with horizontal flow receiving sewage from a residential colony was sampled in Ujjain, India. Size of the wetland is 30m length, 10m width, 0.85m depth, and effective surface area of 300m^2 and has a daily wastewater treatment capacity of 40m^3. Dominant vegetation of locally collected reed grass, *Phragmites karka*, is planted in gravel substrate media. Figure 2.16a is a photograph of the wetland sampled.

Figure 2.16a: Photograph of the wetland sampled at Ujjain, India.

f. Chiang Mai, Thailand (19.5 °N)

Small-scale (1 x 3 x 0.85m^3) experimental subsurface horizontal flow constructed wetland situated in Chiang Mai, Thailand that receives wastewater from a nearby laundry. The wetland was constructed above-ground and filled with gravel and sand substrate planted with *Typha spp.* Figure 2.16b is a photograph of the wetland sampled.

Figure 2.16b: Photograph of the wetland sampled at Chiang Mai, Thailand.

Samples

Water samples for LAS analyses (preserved with 3-5% formaldehyde) and hydrochemistry (anions, DOC, phenolics) were collected (within a max. of 10 days of each other) from the inflow and outflow at each site and the latter filtered (0.2μM). Due to the presence of formaldehyde in the water samples, the first SPE stage had to be conducted before postage. On arriving in the laboratory the final SPE stages were conducted and subsequent analysis conducted as outlined in section 2.1.2.

Statistical analysis of results

Relationships between LAS concentrations and physico-chemical factors for each site were assessed in relation to latitude via correlation analysis (Pearson) using Minitab™ version 13.1 (Minitab Inc. 2000) for data which conformed to the normal distribution. Nonparametric data was assessed using Spearman rank correlation distribution.

2.3.3 RESULTS

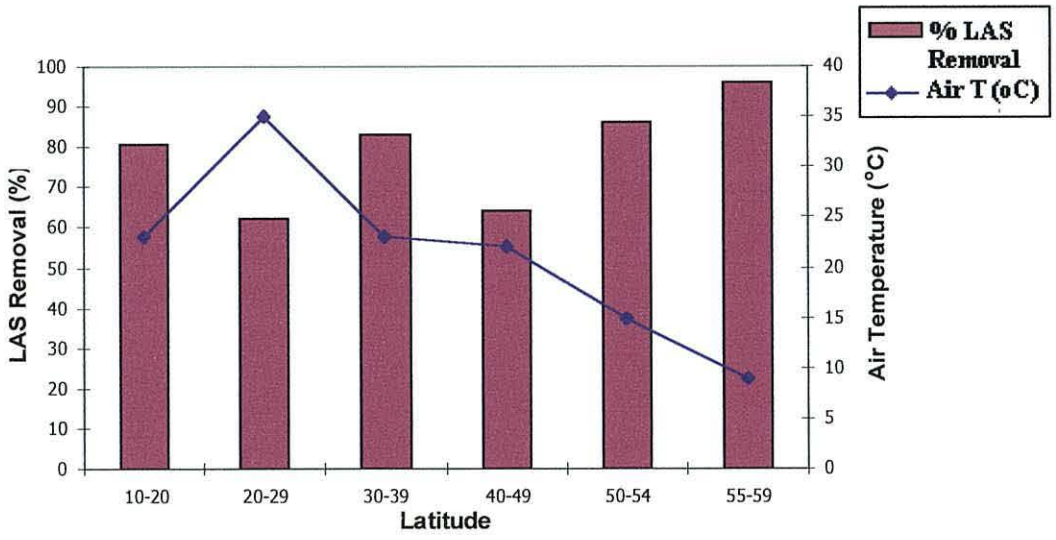
a. LAS

Table 2.10 presents the LAS inflow, outflow and removal for the 6 wetlands sampled. Wetlands located in India and Thailand were sampled on two separate occasions and both results are presented below but mean values used to show the trend with latitude graphically in Figure 2.17a. An extremely high LAS inflow concentration was measured for Chiang Mai, Thailand, at 98.3mg L^{-1} and lowest at 0.03mg L^{-1} for Capel Coch, UK. A minimum of 60% removal was observed at Ujjain, India (23.1°N) and high LAS removal (96%) in cold-climate conditions (Sweden, 58.5°N). Statistical analysis demonstrated that LAS removal was negatively correlated with NO_3 removal ($p<0.01$).

Table 2.10: LAS inflow, outflow and removal data.

Site	Latitude	Inflow (mg L^{-1})	Outflow (mg L^{-1})	% Removal
Nynäshamn, Sweden	58.5°N	0.29	0.01	96
Capel Coch, UK	53.2°N	0.03	0.004	86
Girona, Spain	42.0 °N	0.04	0.015	64
Seoul, Korea	37.0 °N	0.13	0.02	83
Ujjain 1, India	23.1 °N	13.4	4.8	64
Ujjain 2, India	23.1 °N	18.6	7.4	60
Chiang Mai 1, Thailand	19.5 °N	98.3	26.9	73
Chiang Mai 2, Thailand	19.5 °N	33.8	4.0	88

Figure 2.17a: Percentage LAS removal and air temperature measured on a latitudinal gradient.



Generally the alkyl homologue distribution in this study followed the distance principle whereby the longer chain homologues are removed faster than shorter chain homologues (see section 2.1.4). However, Girona (Spain) is the exception as removal distribution was affected by the low removal of the C_{13} homologue.

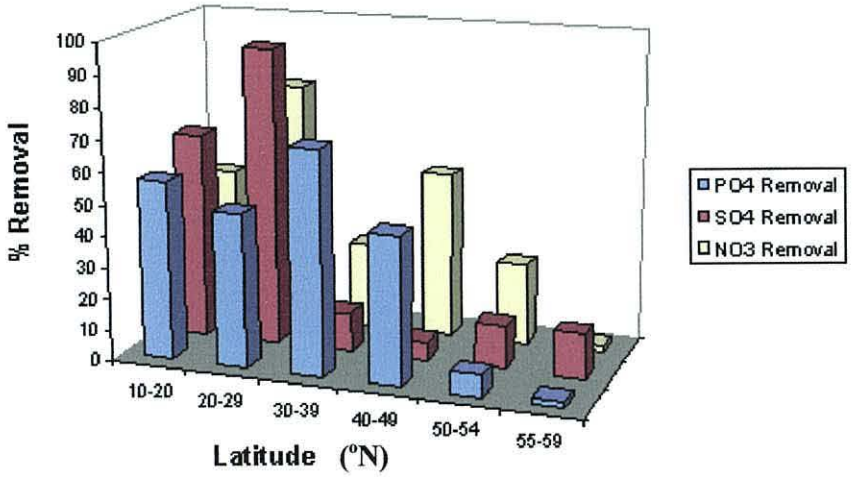
b. Water Chemistry

Anions

Figure 2.17b shows the removal of nitrate, phosphate and sulphate on a latitudinal gradient. The nutrient removal in the lower temperature climates is low in comparison

to the high removal observed in warmer climates. Highest nitrate (78%) and sulphate (95%) removal was observed in India, and phosphate (71%) in Korea. Statistical analysis only recognised a positive relationship between NO_3 removal and air temperature ($p<0.01$). However, an inverse correlation between air temperature and latitude was established ($p<0.05$) and between PO_4 removal and latitude ($p<0.05$).

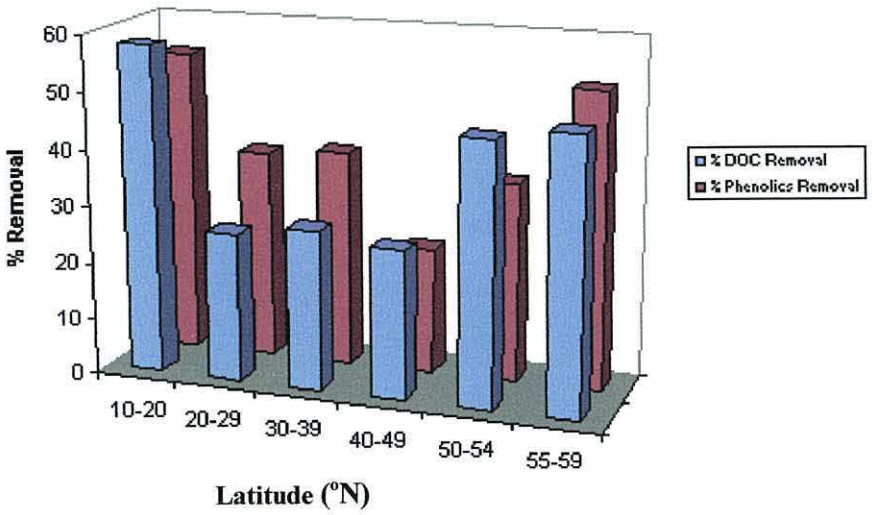
Figure 2.17b: Removal of nitrate, phosphate and sulphate on a latitudinal gradient



DOC and Phenolics

The similar trend in DOC and phenolic removal is highlighted in figure 2.17c with an initial decline followed by an increase. Removal of both parameters were similar with differences in removal within ±10% for each site. Highest removal observed in Thailand (DOC 58%, Phenolics 54%).

Figure 2.17c: DOC and phenolics removal on a latitude gradient



2.3.4 DISCUSSION

a. LAS

Climatic conditions such as temperature and precipitation are largely determined by latitude and can potentially affect water quality amelioration in wetlands. However, results from this study suggest that factors such as loading concentration, wastewater source and wetland design are better indicators for predicting LAS removal efficiencies in wetlands than latitudinal gradients. Mean removal on a global scale was approx. 80%, which was higher than that reported in UK wetlands (55%) in section 2.1.

The greatest LAS removal at 96% was measured unexpectedly for the Nynäshamn wetland, Sweden (min. –3°C). From published data on temperature effects, both from manipulation experiments and seasonal monitoring data (Inaba *et al.* 1988, Inaba 1992, Terzic *et al.* 1992, Dorfler *et al.* 1996), greater LAS degradation in warmer climates would be expected. However, although of the potential problems associated with ice formation in cold-climate wetlands, e.g. hydraulic performance, photoperiods, thermal consequences for biological and microbiological mediated processes (Wittgren & Mæhlum 1997), considerable treatment by wetlands in North America and Scandinavia are reported (Kadlec & Knight 1996, Mæhlum *et al.* 1995). This may be explained by treatment processes often being less temperature sensitive in full-scale wetlands as compared to laboratory studies (Whittgren & Mæhlum 1997). Mæhlum & Stålncke (1999) reported less than 10% differences in removal efficiencies for several parameters between cold (-10°C) and warm periods and anticipated that the temperature effects were partially compensated by the large hydraulic retention time. Hence good design of cold climate wetlands can ensure high LAS removal even at low temperatures with Mæhlum *et al.* (1995) stating that aerobic pre-treatment is essential during the winter when plants are dormant and oxygen supply to the wetland is reduced.

Substantial removal was also observed for warmer climates with the Chiang Mai, Thailand, wetland removing >70% of LAS at extreme inflow concentrations (*c.*100mg L^{-1}). Constructed wetlands are applicable in warm tropical countries with suitable climatic conditions for rapid biological and plant growth throughout most of the year

(Billore *et al.* 1999, Kantawanichkul *et al.* 1999). Developing countries have such favourable conditions and are particularly appropriate for wetland technology application as many villages and rural areas completely lack any form of sewage network and hence direct discharge into rivers and lakes occur (Billore *et al.* 1999). The low cost and low maintenance of wetlands coupled with the high LAS removal and nutrient cycling rate (see below) observed in this study suggest that this sustainable treatment is a practical option for wastewater treatment in these countries.

b. Water Chemistry

Although climatic temperature was not found to be an important factor in determining LAS removal in this study it can act as a major driving force in other biological reactions with higher temperatures generally resulting in greater decomposition and faster nutrient cycling (MacDonald *et al.* 1995). Greater wetland removal efficiencies for temperature mediated processes, e.g. nutrients (Kadlec & Reddy 2001) and higher enzyme activities (Kang & Freeman 1998) in warmer temperature climates have been reported. In this study greater nutrient removal at higher latitudes was generally observed suggesting faster nutrient cycling in warmer climatic temperatures reducing the potential for retention of nutrients in the biomass. Possible influencing mechanisms directly affected by climatological conditions, such as temperature, precipitation and solar radiation, include nutrient plant uptake, decomposition rates and microbial activities (Whittgren & Mæhlum 1997). Indirect effects include physical conditions, including hydraulic retention time (HRT), oxygen availability and freezing/thawing (Whittgren & Mæhlum 1997).

In comparison to sections 2.1.4b and 2.2.4b LAS concentrations as a percentage of DOC were higher at c.10%. Lower latitude sampling sites, i.e. Thailand and India, exhibited considerably higher LAS percentage of DOC possibly suggesting faster decomposition at higher temperatures or greater evapotranspiration resulting in more concentrated wastewater. The curved response of DOC with latitude may suggest that the balance in climatic conditions may be influential in favouring greater removal at low and high latitudes. At high temperature climates (i.e. low latitude) high evapotranspiration would result in more concentrated DOC perhaps being reflected in the greater treatment efficiencies calculated. At low temperature climates (i.e. high latitude) rainfall dilution of the DOC concentrations in the wetland may result in

greater removal efficiencies. However, lower removal efficiencies observed at mid-latitude climates may be due to the more even balance between evapotranspiration and rainfall dilution. However, no rainfall data were available for these sites. The similar trend in DOC and phenolic removal was again observed, comparable to that reported in previous sections, with a similar spatial latitudinal gradient trend.

2.3.5 CONCLUSION

1. No clear relationships between LAS removal efficiency and latitudinal gradient climatic conditions were established, emphasizing that hydrological design parameters, especially loading concentrations and rates, are much more influential.
2. Study has highlighted the potential of constructed wetlands in tropical climates to significantly reduce nutrient and pollutant concentrations, with substantial treatment also observed in cold-climate conditions.
3. Climatic influences were observed on nutrient removal, especially nitrate, with a general decline with increasing latitude.

It is emphasised that LAS removal cited in this chapter, as determined via HPLC measurements, only gives information on the primary biodegradation. However, it is suggested that compounds readily primary degraded will coincide with distinct mineralization (Swisher 1987). Mineralization is investigated in depth in the next chapter.

CHAPTER 3: LAS Removal Processes

The findings presented in chapter 2 were based on field studies where no control over the LAS concentrations, retention times and environmental conditions were possible. In this chapter small-scale laboratory experiments were conducted, using gravel samples collected from the UK constructed wetlands monitored in chapter 2, focusing on quantifying removal by two main processes; biodegradation and adsorption.

3.1 INTRODUCTION

The main removal processes involved in LAS removal are, predominately, biodegradation and adsorption (e.g. Matthijs & De Henau 1985, Matthijs & De Henau 1987, Jensen 1999). Several studies have investigated, and a few quantified, removal by these processes in laboratory (e.g. Swisher 1987) and field (e.g. Inaba 1992) studies. The interactions between these two main processes will determine the bioavailabilty and mobility of LAS in the wetland.

3.1.1 Biodegradation

The term biodegradation refers to the breakdown of an organic substance into inorganic products by microorganisms (Brown 1995). For LAS biodegradation is usually referred to in terms of primary biodegradation and mineralization and is discussed in detail below. Concern expressed after the foaming problems in the 1960s lead to the introduction of legislation regulating the biodegradation of surfactants released into the environment. In 1973 the European Economic Community (EEC) released two directives in relation to the biodegradability of surfactants stating that they must have an average biodegradability of ≥90% (73/404/EEC) and anionic surfactants must be a minimum of 80% degradable in a specified screening test or, if it fails, at least 90% degraded in a second confirmatory test (73/405/EEC) (Steber & Berger 1995). However, these official guidelines for testing the biodegradation of surfactants essentially only provide pass or fail information and no information on the kinetics.

(i) Primary Biodegradation

Primary biodegradation refers to the degradation required to change the identity of a compound (Swisher 1987). It is generally considered that LAS primary biodegradation is rapid under aerobic conditions (e.g. Swisher 1987, Jensen 1999, Scott & Jones 2000) with rates investigated in sewage treatment (Mathijs & De Henau 1987), estuarine waters (Terzic *et al.* 1992) and marine (Kikuchi *et al.* 1986) environments. Factors such as temperature conditions, origin of bacterial cultures and structure of alkylbenzene moiety have been identified to affect the rate (Terzic *et al.* 1992).

(ii) Mineralization

Mineralization (or ultimate biodegradation) refers to the complete conversion of a compound to carbon dioxide (CO_2), water and other inorganic compounds (Swisher 1987). This is a much slower process which involves complex processes and requires a consortium of bacteria to facilitate breakdown (Jiménez *et al.* 1991). Several methods to measure the extent of mineralization have been reported, involving measuring changes in surface tension, absorbance of the benzene ring, DOC removal, oxygen uptake and so on (Callely *et al.* 1977). However, many of these methods are arbitrary. Measurement of CO_2 production has been used but, due to the difficulties and errors in attributing the CO_2 released to LAS mineralization under conventional methods, radiolabelled compounds have been used for quantification based on $^{14}CO_2$ evolution (Gledhill 1975, Swisher 1987, Dorfler *et al.* 1996).

3.1.2 Adsorption

The surface-active nature of LAS results in adsorption existing as an important removal mechanism (de Wolfe & Feijtel 1998). LAS adsorption on sewage sludge is well reported, e.g. 10-35% (Matthijs & De Henau 1985, Matthijs & De Henau 1987, de Wolfe & Feijtel 1998). LAS adsorption is also a factor in determining transport and the residence time of a compound, as greater adsorption will enable more time for biodegradation to occur, hence affecting bioavailability and fate of the surfactant (de Wolfe & Feijtel 1998). Summarised in table 3.1 are the conditions used in previous LAS laboratory adsorption tests.

Sorption is influenced by a wide variety of complex processes dependent on the characteristics of both the surfactant and the substrate. Sorption behaviour is governed by compound properties such as water-solubility, molecular size, hydrophobicity, molecular configuration, concentration and polarity (Swisher 1987). Environmental factors include pH, organic substances, amount and structure of clay minerals and iron (III) oxides (Kuchler & Schnnak 1997).

Table 3.1 : Summary of LAS adsorption test conditions.

Sample	Conc. ($mg\ L^{-1}$)	Duration (hrs)	Temp (^{o}C)	Reference
Mud	0-30	1-3	25	Inaba *et al.* (1988)
Sediment	0.01-0.1	3-8	24	Hand & Williams (1987)
Sediment	0.25-15	6	20	Matthijs & De Henau (1985)
Aquifier material	1	0-25	20	McAvoy *et al.* (1994)

This chapter investigates the main processes involved in LAS removal. The ability of the microbial communities present in operational constructed wetlands to degrade LAS is assessed. The primary biodegradation kinetics of commercial LAS were determined using the HPLC method previously described and microbial mineralization using radiolabelled ^{14}C-LAS. The rate and degree of LAS adsorption is also investigated again using radiolabelled surfactant.

The main aims of this chapter were to:

1. Quantify percentage LAS degradation via primary biodegradation and mineralization
2. Assess LAS adsorption under test conditions
3. Determine the effect of depth on LAS degradation
4. Assess the effect of biofilm adhered to the gravel surface on LAS degradation and adsorption
5. Establish whether history of LAS exposure affects degradation capacity of the microbial community

It is hypothesised that;

1. Since no significant differences were observed between the three constructed wetland sites in section 2.1 in the field, no significant differences will be observed between biodegradation rates (primary and mineralization) and percentage adsorption in the laboratory studies conducted in this chapter.
2. LAS biodegradation will decrease with increasing depth due to the more anaerobic conditions and evidence of poor LAS biodegradation in anaerobic environments (section 1.3.1).

3. Biofilm will greatly affect LAS biodegradation but not adsorption because only the former is a biological process.
4. Prior-exposure will enhance LAS removal as reported in other sewage treatment studies.
5. Alkyl chain length and position of the benzene ring on the alkyl chain will affect biodegradation rates and percentage adsorption as discussed in chapter 2.

3.2 METHODS

3.2.1 Biodegradation

(a) Primary Biodegradation

An initial experiment was conducted to assess whether removal of solution from a flask on each day of sampling would affect LAS removal. A parallel experiment with samples taken from replicate flasks rather than from the same one was conducted. No significant difference was observed between the results and hence the experiment thereafter conducted by removal of small volume from each flask.

High LAS concentration was used in order to easily measure LAS over the test period. 100ml of LAS (100mg L^{-1}) was added to 50g of gravel (top 10cm layer) collected from the 3 constructed wetland sites of Rosset, Brynsiencyn and Clutton constructed wetland sampling sites (Section 2.1) and replicates incubated over a 2 week period at 20°C. Controls of 100ml LAS solution and no sediment were used. On each day of sampling, water samples were taken, preserved with formaldehyde (37% w/v) and analysed for its LAS content via the SPE HPLC method described in section 2.1.

Biofilm effects

Primary biodegradation removal rates were also monitored to assess the importance of the biofilm on the surface of gravel collected from the Brynsiencyn site. The usual gravel samples collected are referred to as the 'control'. A sub-sample of the gravel was washed in order to assess adsorption tendencies. Adaptation of the biofilm microbial community to LAS and also nutrient solution was also assessed by pre-treatment for 4 weeks. Hence, the following treatments were conducted;

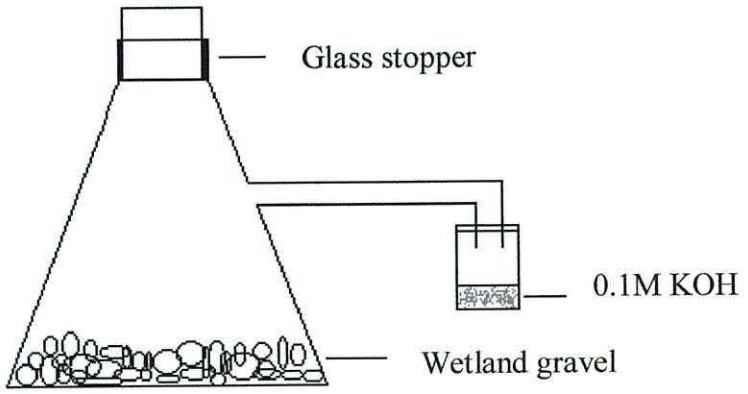
1. no biofilm present (washed and dried at 104°C)
2. control biofilm, i.e. adhered to gravel samples collected from the field
3. biofilm pre-treated with LAS (100mg L^{-1}) in nutrient solution (Appendix C)
4. biofilm pre-treated with nutrient solution (Appendix C)

(b) Mineralization

A 0.5ml solution containing ^{14}C-radiolabelled LAS (compound 3-Dodecyl Benzene Sulphonate (3-DOBS)) with a specific activity of 72.6μCi/mg and purity of 98.3% was added to replicate 50g of gravel (top 10cm layer) collected from each of the three

operational constructed wetlands discussed in section 2.1. Purity was based on Thin Layer Chromatography (TLC) on silica gel 60 A (5x20cm) with a solution of chloroform:methanol:water (65:25:4). The 3-DOBS radiolabelled compound used has the benzene ring attached to the third carbon atom in the alkyl chain. $^{14}CO_2$ was measured by attaching a glass scintillation vial containing 4ml of 0.1M KOH to the side arm of the biometer flask (see figure 3.1) incubated at 20°C. The $^{14}CO_2$ produced was determined daily at the start of the experiment, by replacing the vial with fresh KOH, and at increasing levels over approximately 50 days. On each day of sampling 12ml of Optifluor scintillation liquid was added to the KOH samples, the vial wiped with tissue and alcohol to remove any dirt, and radioactivity determined using a Packard Tricarb 2700TR Liquid Scintillation Analyser. Samples were measured against a KOH blank. Tests were also conducted on [^{14}C] Starch as a comparison for 'normal' biodegradation (data not shown) and with no sediment as a control.

Figure 3.1: Diagram of biometer flasks used for mineralization studies.



Biofilm

Mineralization with biofilm exposed to the four different treatments described in section 3.2.1a was also assessed as described above. In addition for comparison of biofilms with free bacteria 5ml of water collected from the Rosset wetland was placed in a biometer flask and 0.5ml of the radiolabelled surfactant added to assess LAS mineralization.

Depth

Depth analyses were also assessed by collecting gravel substrata cores of 10-20cm and 20-30cm depths in addition to the 0-10cm depth at the Rosset site and LAS mineralization measured as above. Respirometric CO_2 production was measured at the 3 depths to assess microbial activity. 1-3g gravel sub-samples were placed in darkened glass bottles sealed with air-tight caps incorporating a subseal septum through which the headspace gases could be sampled. After 1 hour a 10ml gas sample was taken using gas tight Pressure Lok® syringes and analysed for CO_2 at field temperature (±0.2°C) with an Ai Cambridge model 92 Gas Chromatograph (Porapak QS column at 35°C, N_2 carrier gas $30cm^{-3}$ min^{-1}).

Distance from outflow

Gravel samples were taken at 3, 6 and 9m distances from the outflow at the Brynsiencyn site due to access problems for sampling near the inflow. Mineralization was measured as stated above. Replicate aerial plant shoots were also collected at inflow and outflow locations with morphological characteristics of stem length, numbers of live leaves, basal stem diameter and dry weight assessed.

Isomers

A second LAS radiolabelled isomer was also tested with the benzene ring attached to the second carbon atom in the alkyl chain (2-DOBS). This compound has a specific activity of 6.7μCi/mg (purity 92.8%). Test conditions were as described above.

Scanning Electron Microscopy (SEM)

In order to compare the biofilms attached to the gravel surface representative gravel samples from each wetland were prepared for Scanning Electron Microscopy (SEM). Subsamples of approx. 1g of gravel were preserved in 3% glutaraldehyde in 0.1M phosphate buffer for 3 hours. After fixation, samples were dehydrated in increasing concentration of alcohol (25-100%) followed by acetone concentrations (50-100%), critical point dried and coated with Au (Polaron E5000). The samples were then examined with a Hitachi S-520 SEM and images were taken on an Illford PAN-F 50.

Mass balance

In order to establish a mass balance the gravel at the end of the mineralization tests were frozen and subsequently treated to soxhlet extraction with methanol (200ml) for 4 hours (section 2.2). Replicate samples of 1ml then underwent scintillation counting in 12ml of Optifluor scintillation liquid.

3.2.2 Adsorption

25ml of ^{14}C-radiolabelled LAS (3-DOBS; specific activity 72.6μCi/mg; purity 99.2%) in 0.01M KCl solution was added to 5g of gravel in polycarbonate centrifuge tubes at concentrations of 0.0, 0.04, 0.2, 1.0 and 5.0 mg L^{-1} LAS in duplicate. The samples were agitated at 220 rpm. for 16 hours at 20°C and then centrifuged at 10,000 rpm for 20 minutes. The solution was drained and 1ml aliquots counted on a Packard Tricarb 2700TR Liquid Scintillation Analyser after addition to 10ml of Emulsifier Safe Scintillation Liquid. To establish a mass balance samples were treated as in section 3.2.1b via sohlex extraction and subsequent scintillation counting. The gravel samples used were from the three constructed wetlands monitored in chapter 2 and were washed, sieved (6.7mm) and analysed for LAS adsorption (Table 3.2). To quantify and compare the gravels elemental analysis via X-Ray Microanalysis was conducted at Unilever Research, Coleworth. The gravel samples were ground and pressed prior to analysis using a Jeol 1200 TEM with an ASID 10 scanning attachment, operated at 20kV in SEM mode. The analytical system was an Oxford Instruments INCA with an LZ 5 light element detector.

Table 3.2: Summary of wetland gravel used in adsorption experiments. Measured pore area as analysed via Mercury Intrusion Porosimetry (MIP) using a Micrometrics Autopore IV (Institute of Environmental Science, Bangor).

Wetland gravel	Total pore area (m^2/g)
Rosset	1.523
Clutton	0.792
Brynsiencyn	0.674

Biofilm

Biofilm effects were assessed by running a parallel adsorption test on gravel samples collected from the Brynsiencyn constructed wetland leaving intact the biofilm adhered to the surface. To eliminate degradation of the compound in biofilm presence a biodegradation test was conducted over 16 hours in a biometer flask (fig.3.1), with the same ratio of gravel to radiolabelled compound in KCl solution used in the adsorption test.

Isomers

Adsorption tests using ^{14}C 2-DOBS and 5-DOBS (specific activity 4.8μCi/mg) isomers were also conducted on Rosset and Brynsiencyn gravels (table 3.2) under the same test conditions as above.

3.2.3 Statistical analysis of results

Biodegradation data was assessed statistically via t-tests for comparison of two treatments or one-way ANOVA tests for more than two treatments for data that conformed to the normal distribution and had homogenous variance using Minitab™ version 13.1 (Minitab Inc. 2000).

3.3 RESULTS

3.3.1 Biodegradation

Percentage primary degradation of LAS, as measured via HPLC, for the Brynseincyn, Rosset and Clutton site against the control after 14 days is shown in Figure 3.2a. Degradation occurred with no time lag and continued to increase exponentially until >95% removal was reached. No statistical ($p>0.05$) differences were established between sites. However, the Brynsiencyn site showed an initial faster primary biodegradation rate. Presented in figure 3.2b are the cumulative $^{14}CO_2$ released, as percentage of ^{14}C-LAS initially applied, with gravel sediment from the above 3 sites. Net mineralization of 73.6, 69.3 and 62.2% after 50 days was measured at the Brynsiencyn, Rosset and Clutton sites respectively with a 1-2 day lag phase evident. The control containing no sediment yielded no $^{14}CO_2$ throughout the course of the experiment (data not shown). Again no significant ($p>0.05$) differences between sites were identified. However, the Rosset site showed a faster initial mineralization rate.

Figure 3.2a: Primary biodegradation for Brynsiencyn, Rosset and Clutton wetlands.

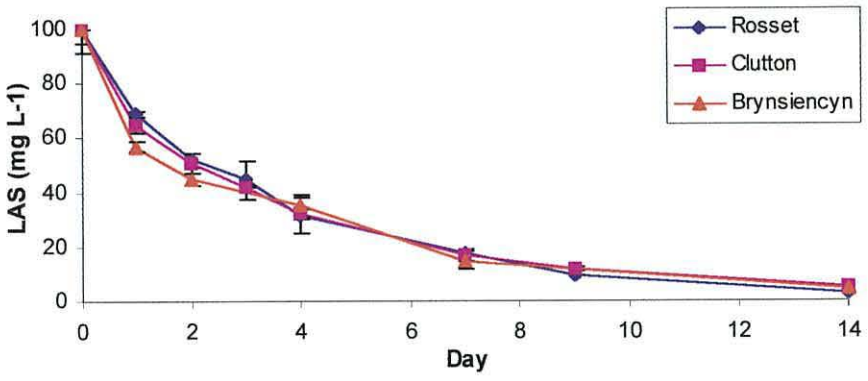
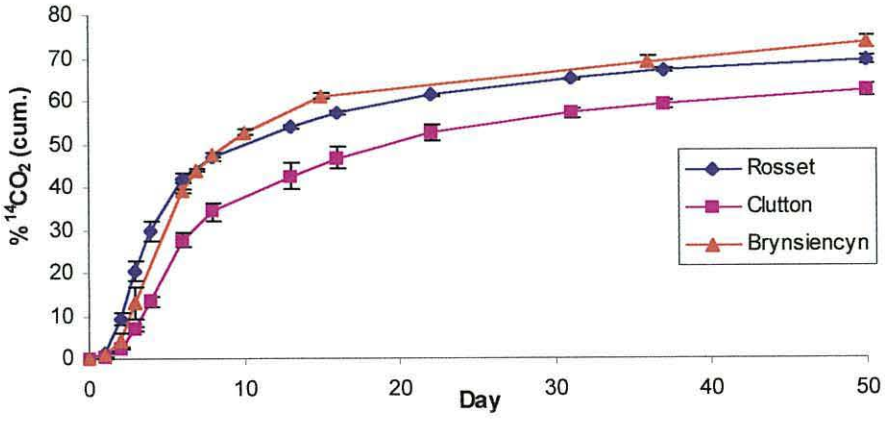


Figure 3.2b: Mineralization for Brynsiencyn, Rosset and Clutton wetlands.



First order kinetics is based on the assumption that a straight line is obtained when the log concentration is plotted against time whereby the slope of the graph is *k*. Primary biodegradation rate constants (*k*) of total LAS (Table 3.3) were calculated assuming first order degradation kinetics (Swisher 1987, Terzic *et al.* 1992) according to the following equation;

$$k = \ln[C_o/C] \times t^{-1}$$

Where C_o and C are the initial concentration and concentration at time t, respectively. The half-life ($t_{1/2}$) of degradation was calculated as;

$$t_{1/2} = \ln 2/k$$

Mineralization kinetics were more complex. First-order kinetics for the initial 8-days were established followed by second-order kinetics for the remainder of the experiment and are summarised in table 3.3. For second-order reactions a plot of 1/concentration against time will give a straight line whereby the slope of the graph is *k*. The second order rate constants were calculated as;

$$[C] = \frac{[C]_o}{1 + kt\,[C]_o}$$

Second order half-life is expressed by;

$$t_{1/2} = \frac{1}{k[C]_o}$$

Table 3.3: Rate constants (*k*) and half-lives ($t_{1/2}$) for LAS primary degradation and mineralization.

Wetland site	**Primary Biodegradation**		**Mineralization 1st order**		**Mineralization 2nd order**	
	k (d^{-1})	$t_{1/2}$ (d)	k (d^{-1})	$t_{1/2}$ (d)	k (d^{-1})	$t_{1/2}$ (d)
Bryn.	0.22	3.1	0.09	7.7	$3x10^{-4}$	33.3
Rosset	0.24	2.9	0.09	7.8	$3x10^{-4}$	33.3
Clutton	0.20	3.3	0.06	11.9	$2x10^{-4}$	50.0

Highest k values were calculated for Rosset, Brynsiencyn and Clutton respectively for primary biodegradation. Expected slower kinetics for mineralization were observed with Brynsiencyn and Rosset of almost equal k and $t_{1/2}$ but Clutton considerably slower. This is also reflected in figure 3.2b where a longer lag phase and lower mineralization was observed for the Clutton site.

The visual analysis conducted via SEM for comparison of the biofilm attached to the surface of the gravel from the three wetlands was unable to distinguish any differences in biofilm composition. As examples, figure 3.3a shows the scanning electron micrograph of heterogeneous bacterial population established at the surface of the Rosset wetland gravel consisting of short rods of various sizes, whereas figure 3.3b shows an example of a diatom identified on the surface of the Brynsiencyn gravel.

Figure 3.3a: Scanning electron micrograph of bacterial populations at the surface of the Rosset gravel. Bar equals 3μm.

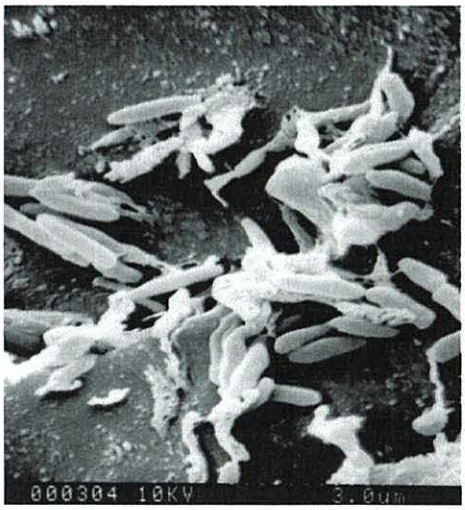
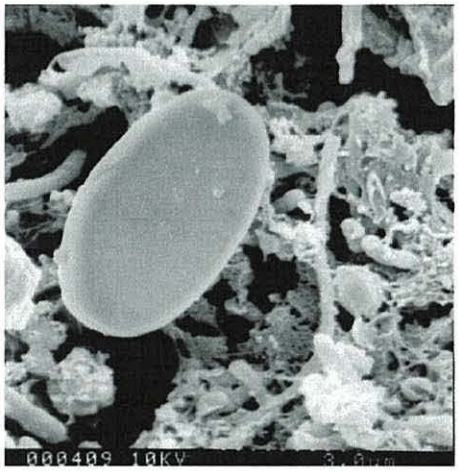


Figure 3.3b: Scanning electron micrograph of microalgae observed on the surface of Brynsiencyn gravel. Bar equals 3μm.



Biofilm effects

Figure 3.4 demonstrates the importance of biofilm microbial processes in LAS degradation. Batch experiments involving no biofilm present resulted in only c.10% reduction in LAS concentration in figure 3.4a and a release of c.1% of $^{14}CO_2$ (fig. 3.4b). The former may be explained by adsorption of LAS onto the gravel and glassware, whereas the latter is within the experimental error. In addition free bacteria only mineralised 1.1% of initial LAS added.

In comparing the LAS unexposed and pre-exposed gravel, primary degradation was initially slower for the latter than observed for the unexposed sample (Fig. 3.4a). However, by the end of the experiment >95% disappearance was observed comparable to the unexposed gravel with no significant differences between treatments ($p>0.05$). Overall similar rates ($k=0.21\pm0.01 d^{-1}$) and half-lives ($t_{1/2}=3.2\pm0.1$days) were calculated for the unexposed and exposed gravel. In contrast, an initial faster rate of mineralization ($k=0.08\ d^{-1}$, $t_{1/2}=9.3$days) and shorter lag phase was exhibited for the gravel pre-exposed to LAS and unexposed gravel ($k=0.07d^{-1}$, $t_{1/2}=10.3$days) than exposed to nutrient solution ($k=0.06d^{-1}$, $t_{1/2}=12.1$days) (Fig. 3.4b). The gravel exposed to nutrient solution (N) exhibited a much longer initial lag phase but by day 15 had exceeded the mineralization of the unexposed gravel to give a 4% higher yield. Significant differences over time between treatments were observed using one-way ANOVA tests ($F=8.247$, $p<0.01$). However, further analysis using

post-hoc Tukey tests revealed that significant differences ($p<0.01$) only exist with 'no biofilm' comparisons and not between biofilm treatments.

Figure 3.4a: Influence of biofilm microbial processes on LAS primary degradation.

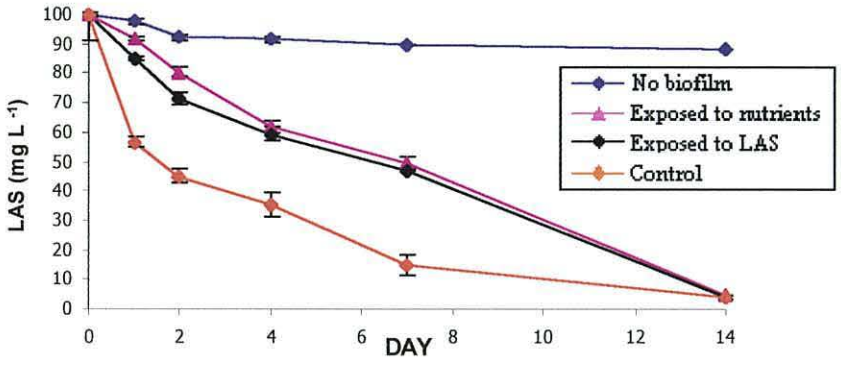
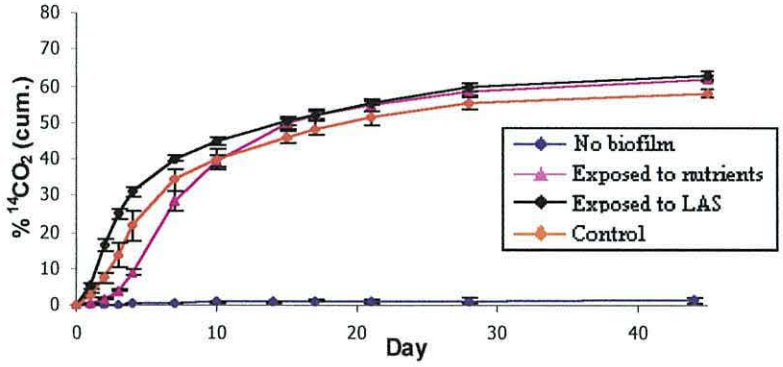


Figure 3.4b: Influence of biofilm microbial processes on LAS mineralization.



Distance from Outflow

Adaptation of the microbial community is demonstrated in figure 3.4c showing the effect of distance from outflow on LAS degradation. The gravel samples collected nearest to the inflow exhibits greater mineralization capacity of net 76% than that closer to the outflow at 74% (6m) and 71% (3m) but with no significant differences ($p>0.05$) and similar k values (0.09-0.1 d^{-1}) and half-lives (6.8-7.1 days). In terms of the plant morphological characteristics measured, substantially taller shoots with greater number of leaves and significantly thicker stems at base were measured near the inflow in comparison to the outflow as given in table 3.4.

Figure 3.4c: Effect of distance from outflow on LAS mineralization.

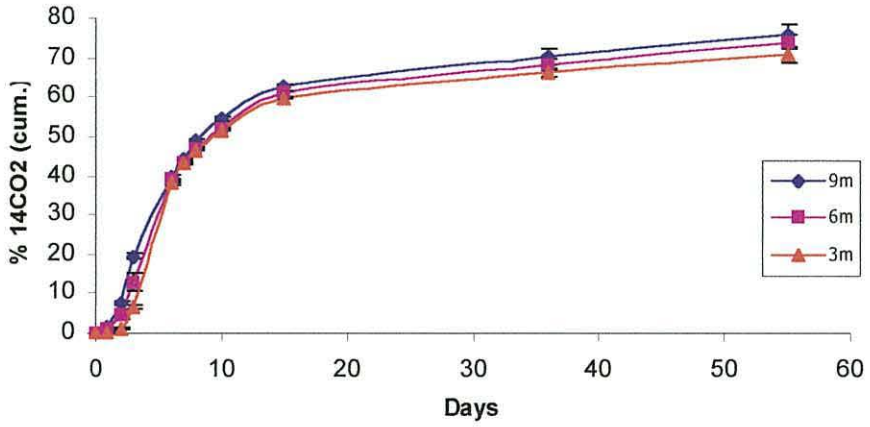


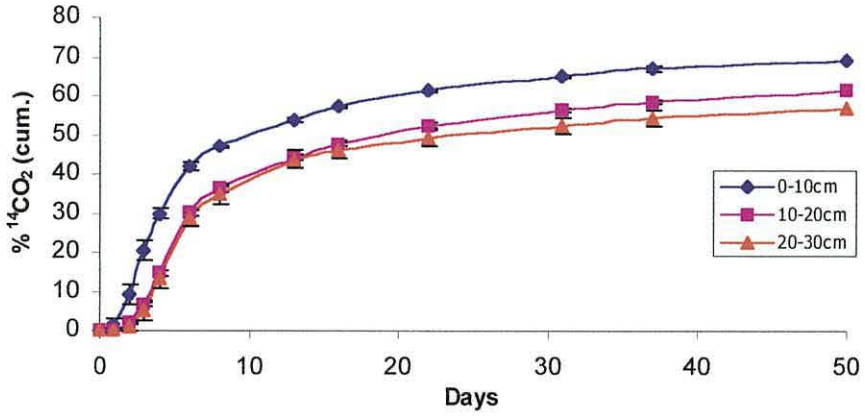
Table 3.4: Morphological characteristics of plans measured near inflow and outflow, Brynsiencyn wetland.

Morphological characteristics	Inflow		Outflow		p
	Mean	SD	Mean	SD	
Length (m)	1.4	0.09	0.8	0.09	<0.001
No. green leaves	7.4	0.54	5.6	0.54	<0.01
Basal stem diameter (cm)	2.02	0.14	1.14	0.29	<0.01
Wet weight (g)	27.7	5.07	11.5	2.86	<0.01
Dry weight (g)	6.0	0.64	2.4	0.73	<0.001

Depth Profile

Figure 3.5 shows the LAS mineralization expressed as total $^{14}CO_2$ evolution over 50 days depth profile (0-30cm). The top 10cm layer exhibited greater LAS mineralization capacity (net 69%) than the 10-20cm layer (62%) and the lower 20-30cm layer (57%). Hence the lowest depth (20-30cm) exhibited net 12% lower mineralization than the upper 0-10cm layer although this was not significant ($p>0.05$). Initial kinetic data exhibited a decreasing trend in k values (0.09-0.06 d^{-1}) and corresponding increasing trend in half-lives (7.7-11.6 days) calculated with greater depth (0-30cm). However, CO_2 respiration as an indicator of microbial activity at the different depths confirms greater activity ($p<0.05$) in the upper 10cm layer (0.22 mg g^{-1} d^{-1}) followed by 10-20cm (0.12 mg g^{-1} d^{-1}) and 20-30cm (0.08 mg g^{-1} d^{-1}).

Figure 3.5: Depth profile (0-30cm) of LAS mineralization.



LAS alkyl homologues and isomers

Table 3.5 demonstrates the higher rate constants (k), and shorter $t_{1/2}$, for the longer chain alkyl homologues in comparison to the shorter chain homologues in the order of $C_{13}>C_{12}>C_{11}>C_{10}$. Significant differences were calculated between the concentrations of the homologues at Rosset ($F=3.108$, $p<0.05$) and Clutton ($F=4.103$, $p<0.01$) but not quite significant for Brynsiencyn ($p=0.06$). However, further analysis using post-hoc Tukey tests revealed that significant differences ($p<0.05$) only existed between C_{11} and C_{13} homologues at both sites.

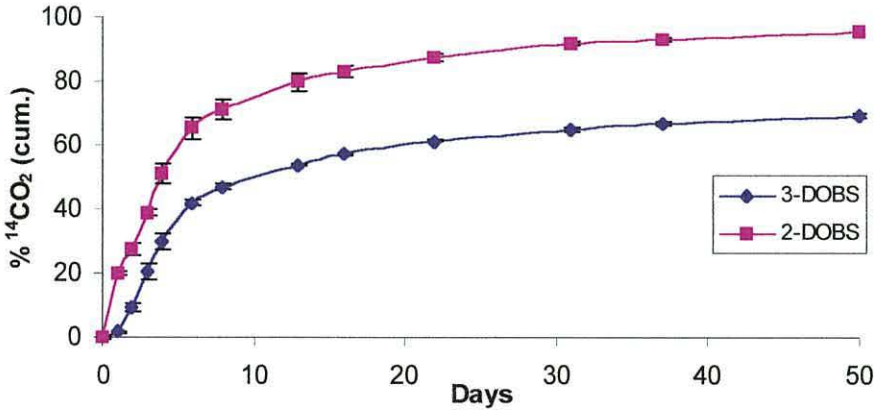
Table 3.5: Rate constant (k, units=d^{-1}) and half-life ($t_{1/2}$, units=day) for LAS alkyl homologues.

Homologue	**Brynsiencyn**		**Rosset**		**Clutton**	
	k	**$t_{1/2}$**	***k***	**$t_{1/2}$**	***k***	**$t_{1/2}$**
C_{10}	0.192	3.6	0.190	3.7	0.182	3.8
C_{11}	0.239	2.9	0.238	2.9	0.211	3.3
C_{12}	0.255	2.7	0.267	2.6	0.217	3.2
C_{13}	0.267	2.6	0.285	2.4	0.211	3.1

Similarly, the rate of mineralization of the isomer positioned closest to the terminal alkyl group (2-DOBS) was faster than that with more central position (3-DOBS) with

net removal of 95% and 69% respectively (figure 3.6) ($T=10.723$, $p<0.001$). First-order kinetics for the initial 8-days with k of 0.09 and 0.16 $days^{-1}$ and $t_{1/2}$ of 7.8 and 4.3 days for the 3- and 2-DOBS compounds respectively. Second order kinetics for the remainder of the experiment with k of 3×10^{-4} and 4.5×10^{-4} $days^{-1}$ and $t_{1/2}$ of 33.3 and 22.2 days for the 3- and 2-DOBS compounds respectively.

Figure 3.6: Mineralization for 2- and 3-DOBS isomers.



Mass Balance

Data obtained from sohlex extraction with hot methanol to determine the ^{14}C-LAS concentration in the sediment phase at the end of the mineralization tests (table 3.6) indicates a minimum mass balance of >90% (n=20).

Table 3.6: Summary of the mass balance data.

	Mass Balance (%)	
	Mineralization	Adsorption
Mean	91.1	91.4
Standard Error	1.08	3.72

3.3.2 Adsorption

The adsorption isotherm for the gravels tested from the three wetlands are plotted in figure 3.7a as the final LAS equilibrium concentration in solution (C_{AW}) against the amount of LAS adsorbed per g of gravel (C_{ASOIL}). The isotherms were linear

(R^2>0.99) for the range of concentrations tested (0-5mg L^{-1}) for each sample and suggests that higher LAS removal capacity was observed in the order of Rosset>Clutton>Brynsiencyn. The characteristics of the gravel used in terms of elemental analysis via quantitative X-Ray microanalysis is summarised in table 3.7 showing that the Brynsiencyn gravel contained the highest Fe and Al content (equal with Clutton for the latter) whereas the Rosset gravel the highest Ca content. Hence this data possibly suggests that the Ca content is most important in determining LAS adsorption.

Table 3.7: Quantitative X-Ray microanalysis of wetland gravel samples

Gravel	**% Element**								
	C	**O**	**Na**	**Mg**	**Al**	**Si**	**K**	**Ca**	**Fe**
Rosset	17.0	49.0	0.5	0.6	3.5	19.9	0.7	6.4	2.2
Clutton	13.9	49.0	0.6	0.5	4.8	26.6	2.5	0.1	2.0
Brynsiencyn	16.6	48.2	1.1	0.9	4.8	20.4	1.3	3.5	3.3

The percentage adsorption (A) determined are summarised in table 3.8, with a similar general decrease observed, and calculated according to the following equation;

$$A = \frac{(C_o \times V) - (C_{AW} \times V_W)}{(C_o \times V)} \times 100$$

Where C_o = dpm/ml in control after agitation

V = volume of test solution (ml)

C_{AW} = dmp/ml in test vessel aqueous phase after agitation and centrifugion (blank corrected)

V_W = total volume of aqueous phase in test vessel (ml)

Table 3.8: Summary of calculated percentage adsorption with 3-DOBS

Wetland Gravel	**% LAS Adsorbed**			
	0.04mg L^{-1}	**0.2mg L^{-1}**	**1.0mg L^{-1}**	**5.0mg L^{-1}**
Rosset	40	40	47	48
Clutton	43	40	40	41
Brynsiencyn	31	26	26	17

Historically sorption has been represented by mathematical equations and plotted as the corresponding isotherms. The Langmuir and Freundlich equations are the most commonly used. The Langmuir equation assumes a surface with a finite number of sorption sites with each one capable of binding a solute molecule. Hence this equation assumes monolayer coverage. The Freundlich equation assumes multilayer adsorption which becomes increasingly difficult with accumulation of adsorbate. However, these adsorption models do not imply any particular mechanism. The Langmuir equation is given as:

$$\frac{1}{v} = \frac{c}{V_m} \times \frac{1}{[S]} + \frac{1}{V_m}$$

Where c is the measure of adsorption intensity (ml μg^{-1}), v is the amount of LAS adsorbed ($\mu g\ g^{-1}$), V_m is a constant for adsorption capacity ($\mu g\ g^{-1}$) and [S] is the equilibrium solution concentration (mg L^{-1}).

Whereas the Freundlich equation:

$$C_{ASOIL} = K_F \times C_w^{1/n}$$

Where K_F is the distribution coefficient (ml g^{-1}), $^1/_n$ is the Freundlich constant, C_w is the amount of test material remaining in solution (mg L^{-1}) and C_{ASOIL} is the amount of LAS sorbed per unit of sorbent ($\mu g/g$).

Figure 3.7a: Isotherms for LAS adsorption by wetland gravel.

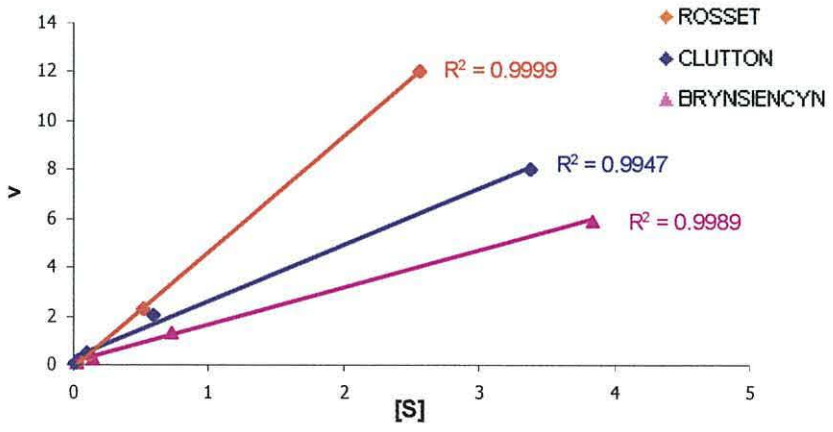


Figure 3.7b: Langmuir isotherms for LAS adsorption by wetland gravel.

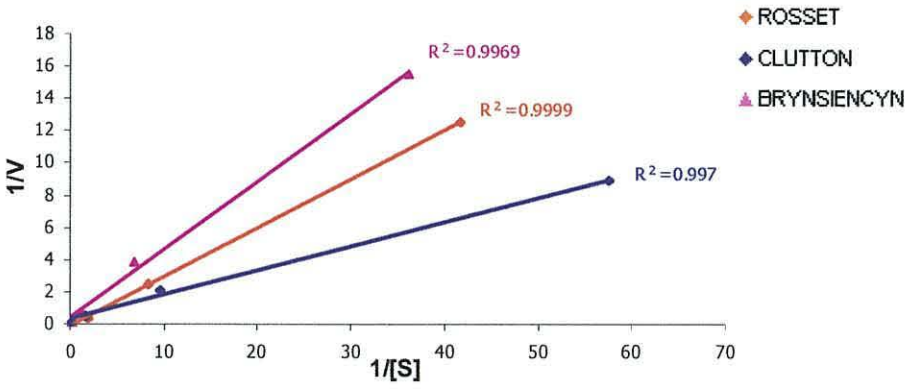


Figure 3.7c: Freundlich isotherms for LAS adsorption by wetland gravel.

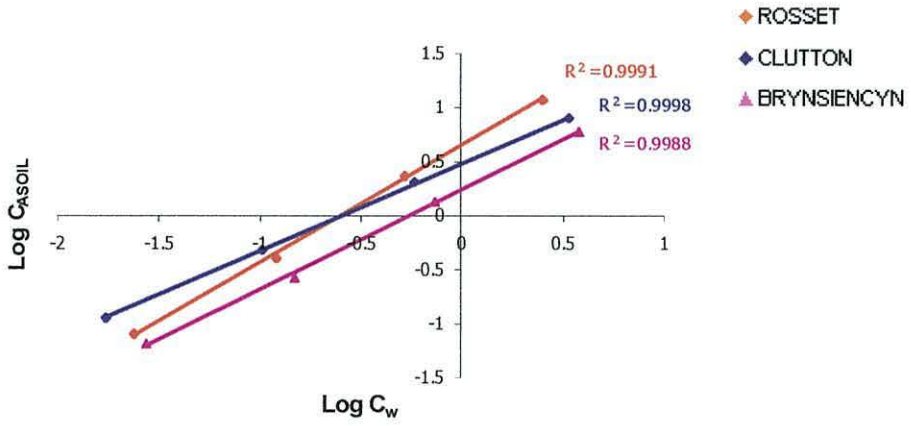


Figure 3.7b and 3.7c shows the Langmuir and Freundlich isotherms respectively for the adsorption of LAS on the gravel collected from the three constructed wetlands. Langmuir parameters, V_m and c, and Freundlich parameters, K and $^1/_n$, were determined by regression analysis (min R^2=0.99) as the intercept and slope respectively. Table 3.9 summarises the Langmuir and Freundlich constants measured for the gravel samples.

Table 3.9: Langmuir and Freundlich Constants for LAS adsorption on gravel.

Gravel	Langmuir Constants		Freundlich Constants	
	V_m	c	*K*	$^1/_n$
Rosset	13.0	3.94	4.4	1.08
Clutton	3.4	0.52	3.1	0.81
Brynsiencyn	2.8	1.18	1.7	0.93

Commonly adsorption data is assessed according to the best fit via regression analysis (i.e. highest R^2 value). Hence from the data plotted in figure 3.7 the data was best described by the Freundlich adsorption isotherm. Although higher K values, indicating greatest adoption capacity, was identified for Rosset, Clutton and Brynsiencyn gravels respectively, the variation is relatively low (mean=3.0, SD=1.35). The values of equal or less than 1 obtained for the slope $^1/_n$ suggests that adsorption is possibly restricted to a monolayer.

Biofilm

Slightly greater adsorption was observed for the Brynsiencyn gravel with biofilm present as indicated in the linear (R^2>0.99) adsorption isotherm in figure 3.8a. Higher K value of 2.1 in biofilm presence in comparison to 1.7 in its absence ($^1/_n$ 0.9883 and 0.9253 respectively) as calculated from the Freundlich isotherm (fig. 3.8b). The amount of LAS degraded with biofilm presence is negligible as indicated by a parallel mineralization test conducted yielding <1% $^{14}CO_2$ after 16 hours.

Figure 3.8a: Isotherms for LAS adsorption by Brynsiencyn wetland gravel with and without biofilm.

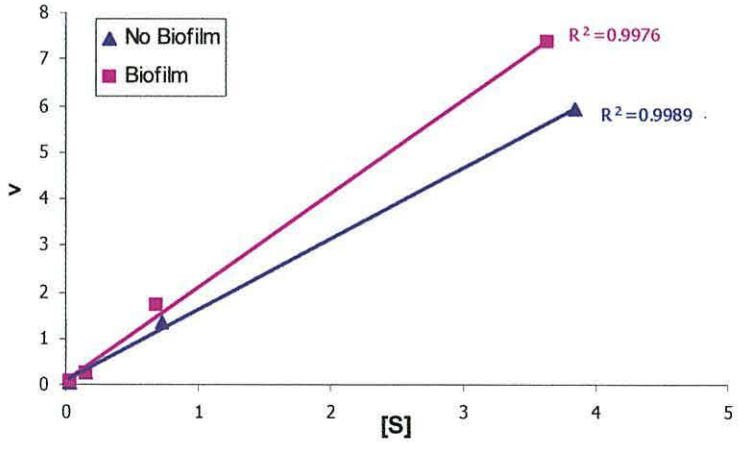
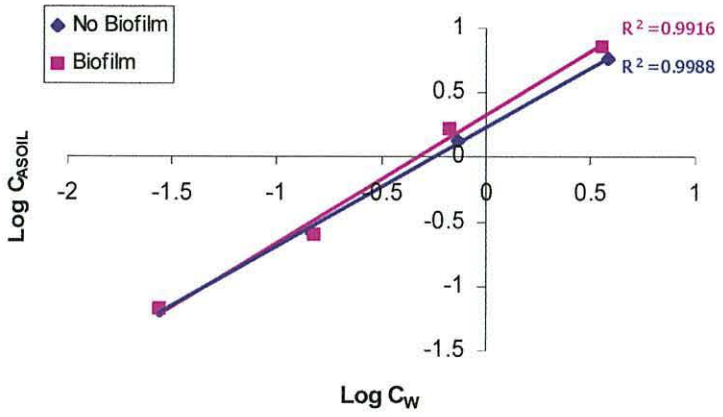


Figure 3.8b: Freundlich isotherms for LAS adsorption by Brynsiencyn wetland gravel with and without biofilm.



Isomer

Adsorption of isomers increased as sulphophenyl position changed from central to more terminal structures, i.e. 5-<3-<2-DOBS as shown in the adsorption isotherms for the Rosset and Brynsiencyn gravel in Figure 3.9. This is also highlighted in the Freundlich adsorption isotherms (R^2>0.99) shown in figure 3.9b with the higher K calculated for 2-DOBS as summarised in Table 3.10.

Figure 3.9a: Isotherms for 2-, 3- and 5-DOBS adsorption by (i) Rosset and (ii) Brynsiencyn wetlands gravel.

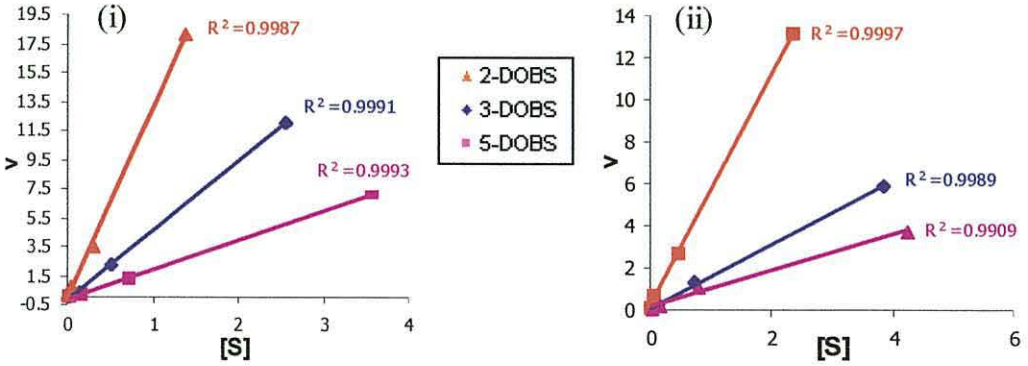


Figure 3.9b: Freundlich isotherms for 2-, 3- and 5-DOBS adsorption by (i) Rosset and (ii) Brynsiencyn wetlands gravel.

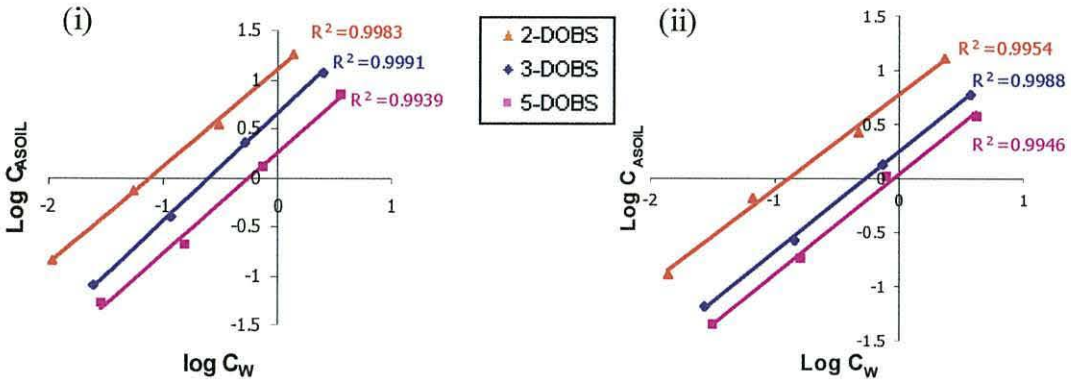


Table 3.10: Freundlich constants for adsorption of different isomer compounds.

Freundlich	Rosset			Brynsiencyn		
Constants	2-DOBS	3-DOBS	5-DOBS	2-DOBS	3-DOBS	5-DOBS
K	12.2	4.4	1.76	5.89	1.70	1.08
$^1/_n$	0.99	1.08	1.03	0.88	0.93	0.92

Mass Balance

Mean data obtained from sohlex extraction was presented in table 3.6 indicating satisfactory mass balance of mean *c.*90%. Table 3.11 summarises the data for the three wetlands indicating that the sohlex extraction procedure was satisfactory and that adsorption onto the tubes accounted for <7% of initial radioactivity added.

Table 3.11: Summary of mass balance for LAS gravel adsorption tests (initial conc. 5mg L^{-1}).

	% Adsorption		
	Rosset	Clutton	Brynsiencyn
Tube	2	7	5
Solution	24	27	48
Gravel	66	58	42
Total	92	92	95

3.4 DISCUSSION

3.4.1 Biodegradation

The biofilm microbial community was identified as the major LAS removal mechanism in constructed wetlands, supported by the negligible degradation observed with biofilm absence and the work of other researchers in different environments (Takada *et al.* 1994, Boeije *et al.* 2000). Hence the hypothesis proposing that biofilm presence will affect LAS biodegradation is accepted. This study also identified that LAS in constructed wetlands is also controlled, in part, by previous exposure history to the microbial community and compound structure. These factors not only affected yield degradation but also the kinetics. The similar rate, half-life and net primary biodegradation confirms the data presented in chapter 2 suggesting that comparable LAS removal between sites is exhibited. However, the mineralization data showing that Clutton exhibited a higher half-life perhaps suggests that the Brynsiencyn and Rosset wetlands are superior for LAS removal.

Primary biodegradation was rapid (>95%) with no statistically significant differences ($p>0.05$) between site comparisons. Other researchers have reported >99% primary biodegradation in similar tests (Branner *et al.* 1999, Terzic *et al.* 1992, Larson & Payne 1981). The substantial removal observed within initial 24 hours suggests the carbon chain is broken down immediately by bacterial attack after inoculation (Zhang *et al.* 1999). Disappearance over time followed first order kinetics with an exponential decay pattern with similar rate constants (k 0.20-0.24 $days^{-1}$) and half-lives ($t_{1/2}$ 2.9-3.3 days). Terzic *et al.* (1992) reported comparable k of 0.100-0.303 $days^{-1}$ and $t_{1/2}$ of 2.3-6.9 days by bacterial cultures originating from estuarine waters (14°C). However, Larson & Payne (1981) measured more rapid kinetics in river water and sediment samples, via MBAS, reporting k of 3.0 $days^{-1}$ and $t_{1/2}$ of 0.23 days. Relatively high LAS concentration was used for these tests approx. 10-fold higher than normally encountered in domestic wastewater treatment. However, this is justified as industrial waters may exceed 300mg L^{-1} LAS (Wagner & Schink 1987) and high initial concentration required for HPLC detection due to the rapid degradation of the surfactant commonly employed in other publications.

The measurement of $^{14}CO_2$ evolved provides unequivocal information on the extent of LAS mineralization. The yield data is comparable with the literature at $^{14}CO_2$

evolution of 50-75%, depending on chain length and test system used, over a minimum of 28-30 days (Swisher 1987) as summarised in table 3.12. However, Huddleston (1979) (in Swisher 1987) reported a 99% evolution in a longer experiment over a 40-80 day period. One-way ANOVA tests revealed no significant differences between treatments further supporting field data in Chapter 2. Similarly, in soil studies, Larson *et al.* (1989) claimed that LAS biodegradation rates are not influenced by soil type. However, de Wolfe & Feijtel (1998) state that overall the evidence indicates that soil type can influence biodegradation. Although a standard concentration was used in this study Dorfler *et al.* (1996) reported that the relative amount of $^{14}CO_2$ released as a function of ^{14}C initially applied was independent of the initial concentration added. This suggests that the bacterial population has a high capacity for LAS degradation even at high substrate concentration.

No visual differences between the bacterial populations incorporated in the biofilm attached to the gravel surface of the three wetlands monitored, as examined via SEM, were observed. This suggests that the lack of significant differences in primary biodegradation and mineralization, and similar percentage removal observed in chapter 2, may be accounted for by the similar composition of the biofilms.

Table 3.12: Summary of mineralization data generated in the literature.

Mineralization (%)	**Duration (days)**	**Sample**	**Reference**
72-80	20	River water & sediment	Larson & Payne (1981)
64-66	60	Soil	Dorfler *et al.* (1996)
37-77	28	Soil & microorganisms	Gledhill (1975)
33.3		Pond sediments	Federle & Pastwa (1988)
25%	13	Activated sludge	Jiménez *et al.* (1991)
9%	69	Soil columns	Branner *et al.* (1999)
ND	87	Anaerobic sediment	Federle & Schwab (1992)

ND = not detectable

In contrast to primary degradation a lag of 1-2 days was evident in the mineralization studies. The lag phase is defined as the period from inoculation until degradation has

reached approx. 10% (Painter 1995). An observed initial lag phase is characteristic of biological tests involving growth and/or adaptation (Dorfler *et al.* 1996) and is observed in several LAS studies (Dorfler *et al.* 1996, Larson & Payne 1981, Branner *et al.* 1999, Federle & Schwab 1989).

Complete mineralization involves more complex mechanism pathways and requires a consortium of bacteria (Jiménez *et al.* 1991). Hršak *et al.* (1982) reported that none of the bacterial strains isolated from a wastewater source could mineralize LAS completely indicating complex interactions based on combined metabolic attack or provision of specific nutrients. Similarly Jimenez *et al.* (1991) reported that pure cultures of a four-membered bacterial consortium were able to primarily degrade LAS but not mineralize unless they were all present. The limited capability of pure cultures to mineralize surfactants is associated with the ability to degrade either the hydrophilic or hydrophobic ends of the molecule (van Ginkel 1996). Hence the data from this study suggest that the bacterial communities in constructed wetlands may co-operatively utilize LAS.

The more complex mineralization mechanism is reflected in the kinetics data. The first order kinetics observed for the initial 8 days is >2-fold slower for Brynsiencyn and Rosset but >3-fold slower for Clutton comparative to primary biodegradation. First-order mineralization kinetics over approx. 20-day test period have been reported (Federle *et al.* 1990, Larson *et al.* 1989, Larson & Payne 1981). However, the second-order kinetics identified for the remainder of the experiment, with slower $^{14}CO_2$ emission, may suggest inhibition of the reaction and may possibly be explained by death or decay of LAS-utilizing bacteria, insufficient compound availability for degradation and/or toxicity over time to microbes. Several authors with similar kinetics data identified 3/2-order kinetics via various non-linear regression computer programs designed for adaptation of such data (Dorfler *et al.* 1996, Knaebel *et al.* 1994). However, in this study, access to such programs was not available and subsequently it was only possible to speculate that the data would be well represented by 3/2-order kinetics. The slower mineralization kinetic rate is also reflected in the longer half-lives. Half lives of 3-35 days have been reported in previous studies with the maximum taking into account slow mineralization facilitated by extreme high test

concentrations (Waters *et al.* 1989). Table 3.13 summarises comparative rates and half-life data demonstrated in the literature.

Table 3.13: Summary of comparative rates (k) and half-lives ($t_{1/2}$) in the literature.

$t_{1/2}$ (days)	k (d^{-1})	Reference
8-27		De Henau *et al.* (1986)
5-25		Litz *et al* (1987)
1.5-3.3		Larson *et al.* (1989)
3.2-16.5	0.042-0.219	Federle & Pastwa (1988)
0.7-2.9	0.5±0.07	Larson & Payne (1981)

Complete mineralization was not achieved over the 50-day experimental period and is evident in the majority of studies (Dorfler *et al.* 1996, Larson & Payne 1981, Knaebel *et al.* 1994, Branner *et al.* 1999). The remainder, approx. 30%, may have been further degraded over a longer experiment, represents a substrate concentration too low to sustain a metabolising microbial community (Dorfler *et al.* 1996) and/or is irreversibly bound resulting in a reduction in availability (Dorfler *et al.* 1996). In addition it is possible that the LAS has been incorporated into the biomass (Branner *et al.* 1999, Swisher 1987, Steber & Berger 1995). Estimates of 10-25% incorporation of LAS into cells are reported (Kölbener et al. 1995, Branner et al. 1999). Hence, a mass balance approach was followed with >90% recovery measured. Degradation products expected are mainly sulfophenyl carboxylates (Swisher 1987). However, identification was not possible due to complex HPLC-MS detection required.

Biofilm effects

As stated above biofilm microbial processes mainly accounted for LAS removal. Takada *et al.* (1994) reported that the existence of riverbed biofilm accelerated biodegradation of LAS with >95% removal in the presence of biofilm in comparison to <50% with negligible accumulation of LAS in the biofilm. The 1% mineralization observed in this study in the presence of free bacteria only confirms the importance of biofilm processes. The *c.*10% removal in the absence of biofilm for primary degradation observed in this study is assumed to be due to adsorption and is further discussed in section 3.4.2. Biofilms are important in any ecosystem and will develop

wherever there is a gas-liquid or solid-liquid interface with several factors affecting biofilm microbial populations and/or activity in wetlands. These include flow, substrate, nutrients, previous exposure, temperature (see chapter 5) and plants (see chapter 4). High flows wash down biofilm and reducing removal efficiency, whereas low flow facilitates LAS removal due to increased residence time and consequently increased contact of LAS with the biofilm (Takada *et al.* 1994). Surface area of wetland gravel substrate is proportional to area available for biofilm growth and hence to biodegradation rate (Boeije *et al.* 2000).

The initial faster mineralization rate and absence of lag period comparative to the unexposed biofilm suggested adaptation and acclimatisation of the biofilm microbial community to LAS. Adaptation is defined as 'a process whereby rate of biodegradation of a chemical is significantly increased as a result of prior exposure to that chemical' (Spain *et al.* 1980). Microbial communities acclimated by pre-exposure to the surfactant are enriched in organisms capable of degrading the compound resulting shifts in community structure with increasing dominance in populations of these organisms (Federle & Pastwa 1988). Previous adaptation accelerated by initial LAS degradation is reported (Larson & Payne 1981, Palmisno *et al.* 1991, Federle & Pastwa 1988, Branner *et al.* 1999, Jensen 1999). Brown (1995) suggests that the bacterial population can increase its capacity to degrade surfactants by, for example, population growth potentially increasing number of degraders, an increase in the amount of enzyme per cell biosynthesised or random genetic mutation increasing biodegradation activity or creating new activity. Terzic *et al.* (1992) reported that the composition of a mixed bacterial culture rather than total number of bacteria determined biodegradation efficiency.

However, there can be various degrees of adaptation (Swisher 1987) with effects on biodegradation rates depending upon, for example, compound studied, concentration, microbial community structure and function and environmental conditions (Palmisano *et al.* 1991, Spain *et al.* 1980, Shimp *et al.* 1989). Larson *et al.* (1989) reported pre-exposure of LAS lead to 17% improved biodegradation response than to unexposed treatments in comparison to the net 5% reported in this study. Hence previous exposure had a more profound effect on initial mineralization rates with shortening of the lag phase widely reported (Shimp *et al.* 1994, Federle & Pastwa 1988, Larson *et*

al. 1989). Knaebel & Vestal (1992) suggested that similar net yield for pre- and un-exposed samples may suggest that extent of mineralization is limited by other factors, e.g. chemical and or physical interactions between soil and chemicals.

LAS removal was affected by pre-exposure to nutrient ions required for microbial growth, namely N, P, S, K, Na, Fe, Ca, Mg at 'macro' levels (Brown 1995). However, as no carbon source was added this may have detrimentally affected the microbial population of LAS-utilizing bacteria. This is suggested by the initial longer lag phase observed. However, a shift in the microbial community or acclimation is suggested by the equal $^{14}CO_2$ released by the tenth day as the unexposed control.

Evidence of adaptation in response to continual and prolonged LAS exposure is proposed from the observed spatial data in the Brynsiencyn wetland. More rapid LAS mineralization was observed for the gravel samples taken from near the inlet. The rate of LAS mineralization then decreased with increasing distance from the inlet. These results suggest adaptation of the microbial community exposed to higher LAS and nutrient concentration near the inflow. Larson & Payne (1991) reported a shorter half-life with a 10-fold faster rate for degradation tests with river sediment collected closest to the vicinity of the effluent from a sewage treatment plant similarly suggesting adaptation for communities receiving higher LAS concentrations. Shimp *et al.* (1989) reported greater number of LAS degrading microorganisms, reduced lag period and higher degradation in water samples collected from an effluent exposed site than from a control pristine site. However, as one-way ANOVA tests revealed an insignificant ($p>0.05$) relationship it is not possible to draw any further conclusions.

The expected higher concentrations of nutrients near the inflow were evident in the significantly greater plant biomass observed in comparison to the outflow. Coleman *et al.* (2001) reported a decline in *Typha* growth with increasing distance from the influent in a small scale constructed wetland. The authors suggested that this was due to limited nutrients or greater toxicity with increasing distance from the inlet. Similar findings and comparable morphological characteristics (Table 3.14) were reported by Dušek & Kvet (2001) who suggested plants near the inflow exhibited enhanced nutrient uptake. However, the authors also state that the inflow plants often show

symptoms of stress induced by an excess of some nutrients and possibly also by heavy organic loading of their habitat (Dušek & Kvet, 2001).

Table 3.14: Comparable morphological parameters of plants reported by Dušek & Kvet (2001).

Morphology	Inflow		Outflow	
	Mean	SD	Mean	SD
Length (cm)	130.0	31.5	124.0	28.9
No. green leaves	6.7	1.3	5.6	1.2
Basal stem diameter (cm)	4.6	1.4	4.8	1.1

Unexpectedly, in contrast to mineralization, primary degradation was initially slower with gravel pre-exposed to LAS or nutrient solution than unexposed gravel. This may suggest that a high concentration of nutrients and LAS results in reduction of bacterial numbers and/or activity responsible for oxidation of the terminal methyl group (Swisher 1987). However, similar kinetic rates and comparable high net removal were observed by the end of the experiment in all treatments.

Depth

Mineralization of LAS as a function of depth in gravel sediment profile (0-30cm) demonstrated that the production of $^{14}CO_2$ in the lower depths, although not quite significant ($p=0.056$), were lower than in the top 10cm. Branner *et al.* (1999) demonstrated greater LAS mineralization in the upper 5cm of soil columns (34cm depth), associated with lower and different biomass due to anoxic conditions at greater depth. Shimp *et al.* (1994) and Federle & Pastwa (1988) also found LAS biodegradation activity highest in the near surface soil samples and decreased with respect to depth with the latter correlating with microbial biomass and activity. Larson *et al.* (1989) reported an increase in half-life from 4 to 20 days with decreasing depth (0-20m). In addition Freeman *et al.* (1995) found soil enzyme activity highest at depths less than 10cm. Respiration data in this study confirm that microbial activity and/or numbers decrease with increasing depth. This supports the hypothesis that LAS biodegradation will decrease with increasing depth.

LAS alkyl chain length and isomer effects

The data presented suggest that LAS degradation depends upon the alkyl chain length and structure of the surfactant. The rate constant for primary biodegradation increases with increasing alkyl chain length. This result is in accordance with previously published data (Swisher 1987, Kikuchi *et al.* 1986). Comparatively, Terzic *et al.* (1992) reported degradation of the C_{10} homologue to be two times slower compared to that of C_{13}, with half-lives of 3.8, 2.4, 2.6 and 2.3 days (23°C) respectively for C_{10}-C_{13} homologues. The faster degradation of the longer chain homologues supports the field data collected in chapter 2.

In terms of structure, it is apparent that mineralization was fastest for 2-DOBS in comparison to 3-DOBS compounds by net *c.*25%, i.e. degradation of isomers with the sulphophenyl group attached more towards the centre of the alkyl chain was significantly ($p<0.001$) slower. Verification of the more rapid disappearance of isomers with the phenyl attached near the end of the chain than those of more central attachment is reported (Terzic *et al.* 1992, Swisher 1987, Marcomini & Giger 1987) and is attributed to greater hydrophobicity of more terminal isomers.

3.4.2 Adsorption

As addressed in table 3.1 various adsorption tests have been conducted over different time series ranging from 1 to 25hrs (e.g. Inaba *et al.* 1988, McAvoy *et al.* 1994) with equilibrium achieved within 3-4 hours (Fytianos *et al.* 1998a, Hand & Williams 1987, Matthijs & De Henau 1985). However, preliminary experiments previously conducted to determine equilibrium confirmed 16 hours was appropriate for this test (Unpublished data, Unilever Research, Port Sunlight). The amount of LAS degraded during this time is negligible as indicated by the blank controls conducted (data not shown).

The adsorption procedure adopted was based on the measurement of the decrease in concentration in aqueous solution in contact with the gravel substrate used in the majority of tests (Matthijs & De Henau 1985, Inaba *et al.* 1988, Hand & Williams 1987). A mass balance approach was followed and the results (>90%) were satisfactory and comparable to previous work (e.g. Matthijs & De Henau 1985). The

use of radiolabelled LAS enabled sensitive tests at low environmentally relevant concentrations to be assessed. Other advantages include simple and rapid measurements in comparison to e.g. HPLC analysis. The KCl and other chemicals used in this study could have caused possible damage to the HPLC columns resulting in inaccurate measurements, whereas by using scintillation counting techniques this was eliminated.

A general decline in percentage adsorption with increasing initial concentration was observed, as was reported elsewhere (Matthijs & De Henau 1985). The increase exhibited for the Rosset gravel is reflected in the larger amount of LAS adsorbed per unit of gravel suggesting that stronger attractive forces exist in the system (Matthijs & De Henau 1985). Linearity of the adsorption isotherms suggests that a similar mechanism is governing adsorption on the gravels tested and reported in similar studies (Inaba *et al.* 1988). However, non-linear isotherms are also reported (Fytianos *et al.* 1998a, Matthijs & De Henau 1985). Although the data could potentially be described by Langmuir or Freundlich isotherms (Matthijs & De Henau 1987), the latter best represented the adsorption data in this study (Litz *et al.* 1987, Hand & Williams 1987, Ou *et al.* 1996, Brownawell *et al.* 1997, Fytianos *et al.* 1998a). As stated above the Langmuir equation assumes that adsorption is limited to a monolayer whereas Freundlich assumes multi-layer sorption. Comparable K values are reported by Ou *et al.* (1996) on natural sediment of 1.23-202 l/kg and are related to the binding energy and represent the amount of LAS adsorbed on the gravel (Fytianos *et al.* 1998a). However, much higher K values were reported for river sediments, e.g. 138-360l/kg for C_{13} homologue (Matthijs & De Henau 1985), 446l/kg for C_{10} (Hand & Williams 1987) and 2089l/kg for C_{14} (Hand & Williams 1987). A low K value indicates that adsorption is still localised in a monolayer at low equilibrium concentration, but a high slope ($^1/_n$) value may indicate greater sorption activity at higher concentration (de Wolfe & Feijtel 1998).

LAS adsorption has been studied in various environments, e.g. soils (Küchler & Schnaak (1997), river (Matthijs & De Henau 1985) and marine (Fytianos *et al.* 1998a) sediments, and in wetlands (Inaba *et al.* 1988, Inaba 1992, Del Bubba 2000). The surfactant properties of LAS indicates that sorption can be understood by 2 different mechanisms, i.e. electrostatic interactions between surfactant molecule and positively

charged surfaces, and hydrophobic interactions with organic matter of soils (Küchler & Schnaak 1997). In this study the origin and geology of the gravel only had a small effect on adsorption, as reported for source and nature of river sediment by Matthijs & De Henau (1985). Physical substrate factors known to affect LAS adsorption include Fe and Al oxides content (Küchler & Schnaak 1997), organic matter content (Küchler & Schnaak 1997), $CaCO_3$ content (Litz *et al.* 1987), pH (Küchler & Schnaak 1997) and redox (Litz *et al.* 1987). The X-Ray Microanalysis results in this study also suggests that Ca content of the gravel may possibly promote LAS adsorption, with Fe and Al less important under the test conditions adopted. This may be related to the positive charge of the Ca^{2+} ion attracting the negatively charged surfactant ion. Overall adsorption coefficients seem to be higher for sediments than for soils. This could be due to differences in composition e.g. more organic carbon in sediments (de Wolfe & Feijtel 1998).

This study demonstrates that the environmental fate of LAS can be significantly affected by adsorption onto gravel substrate in constructed wetlands. A mean *c.*37% adsorption was exhibited for the constructed wetland gravel types assessed. Sorption will also affect degradation kinetics as it is directly related to the residence time (McAvoy *et al.* 1994) and hence bioavailability of LAS. Longer residence times can increase the contact between microorganisms and chemical resulting in significant loss (Federle & Pastwa 1988). Hence the greater the adsorption then subsequently the greater potential for biodegradation in the wetland.

Biofilm

The slightly greater adsorption observed in the presence of biofilm (K=2.1 with biofilm, K=1.7 without biofilm) suggests that published data on LAS adsorption, normally involving sieving, air-drying, pH manipulation and/or water content adjustments (Matthijs & De Henau 1985, Fytianos *et al.* 1998a), may underestimate adsorption under field conditions. The results suggest multiple interactions between surfactant, biofilm bacteria and gravel-liquid interfaces. Although usually chemicals, such as $HgCl_2$ (Matthijs & De Henau 1985), are used to inhibit microbial activity the mineralization test conducted in this study suggested negligible $^{14}CO_2$ release under adsorption test conditions in biofilm presence over 16 hours. Hence this study

indicates that further investigation into the role of biofilm in LAS adsorption is required.

Surfactants are reported to not only adsorb onto sediment surfaces but to also stimulate bacteria attachment in river sediments (White 1995). Anionic surfactant SDS (sodium dodecyl sulphate) is reported to enhance the attachment of known degrading bacteria to sediment surfaces coinciding with rapid biodegradation which was reversed when biodegradation was complete (White 1995). No stimulation of known non-degraders attachment is reported (White 1995). Hence this highlights that biodegradation and adsorption are important parallel interactive mechanisms for LAS removal.

Alkyl chain length and isomer structure

The results suggest that LAS sorption in wetlands is governed by a hydrophobic mechanism as reported elsewhere (de Wolfe & Feijtel 1998, Küchler & Schnaak 1997). Terminally substituted alkyl chain LAS isomers are known to adsorb greater than more centrally substituted isomers on various sediment types (Swisher 1987, Hand & Williams 1987, Marcomini & Giger 1987). Hence the results of this study, i.e. 2->3->5-DOBS, support the distance principle (section 2.1). The negative charge on the sulfonate group attached to the benzene ring of the 2-DOBS compound will have a relatively smaller effect in hydrophobic interactions than the 3- and 5-DOBS phenyl positions (Hand & Williams 1987). Hence within an isomeric series, the isomer containing the longest unsubstituted alkyl chain fragment will be the most sorptive (Hand & Williams, 1987). Similarly longer chain homologues will adsorb greater than shorter chain homologues as found onto wetland gravel in section 2.2. Hand & Williams (1987) reported sorption significantly increased as LAS chain length increased with K values increasing by a factor of 2.8 for each additional methylene group and for isomers increased sorption as phenyl position changed from the 5/6 to the 2 position.

3.5 CONCLUSION

This chapter identified that the activities of the microbial community incorporated in the biofilm attached to the gravel surface to be the major irreversible LAS removal process in constructed wetlands. Previous exposure history and compound structure also controlled LAS degradation and the kinetics. Adsorption was further confirmed as an important LAS reversible removal process. In an operational context, the balance between LAS biodegradation and adsorption would determine the bioavailability and mobility of the surfactant and hence the overall removal observed. It is concluded that:

1. Biofilm microbial activity was identified as the major irreversible LAS removal mechanism in wetlands supporting work from other researchers (Takada *et al.* 1994, Boeije *et al.* 2000).
2. Prior exposure to the surfactant, and subsequent adaptation of the microbial community, promoted greater mineralization.
3. Biofilm presence only slightly increased adsorption.
4. LAS mineralization decreased with increasing depth supporting the current data that LAS is less degradable under oxygen depleted conditions.
5. Hydrophobic mechanisms resulted in greater degradation and adsorption of longer alkyl chain homologues and more terminally substituted isomers.

Other factors such as temperature (Terzic *et al.* 1992), pH (Dorfler *et al.* 1996) and water hardness (Berna *et al.* 1989) will also influence degradation and/or adsorption yield and kinetics. These are discussed in chapter 5 and effect of plants in chapter 4.

CHAPTER 4: Role of Plants in LAS Removal

The laboratory microcosm experiments conducted in Chapter 3 enabled quantification of potential LAS removal via biodegradation and adsorption in constructed wetlands. However, the role of plants in these processes has largely been ignored. Chapter 2 highlighted the springtime enhancement of LAS degradation indicating greater microbial activity and/or degradation capacity when plant growth is most intensive. Hence the high plant biomass in constructed wetlands requires investigation. This chapter will focus on plants with a series of experiments investigating the effects of their presence on LAS degradation.

4.1 INTRODUCTION

Plants are the most obvious component of any wetland ecosystem with their presence and species used as a key in the definition of natural systems, e.g. US Clean Water Act Amendments (Section 404) 1977 (see section 1.4.1). Plants are known to play a role in various physical, chemical and biological processes in a wetland. For example, they serve to stabilize the bed surface, insulate against freezing and frost through litter production, prevent clogging, shield algae from incoming solar radiation, adsorb and store nutrients, and prevent channeled flow (Brix 1997, Kadlec & Knight 1996).

In constructed wetlands several plant species have been used, e.g. *Typha* (Breen 1990), s*choenoplectus validus* (Breen 1997, Tanner *et al.* 1998) and willow (Kowalik & Randerson 1994). However, in the majority of domestic wastewater treatment wetlands the common reed *Phragmites australis* is used (Kadlec & Knight 1996). *Phragmites* naturally grows in chemically reduced, water-logged soils and has the ability to produce zones of oxidation within the soil and consequent aiding in the removal of BOD, COD and nitrogen (Armstrong & Armstrong 1988). The oxygen transport ability of *Phragmites* down the shoot and into the root-zone resulting in oxidised zones in the rhizosphere has been well documented (Armstrong & Armstrong 1988, Brix 1990). Higher populations of strict aerobic heterotrophic bacteria have been found in the *Phragmites* rhizosphere than in aerobic stabilised sewage sludge (Hoffman 1990). Aeration of the *Phragmites* rhizome of younger roots is reported to be higher during the growing season (Armstrong & Armstrong 1988). Estimates of root oxygen release rates vary at, e.g. 0.02g m^{-2} day^{-1} (Brix 1990) and 5-12 g m^{-2} day^{-1} (Armstrong *et al.* 1990) depending on the methods used for measurements. However, critics have questioned the ability and/or quantity of oxygen released in the root zone by *Phragmites* and whether this has any significant effect on effluent purification. Brix (1994) reported that the amount of oxygen transferred to the root-zone is minimal in most systems.

Nutrient uptake is well reported plant mechanism for improving water quality (Brix 1997, Breen 1990) and is influenced by growth rate of plants, hydraulic loading rate and concentration of stored nutrients in plant tissue. Greater nutrient uptake has been observed during growth seasons followed by a decrease or even a cessation in the

autumn and winter (Kadlec & Reddy 2001). Gumbricht (1993) found that the uptake capacity of emergent macrophyes for phosphate was approximately 30-150kg P ha^{-1} yr^{-1}, whereas Brix (1997) found an uptake capacity for nitrate of 200-2500 kg N ha^{-1} yr^{-1} when the plants were harvested. However, Tanner (2001) found nutrient uptake insufficient to account for significant nutrient removal. Debate surrounding harvesting is currently undecided with researchers in support (Breen 1990) and others questioning the justification in terms of wetland performance and cost (Lin *et al.* 2002, Kim & Geary 2001, Tanner 2001, Brix 1994).

Macrophytes and their roots facilitate microbial activity by providing greater attachment sites for growth of large colonies of bacteria and supplying carbon in the rhizosphere (Brix 1994, Brix 1999). The leaching of dissolved organic carbon material and e.g. amino acids, exudates and sugars via the plant roots and rhizosphere can aid in sustaining and influencing microbial activity (Coleman *et al.* 2001) and extracellular enzyme activity. Plants also play important secondary roles such as providing a habitat for a variety of wildlife, sustainability, improving odor control and wetland aesthetics (Brix 1997). These additional factors may be equally as important especially if serving small communities or single households. The main roles of plants, both above and below ground, are summarized in figure 4.1.

Figure 4.1: Summary of above and below ground plant mechanisms in wetlands.

- Uptake of nutrients and metals
- Insulate against freezing
- Shading algae from solar radiation
- Habitat for wildlife
- Aesthetically pleasing
- Stabilise bed surface

- Root and rhizosphere oxygen release
- Surface area for microbial attachment
- Prevents clogging
- Organic matter root release
- Filtration

However, there remains a lack of knowledge and quantitative data on the role of plants in wastewater treatment with information mainly centred on nutrient rather than pollutant removal. Debate has arisen over the necessity of plants and adverse impacts reported in some cases e.g. acid mine drainage treatment (King & Garey 1999). However, the impact plants will have will depend on the individual constructed wetlands in terms of their design, loading, type of treatment and environmental conditions.

The main aim of this chapter is to assess the role of plants in LAS removal mechanisms in constructed wetlands. This is achieved by investigating the following;

1. Differences in LAS removal between both planted and unplanted mesocosms and plant species
2. Whether plant biomass affects LAS removal and quantification of LAS degradation by rhizosphere microbial communities
3. If plant uptake of LAS is a valid removal mechanism
4. Whether oxygen root release results in greater aerobic LAS degradation
5. If plant DOC exudates promote LAS biodegradation

In section 2.1 greater LAS removal was observed during the spring, coinciding with the plant growth season, possibly indicating plant processes facilitating removal. In addition published research suggests that greater LAS removal occurs when plants are present (Federle & Schwab 1989). Hence it is hypothesised that;

1. Planted mesocosms will show greater LAS and nutrient removal.
2. Greater plant biomass per unit area will result in greater LAS removal.
3. Oxygen root release (Federle & Schwab 1989), plant DOC exudates (Knaebel *et al.* 1990) and rhizosphere microbial communities (Knaebel & Vestal 1994) will facilitate LAS removal. However, plant uptake of LAS will be negligible as suggested by Knaebel & Vestal (1992).
4. Significant differences will be observed between plant species because of the variability in their capacity for the above plant mechanisms.
5. Greater enzyme activity will be exhibited in planted than unplanted systems as reported elsewhere (e.g. Kiss *et al.* 1974 as quoted in Speir & Ross 1978).

Separate experiments were conducted to test the hypothesis above. A 6-month field based study was conducted comparing planted and unplanted systems in mesocosm experimental wetlands. Plant biomass (zero, low and high) effects were assessed at the same field site over a 15-day experiment. Microcosm laboratory experiments were conducted to assess effect of plant species and oxygen root release. Finally, a series of HPLC based and radiochemical tests were conducted to assess plant uptake, rhizosphere degradation and DOC exudates on LAS removal.

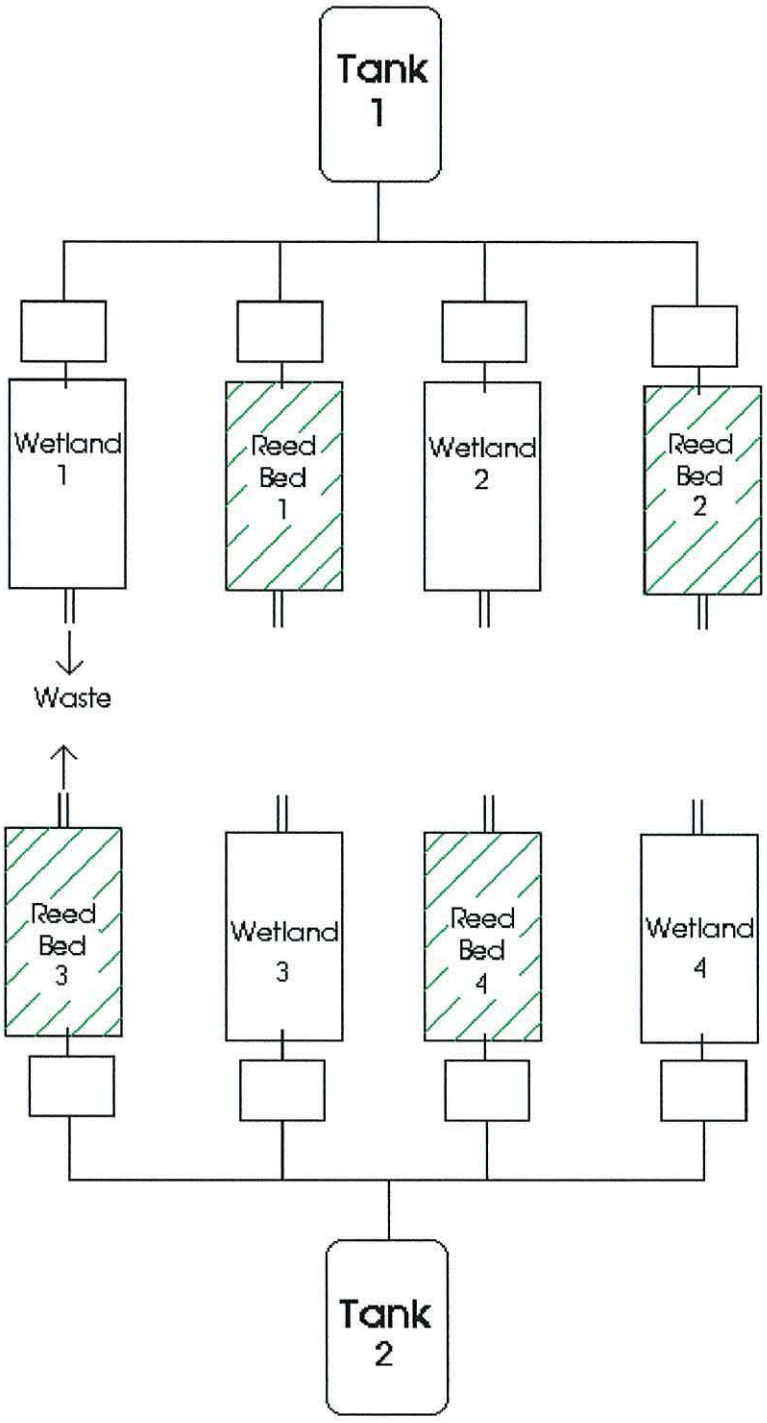
4.2 METHODS

4.2.1 LAS removal in field planted and unplanted systems

Eight purpose built sub-surface flow constructed wetland mesocosms (4 planted, 4 unplanted) were used to control loading, concentration and flow. The mesocosms, located at Henfaes in Abergwyngregyn (Grid Ref. SH 6573), measured at 1.95m (l), 0.65m (w) and 0.4m (d) and were filled with gravel (approx. 5-10mm diameter) to a depth of 0.38m. Four of the mesocosms were planted with *Phragmites australis* at a density of 4 plants m^{-2}. Figure 4.2 shows a photograph and plan of the site.

Figure 4.2a: Photograph of constructed wetland units at Henfaes, Abergwyngregyn.

Figure 4.2b: Diagram of the constructed wetland units at Henfaes, Abergwyngregyn.



Inflow rates of 35 L day^{-1} of 5mg L^{-1} LAS was deigned to mimic full-scale operational constructed wetlands flow and expected LAS loading. Planted and unplanted microcosms were monitored in parallel over a 6-month period (Sept-Feb). LAS addition began at the start of September 2000 with the first samples taken at the end of the month. The experiment was initially planned for a 12-month period.

However, due to the Foot and Mouth outbreak and legal restrictions in the UK at the start of 2001 it was impossible to continue the monitoring due to the location on farmland of the wetlands for more than 6-months.

Monthly sampling of each wetland consisted of inflow and outflow water samples for LAS (preserved 3.5% formaldehyde) and hydrochemistry (filtered 0.2μm (section 2.1.2). Gravel substrate samples ($150cm^3$) were collected for subsequent enzyme (section 2.1.2) and microbial activity respiration (section 3.2) analyses. In addition pH (Orion pH electrode), air (1m above ground) and soil (10cm depth) temperature were taken for completion. Rainfall data was obtained from the field monitoring station at Henfaes Farm, Abergwyngregyn.

4.2.2 Effect of plant biomass on LAS removal

Further work using the mesocosms investigating the effect of plant biomass (zero, low and high) was conducted over a 15-day period (6-20^{th} June 2001) with the same sampling parameters assessed as described in section 4.2.1 above. An initial LAS concentration of 10mg L^{-1} in artificial sewage (Appendix D) was added (25L). KBr (1.5mg L^{-1} Br^-) was used as a chemical tracer to assess the hydraulic retention time and measured using the DX-120. At the end of the experiment morphological characteristics of stem length, basal stem diameter, dry weight, number and length of leaves were assessed in aerial shoots of *Phragmites australis* growing in the low and high-biomass mesocosms. The unplanted gravel units served as the control at zero biomass.

4.2.3 Effect of plant species on LAS removal

Small-scale laboratory wetland replicate microcosms (11.5cm(w) x 13cm(d)) to compare and quantitatively assess differences between 5 plant species (*Phragmites australis*, *Typha latifolia*, *Salix viminalis*, *Iris* and *Juncus effusus*) against a gravel control were investigated at 12°C. 350ml of a 10mg L^{-1} LAS solution, simulating normal to high LAS loading conditions, mixed in artificial sewage (Appendix D) was added to each microcosm and samples for LAS and hydrochemistry monitored over a 12 day period. Filters (cut off 2.5ml Plastipak™ syringes packed with glass wool) were inserted as a sample port in each microcosm.

4.2.4 LAS uptake by Plants

100ml LAS solution containing ^{14}C radiolabelled 3-DOBS compound (see section 3.2 for details) was added to four replicate *Phragmites* and left for 14 days. The experiment was only conducted for this length of time due to the laboratory constraints. The stem and leaves of the plants passed through the flask lid, with only the roots exposed to the radiolabelled compound and the lid was sealed with rubber stoppers and greased. At the end of the experiment the roots were washed in deionised water, the plants separated into root and stem parts and oven dried (70°C for 72hrs). Each plant part was then combusted using a Harvey biological sample oxidiser model OX400. The liberated $^{14}CO_2$ was collected in 15ml OXOL ^{14}C scintillant and measured using a Wallac Winspectral Model 1414 liquid scintillation counter. Control plants not exposed to LAS were used as background samples.

4.2.5 Effect of root oxygen release on LAS removal

Three replicate microcosms (11.5 x 13cm) of unplanted, planted (*Phragmites australis*) and oxygen-free planted (*Phragmites australis*) systems, fitted with filters as above, were compared over a 48hr period. The oxygen-free planted systems were subjected to a constant flow of nitrogen exposed to the aerial plant part so that no oxygen was transported to the root-zone. Water samples were taken at 6, 24 and 48hr and analysed for LAS content via the HPLC method (section 2.1.2).

4.2.6 Effect of plant DOC exudates and glucose on LAS removal

Natural DOC release from *Phragmites australis* was collected by placing the plants in water and periodically measuring the DOC concentration until >150mg L^{-1} was reached. This solution was used to dissolve 100mg L^{-1} LAS (primary biodegradation, section 3.2) with 100ml added to 50g of gravel collected from the Brynsiencyn site. The ^{14}C-3-DOBS (mineralisation, section 3.2) was added to the solution and 0.5ml of the radiolabelled mixture added to 50g of gravel. In comparison, 150mg L^{-1} solution of glucose dissolved with LAS, commercial and radiolabelled mixture, was used as an additional carbon and energy source as above.

4.2.7 LAS degradation by rhizosphere bacteria

Phragmites roots were collected from the field (Brynsiencyn wetland) with 5g placed in a biometer flask and mineralization monitored over a 45-day period via

scintillation counting of the KOH trap (section 3.2). Mineralization was also assessed on roots (5g) washed with water prior to the test so as to remove some of the biofilm.

4.2.8 Statistical Analysis of Results

Data for the field experiments were analysed via correlation matrices (Pearson correlation for data conformed to the normal distribution; Spearman correlations for non-conforming data). Differences between planted and unplanted treatments were assessed via paired t-tests. However, for differences between more than two treatments, e.g. species or biomass, repeated measures ANOVA tests were applied. This type of ANOVA is appropriate in an experiment where the data derived from normal population are taken on the same subjects repeatedly over a period of time (Manly 1992, Gray *et al.* 2000). Data of this type is not independent and therefore t-tests or one-way ANOVA tests are not suitable.

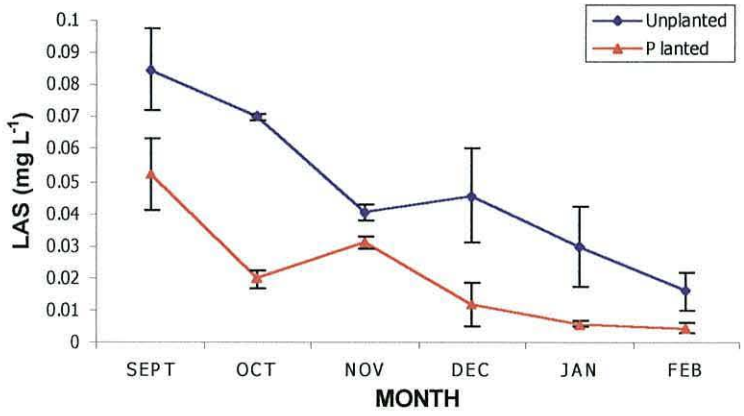
4.3 RESULTS

4.3.1 LAS removal in field planted and unplanted systems

LAS

LAS inflow remained stable throughout the experiment with an average concentration of 4.9mg L^{-1} (±0.4mg L^{-1}). High LAS degradation was observed from the start of the experiment and increased with time. Figure 4.3 shows the LAS concentration in the outflow with a higher mean calculated for the unplanted (0.05mg L^{-1}) than planted (0.02mg L^{-1}) mesocosms. Significant differences ($p<0.01$) between treatments was observed. However, >95% removal was observed in both systems. In addition, as in chapter 2, an inverse correlation between LAS and NO_3 concentration was established for the unplanted system ($p<0.01$).

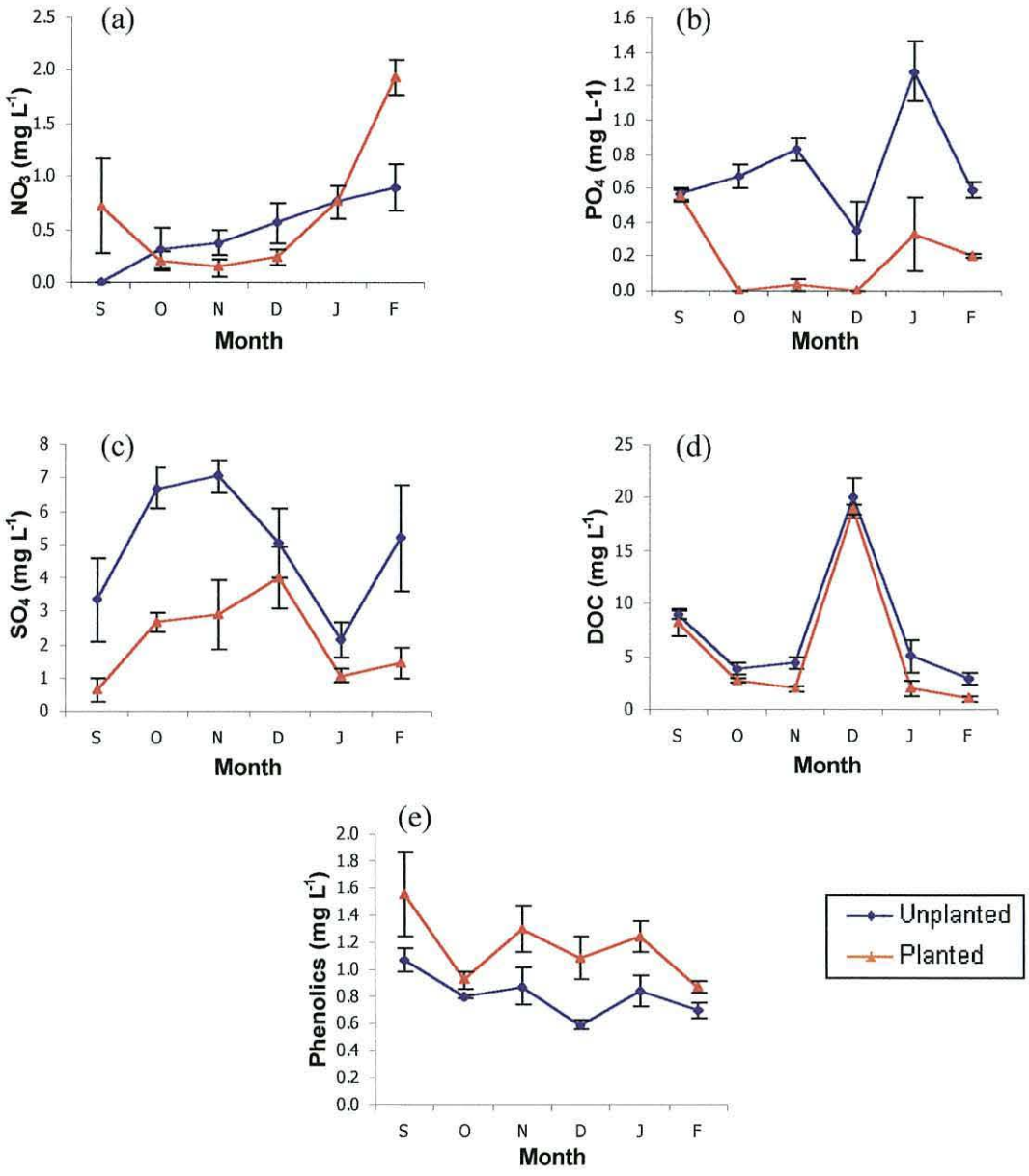
Figure 4.3: LAS concentration at the outflow in planted and unplanted mesocosms.



Hydrochemistry

Nutrient anion concentrations were low as expected with planted systems generally exhibiting lower outflow concentrations (except for NO_3 in Sept. and Feb.) as shown in figure 4.4. NO_3 in the unplanted mesocosms exhibited the only relationship (inverse) with temperature ($p<0.01$). DOC concentration in the outflow of the unplanted microcosms were significantly ($p<0.01$) higher than in the planted with a mean of 7.5mg L^{-1} and 5.8mg L^{-1} respectively (figure 4.4d). Both systems showed a sharp peak in December but were otherwise relatively stable. The outflow phenolics concentration in both the planted and unplanted beds showed a similar pattern with time (figure 4.4e) but was slightly higher in the former. Average pH of 7.0(±0.2) for the planted beds and significantly ($p<0.05$) higher at pH 7.3(±0.5) for the unplanted were observed.

Figure 4.4: Hydrochemical characteristics in planted and unplanted mesocosms for (a) NO_3, (b) PO_4, (c) SO_4, (d) DOC and (e) Phenolics.

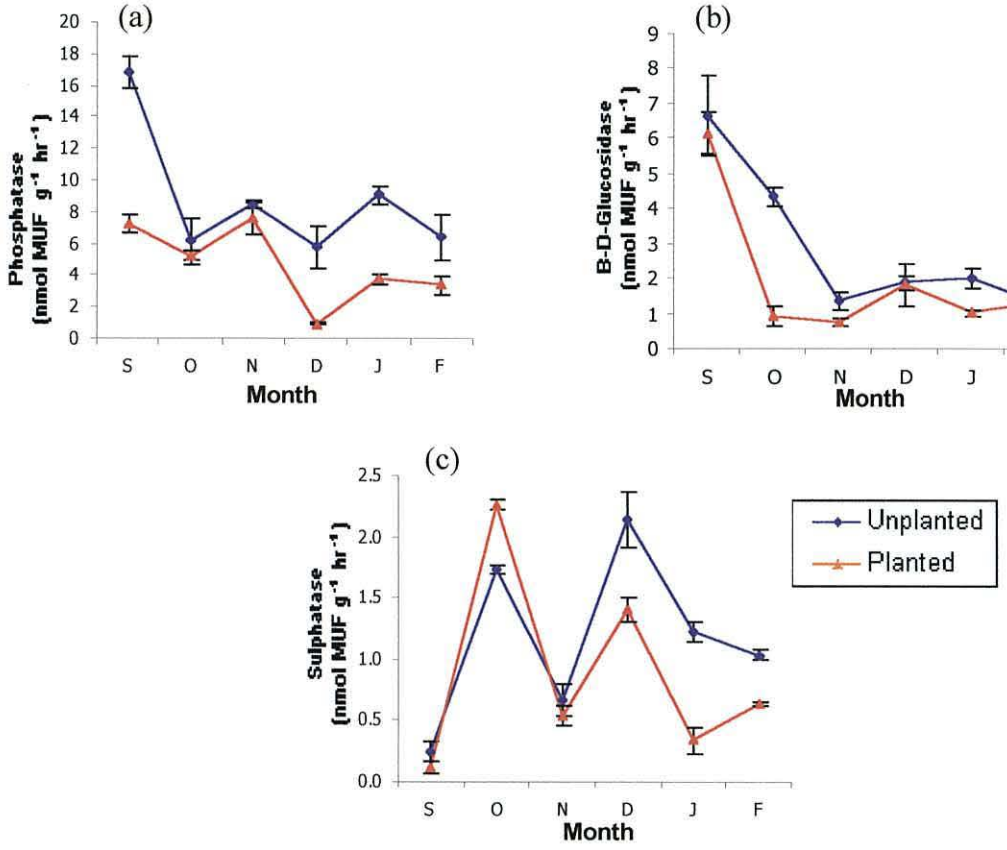


Enzyme activity

Figure 4.5 shows that the enzyme activity was significantly higher for phosphatase, then β-glucosidase and sulphatase, by a minimum of a 2-fold factor in the planted ($F=6.04$, $p<0.01$) and unplanted ($F=12.79$, $p<0.001$) mesocosms. Unplanted systems exhibited greater enzyme activity in comparison to planted microcosms but this was only significant ($p<0.05$) for phosphatase. Both phosphatase and β-glucosidase

activity decreased from the initial activity measured in September, especially for the unplanted systems. In contrast an increase in sulphatase activity from initial levels after LAS addition was observed.

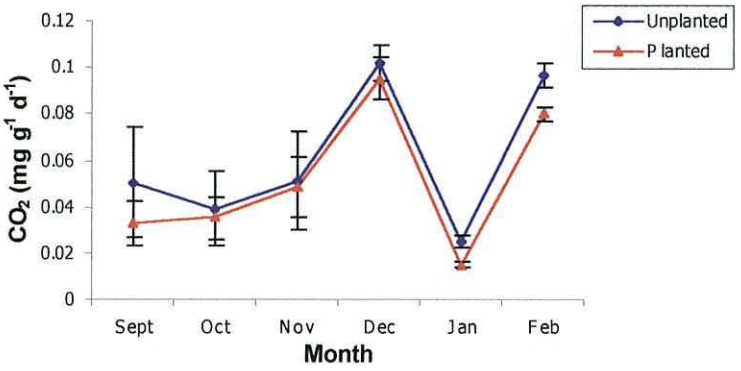
Figure 4.5: Enzyme activity for planted and unplanted microcosms for (a) phosphatase, (b) β-glucosidase and (c) sulphatase.



Microbial Respirometry

Figure 4.6 shows the CO_2 microbially respired with a substantial decrease observed in January followed by recovery in February. The largest positive flux in CO_2 occurred in December for both unplanted (0.1 mg g^{-1} d^{-1}) and planted (0.095 mg g^{-1} d^{-1}) microcosms with significant differences ($p<0.05$) between treatments.

Figure 4.6: CO_2 microbial respiration in planted and unplanted microcosms.



Environmental conditions

The bed temperature of both treatments were positively correlated ($p<0.01$) to air temperature (mean 11.7°C, range 5.7-21.3°C). Average bed temperature of 8.3°C for the planted and 9.2°C for unplanted beds were observed but with no significant statistical difference observed ($p>0.05$). Although the lowest air temperature measured was only 5.7°C in January, freezing of the wetland had occurred during the night and had not defrosted at the time the temperature was measured. Mean rainfall recorded was 5.5mm (range 2.0-10.8mm) with greatest rainfall overall observed in November.

4.3.2 Effect of plant biomass on LAS removal

LAS

High LAS removal (>95%) was observed in all treatments with LAS concentrations increasing initially, corresponding with the tracer study (see below), and then decreasing in the last 7 days (figure 4.7). Plant biomass affected LAS removal with significant differences ($F=8.26$, $p<0.01$) between treatments occurring and the order of decreasing removal as high-biomass(0.08mg L^{-1})>low-biomass(0.36mg L^{-1}) >unplanted (0.45mg L^{-1}). However, post hoc Tukey test revealed no significant differences between the unplanted and low biomass planted treatments. Aboveground plant biomass was measured with the results in table 4.1 showing significantly greater biomass in the high than low-biomass mescosms in terms of the morphological characteristics measured.

Figure 4.7: LAS concentration in plant-biomass treatments

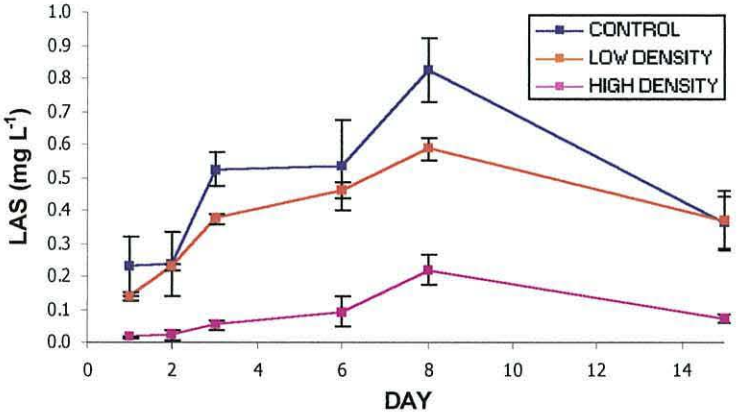


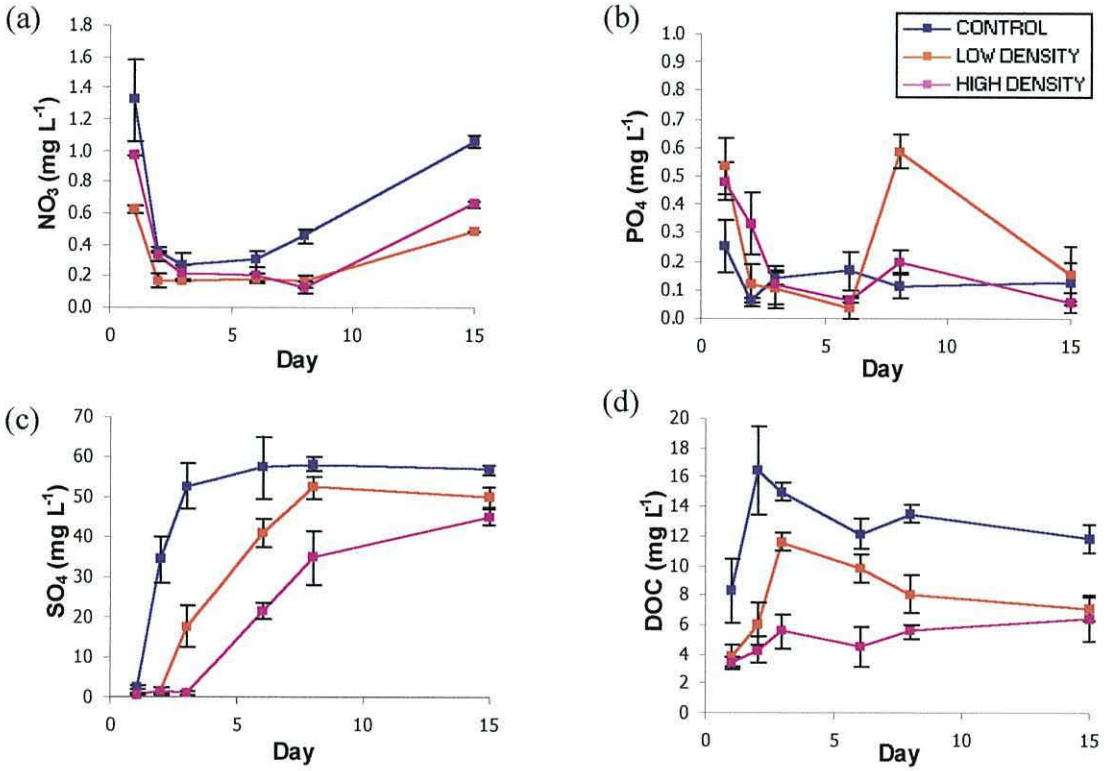
Table 4.1: Plant morphological characteristics.

Plant Characteristic	Low-Biomass	High-Biomass	p
No. of leaves	8.1 (±0.6)	10.1 (±0.4)	<0.01
Shoot Length (cm)	57.0 (±12)	91.3 (±9)	<0.01
Stem Diameter (cm)	0.74 (±0.1)	1.0 (±0.1)	<0.05
Dry Mass (g)	4.1 (±1.5)	10.6 (±1.0)	<0.001

Hydrochemistry

NO_3 concentration was generally lower in the planted than unplanted systems with significant ($F=8.837$, $p<0.01$) difference between the unplanted and low-biomass planted treatments observed (Fig. 4.8a). PO_4 concentrations were low with no significant ($p>0.05$) differences between treatments (Fig. 4.8b). On the other hand, SO_4 did show a distinctive pattern with time with significant ($F=10.351$, $p<0.01$) differences identified in comparing the planted and unplanted treatments, but not between the low- and high-planted treatments (Fig. 4.8c). Correlation between SO_4 and LAS concentrations was established for the two planted treatments ($p<0.05$). The unplanted control exhibited the highest overall DOC concentration followed by the low then high biomass treatments with again significant differences ($F=28.182$, $p<0.001$) only between unplanted and planted treatments identified (Fig. 4.8d). In addition an inverse correlation ($p<0.05$) was observed for DOC and NO_3 concentration in the unplanted control.

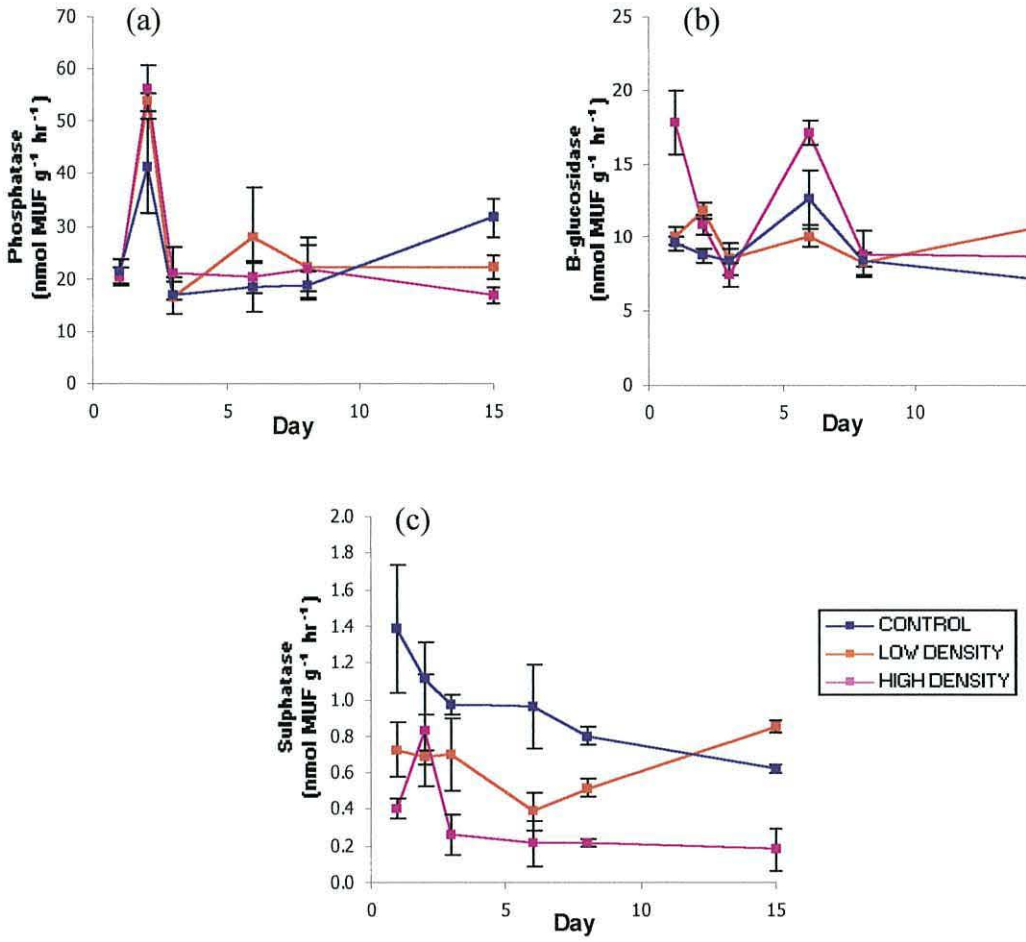
Figure 4.8: Hydrochemistry characteristics in plant-biomass treatments for (a) NO_3, (b) PO_4, (c) SO_4 and (d) DOC.



Enzyme activity

Highest activity in all treatments was observed for phosphatase followed by β-glucosidase and sulphatase respectively (figure 4.9). Activities between enzymes in each treatment or between treatments were not correlated, except for phosphatase ($p<0.05$) reflecting the large fluctuations in activity measured. Significant differences ($F=15.192$, $p<0.001$) between planted and unplanted treatments was observed for sulphatase only. In comparison to the levels reported in section 4.3.1, approximately 4-fold higher phosphatase and β-glucosidase activity was observed. However, in contrast, a reduction in sulphatase was observed. The reduction was more profound in the planted (low-biomass -30%, high-biomass -60%) than unplanted (-20%) mesocosms. An inverse relationship between bed temperature and sulphatase activity in the unplanted mesocosms ($p<0.01$) and with SO_4 concentration ($p<0.05$) was established possibly suggesting inhibition.

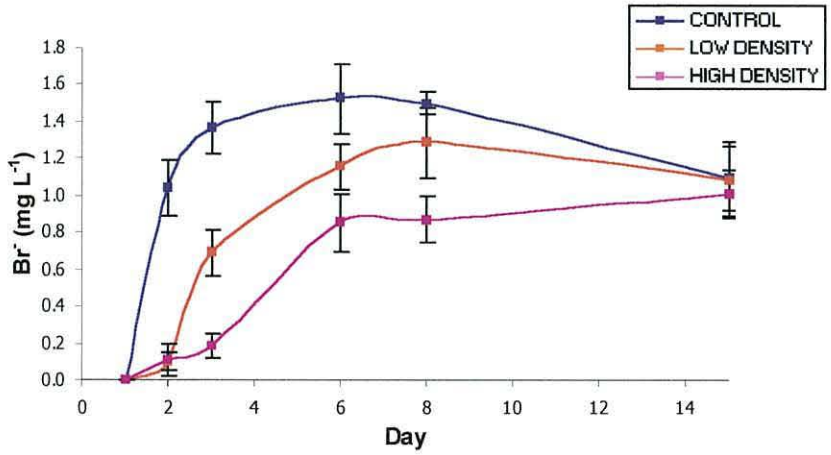
Figure 4.9: Enzyme activities in plant-biomass treatments for (a) phosphatase, (b) β-glucosidase and (c) sulphatase.



Tracer study

Figure 4.10 shows the tracer study results from which an indication of the diffusion rate can be observed with faster recovery in the unplanted, low and high-biomass mesocosms respectively. However, a significant ($F=7.756$, $p<0.01$) difference was only observed between the control and high-biomass planted treatments. The plots show typical tracer curves for a low rainfall period with a longer initial slow diffusion exhibited for the planted treatments in comparison to unplanted. A positive correlation ($p<0.01$) between LAS and Br^- concentration was established for the low biomass treatment. In addition correlations with SO_4 was also established ($p<0.01$).

Figure 4.10: Br^- tracer study for the plant-biomass treatments.



Environmental conditions

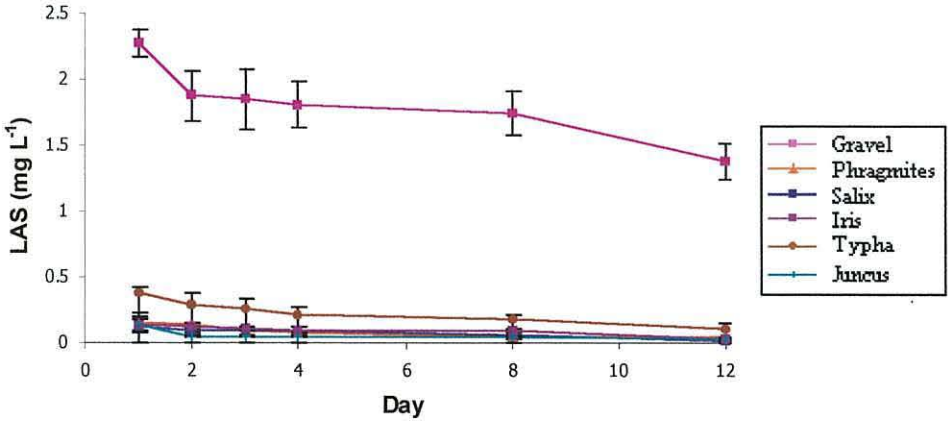
Again bed temperature was warmer in unplanted than planted mesocosms ($F=4.06$, $p<0.05$). Rainfall was low during this study with a total of 11.5mm rainfall measured over the 15 days with 9mm in the last week.

4.3.3 Effect of plant species on LAS removal

LAS

The mean concentrations measured after 12 days in the six treatments were, in descending order, the gravel control (1.83mg L^{-1}); *Typha* (0.23mg L^{-1}); *Iris* (0.12mg L^{-1}); *Juncus* (0.10mg L^{-1}), *Salix* (0.09mg L^{-1}) and *Phragmites* (0.08mg L^{-1}). The disappearance of LAS with time is shown in figure 4.11. There is a marked significant difference in the observed disappearance of LAS concentration with time between the unplanted and planted treatments ($p<0.001$). However, post hoc Tukey test revealed no significant differences between planted treatments.

Figure 4.11: LAS concentrations for different of plant species and gravel control.



Hydrochemistry

Generally PO_4 concentrations initially increased in the first 4 days of the experiment (figure 4.12a). Extremely significant differences between treatments was observed ($F=34.342$, $p<0.001$) with levels in the *Juncus* treatment significantly higher (mean 8.8mg L^{-1}) and gravel treatment control significantly lower (mean 1.9mg L^{-1}). The trend in SO_4 concentrations was more variable with an initial increase followed by a sharp decrease on day 3 and then a general rise in concentration (figure 4.12b). Significant differences in treatment was again observed ($F=30.092$, $p<0.001$) with *Juncus* treatment exhibiting the highest concentration and gravel control the lowest. DOC also varied between treatments ($p<0.001$) with levels lowest in the gravel control (figure 4.12c). *Phragmites* exhibited the lowest DOC concentration (mean 39.0mg L^{-1}) of the planted treatments throughout the course of the experiment, whereas *Salix* exhibited the highest (mean 70.1mg L^{-1}). The DOC percentage removal observed was of the order: Gravel (G), *Phragmites* (P), *Typha* (T), *Juncus* (J), *Iris* (I), *Salix* (S). The significant differences between treatments as analysed via post hoc Tukey test are summarised below with the lines drawn to indicate groups that are not significantly different.

For SO_4:

J G T P I S

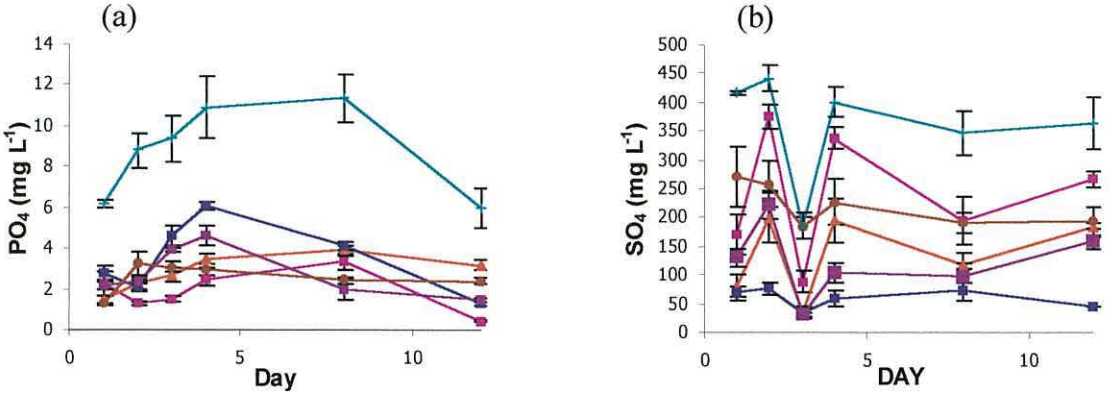
For PO_4:

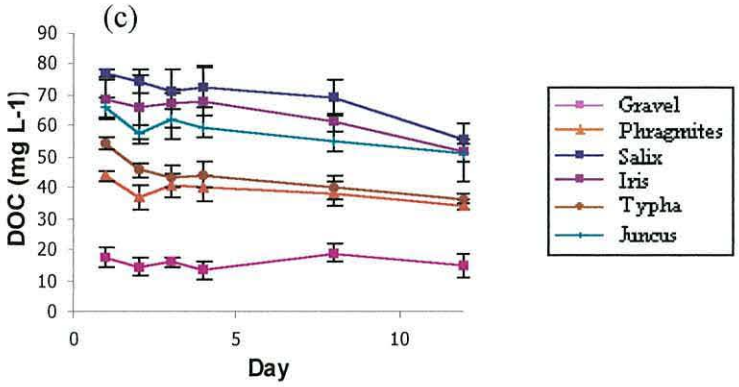
J S P I T G

For DOC:

J S P I T G

Figure 4.12: Hydrochemistry characteristics of plant species and gravel control for (a) PO_4, (b) SO_4 and (c) DOC.





4.3.4 LAS uptake by Plants

Uptake of LAS, measured via radiolabelled methods, showed minimal shoot uptake as demonstrated with markedly greater radioactivity measured in the root (98.6±0.4%) than shoot (1.4±0.2%). In terms of the percentage removed in relation to initial radioactivity added a mean of 3.1(±0.2)% was associated with the plant (2.8% roots, 0.3% shoot).

4.3.5 Effect of root oxygen release on LAS removal

Figure 4.13 shows the LAS concentration measured for the three treatments with greatest removal observed for the planted system. Again gravel exhibited significant removal but plants enhanced treatment efficiency. The plants exposed to nitrogen exhibited greater LAS removal than the gravel control but significantly ($F=17.954$, $p<0.001$) less than those exposed to the air. In terms of the hydrochemistry no significant differences between treatments were observed for NO_3 or PO_4, but was significant for SO_4 ($F=8.011$, $p<0.01$) (figure 4.14). DOC levels were slightly but not significantly ($p>0.05$) higher in the planted treatments than the gravel control.

Figure 4.13: LAS concentrations measured for planted (air and nitrogen exposed) and unplanted microcosms

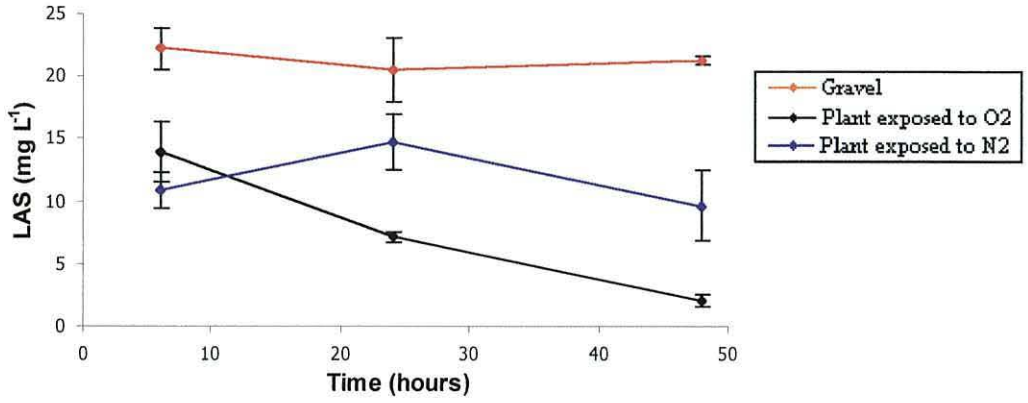
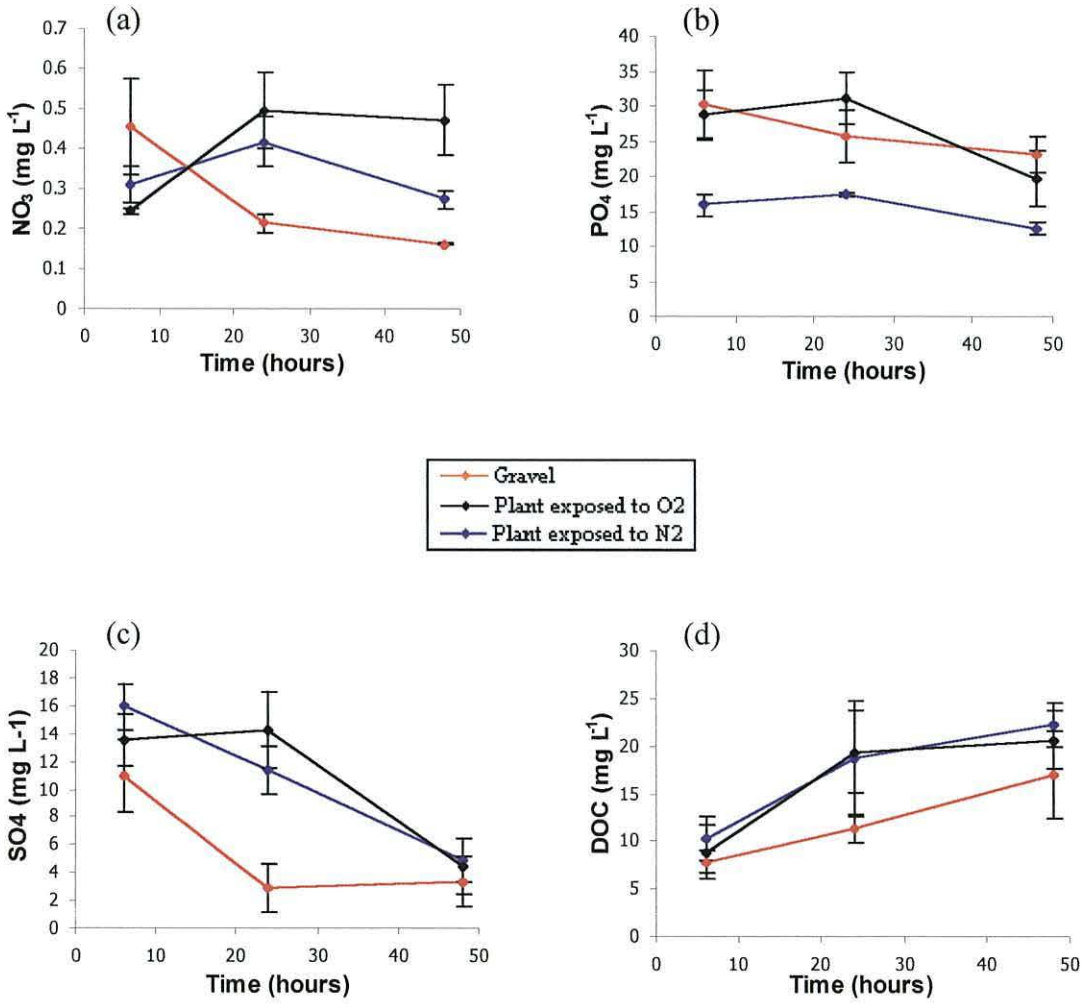


Figure 4.14: Hydrochemistry characteristics of planted (air and nitrogen exposed) and unplanted microcosms for (a) NO_3, (b) PO_4, (c) SO_4 and (d) DOC.

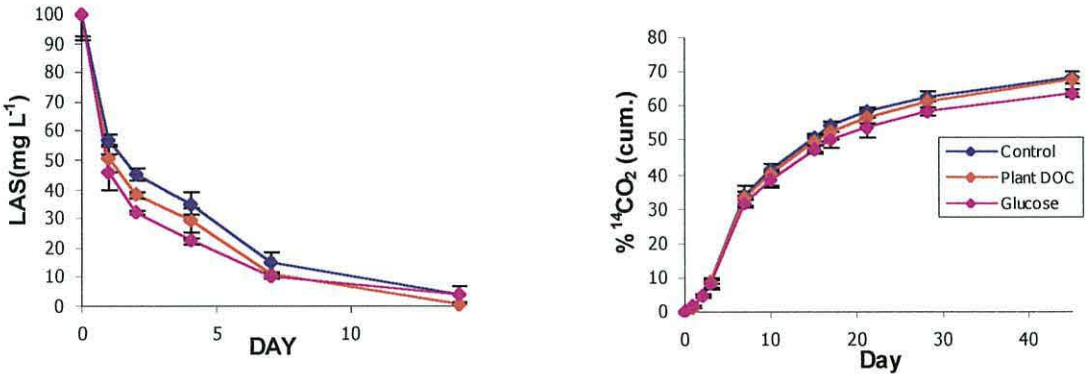


4.3.6 Effect of plant DOC exudates on LAS removal

Additional carbon treatments did not significantly affect primary biodegradation or mineralization in comparison to the control ($p>0.05$) as shown in figure 4.15. Similar net primary biodegradation of 95, 96 and 99% was observed for the control, glucose and plant DOC treatments respectively. In contrast, the order of net mineralization was 64, 67 and 69% for the glucose, plant DOC and control respectively.

Figure 4.15: LAS removal with additional carbon for:

(a) Primary biodegradation (b) Mineralization

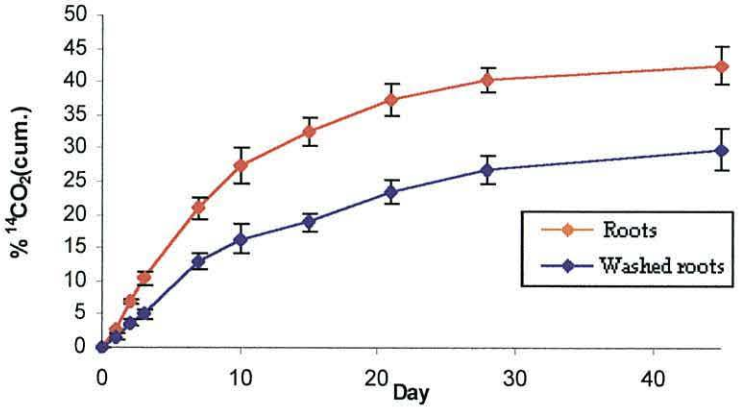


4.3.7 LAS degradation by root/rhizosphere bacteria

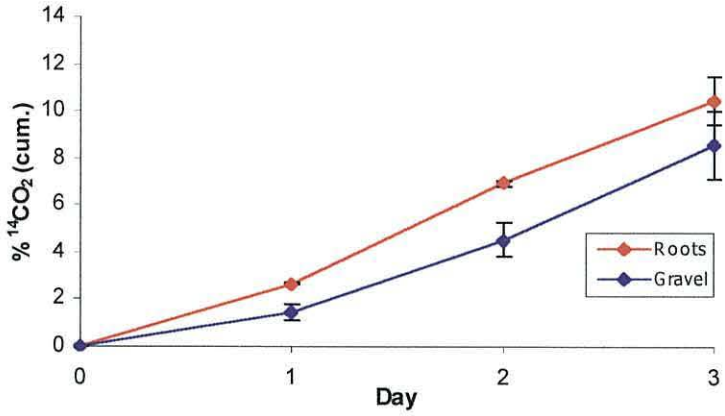
LAS mineralization by rhizosphere biofilm bacteria was considerable with net 43% after 45 days, as cumulative $^{14}CO_2$ released, in comparison to net 30% for the washed roots as shown in figure 4.16a. The differences between treatments were statistically extremely significant ($p<0.001$). If the lag phase during the first 3 days of the test for the Brynsiencyn gravel biofilm is compared with that for the roots biofilm from the same sampling site, as shown in figure 4.16b, greater initial mineralization rate is observed for the latter.

Figure 4.16: LAS mineralization by rhizosphere biofilm microbial community in comparison to:

(a) washed roots



(b) gravel biofilm.



4.4 DISCUSSION

4.4.1 LAS removal in field planted and unplanted systems

LAS

The high removal observed (>95%) suggests that constructed wetlands have high potential for LAS degradation under optimal conditions. Significant differences were observed in LAS outflow concentration between planted and unplanted systems ($p<0.01$). Hence, although the unplanted control provided considerable treatment, vegetation presence further improved LAS treatment efficiency. This is consistent with other studies, e.g. organic matter (Allen *et al.* 2002), nutrients (Heritage *et al.* 1995), heavy metals (Doyle & Otte 1997) and ammonia (Sikora *et al.* 1995) removal. Federle & Schwab (1989) reported a higher rate of LAS mineralization with microbiota associated with aqueous plants in the rhizosphere than in nearby root free sediment. This may be explained by several possible plant mechanisms facilitating microbial activity, including rhizophere oxygen release, rhizosphere and root attachment sites for bacterial growth, DOC root release enhancing bacterial activity and plant uptake (Brix 1994, Brix 1997). However, several factors may affect plant growth and consequently LAS removal in a wetland including loading concentration and rate, water depth, aeration, presence of pollutant, air and water temperature. The ability of the gravel alone for LAS treatment may suggest related physical processes (e.g. adsorption) or formation of biofilm on the gravel surface.

Higher temperature was observed in the unplanted than planted systems. However, although this was not significant ($p>0.05$), the data appear to confirm the greater insulation and resulting winter bed temperature characteristics reported elsewhere for planted systems (Smith *et al.* 1997) does not occur in this study and cannot account the difference in LAS removal between planted and unplanted systems.

Cold temperatures (<3-4°C) have been reported to detrimentally affect LAS degradation in laboratory studies (e.g. Inaba *et al.* 1988) and in the field (e.g. Terzic *et al.* 1992). Low temperatures can also affect plant mechanisms in wetlands that may, in turn, detrimentally affect LAS degradation. However, although freezing occurred in this study in January high LAS removal was observed with flow continuing below the ice layer as observed elsewhere (Whitgren & Mæhlum 1997).

This is supported by the high LAS removal (96%) observed at the Nynäshamn wetland in Sweden at low temperature as reported in Chapter 2.

The observed general decline in LAS concentration with time may possibly be explained by acclimatisation of the microcosm bacterial community to the surfactant. Enhanced LAS degradation with time was reported in section 2.2 for the Brynsiencyn wetland monitored over a 30-month period. Acclimatisation and adaptation has been observed in various experiments (e.g. Federle & Pastwa 1988, Branner *et al.*1999) and previously discussed in section 3.4. In contrast to that reported in Chapter 2 and 3, no significant differences were recognised between the alkyl chain homologues in this system as extremely high removal for all homologues were observed.

Hydrochemistry

As in chapter 2, an inverse correlation between LAS and NO_3 concentration was established for the unplanted system ($p<0.01$) and is again attributed to nutrient limitation of LAS-utilizing bacteria (see section 2.1.4). Section 6.5 further investigates the effect of LAS on the N cycle. However, no relationships were established for LAS with PO_4 or SO_4 even though the latter is a known end product of LAS mineralization (Swisher 1987).

The lowest rate of nutrient ion removal occurred in January, corresponding to the period of lowest air and bed temperature recorded during the study. Temperature associated removal of nutrients is well documented (Kadlec & Reddy 2001). Although low nutrient conditions were present in this study higher outflow concentrations were observed for the unplanted rather than planted mesocosms. This was more marked for PO_4 and SO_4 than NO_3 and is confirmed by the statistical data showing no significant differences for the latter. The particular difference in PO_4, following the poor initial removal, may suggest plant uptake (Brix 1997, Breen 1990, Gumbricht 1993) or greater degradation by rhizosphere microorganisms, with e.g. 50% removal attributed to plants in some studies (Luderitz & Gerlach 2002). However, evidence of plant nutrient uptake, especially during the dormant season, is controversial (Tanner 2001). The expected higher DOC in the planted systems due to root release was not observed. The low DOC observed in both systems is characteristic of the low enzyme activity also exhibited.

Enzyme activity

Greatest activity was measured for phosphatase>β-glucosidase>sulphatase confirming that reported in Chapter 2 and by other authors (Freeman *et al.* 1995, Chappell & Goulder 1992), with no significant correlations between activities ($p>0.05$). The low enzyme activity observed may reflect the low nutrient and organic carbon availability in the system. A positive correlation between β-glucosidase and air temperature was observed for the planted microcosms ($p<0.05$). This was the only relationship with temperature observed, and this phenomenon has been reported elsewhere (Shackle *et al.* 2000a). In addition both phosphatase and β-glucosidase activity decreased from the initial activity measured in September, especially for the unplanted systems that may suggest toxicity or lack of suitable substrate and nutrients. The possible toxicity of LAS to enzyme activity is investigated in section 6.2.

The presence and species of plants can have a marked effect on enzyme activity, e.g. an increase in phosphatase activity (Khan 1970, Neal 1973, Kiss *et al.* 1974 as quoted in Speir & Ross 1978). This effect may be indirect and caused by changes on organic matter and microbial populations brought about by the plants with highest activity when growth was most intensive (Speir & Ross 1978). However, the hypothesis outlined in section 4.1 stating that planted systems would exhibit greater enzyme activity than unplanted was unfounded as the opposite was observed. T-test analysis showed significantly ($p<0.05$) higher phosphatase activity in unplanted than planted microcosms but not for sulphatase and β-glucisidase ($p>0.05$).

Although pH is reported to have considerable effect on enzyme activity in soils (Speir & Ross 1978, Nannipieri *et al.* 1982) no correlation was evident in this study. However, as pH was relatively stable (approx. pH7) any regulatory effect on enzyme activity may not be detectable over such a narrow range.

In comparison to the enzyme activities reported in Chapter 2 measured in operational constructed wetlands, considerably lower activities were exhibited in this study by approx. 5-fold. However, the mesocosms were exposed to low nutrient inputs, low

organic matter and had only a short period to establish in comparison to the years of operation for the wetlands in chapter 2. Shackle (2000) in an experiment looking at the role of plants in water quality improvement in nutrient rich conditions in the mescocosms reported much higher activities but again higher in unplanted than planted mesocosms.

Microbial Respiration

Soil temperature has been reported as being the most important variable in predicting soil CO_2 emissions (Bridgeham & Richardson 1991) with a decrease in CO_2 evolution to dormant levels at temperatures below 4°C (Moore & Knowles 1989). In this study no significant correlations with bed nor air temperature was established. However, a sharp reduction in CO_2 evolved was evident in January, when temperature declined and mesocosm units frequently froze. LAS appeared not to be toxic to CO_2 respiration by microbes in the mesocosms. Soil respiration is reported as not sensitive to LAS exposure (Elsgaard *et al.* 2001a) and further investigated in chapter 6.

4.4.2 Effect of plant biomass on LAS removal

LAS

Significantly ($p<0.01$) greater LAS removal in planted than unplanted systems was observed in this experiment. Greater LAS removal efficiency by the higher plant biomass treatment further proves that plants play a significant role in enhancement of LAS degradation. This suggests that wetlands with a high plant biomass ratio will promote LAS removal to a greater degree than comparative low plant biomass ratio wetlands. Knaebel & Vestal (1992) reported that the amount of above ground plant biomass correlated with the initial rates of mineralization. Wiessner *et al.* (2002) reported that the total size of the root system did not significantly affect the amount of oxygen root release but was governed by the size of the above ground biomass. This suggests that if the plant biomass had been greater then enhanced rates of LAS biodegradation would likely be observed. Hence the hypothesis that greater plant biomass will result in greater LAS removal is accepted.

KBr was chosen as a tracer in this study due to its stability and ease of analyses (Tanner *et al.* 1998). Tanner *et al.* (1998) reported similar curves in a rain-free tracer study using bromide. Data from the tracer study conducted suggest slower diffusion rates in the planted mesocosms, especially in the high biomass treatment, and this is reflected in the correlation ($p<0.01$) established with LAS concentration in the low biomass treatment. The slower the diffusion rates then the greater the contact time between microorganisms and LAS within the wetland, promoting greater biodegradation. Greater removal has been observed in wetland systems with longer retention times. For example, greater removal of TN, TP and COD is reported with a 5 day retention time than a 2.5 day retention time (Breen 1997). Tanner (1994) reported a positive correlation between removal and retention time. Fisher (1990) and Marstener *et al.* (1996) stated that plant roots markedly affected the hydraulic flow profiles in the upper layers of gravel wetlands in comparison to unplanted controls (as quoted in Tanner *et al.* 1998). On the other hand, the faster diffusion rate for the unplanted control may suggest potential short-circuiting. Gravel substrate can cause problems with non-uniform and short-circuiting flow of wastewater through the wetland (King *et al.* 1997). Factors, such as clogging by solid particles, can lead to preferential flow paths occurring. However, channelling within planted wetlands has also been reported (Bavour *et al.* 1988 as quoted in King *et al.* 1997).

It is difficult to compare the continuous (Section 4.4.1) and batch loading methods used at this site due to the different volumes used. Breen (1997) reported statistical differences between continuous and batch loading treatments. However, the author reported that differences would be small in an operational context. In this study high removal of LAS was observed by both loading methods here with no evidence to recommend one method in particular, except for the potential lower costs and operation of batch loading. Hence, as reported above, the diffusion and retention time is more influencial than loading method.

Hydrochemistry

A similar trend with time was established between SO_4 and LAS concentrations for the two planted treatments ($p<0.05$). As shown in figure 1.7 SO_4 is released during LAS mineralization (Swisher 1987, Steber & Berger 1995). Hence LAS can act as a source of SO_4 in the wetland. This relationship was not identified in chapter 2

presumably due to the various other unidentifiable sources contributing to the SO_4 concentration measured. However, no relationship between LAS and NO_3 was identified in this study even though correlations were established in sections 2.1-2.3 and above in 4.4.1.

The general lower NO_3 concentration in the planted than unplanted systems may suggest plant uptake as an important removal mechanism with higher nutrient uptake during the growth season (Kadlec & Knight 1996). However, plant uptake was not an important PO_4 removal mechanism with concentrations again variable as reported in chapter 2, requiring further investigation as described in chapter 6. The distinct increasing temporal trend in SO_4 concentration peaks similarly to that of the tracer study as reflected in the correlation ($p<0.01$) established with Br^- ($p<0.01$) and LAS ($p<0.05$). Plant SO_4 uptake may be a possible influencing factor (Speir & Ross 1978) for the lower concentration observed in the planted control. The higher DOC concentration in the unplanted control may suggest that the DOC in the planting treatments may be utilized at a faster rate by the rhizosphere microbial community which is reported to have high and/or more active microbial populations (Knaebel & Vestal 1994).

Enzyme activity

Compared to section 4.4.1 no significant difference between treatments in enzyme activity was observed, except for sulphatase in the favour of unplanted mesocosms. This contradicts the hypothesis that planted mesocosms would exhibit greater activities and is thus rejected. The order of greatest enzyme activity again supports the data reported in chapter 2, section 4.4.1 above and elsewhere (Freeman *et al.* 1995), i.e. phosphatase>β-glucosidase>sulphatase. However, in comparison to section 4.4.1 an approximate 4-fold higher activities for phosphatase and β-glucosidase was exhibited associated with the higher nutrient input with levels more reflective of operational wetlands reported in chapter 2, especially Clutton. The stimulated activity may also reflect the warmer temperature (Kang & Freeman 1998), although no correlation was established. Higher activity associated with plant growth mechanisms reported elsewhere (Speir & Ross 1978) is unlikely due to the increase observed in both planted and unplanted mesocosms.

In contrast, the drop in sulphatase activity may suggest inhibition of this enzyme. The inverse relationship with temperature ($p<0.01$) further supports inhibition assumption. The high S availability may suppress sulphatase activity. Inhibition by SO_4 has been reported on phosphatase activity (Dinesh *et al.* 1995) and may be a possible limiting factor of sulphatase activity. However, it has been reported that sulphatase is not directly inhibited by sulphate but by sulphite (Tabatabai & Bremner 1970). Similarly inhibition due to PO_4 availability has been reported as a limiting factor (Tabatabai & Bremner 1970). However, no correlation was established in this study. Inhibition may be due to specific effects on microbial growth and subsequent enzyme synthesis or possible modification of the active site of the enzyme protein (Dinesh *et al.* 1995). Galstyan & Bazoyan (1974) (quoted in Speir & Ross 1978) demonstrated that sulphatase activity did not significantly correlate with SO_4 content of soil but with the organic fraction. Although no correlation was found in this study it is not possibly to confirm any relationship between sulphatase activity and substrate organic matter content. Possible inhibition of enzyme activity and subsequent nutrient cycling by LAS is suggested elsewhere (Jensen 1999) and is further investigated in chapter 6. The greater reduction observed in the planted than unplanted mesocosms may suggest plant mechanisms enhancing the inhibitory effect. However, from the data no further conclusions may be drawn.

4.4.3 Effect of plant species on LAS removal

LAS

The high LAS removal rates observed in this laboratory-scale experiment (>98% in planted systems) again highlights the potential for high LAS removal in constructed wetland systems as above. This study also confirms greater LAS removal in planted treatments in comparison to the unplanted gravel control. However, the difference between planted systems in terms of net percentage removal was small within an operational context (±2%) and significant ($p<0.001$). Greatest removal was observed in the following order;

Phragmites>*Salix*>*Juncus*>*Iris*>*Typha*>Unplanted

Variations in treatment by different plant species are published (Allen *et al.* 2002, Zhu & Sikora 1995) and several confirm the order of treatment efficiency reported in this study for some species. Greater removal by *Phragmites* than *Typha* for ammonia and BOD (Gersberg *et al.* 1986), ammonium and nitrate (Zhu & Sikora 1995) and TN and TP (House *et al.* 1994) are reported. Gersberg *et al.* (1986) attributed the better ability of *Phragmites* to pass oxygen into the root-zone to account for this occurrence. However, Burgoon *et al.* (1990) found *Typha* removed a significantly larger percentage of BOD_5 and total phosphate than the *Phragmites* and Coleman *et al.* (2001) reported *Typha* outperformed *Juncus* in wastewater treatment.

Although this experiment was conducted under controlled conditions it is recognised that environmental conditions may cause differences amongst plant species treatment performances. For example, Allen *et al.* (2002) reported different plant effects among species, including *Typha* spp. and bulrush, on organic matter removal was much greater at 4°C, during dormancy, than at 24°C (growing season). Other factors such as pH may also be influencial. *Phragmites* has an optimal pH of 2-8, *Typha* pH 4-10 and *Juncus* pH 5-7.5 (Reed *et al.* 1995). Hence pH of the input wastewater will largely affect treatment. In addition, as highlighted in section 4.4.2, plant biomass will also affect treatment. Hence the growth, above and below ground, and root penetration will also affect the outcome with *Phragmites* reported to have a much deeper root penetration in gravel than *Typha* (Reed *et al.* 1995).

Effect of treatment by a mixture of species has also been investigated with some evidence in support of mixtures exhibiting better treatment performance than monoculture (Coleman *et al.* 2001). However, competition between species requires further investigation for compatibility as, over time, one species may dominate the wetland to the detriment of growth of others, e.g. Coleman *et al.* (2001) found *Typha* was the superior competitor in plant mixture mesocosms, whereas *Juncus* is unlikely to be competitive (Tanner 1996).

Hydrochemistry

In this experiment the order of PO_4 removal, i.e. Gravel>*Typha*>*Iris*>*Phragmites*> *Salix*>*Juncus*, suggests that adsorption and precipitation rather than plant uptake is the main removal mechanism (e.g. Mann 1990) confirming earlier work in section

2.1. However, for SO_4, planted treatments, except for *Typha* and *Juncus*, enhanced treatment performance than the gravel control in the order of *Salix*>*Iris*>*Phragmites*>*Typha*>Gravel>*Juncus*, with plant uptake a possible mechanism (Speir & Ross 1978).

Percentage DOC removal in the unplanted control was significantly ($p<0.001$) higher than planted treatments of the order Gravel>*Phragmites*>*Typha*>*Juncus*>*Iris*>*Salix*. The lower removal by planted treatments may be reflective of the considerable higher DOC concentration measured in the opposite order to the above (i.e. *Salix*>*Iris*>*Juncus*>*Typha*>*Phragmites*>Gravel). This may be indicative of DOC exudates from the plant roots resulting in increases of the observed concentration and hence decreasing DOC removal.

4.4.4 LAS uptake by Plants

Plant uptake was not a significant LAS removal mechanism in this study over the 14-day period of the experiment. The sensitive radiochemical technique adopted to quantify uptake allowed trace amounts of the surfactant to be measured in the aerial plant tissue (<1% of initial radioactivity added). Due to the low amount of surfactant uptake loss of $^{14}CO_2$ via plant respiration considered as negligible. Hence the significant differences proposed between planted and unplanted wetlands in previous sections cannot be accounted for by plant uptake and the hypothesis that plant uptake of LAS is negligible is accepted.

Due to the presence of LAS in sewage sludge, via adsorption and poor anaerobic degradation (chapter 5), and subsequent use as fertiliser, a substantial proportion of plant uptake research with relation to LAS has focused on agricultural plants. Knaebel & Vestal (1992) measured similar low uptake (<1% initial activity) of LAS as ^{14}C in soybean and corn plant biomass. Figge & Schöberl (1989) in an investigation involving mesocosms containing bush beans, grass and radish, and another with potatoes found limited plant uptake of measurable amounts of ^{14}C (5.9-6.6% initial activity). Although Federle & Schwab (1989) investigated biodegradation of LAS by microbiota associated with *Typha* no attempt to quantify stem uptake was reported. The authors did note 1-5% of initial activity associated

with the roots, comparable to the 2.8% measured in this study. Uptake by wetland plants of other man-made compounds has been reported, e.g. ^{14}C radiolabelled explosives (Best *et al.* 1999), and of heavy metals, e.g. As (Otte *et al.* 1991) has been investigated.

Possible mechanisms to account for the detection of ^{14}C-LAS in the aerial plant include the uptake of the compound, or metabolites, by the roots and/or uptake through the foliage of $^{14}CO_2$ generated by the mineralization of the surfactant by rhizosphere microbes (Knaebel & Vestal 1992). In this study it is not possible to distinguish between exposure route or radiolabel form of the radioactivity detected in the aerial plant tissue. Other authors have also been unable to identify the radiolabel form encountered (Figge & Schoberl 1989, Knaebel & Vestal 1992).

Plant uptake studies in wetlands have mainly focused on nutrients with N uptake of 7% (Frankenbach & Meyer 1999) and 4-11% (Lin *et al.* 2002) quoted. However, several studies conclude that insufficient nutrient removal via plant uptake occurs as it only accounts for only a fraction of the overall removal (Tanner 2001, Brix 1997). Possible plant decomposition release of stored nutrients (Kadlec & Reddy 2001) has led to a debate on harvesting with no conclusive evidence on whether this is a significant and viable option. Although, according to the results of this study, irreversible LAS removal via harvesting is not a significant removal mechanism not harvesting may cause other problems that may affect treatment efficiency. For example, in constructed wetlands plants reach heights well over 6ft leading to flattening by wind and heavy rain (observed at the wetlands monitored in chapter 2). This may result in damage to the *Phragmites* resulting in possible impact on oxygen transport and nutrient uptake. However, support for the plants to limit damage can be incorporated in the design, e.g. thick string at sections across the wetland.

4.4.5 Effect of root oxygen release on LAS removal

Oxygen diffusion to the roots is a well established physiological characteristic observed in *Phragmites* with evidence from high numbers of aerobic bacteria in the rhizosphere (Hoffmann 1990) and quantitative analysis via electrodes (Armstrong *et al.* 1990, Brix 1990). *Phragmites* is characterised by aerenchyma that facilitates

internal gas transport of oxygen resulting in the aeration of rhizome and roots and release of oxygen to sediment (Armstrong & Armstrong 1988). In this study, significantly ($p<0.001$) greater LAS removal was observed which is possibly due to aeration of the rhizome in the *Phragmites* exposed to air in comparison to oxygen depleted conditions and the gravel control. Similarly, in a study on LAS in a wastewater pond, Federle & Schwab (1989) reported that oxygen levels were higher and surfactant concentration lower in water and sediment portion of the pond colonised by macrophytes. Allen *et al.* (2002) attributed differences in organic matter removal to apparent abilities of different species to increase root oxygen supply. Although *Phragmites* was the only macrophyte investigated in this study the relatively high oxygen transport ability of this species may explain the significantly higher LAS removal observed in section 4.4.3. However, as the oxygen level in the rhizome was not measured in this study further work is required to investigate the level of aeration by *Phragmites*.

In the field several factors could affect the oxygen transport and consequently LAS degradation. Factors affecting plant growth will affect treatment with greater oxygen transport reported in the growth season (Armstrong & Armstrong 1990). Oxygen release rate also depends on oxygen demand of biological and chemical processes in the surrounding medium, internal oxygen concentration, rhizosphere redox state and permeability of root-walls (Sorrell & Armstrong 1994, Wiessner *et al.* 2002) with a decrease in rates with tissue age (Brix 1997). As discussed in section 4.4.4 damage to the plants by extreme winds and rainfall could also potentially limit oxygen supply to the root zone.

Plant species may also affect oxygen transport (Brix 1997, Michaud & Richardson 1989). As reported above Gersberg *et al.* (1986) attributed greater ammonia and BOD removal by *Phragmites* than *Typha* to the better ability of *Phragmites* to pass oxygen into the root-zone. Several investigations have focused on oxygen transport in plant species, e.g. Michaud & Richardson (1989) reported clear differences between unplanted controls and planted systems with the latter exhibiting much greater oxygen levels with *Typha* greater than *Juncus*. *Typha* has also been investigated in relation to LAS and was reported to readily mineralize the surfactant whereas Duckweed did not (Federle & Schwab 1989). The authors suggested that

plant species determined the composition and biodegradation capacity of the microbial community associated with the roots and rhizosphere (Federle & Schwab 1989).

However, factors other than plant oxidation capacity alone would influence rhizome oxygenation. Oxygen transfer per unit of substrate requires consideration. Depth of root penetration, growth rate and biomass per unit area would affect species selection. *Phragmites* has a high potential productivity, deep rhizome and root system, and rapid establishment, spread and increase in shoot density (Tanner 1996). Significantly greater growth of *Phragmites* than *Juncus* (Tanner 1996) is reported. Parr (1990) reported no apparent difference in biomass of *Phragmites* growing in soil or gravel substrate. However, the author reported shallower root penetration in gravel, similar to Adcock & Ganf (1994) (as quoted in Tanner 1996) at 25cm. However, in contrast, Gersberg *et al.* (1986) reported penetration at over 60cm depth. Zhu & Sikora (1995) attributed greater treatment performance by *Phragmites* than *Typha* to the density of the root biomass. However, in comparison to *Juncus*, *Typha* grows deeper (Michaud & Richardson 1989) and hence, coupled with its greater rhizosphere oxygenation (Wiessner *et al.* 2002), may enhance treatment. However, species of similar oxygenation capacity but greater stand density would have a greater effect.

Degradation under oxygen-poor conditions (see chapter 5) or possible release of oxygen storage in the roots may explain the relatively high LAS removal observed for the *Phragmites* exposed to nitrogen. Similarly Michaud & Richardson (1989) tested dead plants and cut off plants covered with paraffin to seal aerenchymal tissue to determine if the oxygen presence was due to diffusion or plant enzymes. The dead plants had much less oxygen in comparison to live-planted treatments, however, the waxed plants exhibited greater oxygen than the unplanted controls. The authors attributed this to oxygen storage in the roots and subsequent diffusion into solution (Michaud & Richardson 1989).

Although critics question whether oxygen release in the rhizospohere makes a significant difference in wastewater treatment, this study may suggest that the rhizosphere oxygenation by plants enhanced LAS degradation in wetlands. However,

the results in section 4.3.3 indicate that *Juncus* enhances LAS removal more than *Typha* even though the former is reported to facilitate lower rhizosphere oxygenation (Michaud & Richardson 1989). Hence this may suggest that other plant mechanisms are also involved in LAS degradation and further work is required.

The hydrochemical data was variable with significant differences between treatments only observed for SO_4 ($p<0.01$) suggesting that plant mechanisms do not enhance PO_4 removal (Tanner 2001). The slightly higher DOC levels in the planted treatments possibly suggests DOC leakage from the plants roots. This is in conflict to the data in figure 4.8 whereby higher DOC concentrations were measured in the unplanted control in the field study. However, as the difference was not significant ($p>0.05$) in this study in comparison to the gravel control, coupled with the data reported in section 4.4.6 below, this is not responsible for the differences between treatments in LAS removal in this study.

4.4.6 Effect of plant DOC exudates on LAS removal

The effect of plant DOC exudates on LAS removal was addressed in terms of co-metabolism, which refers to degradation of a compound by an organism in the presence of a second substrate that supports growth. It has been suggested that co-metabolism could be more profound in LAS mineralization than primary biodegradation (Brown 1995). Knaebel *et al.* (1990) reported that organic plant material reaching the soil induced synthesis of LAS degrading enzymes and sustained LAS degrading microorganisms. Drewes & Jekel (1998) found elimination of halogenated organic compounds under anoxic conditions probably based on co-metabolism with DOC functioning as a co-substrate. Zhu & Sikora (1995) reported greater NO_3 removal in wetlands with additional carbon source, whereas Gersberg *et al.* (1983), using mulched plant biomass as a cheap source of carbon, observed greatly enhanced TN removal. May *et al.* (1990) attributed the higher population bacteria densities in rhizosphere biofilm to exudation of soluble organic components from rhizomes and decay of dead plant material.

However, in this study supplemental plant-derived DOC or glucose did not enhance greater primary degradation or mineralization. Hence, from these data, the hypothesis

that plant DOC exudates will have a significant effect on LAS degradation is rejected. Although glucose has been reported elsewhere as a source of carbon for bacteria, its addition has the disadvantage of being expensive and is reported to only have a slight effect on N removal in wetland soils (Davidsson & Stahl 2000). Hence this suggests that the sub-optimal conditions for LAS removal observed in chapter 2 comparative to the high removal in the small scale experiments thereafter conducted cannot be explained by DOC facilitated microbial metabolism.

Possible explanations for the lack of co-metabolism include that it is not an important process in LAS degradation or that the bulk addition of high concentration of carbon, either as DOC or glucose, could to be toxic to LAS-utilizing bacteria. Under in-situ natural conditions DOC would be leached slowly into the system and may have a possible lower toxic effect. Alternatively, the DOC collected which is utilized by micro-organisms may have degraded before the solution was added to the gravel biofilm samples. However, a co-metabolising effect was not observed when glucose was added. Hence, from the data presented the hypothesis is rejected.

Additional DOC may also affect other processes in the wetland not investigated in this study. For example, Shackle *et al.* (2000b) investigated the contribution of carbon supply as a possible regulator for rates of activity of extracellular enzymes involved in organic matter decomposition. The authors reported manipulation of the carbon supply can affect enzyme activity both positively (β-glucosidase) or negatively (sulphatase). In addition plant exudates will vary according to season, plant species, age and development, temperature, pH, CO_2 concentration and light intensity (Grayston *et al.* 1996).

4.4.7 LAS degradation by rhizosphere bacteria

The significant difference between LAS mineralization by intact rhizosphere biofilm comparative to the washed roots suggests that the rhizosphere bacteria play a crucial role in LAS biodegradation. The rhizosphere has a higher and more active microbial biomass than the surrounding bulk soil (May *et al.* 1990, Knaebel & Vestal 1994), is highly active and is possibly more so towards detoxification of pollutants in soil (de

Wolfe & Feijtel 1998). Whipps & Lynch (1986) reported greater microbial population by 1-2 orders of magnitude than in soil without roots, whereas Federle & Schwab (1989) reported rhizosphere microbes tend to have faster growth rates and differ in nutritional requirements and metabolic capacities. In addition, although not investigated in this study, biofilms can also form on the submerged lower stem and leaves and provide even greater surface area for attached microbial growth (Chappell & Goulder 1994). Differences between *Phragmites* rhizosphere biofilms and gravel biofilms in wetlands have been reported (Williams *et al.* 1994) with the former supporting higher potential rates of N transformations per unit surface area.

In section 4.4.5 oxygenation of the rhizosphere via plant transport was possibly suggested as a significant mechanism in the enhancement of LAS removal. This experiment has suggested that the rhizosphere also enhances treatment via providing attachment sites for greater microbial activity as no aerial plant tissue was included in this experiment hence excluding oxygen transport. The different and/or more active microbial community in the rhizosphere biofilm comparative to that on the gravel was highlighted by the less distinctive lag phase in the presence of roots as compared to the gravel. The lag phase was much less distinctive in the presence of roots in comparison to that observed with gravel biofilm in mineralization tests in chapter 3. Thus the hypothesis that rhizosphere microbial communities will enhance LAS degradation is accepted. Knaebel & Vestal (1994) examined LAS biodegradation with and without plants and concluded that the rhizosphere increased initial rates of mineralization without affecting total amount of $^{14}CO_2$ emitted. The authors state this implies rhizosphere microbial communities metabolise LAS at greater rates than those in the bulk soil. However, the similarity in net mineralization may suggest limitation by other soil-chemical interactions. The removal observed for the washed roots suggests that the bacterial community in the biofilm was able to recover and re-establish on the root and rhizosphere surface over time.

In chapter 3 prior exposure was identified as a potential factor influencing LAS removal with history of exposure resulting in more rapid and greater LAS degradation. This was not explored as a factor with respect to rhizosphere microbial communities. However, Federle & Schwab (1989) reported that prior exposure does not determine rhizosphere LAS mineralization. The authors suggested that

acceleration of mineralization could result from elevated oxygen levels near the roots and/or composition and activity of rhizosphere inhabitants. Hence the plant itself rather than previous exposure to the surfactant determines the composition and biodegradation capacity of the rhizosphere microbiota (Federle & Schwab 1989).

4.5 CONCLUSION

Although in recent years the emphasis on the importance of plants in constructed wetlands has become debatable, this study suggests that plant-microbes interactions in the rhizosphere can significantly enhance LAS removal. Gravel unplanted controls exhibited significant LAS removal but a marked improvement in the presence of plants were observed. From the series of experiments conducted on the possible plant mechanisms facilitating LAS degradation, the following conclusions can be drawn;

1. Greater plant biomass significantly enhanced LAS removal
2. Plant species affected LAS removal with *Phragmites* promoting greatest and *Typha* the lowest removal
3. LAS removal was not enhanced by increased DOC exudation nor plant uptake. The most likely impact of plants appears to be through oxygenation of the root zone and supporting LAS degrading microbial communities in the rhizosphere.

However, plant effects will depend on the species, wetland design, wastewater characteristics and loading rate.

CHAPTER 5: Manipulation of Factors Affecting LAS Removal

In chapters 3 and 4, factors influencing LAS removal such as biofilm presence and activity, compound structure and plant effects were examined. In this chapter several other factors were investigated and manipulated for possible effects on LAS removal efficiency. Environmental parameters of temperature, pH and oxygen availability are discussed along with effects of water hardness, presence of other surfactants and desorption. These conditions are highly variable in wetlands and thus several laboratory-based experiments were conducted to determine the effects of these factors on LAS removal. In this chapter, extreme changes in conditions are evaluated in order to assess LAS treatment efficiency.

5.1 TEMPERATURE

5.1.1 Introduction

Conventionally it is perceived that microbial activity increases with increasing temperature until an optimum is reached (Kadlec & Reddy 2001). In wetlands, water and substrate temperature variations cause changes in microbial activity that, in turn, affect water quality amelioration (Kadlec 1999). Several studies have examined the effect of temperature on LAS degradation under normal environmental conditions (e.g. Inaba *et al.* 1988) and manipulative laboratory experiments (e.g. Terzic *et al.* 1992). Kikuchi (1985) reported that water temperature affected LAS biodegradation in a die-away test using river water with a significant reduction at low water temperature (10°C). Terzic *et al.* (1992) measured higher primary biodegradation rate at 23°C than 14°C in estuarine waters (e.g. C_{12} $t_{1/2}$ of 4.3 days at 14°C and 2.6 days at 23°C). Litz *et al.* (1987) reported warming of soil in the spring caused acceleration of degradation. However, interactive factors may affect the optimum temperature for microbial activity in a wetland such as the hydraulic loading rate, effluent quality, vegetation and substrate (Kadlec & Reddy 2001). In terms of adsorption, however, temperature has not been thoroughly investigated for LAS. Adsorption would be expected to be less affected by changes in temperature as it is not a biological mechanism in contrast to biodegradation.

The effect of temperature on an annual and latitudinal basis was approached in chapter 2. Although it is not possible to influence climatic temperature it is possible to alter the temperature at which wastewater is treated in wetlands. This study further investigates temperature and LAS removal (primary degradation and mineralization) and adsorption in a series of laboratory manipulated experiments. It was hypothesised that LAS degradation would increase with higher temperature but that adsorption would largely be unaffected.

5.1.2 Methods

Gravel collected from the Brynsiencyn wetland (top 10cm) was monitored for LAS primary degradation (section 3.2.1.a) and mineralization (section 3.2.1.b) at 5, 20 and 30°C. In addition adsorption tests as described in section 3.2.2 were conducted at 5 and 20°C for the Brynsiencyn, Rosset and Clutton gravel. Repeated measures

ANOVA tests were applied to data that conformed to the normal distribution and had homogenous variance using Minitab^{TM} version 13.1 (Minitab Inc. 2000).

5.1.3 Results

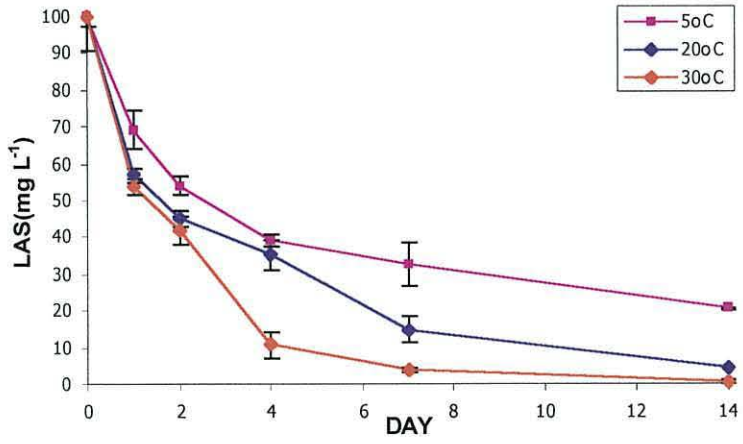
Biodegradation

Figure 5.1a shows primary degradation of LAS at the three different temperatures investigated with greatest removal observed at a rate increasing with temperature. First order kinetics were observed at each temperature with the calculated k and $t_{1/2}$ values expressed in table 5.1 below. The statistical analysis suggests significant differences between treatments ($F=11.07$, $p<0.05$), however, post hoc Tukey test revealed that this was only significant for comparison of data for 5 and 30°C.

As shown in figure 5.1b an initial faster and higher maximum yield mineralization was observed at 30°C. An apparent longer lag phase (4-5 days) of $^{14}CO_2$ production at 5°C is evident after which mineralization proceeded at a low rate indicating that mineralization was limited at this temperature, as indicated by the high half-life. The first order kinetics calculated for the initial 8 days (see section 3.3.1) demonstrating the faster initial mineralization rate at higher temperature is shown in table 5.1. Statistical analysis suggested that significant differences between treatments were observed ($F=48.139$, $p<0.01$). Figure 5.2 shows the linear relationship between increasing temperature with primary biodegradation and mineralization.

Figure 5.1: Temperature effect on LAS removal for:

(a) Primary biodegradation



(b) Mineralization

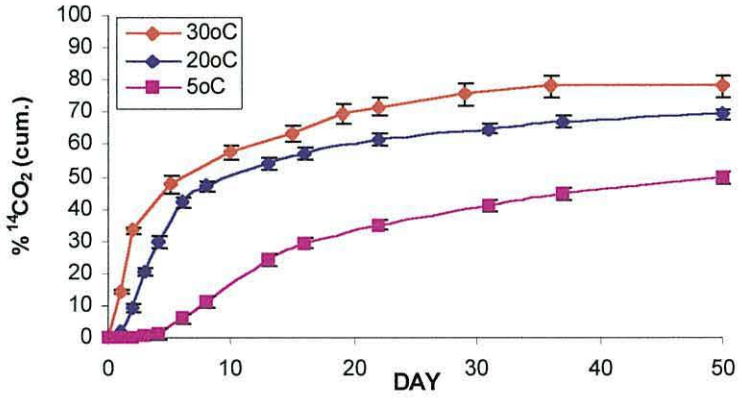


Figure 5.2: Linear relationship of temperature with primary biodegradation and mineralization.

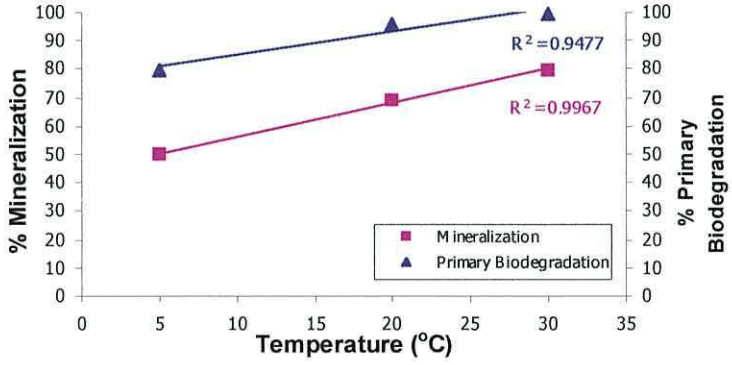


Table 5.1: Rate constants (k) and half-life ($t_{1/2}$) for LAS primary degradation and mineralization (units = d^{-1}) at 5, 20 and 30°C.

	5°C	20°C	30°C
Primary			
k (d^{-1})	0.10	0.22	0.38
$t_{1/2}$ (d)	6.93	3.15	1.83
Mineralization			
k (d^{-1})	0.02	0.09	0.127
$t_{1/2}$ (d)	46.8	7.80	5.44

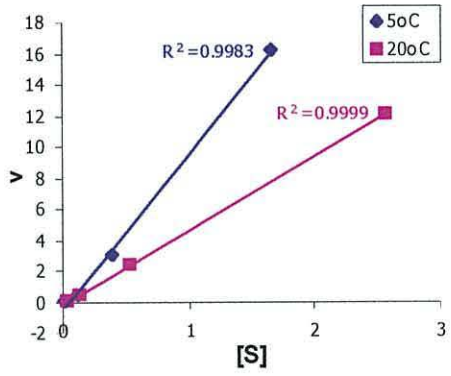
Adsorption

The adsorption isotherm conducted at 5 and 20°C for the Rosset, Brynsiencyn and Clutton gravel respectively are plotted in figure 5.3a-c. The isotherms were linear

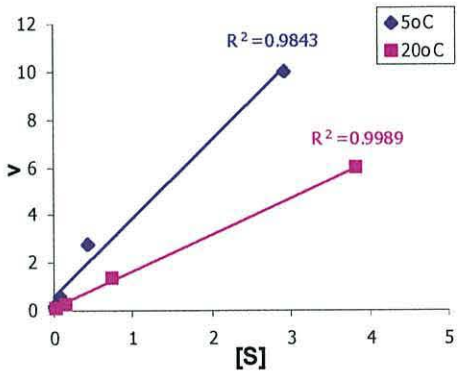
(R^2>0.99) for the range of concentrations tested (0-5mg L^{-1}) for each sample and suggest that greater adsorption was observed at lower temperature. Figure 5.4 shows the Freundlich isotherms calculated as described in section 3.3.2. Freundlich parameters, K and $^1/_n$ determined by regression analysis (min R^2=0.99), are summarised in table 5.2 demonstrating the greater LAS adsorption capacity at lower temperature.

Figure 5.3: LAS adsorption isotherms at 5 and 20°C for:

(a) Rosset



(b) Brynsiencyn



(c) Clutton

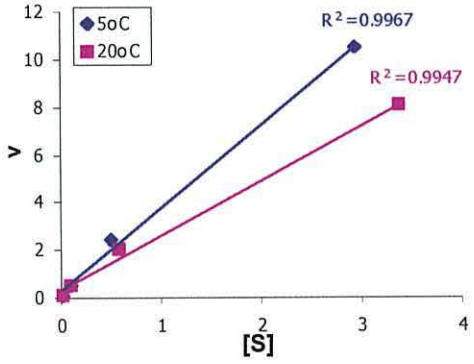
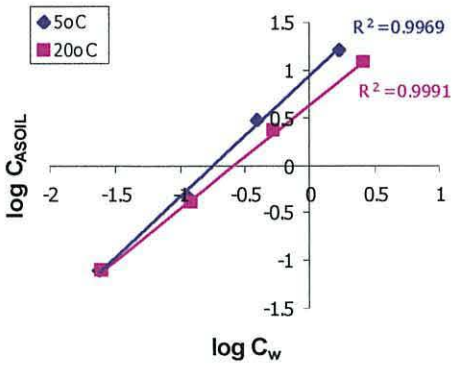


Table 5.2: Freundlich Constants for LAS adsorption on gravel at 5 and 20°C.

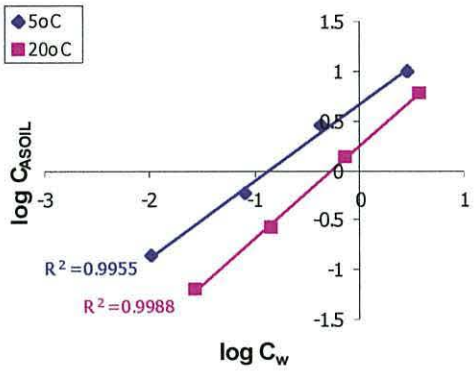
Temp.	Rosset		Brynsiencyn		Clutton	
(°C)	*K*	$^1/_n$	*K*	$^1/_n$	*K*	$^1/_n$
5	8.7	1.27	4.6	0.78	4.2	0.92
20	4.4	1.08	1.7	0.82	3.1	0.93

Figure 5.4: Freundlich isotherms at 5 and 20°C for:

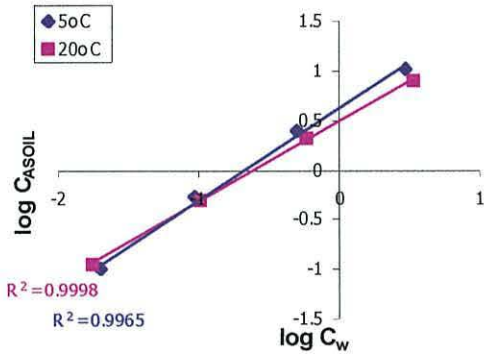
(a) Rosset



(b) Brynsiencyn



(c) Clutton



5.1.4 Discussion

Biodegradation

The results suggest that LAS biodegradation is dependent on temperature. Temperature change from 5 to 20°C can induce 20% greater net LAS mineralization, with a further 10% at 30°C. This was more evident in the $t_{1/2}$ values with, for example, 6.9, 3.1 and 1.8 days calculated for LAS primary biodegradation for 5, 20 and 30°C respectively. However, the percentage increase with 6-fold increase in temperature was possibly lower than expected. This reflects the relatively high removal observed at 5°C for both primary biodegradation and mineralization which is discussed below. Optimum temperature for LAS degradation in wetlands has been reported at 20-30°C (Inaba *et al.* 1988). In this study, of the range tested, 30°C promoted greatest LAS degradation and is thus in agreement with previous work. Dorfler *et al.* (1996) investigated LAS mineralization at temperatures between –2 to 20°C and demonstrated good correlation with increasing temperature. Inaba *et al.*

(1988) monitored the activity of LAS-utilizing bacteria under different temperatures (5-30°C) using a plate cultivation method with LAS as the only C source and reported higher growth at higher temperatures.

In this study low temperature effectively limited mineralization, with an increased lag phase and a net 30% lower yield at 5°C in comparison to 30°C. Dorfler *et al.* (1996) and Branner *et al.* (1999) reported temperature limited LAS removal with negligible mineralization at 3°C or below. Dorfler *et al.* (1996) observed an extension of the lag phase at low temperature in soils. Inaba (1992) reported no LAS biodegradation at 5°C after 2 weeks. A negative effect on LAS degradation by low temperature has also been reported by Litz *et al.* (1987), Palmisano *et al.* (1991) and Quiroga *et al.* (1999). However, in this study, primary biodegradation was less affected by low temperature with net c.80% removal observed at 5°C. Del Bubba *et al.* (2000) also reported high LAS primary biodegradation rates in a wetland field study at temperature values as low as 5-9°C. This is in contrast to Quiroga *et al.* (1999) who reported that only 5% primary degradation at 5°C in comparison to almost 100% at 20°C and Inaba (1992) who reported that above 7°C bacteria can degrade LAS. However, a 2- or 3-fold increase in the half-life at the lower temperature in comparison to 20 and 30°C respectively were observed in this study. The hypothesis that LAS degradation will increase with higher temperatures is accepted.

Although no statistical trend was observed between LAS removal and air or bed temperature in Chapters 2 and 4 this study suggests that temperature can directly influence LAS degradation. A positive correlation of removal with temperature has been reported elsewhere (e.g. Dorfler *et al.* 1996, Palmisano *et al.* 1991). Air temperature at the Brynsiencyn site reached a mean of 10.7°C which is well below the 30°C that promoted greatest LAS removal in this study and may be a limiting factor resulting in the lower removal observed in the field than in laboratory studies in chapters 3 and 4. Hence this indicates that warmer bed temperatures could enhance LAS removal. Since no insulation advantage was found for the planted than unplanted mescosms in chapter 4 this may suggest that other forms of insulation may benefit LAS removal, e.g. straw (Mæhlum *et al.* 1995). However, in large scale wetlands not harvesting the plants may provide extra insulation (Mæhlum *et al.* 1995).

Adsorption

Greater adsorption was exhibited at 5 than 20°C in this study by >10%. This was unexpected due to the lower sensitivity of such reactions in comparison to biological processes. Hence the hypothesis that LAS adsorption would be largely unaffected by temperature is rejected. This is in contrast to Inaba *et al.* (1988) who reported adsorption was not altered by temperature changes in the range of 5-25°C. However, in wetlands temperature has been reported to affect P adsorption (Kadlec & Reddy 2001) but with the opposite trend reported, i.e. a decrease in adsorption as temperature was lowered from 20 to 5°C (Gardner & Preston-Jones 1973). Possible explanations for the increased adsorption include possible slower movement of molecules due to the lower temperature resulting in greater contact with the gravel surface. However, due to the lack of research in this area no firm conclusions can be drawn. Hence this phenomenon requires further investigation.

5.2 pH

5.2.1 Introduction

It is well known that pH can affect microbial activity in the environment. The low pH in many natural wetlands (e.g. bogs) has been proposed as a dominant factor in the low decomposition rates (Valentine *et al.* 1994). Wetland pH related research has mainly focused on soil enzyme activity (e.g. Nannipieri *et al.* 1982) and subsequent effects on nutrient cycles. In constructed wetlands, pH can also influence microbial activity involved in the degradation of pollutants. Hence, under varying pH conditions LAS biodegradation may be potentially affected, with reports of significant changes in removal efficiency with pH manipulations (e.g. Litz *et al.* 1987, Dorfler *et al.* 1996, Küchler & Schnaak 1997). Similarly, in terms of LAS adsorption, pH is reported as an influencing factor. Litz *et al.* (1987) demonstrated that soil characteristics of pH, as well as Fe oxide and humic content, influenced sorption of LAS. Küchler & Schnaak (1997) investigated LAS adsorption in relation to the pH of soils and reported that under more acidic conditions adsorption is higher.

The pH in constructed wetlands is largely determined by the wastewater source. However, it may be possible to alter the influent pH via buffering in order to maximise LAS degradation and/or adsorption. In this study addition of acid or alkali to the LAS solution exposed to wetland derived gravel was used to assess manipulation of extreme pH changes in primary degradation, mineralization and adsorption studies. It was hypothesised that degradation would decrease with extreme changes in pH.

5.2.2 Methods

Gravel collected from the Brynsiencyn wetland (top 10cm) was monitored for LAS primary degradation, mineralization and adsorption (see section 3.2) at pH 2, 7 and 12. An Orion 720A pH meter was used to determine pH, following the addition of concentrated hydrochloric acid or sodium hydroxide, for the appropriate solutions. Repeated measures ANOVA tests were applied to data that conformed to the normal distribution and had homogenous variance using Minitab™ version 13.1 (Minitab Inc. 2000).

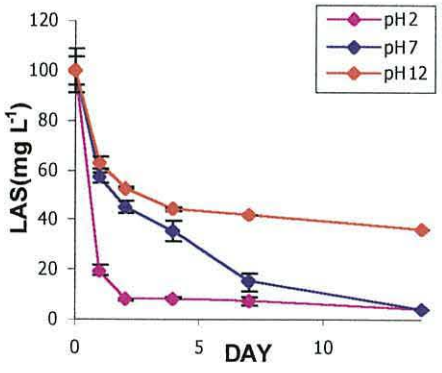
5.2.3 Results

Biodegradation

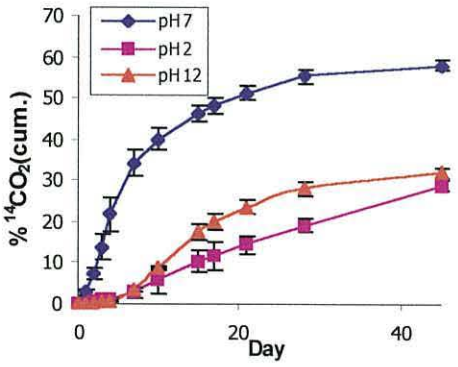
Figure 5.5 shows LAS degradation in response to changes in pH. Primary degradation at the higher pH was significantly less than at pH 7 suggesting that microbial inhibition potentially occurred (Fig. 5.5a). However, at low pH an initial lower LAS concentration was measured but a similar end yield, possibly suggesting precipitation. Both low and high pH lowered the mineralization rate and yield in comparison to the neutral control (Fig. 5.5b) by 25-30% and an increased initial lag phase of approximately 10 days. The data were analysed using repeated measures ANOVA to look for significant differences between treatments, and within treatments, over time. Highly significant differences for both primary biodegradation ($F=12.632$, $p<0.01$) and mineralization ($F=33.473$, $p<0.001$) were observed. Post hoc Tukey tests showed that no significant differences between the pH 7 and 12 treatments for primary biodegradation, whereas no significant differences between pH 2 and pH 10 was recognised for the mineralization tests.

Figure 5.5: LAS response to pH for:

(a) Primary biodegradation



(b) Mineralization



Adsorption

The linear ($R^2>0.99$) adsorption isotherm conducted at pH 2, 7 and 12 is plotted in figure 5.6 and suggests greatest adsorption was observed in the order of pH2>pH12>pH7. This is reflected in the Freundlich isotherms (min $R^2=0.99$) (Fig. 5.7), calculated as described in section 3.3.2, and order for K as summarised in table 5.3.

Figure 5.6: LAS adsorption isotherms at pH 2, 7 and 12.

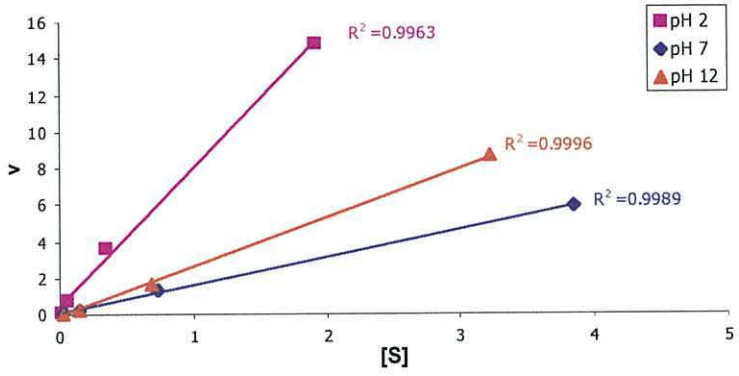


Figure 5.7: Freundlich adsorption isotherms at pH 2, 7 and 12.

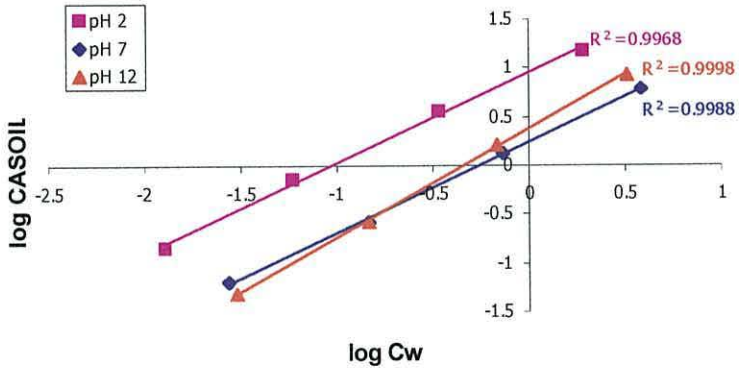


Table 5.3: Freundlich Constants for LAS adsorption at pH 2, 7 and 12.

pH	Freundlich Constants	
	K	$^1/_n$
2	8.9	0.926
7	1.7	0.925
12	2.4	1.117

5.2.4 Discussion

Biodegradation

LAS degradation exhibited a distinct response to changes in pH, with extreme low or high pH (pH 2 and 12) detrimental. Characteristically, microorganisms will have a pH value at which optimal rate is exhibited. At either side of this optimum pH biodegradation of the surfactant will be lower. Hence, pH can control the activity of surfactant degrading microorganisms (Fytianos *et al.* 1998b). This may be explained

by pH influencing the ionisation groups of enzymes or causing irreversible inactivation of enzymes (Lehninger 1982). Dorfler *et al.* (1996) investigated the influence of soil pH on LAS mineralization and reported higher degradation capacity for soils exhibiting a higher pH (7.2) in comparison to the other two soils at pH 3.4 and 4.5. This is in agreement with the finding of greater mineralization at pH 7 than pH 2 in this study. The Brynsiencyn wetland studied usually received wastewater with pH of 6.6±0.3 (section 2.2). Thus the microbial community would be well adapted to the conditions which exhibited greatest overall degradation in this study.

The apparent rapid disappearance of LAS measured via HPLC at low pH, in comparison to the slow mineralization observed at the same pH, may suggest possible precipitation of the surfactant or chemical dissociation under these extreme conditions. Hence this would result in a lower concentration measured via HPLC.

The results from this study suggest that any form of extreme pH manipulation would be detrimental to LAS removal mechanisms facilitated by microorganisms in a wetland. It is also essential to consider other processes that may be affected. Substantial research has been conducted on effect of pH on enzyme activity involved in nutrient cycling (Speir & Ross 1978, Tababatai 1982, Sinsabough *et al.* 1991). A high hydrogen ion (pH 1-2) or hydroxyl ion (pH 12-14) concentration tends to disrupt the ionic and hydrogen bonds required to maintain the active conformation if enzyme proteins (Frankenberger & Johanson 1982). Hence detrimental effects on wetland enzyme activity would be expected at such extreme pH values.

Adsorption

Greater adsorption was exhibited at pH2>pH12>pH7 in this study. pH has been identified as an influencing factor on LAS adsorption in several publications (Brownawell *et al.* 1997, Küchler & Schnaak 1997, Fythianos *et al.* 1998b). Greater LAS adsorption at low pH has been reported (Küchler & Schnaak 1997, Fytianos *et al.* 1998b). Küchler & Schnaak (1997) found a positive relationship between acidic soil pH and adsorption suggesting that the higher amount of positively charged surfaces at low pH values resulted in greater adsorption of the anionic substance. Fytianos *et al.* (1998b) investigated LAS adsorption at pH 2-12 and reported an

increase in adsorption with decreasing pH due to higher positive charge of colloidal surfaces. In addition, at low pH the LAS molecule is undissociated and thus less hydrophilic than at high pH resulting in possible greater adsorption.

Decreasing LAS sorption on soils with increasing pH is reported (Fytianos *et al.* 1998b). Similarly this has been observed for non-ionic surfactant APE on materials with pH-dependent surface charges, e.g. quartz and silica (Brownawell *et al.* 1997). However, in this study greater adsorption was found at pH 12 than pH 7. This may possibly be explained by a similar phenomenon described in chapter 6 for PO_4 adsorption. Increased PO_4 sorption at elevated pH due to formation of stable Ca-P complexes is reported (Mann 1990) and hence may possibly be also occurring for LAS. Evidence of formation of Ca-LAS compounds is reported below (section 5.4) with regard to water hardness (Ferrer *et al.* 2000).

5.3 OXYGEN AVAILABILITY

5.3.1 Introduction

Degradation of LAS can also be affected by dissolved oxygen concentration. High aerobic LAS removal is well documented and forms the basis of statutory regulations for surfactant degradation (Section 3.1). Since the major part of the biosphere is aerobic, priority has been allocated to the assessment of degradation under oxygenated conditions. However, LAS is also encountered under anaerobic conditions, either permanently or temporarily, in several environments, e.g. river sediments, anaerobic sludge digestors and wetlands. Currently there is debate in the literature on whether LAS degradation occurs under oxygen poor conditions.

It is argued that ω-oxidation initial attack of the alkyl chain and cleavage of the benzene ring requires molecular oxygen to occur (Scott & Jones 2000, Steber & Berger 1995). Hence under oxygen poor conditions degradation via these pathways is unlikely and consequently several authors conclude that current evidence suggests LAS degradation does not occur in these circumstances. This is supported by Jensen (1999) who examined results from 10 studies of LAS treated sewage sludge and found anaerobically treated sludge had LAS concentrations considerably higher than the aerobic treatments. Federle & Schwab (1992) examined the mineralization of ^{14}C-LAS in anaerobic sediments of wastewater ponds and demonstrated that the anaerobic microbial community was not able to mineralize LAS.

However, on the other hand, it is argued that degradation does occur under oxygen poor conditions with supporting evidence for alternative pathways being proposed. For example, Denger & Cook (1999) suggested that not all desulphonation reactions require oxygen with desulphonation of LAS by anaerobic bacteria reported. In addition, generally, low LAS concentrations are observed in oxygen limited environments in the field, e.g. river sediments (Waters & Feijtel 1995). The majority of research against LAS degradation in oxygen poor conditions is based upon treatment of digested sludge due to the adsorption of the surfactant onto sludge during the primary settling process. However, the current methodology may not be representative of environmental conditions and consequently may be underestimating degradation capacity. In addition, due to the high test compound concentrations

commonly employed in anaerobic tests for analytical purposes, inhibitory effects may be present (Garcia-Morales *et al.* 2001).

Thus the occurrence and extent of LAS degradation under oxygen poor conditions is currently a topical subject in the literature. It is an applicable concern for wetlands due to the anoxic or anaerobic environments within the system. This study examined the effect of oxygen availability on LAS removal by biofilm microbial communities in constructed wetlands. Section 4.4.5 highlighted greater LAS removal for planted systems exposed to air in comparison to nitrogen exposure. In addition a decrease in LAS mineralization was observed with increasing depth in section 3.2.1, corresponding to a decrease in oxygen availability. Hence in this study, degradation under low and high oxygen conditions were examined with gravel samples from the Brynsiencyn wetland bubbled with air or oxygen-free nitrogen and LAS removal monitored. It was hypothesised that LAS degradation would be higher in the oxygenated treatment and that lower degradation would be exhibited for the low oxygen treatment.

5.3.2 Methods

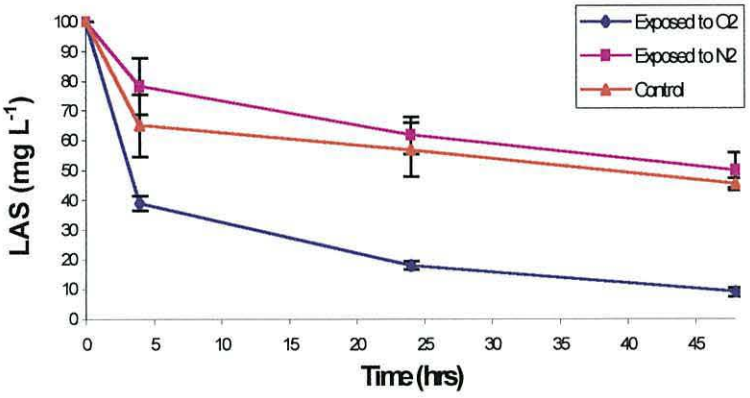
Three replicate 50g of gravel collected from the Brynsiencyn wetland were placed in plastic containers and 100ml of 100mg L^{-1} LAS solution added and bubbled separately with air or oxygen-free nitrogen for 48 hours. A control with gravel and LAS solution with no additional gas introduction was also incorporated. Samples were taken at 4, 24 and 48hr intervals and preserved with formaldehyde prior to HPLC analysis as described in section 2.1.2. Repeated measures ANOVA tests were applied to data that conformed to the normal distribution and had homogenous variance using Minitab™ version 13.1 (Minitab Inc. 2000).

5.3.3 Results

Figure 5.8 demonstrates greater LAS disappearance with the treatment subjected to oxygen rich conditions with net >90% removal observed after 48 hours. In comparison, the control and the oxygen depleted treatments exhibited similar lower levels of LAS removal of net 55 and 50% respectively. Greatest removal was observed during the first 4 hours in all three treatments. Repeated measures ANOVA revealed significant differences between treatments and within treatments over time

(F=13.888, $p<0.01$), with post hoc Tukey test revealing no significant differences between the oxygen depleted treatment with the control.

Figure 5.8: LAS removal for the unexposed gravel control, oxygen-free gravel and oxygenated gravel treatments.



5.3.4 Discussion

Oxygen appears to play a critical role in LAS primary biodegradation by wetland microbial communities. In constructed wetlands oxygenation is largely dependent upon wastewater source, flow rate, type of substrate, plant root release as well as climatological influences. The greater removal of LAS found in this study under more oxygen-rich conditions was as expected due to the well established rapid aerobic degradation of the surfactant. Krueger *et al.* (1998) reported increased LAS degradation rate in sewage contaminated groundwater with increased dissolved oxygen concentration. Comparison of the effect of oxygen levels on mineralization would be proved useful in this study. However, due to the hazards associated with radiochemicals this was not possible.

While oxygen serves as an electron acceptor in an aerobic environment, alternative acceptors such as sulphate, nitrate or carbonate are required in oxygen depleted conditions to eventually yield H_2S, N_2 and/or NH_3, and CH_4 respectively. Hence such reactions will depend upon the availability of organic and inorganic substrates, redox potential and types of bacteria present. This study suggests that LAS degradation in constructed wetlands may proceed at a faster rate in aerobic rather than oxygen depleted environments. Chapter 4 concluded that greater LAS degradation in the rhizosphere would be greater due to raised oxygen levels and is support by other authors (e.g. Federle & Schwab 1989). A similar phenomenon has been noted for

some soil enzymes involved in nutrient cycling in wetlands with greater activity under aerobic than oxygen poor conditions (McLatchey & Reddy 1998, Reddy & Patrick 1975).

Under O_2 depleted conditions c.50% LAS removal was observed supporting other work (Denger & Cook 1999, Federle & Schwab 1992). This is in contrast to several authors, such as McEnvoy & Giger (1986) and Matthijs & De Henau (1987), who associated no LAS degradation under oxygen depleted conditions in sludge. In wetlands the presence of standing water may result in oxygen-poor conditions in the substrate creating a reducing environment. Significant LAS removal in wetlands has been reported in previous chapters. The data from this study may support the hypothesis that LAS degradation can occur in oxygen depleted as well as aerobic environments in a wetland, although greater degradation would be expected in the more oxidised zones (e.g. rhizosphere and substrate surface). Oxygen availability may also possibly affect LAS adsorption and have possible indirect consequences on biodegradation via bioaccumulation influences. Litz *et al.* (1987) reported a slight increase in adsorption coefficient measured at low redox potential in comparison to more aerobic conditions. However, this may be related to a slower flow rate which would be likely to result in a more reduced environment in the substrate.

Manipulation of the oxygen level prior to or during wetland treatment may be possible in order to promote greater and faster LAS degradation. For example, aeration of the influent wastewater would introduce higher oxygen levels to the wetland promoting oxygen transport to deeper more anaerobic environments. Larson *et al.* (1993) reported that initial exposure to oxygen would enable continuation of LAS breakdown in oxygen poor environments. It is possible in the design of a wetland to incorporate perforated down-tubes installed into the substrate in order to oxygenate deeper layers. This technology has been widely employed in constructed wetlands for sludge drying (e.g. Cooper 2001). Alternatively, as indicated in chapter 4, greater planting density of *Phragmites* may increase oxygenation in the rhizosphere and also enhance degradation. Hence for treatments designed for high LAS concentration (e.g. raw untreated sewage or industrial source) the results from this study suggests that the technologies described would be applicable for increased oxygenation and consequent enhancement of LAS degradation.

5.4 WATER HARDNESS

5.4.1 Introduction

Water harness is another influencing factor on LAS removal and bioavailability in sewage treatment (Berna *et al.* 1989). LAS can form highly insoluble compounds with calcium ions (Ferrer *et al.* 2000). LAS precipitation from the water column decreases the bioavailable fraction for microorganisms and consequently a reduction in toxicity. For example, Lewis & Perry (1981) reported significant variation in aquatic toxicity of LAS to *Daphnia* with water hardness increasing from 25 to 150mg L^{-1} $CaCO_3$. The effect of water hardness on LAS removal is reported as variable depending on chain length. Verge *et al* (2001) reported a higher precipitation rate for longer chain homologues, with C_{14} precipitated much faster than C_{10} homologue.

From the literature it is hypothesised that high levels of water hardness would result in greater elimination of LAS via precipitation but lower degradation due to reduction in bioavailability. The level of water hardness is dependent upon the wastewater source and geographical area but could be influenced via addition of $CaCO_3$. In this study, effects of high Ca^{2+} concentrations compared to that of soft water encountered in the wetlands monitored on LAS mineralization was investigated.

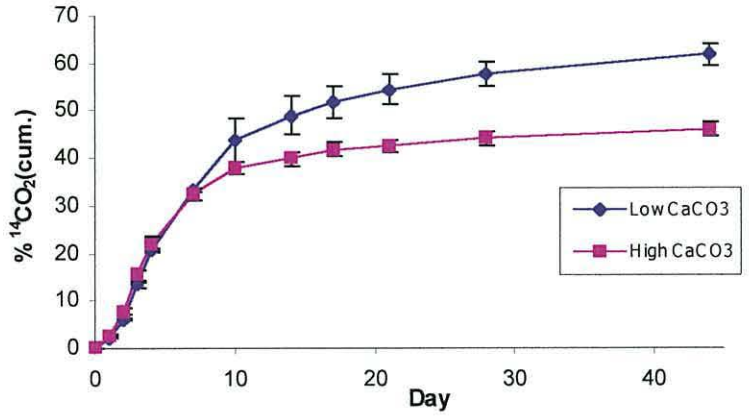
5.4.2 Methods

Mineralization with 50g of gravel collected from the Brynsiencyn wetland as followed in section 3.2.1 was applied to this study with 200mg L^{-1} $CaCO_3$ added to the radiolabelled solution and compared to the control with no addition. Statistical differences were evaluated via paired T-tests for the data that conformed to the normal distribution using Minitab™ version 13.1 (Minitab Inc. 2000).

5.4.3 Results

Figure 5.9 shows that initially no difference was observed due to the additional $CaCO_3$ in the initial 7 days of the experiment. However, with time a significant ($T=2.653$, $p>0.05$) reduction in $^{14}CO_2$ production is observed with net c.15% reduction in the additional $CaCO_3$ system.

Figure 5.9: LAS mineralization under soft and hard water conditions.



5.4.4 Discussion

The lower net mineralization observed in the high $CaCO_3$ treatment observed may be explained by two possible factors. Firstly, the higher Ca^{2+} concentration may be toxic to microbes responsible for LAS mineralization. Secondly, precipitation with Ca^{2+} ions may reduce the amount of LAS available for degradation and hence consequently reduce the net yield. Berna *et al.* (1989) reported that the higher the water hardness then the higher the LAS elimination through physical removal in primary settling, i.e. the higher the Ca^{2+} concentration (hard water) then the greater amount of precipitated LAS Ca-salts and hence greater removal. In soft waters ($CaCO_3$ <100mg L^{-1}, World Health Organization (WHO)) approx. <10% LAS may be precipitated, however, in hard waters ($CaCO_3$ >200mg L^{-1}, WHO) >30-35% (Berna *et al.* 1989). Elimination of LAS via precipitation also lowers the bioavailability of the surfactant and hence reduces the in situ toxicity, as reported for *Daphnia* (Verge *et al.* 2001). Effect of water hardness has also been investigated on LAS adsorption with a correlation reported (Jensen 1999).

However, from the data, it is unclear why similar mineralization rates were observed for both the control and hard water treatments for the first seven days before a reduction in observed in the latter. This may possibly be explained by the gradual increase in Ca-LAS precipitation resulting in a decrease in LAS bioavailbility and/or a gradual decline in the population of LAS-utilizing bacteria due to toxicity.

5.5 PRESENCE OF OTHER SURFACTANTS

5.5.1 Introduction

The majority of the experiments in this thesis have focused on LAS biodegradation alone with and without nutrients present. However, in the environment the presence of other surfactants is inevitable with several other anionic, cationic, non-ionic or amphoteric surfactants in detergent formulations. Hence it is important to evaluate the effect of other surfactants in terms of possible reduction or stimulation in the population and/or activity of LAS-utilizing bacteria. This study monitored LAS mineralization in the presence of cationic surfactant cetyl trimethylammonium bromide (CTAB) and non-ionic surfactant Synperonic A7, an alcohol ethoxylate.

Cationic surfactants have a positively charged ion in aqueous solution with the majority used in detergents based on quaternary ammonium salts (Swisher 1987). However, due to cost considerations, they represent only a minor fraction of the surfactants used (Swisher 1987). Positively charged cationic surfactants have a strong affinity for the surface if particulates in sewage sludge (Scott & Jones 2000) and hence may affect the surface properties possibly affecting LAS bioavailiabilty. The literature on the fate and effects of the CTAB surfactant in sewage treatment is limited. Data on cationic surfactants with close structure with ammonium group include >80% mineralization of alkylbenzyldimethylammonium chloride (Krzeminski *et al.* 1973 as cited in Scott & Jones 2000) and half-life of 2.5h for octadecyltrimethyl -ammonium chloride (Games *et al* 1982 as cited in Scott & Jones 2000).

Nonionic surfactants contain hydrophilic groups that do not ionise appreciably in aqueous solution (Swisher 1987). These surfactants are of great commercial importance and include alcohol ethoxylates (AE), alkyl phenol ethoxylates (APE) and nonylphenol (NP). In contrast to the high degradability of LAS, non-ionic surfactants such as APE are less biodegradable with values of 0-20% reported (Swisher 1987). The non-ionic surfactant used in this experiment is classed as an alcohol ethoxylate (AE) and is a highly biodegradable replacement for APE and NP surfactants. Problems with NP arose from its mimic effect of the hormone oestrogen affecting sex determination and development in organisms, e.g. *Daphnia* (Scott & Jones 2000). The environmental problems associated with APE relate more to the biodegradation products than the intact surfactant (Scott & Jones 2000). In contrast, linear AE is

readily biodegradable with >80% primary degradation in 28 days reported (Kravetz *et al.* 1991).

Hence this study focused on LAS mineralization in the presence of cationic or non-ionic surfactants at two different concentrations. It was hypothesised that mineralization would be reduced in the presence of other surfactants with the cationic surfactant affecting the bioavailaibilty of LAS.

5.5.2 Methods

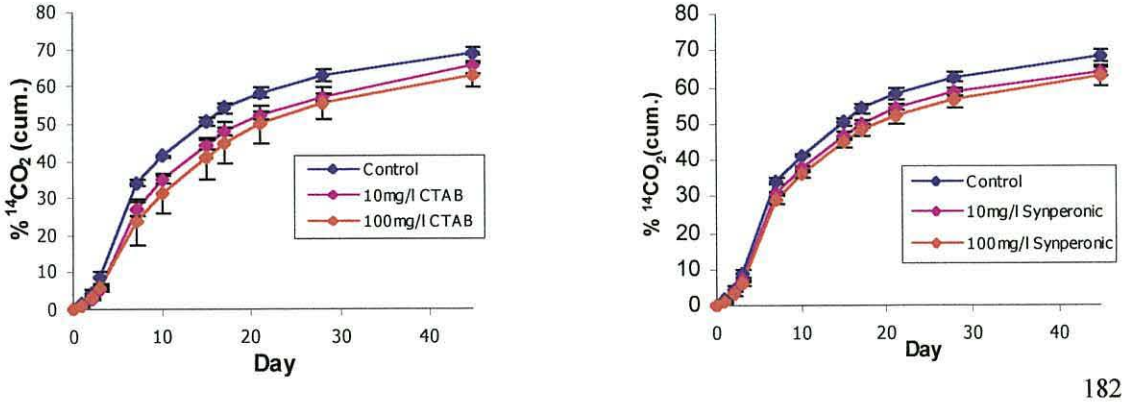
Mineralization with 50g of gravel collected from the Brynsiencyn wetland as followed in section 3.2.1 was applied to this study with 10mg L^{-1} and 100mg L^{-1} for both cationic (CTAB, cetyl trimethylammonium bromide, Mr. 364.46) and non-ionic (Synperonic A7, chain length of 13.7) surfactants present as compared to the control. Statistical variations between treatments were evaluated via Repeated Measures ANOVA for the data that conformed to the normal distribution and had homogenous variance using Minitab^{TM} version 13.1 (Minitab Inc. 2000).

5.5.3 Results

As shown in figures 5.10a and b the presence of cationic or non-ionic surfactant only slightly hindered LAS mineralization. However, repeated measures ANOVA, followed by post hoc Tukey tests, revealed that significant ($p<0.05$) differences existed for the 100mg L^{-1} surfactant treatments in comparison to the control. An initial 3-day lag period was evident with the slight detrimental effect more noticeable for the cationic surfactant.

Figure 5.10: LAS mineralization with the presence of:

(a) cationic (b) non-ionic surfactants.



5.5.4 Discussion

LAS generally accounts for approximately 5-10% part of the washing agents in detergents (Litz *et al.* 1987). Hence mixtures of surfactants at various concentrations are encountered in the environment. In this study the presence of either cationic or non-ionic surfactants at concentrations of 10 or 100mg L^{-1} only slightly detrimentally affected LAS mineralization. Concentrations of cationic and non-ionic surfactants in sewage treatment would not be expected to exceed 10mg L^{-1} (Swisher 1987). Hence for tertiary treatment by wetlands much lower concentrations are likely with possibly a lesser effect on LAS mineralization observed. However, literature concerned with possible effects of other surfactants on LAS biodegradation is scarce and hence comparable data for this study are minimal.

The cationic surfactant at the highest concentration (100mg L^{-1}) was found to have a slightly greater negative effect on LAS mineralization than the non-ionic surfactant. Possible explanations include a reduction in LAS bioavailability and toxicity to LAS-utilizing bacteria. Cationic and anionic surfactants neutralise each other when present in the same solution together due to the respective positive and negative charge (Swisher 1987). The opposite charged surfactant ions form a water insoluble salt that would reduce LAS bioavailability for bacteria to utilize. LAS biodegradation affected by cationic surfactants have been reported (Utsunomiya *et al* 1998, Utsunomiya 1997 both as quoted in Scott & Jones 2000). Formation of LAS complexes with cationic surfactants alkyltrimethylammonium chloride (TM) and dialkyldimethyl ammonium chloride (DM) was reported to affect LAS biodegradation rates. The ratio of cationic surfactant to LAS was influential with greater biodegradation observed when ratio was 2:1 than 1:1 in favour of LAS. Alternatively the additional surfactant may be toxic to LAS-utilizing bacteria. Cationic surfactants are known to be much more toxic than anionic surfactants (Scott & Jones 2000). In addition it may be that the bacteria involved in LAS mineralization are also involved in the degradation of the cationic surfactant hence reducing the former. Similar mechanisms are suggested for the decline in LAS mineralization due to presence of the non-ionic surfactant.

5.6 DESORPTION

5.6.1 Introduction

Desorption is an important process involved in determining the bioavailability of LAS in the environment via returning the adsorbed LAS to the aqueous phase (Fytianos *et al.* 1998a). Adsorption and desorption are reversible processes with the balance between these processes determining overall irreversible removal of the surfactant. Publications on the desorption of LAS in environmental samples are limited. However, from adsorption studies, it is possible to identify possible influencing factors, e.g. pH (section 5.2, Kuchler & Schnnak 1997) and presence of other surfactants (section 5.5), that may similarly affect desorption. This work addresses the potential effect of LAS desorption in wetlands in terms of quantification and possible influencing factors in terms of extreme events. These factors were assessed in order to evaluate what conditions would be required to cause a large LAS desorption event.

5.6.2 Methods

Desorption was investigated via two separate methods. The first experiment involved desorbing the ^{14}C-LAS that had been adsorbed on the gravel in section 3.2.2. Hence 20ml of 0.01M KCl was added to the gravel samples from the Rosset, Brynsiencyn and Clutton wetlands used in section 3.2.2 and shaken for 16 hours at 220 rpm (20°C). The samples were then centrifuged at 10,000 rpm for 20 minutes, the solution drained and 1ml aliquots counted in 10ml of Emulsifier Safe Scintillation Liquid on a Packard Tricarb 2700TR Liquid Scintillation Analyser. This was then repeated for a second 16-hour period.

However, it was recognised that tests involving KCl may not be representative of actual desorption in the wetland. Hence the second test involved collecting 50g aliquots of gravel from the Brynsiencyn wetland and shaking with appropriate solution (220rpm) at room temperature for a total of 48 hours. 100ml water was added as the control. To assess extreme pH effects, 100ml of solutions amended to pH 2 and pH 12, by the addition of concentrated hydrochloric acid or sodium hydroxide addition respectively, were added. The effect of cationic surfactant CTAB and non-ionic surfactant Synperonic A7 (both 100ml at 100mg L^{-1}) on LAS

desorption were also tested. At 24 and 48-hour intervals a portion of aqueous sample was removed and preserved with formaldehyde and subsequently analysed via HPLC.

5.6.3 Results

Table 5.4 shows the total percent desorption of the adsorbed LAS reported in section 3.2.2 for the Rosset, Brynsiencyn and Clutton gravels after two successive steps of desorption with 0.01M KCl, i.e. after 48 hours. The Rosset gravel exhibited greatest desorption (45.7%), followed by Clutton (35.3%) and Brynsiencyn (33.3%).

Table 5.4: Mean percent desorption of the adsorbed LAS in section 3.2.2 after two successive steps of desorption with 0.01M KCl.

Gravel	Mean % Desorption	St error
Rosset	45.7	3.8
Brynsiencyn	33.3	3.3
Clutton	35.3	3.5
Average	38.0	3.5

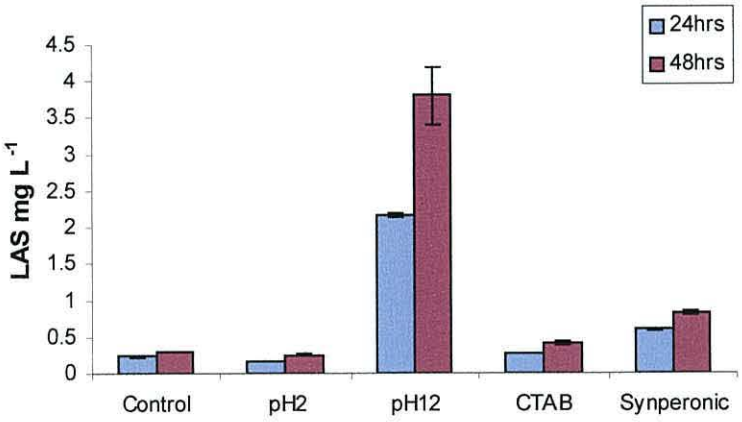
These data, as compared to those cited for adsorption in section 3.2.2, can be used to calculate the amount of reversible and irreversible LAS sorption (table 5.4). The percentage desorption shown above in table 5.4 can be subtracted from the percentage total adsorption summarised in table 5.5 (original data from table 3.8) to give the amount of LAS that is irreversibly bound to the gravel. Table 5.5 demonstrates that LAS is most strongly adsorbed to the Rosset gravel, whereas the Brynsiencyn gravel exhibited the greatest sorption reversibility. In terms of mean total and irreversible sorption percentages of 37% and 23% were calculated for the three sites.

Table 5.5: Mean LAS percentage total and irreversible sorption.

Gravel	% Total Adsorption	% Irreversible Adsorption
Rosset	44	24
Clutton	41	27
Brynsiencyn	25	17
Average	37	23

The second experiment indicated that extreme changes in pH and the presence of other surfactants (100mg L^{-1}) can influence LAS desorption in wetlands. Figure 5.11 shows that high pH may promote desorption with a 10-fold increase in comparison to the control. However, extreme lowering the pH had an opposite effect with a slight decrease in LAS desorption observed. The presence of a non-ionic surfactant had a greater effect of promoting LAS desorption than the cationic effect.

Figure 5.11: Influencing factors on LAS desorption measured via HPLC analysis.



5.6.4 Discussion

Knowledge of desorption is a key factor in understanding the reversibility or irreversibility of LAS adsorption in the wetland systems monitored. This current study, as compared to the adsorption data reported in section 3.2.2, has provided information of the bioavailability of LAS in both the water and solid phase. Mean of 33.3-45.7% desorption was observed which is comparable to that reported elsewhere, e.g. 35.3-65.9% (Matthjis & De Henau 1985). The higher sorption capacity and stronger binding capacity of LAS with the Rosset gravel may suggest that the Ca content of the substrate is more influential than Fe or Al (table 7, section 3.2.2). However, further analysis on a range of gravel varying in geology would be required to confirm this.

The data indicated that a mean of 23% of LAS adsorbed is irreversible. Hence the results indicate that the gravel is a sink for LAS. Desorption is commonly reported in terms of further tests on samples subjected to previous adsorption of radiolabelled

surfactant (Matthijs & De Henau 1985, Fytianos *et al.* 1998a). Matthijs & De Henau (1985) reported slightly higher total and reversible adsorption of LAS average 57% and 31% respectively for river sediments. Branner *et al.* (1999) reported low irreversible LAS desorption and attributed this to the low organic content of the soil investigated. Similarly Hand & Williams (1987) reported nearly complete desorption from river sediments.

The second experiment investigating influencing factors on desorption indicates that extreme changes in pH and the presence of other surfactants can affect LAS desorption. Possible explanations include changes to the solid surface resulting in a lower binding capacity between LAS and the gravel. LAS biodegradation and adsorption is reported to be affected by pH (Litz *et al.* 1987, Dorfler *et al.* 1996, Küchler & Schnaak 1997). Hence it is not surprising that LAS desorption is also affected by extreme changes in pH. Low pH was reported to enhance LAS adsorption in section 5.2 with no substantial subsequent effect on desorption observed in this study. This may possibly be explained by greater stability of surface charges at low pH or greater attraction of charges resulting in less desorption. The increase in LAS adsorption reported by Fytianos *et al.* (1998b) with decreasing pH due to higher positive charge of colloidal surfaces may suggest that the greater attraction observed may result in less desorption over time. In terms of the effect of other surfactants possible attraction of charges and subsequent precipitation or competition for adsorption sites with LAS may be additional explanations for the greater desorption observed. However, due to the extremely limited literature available in this area it is not possible to draw any firm conclusions.

5.7 CONCLUSION

The manipulation experiments conducted in this chapter demonstrated that there are several possible factors that control or influence LAS removal in constructed wetlands that may be possible to modify so as to enhance treatment.

1. Temperature affected LAS removal with higher temperature promoting greater degradation. Unexpectedly greater adsorption was observed at lower temperature.
2. Neutral pH was the best conditions for LAS biodegradation of the range tested. However, more acidic or alkaline conditions promoted LAS adsorption.
3. Additional oxygen exposure increased LAS degradation. However, the results also indicate that LAS degradation can occur under oxygen depleted conditions, although this is substantially lower than under oxygenated conditions.
4. Water hardness affected LAS bioavailability possibly by promoting greater precipitation of Ca-LAS.
5. Presence of cationic or non-ionic surfactants slightly decreased LAS mineralization with the former having the greatest effect.
6. The lower LAS desorption measured in comparison to the adsorption study in section 3.3.2 indicates that wetland gravel is a sink for LAS. However, the balance may be affected by factors such as pH and the presence of other surfactants.

CHAPTER 6: Effect of LAS on Constructed Wetlands Processes

In previous chapters LAS removal under field and laboratory conditions was investigated and various factors identified as influential on removal efficiency. However, LAS is a xenobiotic compound and the effect of its presence in varying concentrations on the natural processes occurring in constructed wetlands must also be addressed. In this chapter several independent experiments were conducted to assess possible effects of LAS on phosphate adsorption, microbial respiration, enzyme activity, plant germination and growth, and greenhouse gas emissions. These processes were also assessed in terms of natural wetland performance. In section 2.1 mean wetland inflow LAS concentration was measured at 1.1 mg L^{-1} (max. 3.2mg L^{-1}) which was within the range expected (1.10-5.58mg L^{-1}, Holt *et al.* 1998). Hence in this chapter the 1-5mg L^{-1} range was assumed as being representative of LAS concentrations expected in wastewater entering wetlands. However, high LAS concentrations (100-1000mg L^{-1}) were also investigated to assess the effect of any extreme events that could cause high LAS concentrations to enter the wetland, e.g. industrial sources (Painter & Zabel 1989), foot and mouth epidemic and failure of sewage treatment stages.

6.1 PHOSPHATE ADSORPTION

6.1.1 Introduction

Variable PO_4 removal was observed in the operational constructed wetlands monitored in Chapter 2. A possible general decline in removal with time attributed to the filling of adsorption sites on the substrate media was suggested to confirm research of other authors (Tanner *et al.* 1999, Mann 1990). Possible PO_4 removal mechanisms in wetlands include adsorption, precipitation and complexation reactions, incorporation into biofilms and plant uptake (Mann 1990, Tanner *et al.* 1999), although significance of the latter is debatable accounting for low overall removal, e.g. 5% (Kim & Geary 2001), 9-14% (Tanner *et al.* 1999).

Adsorption is reported as the major removal mechanism and is highly sensitive to loading rate (Brix *et al.* 2001). Gravel is commonly used as a substrate in wetlands, due to filtration and hydraulic conductivity considerations. However, it has been found to generally have a low P adsorption capacity (Gray *et al.* 2000). Artificial media have been investigated, e.g. industrial waste products (Mann 1990), calcite and crushed marble (Brix *et al.* 2001a), LECA (Light expanded clay aggregates) (Mæhlum *et al.* 1995) and shale (Drizo *et al.* 1997). Table 6.1 shows the comparable test conditions in similar adsorption studies. Selecting a substrate medium with a high P adsorption capacity can improve and maintain removal efficiency. However, several factors affecting PO_4 adsorption capacity have been identified, including Al, Fe or Ca-content, surface area of substrate, pH, Eh, temperature and hydraulic conductivity (Gray *et al.* 2000, Mann 1990, Kadlec & Reddy 2001, Brix *et al.* 2001).

However, these studies are normally performed with only PO_4 present. This is somewhat unrealistic due to the presence of other anions in sewage received by the wetlands. Hence in this study possible competition by LAS for PO_4 adsorption sites was investigated. Adsorption tests were conducted on gravel and the P sorption capacity determined with and without LAS present at various concentrations.

Table 6.1: Comparable P sorption test conditions

Sample	Conc. (mg L^{-1})	Duration (hrs)	Temp (^{o}C)	Reference
Sands	0-320	20		Brix *et al.* (2001)
Sands	0-320	20	20	Arias *et al.* (2001)
Gravel	0-5000	72		Gray *et al.* (2000)
Soils, slags, zeolites		48	25	Sakadevan & Bavor (1998)
Gravel, industrial by-products	5-100	24-30	25	Mann & Bavor (1993)
Gravel, industrial by-products	5-100	30	25	Mann (1990)

6.1.2 Methods

Gravel samples from the Brynsiencyn wetland were washed, air-dried and sieved (6.7mm mesh). To determine PO_4 sorption capacity aliquots of 40ml of PO_4 solution (0, 1, 2, 3, 4, 5, 10 and 20mg L^{-1} P as KH_2PO_4) in 0.01M KCl was added to 20g of gravel in a conical flask (Mann 1990). To establish effect of LAS 0.01M KCl solutions containing LAS at 0, 10 and 100mg L^{-1} were used to make up the appropriate PO_4 concentrations with KH_2PO_4 and added to the gravel. Two drops of toluene were added to each flask to inhibit microbial growth and the flasks then shaken for 30 hours at 150rpm (20°C). The solutions were then filtered through Whatman 0.2μm cellulose acetate syringe filters. Molybdate-reactive PO_4 in solution was then determined using a Skalar 5100 autoanalyser. Blank tests with no gravel present were also conducted.

6.1.3 Results

Figure 6.1 shows the non-linear PO_4 adsorption isotherm observed with the concentration of PO_4 in solution related to that adsorbed. The latter was determined by the measurement of disappearance of PO_4 from solution. In comparison to the control, low LAS presence generally seemed to promote PO_4 adsorption but reduce adsorption at higher concentration. For all treatments, at low PO_4 concentrations (1-5mg L^{-1}) generally >80% was adsorbed, however, at higher concentration (10-20%)

this declined to 50-60%. Hence removal was less efficient at higher PO_4 initial concentration applied in all treatments. Table 6.2 shows the percentage adsorption measured.

The data were best represented by the Langmuir isotherm that is shown in figure 6.2 at low PO_4 concentrations (see section 3.3.2 for equation). These data were used to estimate the PO_4-sorption capacity as summarised in table 6.3 that reflects the effect of the surfactant. However, differences between treatments were narrow.

Figure 6.1: PO_4 adsorption isotherm for gravel.

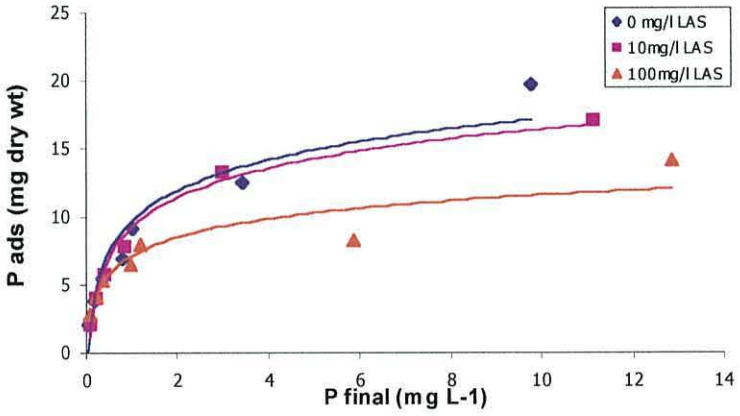


Figure 6.2: PO_4 Langmuir adsorption isotherm for gravel, where v is the amount of LAS adsorbed ($\mu g\ g^{-1}$) and [S] is the equilibrium solution concentration ($mg\ L^{-1}$).

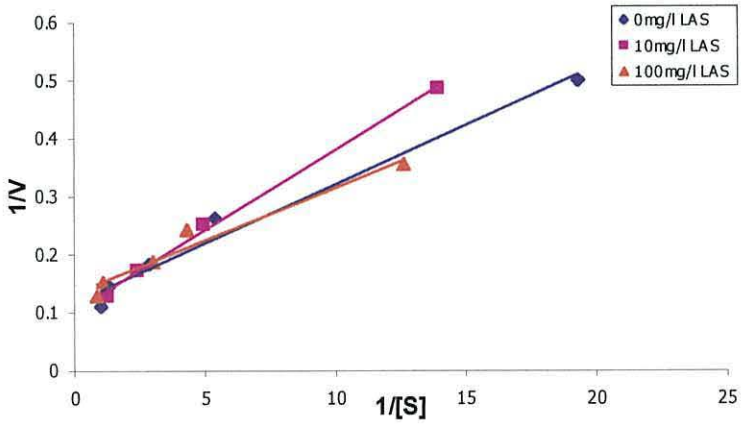


Table 6.2: Percentage PO_4 adsorption with LAS present at 0, 10 and 100mg L^{-1} (± standard error).

	% PO_4 Adsorption		
Initial P (mg L^{-1})	**LAS 0mg L^{-1}**	**LAS 10mg L^{-1}**	**LAS 100mg L^{-1}**
1	95 (±0.1)	93 (±0.1)	94 (±0.9)
2	91 (±0.3)	87 (±0.3)	82 (±0.7)
3	88 (±0.2)	83 (±0.2)	82 (±0.5)
4	81 (±0.1)	75 (±0.4)	81 (±0.3)
5	81 (±0.2)	72 (±0.2)	88 (±0.4)
10	64 (±0.3)	47 (±0.5)	62 (±0.2)
20	50 (±0.7)	31 (±0.4)	49 (±0.2)

Table 6.3: Langmuir constants where V_m is the P sorption capacity (mg kg^{-1}) and c the measure of adsorption intensity.

LAS Treatment	**Langmuir Constants**		
	V_m	c	R^2
0mg L^{-1}	8.389	0.160	0.98
10mg L^{-1}	9.587	0.265	0.99
100mg L^{-1}	7.519	0.138	0.96

6.1.4 Discussion

This laboratory study on PO_4 adsorption onto gravel has given valuable information on the adsorption characteristics in constructed wetlands. PO_4 is present in detergents as tripolyphosphate builders and hence is commonly associated with surfactants (Scott & Jones 2000). LAS was found not to have a substantial effect on PO_4 sorption with only small variations in P sorption capacities measured (7.5-9.5mg Kg^{-1}). The data was best represented by the Langmuir equation as reported elsewhere (Arias *et al.* 2001).

However, contrasting slight effects at the two different LAS concentrations applied were observed. Effects due to microbial activity can be eliminated due to the addition

of toluene at the start of the experiment. Bohn *et al.* (1979) proposed two possible reactions for P sorption. Firstly rapid specific adsorption or ligand exchange where PO_4 anion replaces hydroxyl ion on crystal of hydrous Al of Fe structures. Alternatively non-specific adsorption mediated through protonation of hydroxyl surface that creates positive charges and attracts negative charged anions such as PO_4^{2-}. The slight enhancement in PO_4 sorption observed with LAS at 10mg L^{-1} may suggest that at low surfactant concentration there are enough adsorption sites for both anions with the surfactant facilitating PO_4 adsorption via altering the surface properties of the gravel. However, the detrimental effect exhibited at 100mg L^{-1} LAS may suggest saturation and subsequent competition of available sorption sites. LAS may compete for adsorption sites directly or indirectly via the production of SO_4^{2-} during desulphonation. SO_4^{2-}, along with F^- and some organic acids, are known to compete with PO_4 for adsorption sites (Iyamuremye & Dick 1996). However, according to the slow mineralization reactions discussed in chapter 2 in relation to the short time frame of this adsorption test, it is more likely that the intact surfactant is the competitor.

It is recognised that laboratory tests are not always characteristic of field conditions. Possible factors influencing rate or total PO_4 sorption capacity in wetlands include available surface area, pH (Gray *et al.* 2000), Ca, Mg, Fe, Si and S substrate content (Mann 1990), temperature (Millero *et al.* 2001, Mahi *et al.* 2001) and water hardness (Maurer *et al.* 1999). In addition, as highlighted in section 3.3.2, biofilm presence may affect PO_4 adsorption similar to LAS.

In terms of general wetland performance, this study confirms that gravel has a low P sorption capacity (Breen 1990, Mann & Bavor 1993, Gray *et al.* 2000). The PO_4 sorption capacity measured in this study is fairly low in comparison to other studies, e.g. 25.8-47.5µg/g (Mann & Bavor 1993), <50mg Kg^{-1} (Breen 1990). However, without available data on the geology of the gravel these values are difficult to compare. Much higher P adsorption capacities have been reported for industrial by-products, e.g. 160-44,000mg Kg^{-1} blast furnace slag (Mann & Bavor 1993, Sakadevan & Bavor 1998) and 260mg Kg^{-1} fly ash (Mann & Bavor 1993).

The low sorption suggests that further investigation is required into design considerations of constructed wetlands for enhanced PO_4 removal. Current research into improving P-sorption includes mixing high sorption capacity artificial media within the gravel matrix, e.g. Al and Fe (Luderitz & Gerlach 2002) and lime (Willadsen *et al.* 1990). Alternatively units containing high P-sorption artificial media can treat wastewater before it enters the wetland. These units can then be replaced when P-binding capacity is reached (Brix *et al.* 2001a). Constant high PO_4 removal has been observed in shale (98-100%), maerl (98%), and iron-ore and blast furnace slag (98%) based constructed wetlands (Drizo *et al.* 1997, Gray *et al.* 2000, Gruneberg & Kern 2001). Substrates with high PO_4 sorption capacities can therefore be in operation for longer or at higher loading rates. Subsurface flow wetlands can also be used to maximise adsorption via increasing time of contact of wastewater and substrate surfaces. Similarly horizontal rather than vertical flow wetlands seem to be more effective in P removal due to the longer flowing distance and treatment time of the former (Luderitz & Gerlach 2002). The poor PO_4 removal results from chapter 2 coupled with the low adsorption capacity of gravel observed in study suggest that design alterations are required for consistent PO_4 removal over time in wetlands.

However, employed changes will be dependent on substrate price, availability and overall maintenance. Consideration of the suitability of a substrate is also required with relation to other treatment processes within the wetland. Biodegradation, adsorption and precipitation of LAS may be adversely affected. Detrimental effects on the cycling of other nutrients may occur. The effect of the substrate on plant growth and establishment is also important as it may influence LAS removal (chapter 4). However, Gruneberg & Kern (2001) reported that the use of iron-ore and blast furnace slag as a substrate did not adversely affect reed growth. Similarly Gray *et al.* (2000) reported reed growth was sustained throughout the experiment with maerl as substrate. The hydrology may be detrimentally affected as substrates with a high surface area will have low hydraulic conductivity leading to clogging (Kadlec & Knight 1996). Hence this raises several questions on the use of alternative substrates in wetlands and further research is required in this area.

6.2 ENZYME ACTIVITY

6.2.1 Introduction

Extracellular enzymes are involved in various biogeochemical cycles in wetlands via compound biodegradation or biotransformation and have been discussed in depth in previous chapters. Nutrient cycling in wetland ecosystems involves enzymes such as β-glucosidase to facilitate cellulose breakdown in the carbon cycle (Eriksson & Wood 1985), organic phosphorus transformation to microbially available forms by phosphatases and biochemical reactions of sulphur by sulphatase (Killham 1996). Effect of various environmental factors, e.g. soil type, temperature (Kang & Freeman 1998) and climate (Kang & Freeman 2000), and on wastewater characteristics, e.g. pH (Sinsabough *et al.* 1991) and nutrient availability (Chróst 1991), on enzyme activities have been published.

Research from previous chapters suggests that some form of enzyme inhibition may have occurred in the mesocosm wetlands monitored. It is possible that LAS is the cause of the inhibitory effect. In section 4.3.1 a decrease in phosphatase and β-glucosidase activity was observed after LAS addition. However, it was inconclusive whether this may be due to the low nutrient availability or organic carbon availability. In addition enzyme inhibition is suggested by the few relationships established with temperature in chapter 4, with an inverse correlation established for sulphatase in section 4.3.2. LAS can be inhibitory to biological activity that is important for nutrient cycling (Jensen 1999). Hence in this study the effect of LAS concentrations over the range of 0-1000mg L^{-1} in microcosm constructed wetlands was addressed and the activities of β-glucosidase, phosphatase and sulphatase enzymes measured, and resultant nutrient ion concentration, to assess inhibitory effects.

6.2.2 Methods

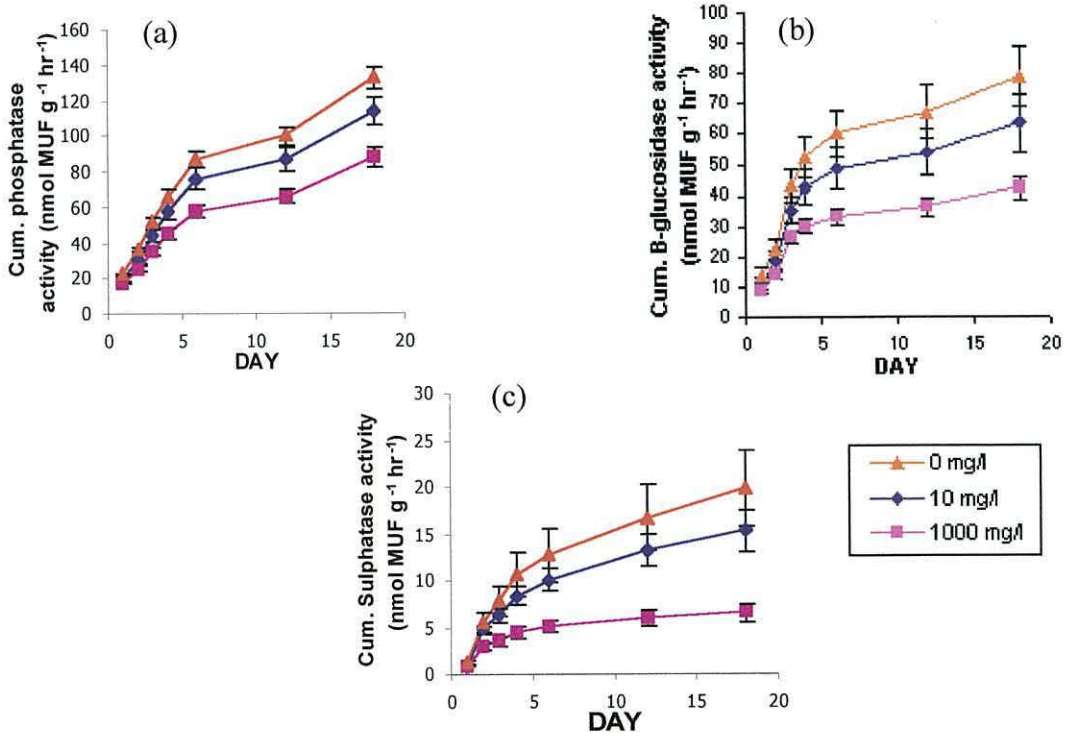
Phragmites australis were planted with gravel collected from the Rosset wetland (section 2.1.2.1) in 1.2L containers (11.5 x 13cm, diameter x depth). Filters packed with glass wool and attached to plastic tubing were inserted into the gravel for water samples to be taken. LAS concentrations of 0, 10 and 1000mg L^{-1}, dissolved in artificial sewage media (Appendix D), were added (400ml) to four replicates for each treatment. Gravel samples were taken from each treatment at regular intervals over an

18-day period and sulphatase, phosphatase and β-glucosidase (200μM L^{-1}) enzyme assays conducted using MUF fluorescent substrates as reported in section 2.1.2.3. Calibration using 0-100μM MUF-free acid solution was used to correct quench interference of phenolics (Freeman *et al.* 1995). Water samples for anion analysis were also analysed (section 2.1.2). Comparisons between treatments were assessed with Repeated Measures ANOVA tests for the data that conformed to the normal distribution and had homogenous variance using Minitab™ version 13.1 (Minitab Inc. 2000).

6.2.3 Results

Figure 6.3 demonstrates the cumulative enzyme activity response to LAS. A distinct response in enzyme activity with LAS concentration was observed. Generally the higher the LAS concentration then the lower the enzyme activity. However, the statistical data suggest that the inhibitory effects was only significant for the 1000mg L^{-1} LAS treatment for all enzymes monitored (phosphatase $F=19.272$, $p<0.001$, sulphatase $F=14.071$, $p<0.001$) and also at 10mg L^{-1} LAS for β-glucosidase in comparison to the control ($F=23.698$, $p<0.001$).

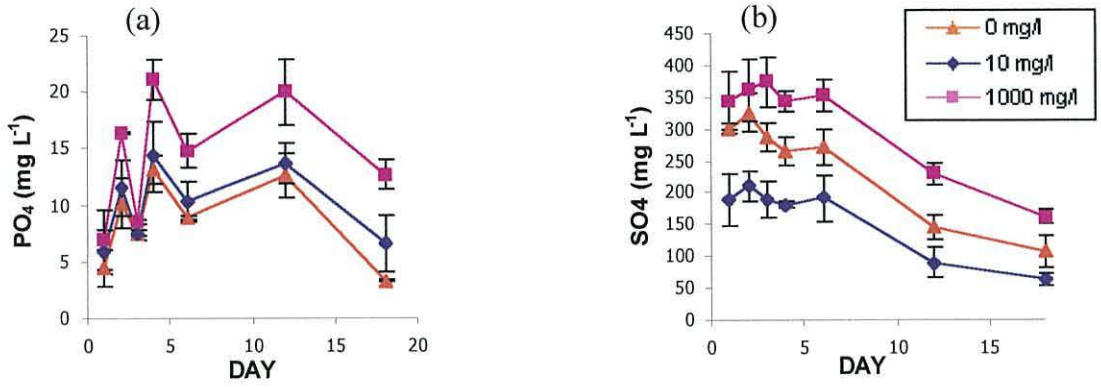
Figure 6.3: Cumulative enzyme activity response to LAS for (a) Phosphatase, (b) β-glucosidase and (c) Sulphatase.



The order of greatest activity was for phosphatase>β-glucosidase>sulphatase with significant differences observed for the control ($F=24.204$, $p<0.001$), 10mg L^{-1} LAS ($F=23.505$, $p<0.001$) and 1000mg L^{-1} LAS ($F=24.570$, $p<0.001$) treatments. However, post hoc Tukey test showed that the difference was not significant between phosphate and β-glucosidase activities in the control treatment.

The PO_4 and SO_4 results are shown in figures 6.4a and b respectively. The former shows that the PO_4 concentration was highest for the 1000mg L^{-1} LAS treatment. SO_4 concentrations showed a distinct decreasing pattern with time in the order of 1000mg L^{-1}>10mg L^{-1}>0mg L^{-1} LAS treatments.

Figure 6.4: Concentrations of (a) PO_4 and (b) SO_4 measured at initial LAS concentrations of 0, 10 and 1000mg L^{-1} added.



6.2.4 Discussion

The order of greatest enzyme activity (phosphatase>β-glucosidase>sulphatase) for all treatments again supports that reported in chapters 2 and 4 and elsewhere (Freeman *et al.* 1995, Chappell & Goulder 1992). The results of this study suggest that at LAS concentrations expected in constructed wetlands, no adverse effects on the enzyme activities will be exhibited. Both phosphatase and sulphatase were largely insensitive of LAS even at 10mg L^{-1}. Although β-glucosidase was statistically more sensitive, figure 6.3b shows that the error bars overlap for the control and 10mg L^{-1} LAS treatment. Elsgaard *et al.* (2001a and 2001b) reported that β-glucosidase and sulphatase activities were insensitive to LAS. Similarly using MUF substrates, only a 25% β-glucosidase inhibition at the highest LAS test concentration (793mg Kg^{-1}) was

reported (Elsgaard *et al.* 2001a). In addition insensitivity of phosphatase to LAS has been reported (Kowalczyk 1992 as quoted in Elsgaard *et al.* 2001a). Even a stimulation of enzyme activity at environmentally realistic levels has been observed followed by a decrease at higher LAS concentration (Elsgaard *et al.* 2001a and 2001b).

In terms of the hydrochemistry the higher PO_4 concentration in the 1000mg L^{-1} LAS treatment can possibly be explained by LAS competition for adsorption sites (see section 6.1), toxicity to microbes (see section 6.3), toxicity to plants (see section 6.4) resulting in less nutrient uptake or possible desorption of PO_4. The general decreasing trend for the 10mg L^{-1} and 1000mg L^{-1} LAS is possibly reflective of the release of SO_4 as LAS is degraded (Swisher 1987). As shown previously in chapter 1 (figure 1.7), SO_4 is produced as a by-product during desulphonation. However, as a similar trend is observed for the control this may be more reflective of sulphatase activity.

6.3 MICROBIAL RESPIRATION

6.3.1 Introduction

CO_2 respiration can be used as an indicator of microbial activity with higher emissions indicative of greater activity. Hence the impact of LAS toxicity on the C cycle can be conveniently determined by measuring microbial respiration. The surface-active properties of LAS can cause negative influence on the activity of microorganisms (Fytianos *et al.* 1998a). Hence LAS may be toxic to microorganisms involved in nutrient cycling and other important wastewater treatment processes within a wetland ecosystem. Toxicity data suggest that such detrimental effects occur to microorganisms at LAS concentrations of approx. 10-50mg Kg^{-1} (Welp & Brumner 1985 as reported in Jensen 1999, Elsgaard *et al.* 2001a). However, effect of LAS on soil microorganisms has been reported to depend on inherent toxicity, concentration and external factors such as pH, temperature, moisture and interactions with other chemicals (Welp & Brummer 1999, as reported in Jensen 1999).

In this study, CO_2 respiration was used as an indicator of the impact of LAS at various concentrations (0-1000mg L^{-1}) on wetland microbial activity. It is hypothesised that no inhibitory effect is observed at environmentally realistic concentrations encountered in sewage treatment. However, at higher concentrations, approaching and in excess of the critical micelle concentration (CMC), inhibitory effects may be evident.

6.3.2 Methods

Triplicate gravel samples (approx. 5g dry weight) were amended with 5ml LAS solution (0, 0.1, 1, 5, 10, 100 and 1000 mg L^{-1}) incubated at 20°C in sealed darkened glass bottles incorporating a subseal septum for headspace gas samples ($10cm^3$). Samples were taken at 1, 2 and 4 hours to test for linearity and CO_2 quantified via GC analysis (section 3.2.1.b).

6.3.3 Results

Figure 6.5 demonstrates as an example the linear relationship (R^2>0.994) with time for the control. Figure 6.6 shows the mean CO_2 respiration measured for the LAS concentrations investigated. An initial 2-fold stimulation of microbial activity in comparison to the control was observed followed by progressive inhibition with

increasing concentration. In comparison to the highest CO_2 respiration measured (0.1mg L^{-1} LAS) microbial activity is inhibited by >50% at 10mg L^{-1} LAS and by >90% at 1000mg L^{-1}.

Figure 6.5: Linearity of CO_2 respiration with time for 0mg L^{-1} LAS.

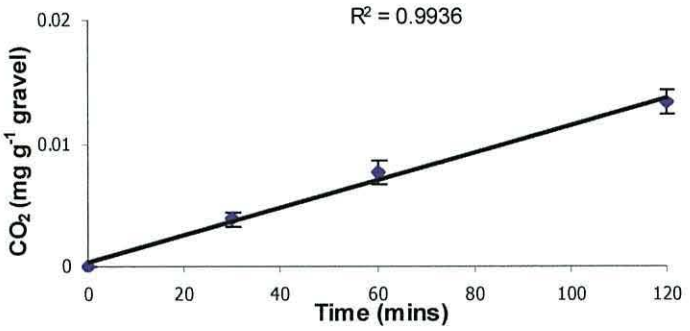
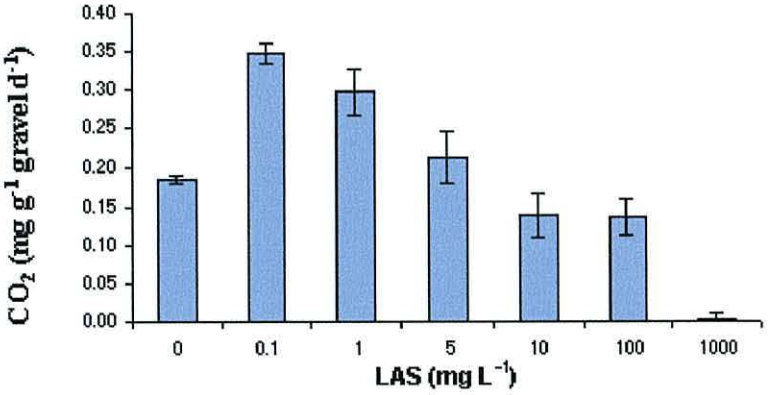


Figure 6.6: Mean CO_2 respiration measured for 0-1000mg L^{-1} LAS concentrations.



6.3.4 Discussion

LAS present at low concentrations encountered in wetlands is not inhibitory to the microbial population activity measured as CO_2 respiration. In chapter 2 the mean wetland inflow LAS concentration was measured at 1.1mg L^{-1}. In this study this concentration range would not be expected to inhibit microbial activity, with in fact a 2-fold stimulation suggested in comparison to the control. Even at the maximum LAS inflow concentration measured at the Rosset site of 3.2mg L^{-1} (section 2.1.3) again no inhibitory effect is suggested as stimulation is still observed at 5mg L^{-1}.

Other researchers support the proposed absence of inhibition at low LAS concentrations. Litz *et al.* (1987) reported soil respiration rates at 1 and 14 days after LAS addition at 0.8-50g/m^2 in a field study and reported an absence of LAS inhibition on soil respirometry. Elsgaard *et al.* (2001a) investigated LAS effect on several microbial parameters in soil and reported inhibition on ethylene degradation, potential ammonium degradation, dehydrogenase activity and iron reduction. However, soil respiration was insensitive to LAS with applied concentrations of 0-793mg Kg^{-1} not inhibitory (Elsgaard *et al.* 2001a). Similarly the authors reported a stimulation in respiration at low LAS concentrations and this was interpreted as a combined response of inhibition and stimulated compartments of microbial community. Several mechanisms may explain this phenomenon. Elsgaard *et al.* (2001a) suggests a possible increase in CO_2 production by LAS resistant microorganisms resulting in degradation of LAS or of microbial carbon from LAS-susceptible cells. Alternatively the adsorption-desorption properties of LAS may cause a mobilisation of nutrients and degradable organic matter in the soil facilitating greater microbial activity (Malkomes & Wohler 1983). Physiological mechanisms, such as stressed-induced metabolism by LAS-susceptible cells, may also result in elevated respiration rates (Elsgaard *et al.* 2001a). For such reasons, a partial or complete compensation of inhibition LAS effects may occur in terms of CO_2 production, even though a decrease in biomass or changes in microbial community structure and stability have taken place (van Straalen & van Gestel 1993).

However, at high LAS concentrations, possibly encountered in wetlands receiving industrial effluents, toxicity to microbial activity is observed with longer chain homologues and more terminal isomers most toxic (Kimeler & Swisher 1977). LAS toxicity to microbes is reported and generally attributed to interactions with cell membranes and disruption of their functioning (Schwunger & Bartnik 1980). Possible harmful effects reported in the literature include delay of development and reproduction and death of microorganisms (Jensen 1999). Welp & Brummer (1985) observed an impairment of microbial activity in soils in which higher concentrations of LAS were applied (as quoted in Jensen 1999). Data reported by Jensen (1999) indicated that LAS can have an adverse effect in soil at initial concentrations of 10-50 mg Kg^{-1} for microorganisms. Malkomes & Wöhler (1983) observed an overall negative effect on soil respiration at LAS concentrations of 10kg LAS ha^{-1}. The main

toxic effect of surfactants on microbes may be attributed to a reduction in surface tension (Jensen 1999). Surfactants generally disrupt biomembranes and denaturation of proteins (Swisher 1987, Schwunger & Bartnik 1980). Toxicity of surfactants affects reactions at the cell surface. Surfactant adsorption may cause depolarisation of cell membranes which may result in a decrease in absorption of essential nutrients and oxygen consumption or a decrease in the release of toxic metabolic products from the cell leading to build up (Jensen 1999). Elsgaard *et al.* (2001a) found that pure LAS was more toxic to the soil microbial processes than LAS-spiked sewage sludge.

6.4 TOXICITY TO SEED GERMINATION AND PLANT GROWTH

6.4.1 Introduction

Plants are the most obvious component in most wetland systems. Interactions between plants and microbes were highlighted in chapter 4 with greater LAS removal observed when plants were present. However, the possible toxic or inhibitory effect of LAS on the plant growth and development was not addressed. The effect of LAS on wetland plants is an important consideration in terms of mechanisms such as oxygen diffusion to the roots, support of rhizosphere bacterial communities and nutrient uptake. Published LAS toxicity studies have mainly focused on crop plants due to application of sludge or pesticide, both of which can contain LAS, commonly used on agricultural land (Jensen 1999). Data reported by Jensen (1999) indicated that LAS can have an adverse effect in soil at initial concentrations of 90mg Kg^{-1} for plants with terrestrial studies, suggesting LAS does not pose a risk to plants (Mieure *et al.* 1990).

However, little is known of the effect of LAS on macrophyte plant species commonly used in constructed wetlands. This experiment investigates the toxicity of LAS on *Phragmites australis* at various concentrations (0-1000mg L^{-1}) in terms of seedling germination and plant growth. From visual assessment conducted in previous experiments on plant growth in the presence of LAS, it was hypothesised that no detrimental effect would be observed at low concentrations.

6.4.2 Methods

Phragmites australis seeds (pack of 100, purchased from the Agroforestry Research Trust, Devon) were placed in petri dishes on tissue soaked in LAS concentrations of 0, 1, 10, 100 and 1000mg L^{-1}. Four replicates for each treatment were used at 20°C. The number of germinated seeds were monitored and counted after 7 days. In a separate experiment replicate plants were grown in John Innes compost for 6 weeks before being exposed to LAS at concentrations of 0, 1, 10 and 1000mg L^{-1}. After 28 days of exposure the development of the plants were established via measuring stem length, number of leaves and dry weight (g). Repeated measures ANOVA tests were applied to data that conformed to the normal distribution and had homogenous variance using Minitab™ version 13.1 (Minitab Inc. 2000).

6.4.3 Results

The germination of seeds was sensitive to LAS exposure. A general decline in the percentage germination was observed with increasing LAS concentration as shown in figure 6.7. Significant differences were observed between treatments ($F=34.16$, $p<0.01$). However, post hoc Tukey tests revealed that this was not significant ($p>0.05$) for comparisons of the 1 and 10mg L^{-1} nor 10 and 100mg L^{-1} LAS treatments.

Figure 6.7: Percentage germination of *Phragmites australis* seeds with LAS exposure.

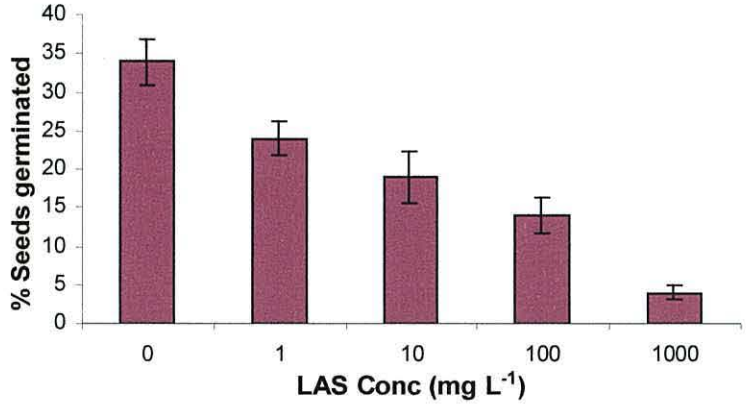
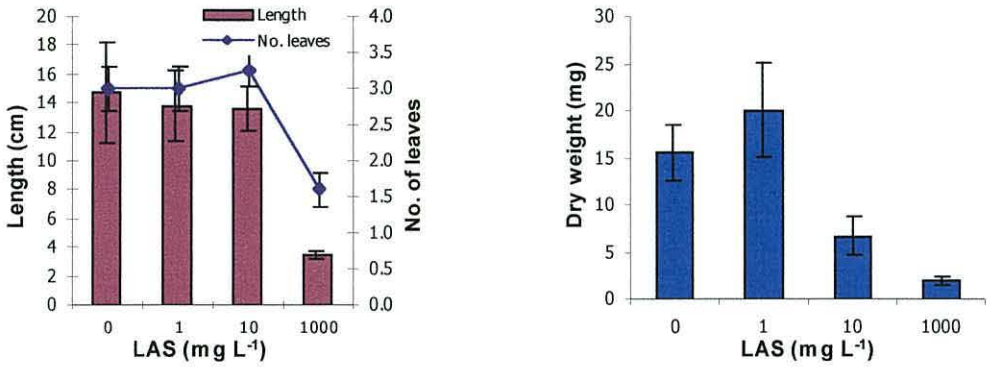


Figure 6.8a demonstrates that no considerable adverse effects were observed in the stem length nor number of leaves characteristics monitored at LAS concentrations of 0-10mg L^{-1}. Figure 6.8b shows that the dry weight was stimulated at 1mg L^{-1} by approx. 25% but adversely affected at 10mg L^{-1} LAS concentration by a decrease of >50%. At 1000mg L^{-1} LAS concentration all plant characteristics monitored exhibited a marked reduction. Statistical data followed by Tukey post hoc tests showed that significant differences were observed for length ($F=6.919$, $p<0.05$), number of leaves ($F=9.939$, $p<0.01$) but not for dry weight between the 1000mg L^{-1} treatment in comparison to the others.

Figure 6.8: Physiological response to LAS exposure to *Phragmites australis*
(a) stem length and number of leaves (b) dry weight.



6.4.4 Discussion

The tests conducted to assess the potential toxicity of LAS to seed germination and plant growth were adequate, with other researchers applying similar LAS concentrations (Holt *et al.* 1989, Marschner 1992 in Jensen 1999) as recommended in the OECD Terrestrial Plant Test 2000. Holt *et al.* (1989) followed this test and reported that LAS passed with relation to the emergence of seedlings and early stages of growth of sorghum, sunflower and mung bean. Figge & Schroberl (1989) reported no adverse effects on growth or yield of the crops monitored with LAS present.

The germination of seeds was more sensitive to LAS exposure than subsequent growth of plants in soil. Germination in hydroponic solution has also been investigated (Jensen 1999). However, pure LAS was used in this study to eliminate any external nutrient effect and because of reports that such tests are most sensitive for plants grown in hydroponics (Mieure *et al.* 1990). Lopez-Zavala *et al.* (1975) observed a delay in the germination of beans and barley in soil with LAS concentrations of 25 or 40mg L^{-1} with an overall inhibition effect on biomass of barley with LAS presence (as quoted in Jensen 1999). However, on the other hand a stimulative effect on biomass of beans and tomatoes was observed when exposed to LAS. Similarly a stimulative effect was observed in this study for the dry weights measured for the 1mg L^{-1} LAS treatment in comparison to the control.

However, at high LAS concentration, detrimental effects on plant growth were observed. The surface-active properties of LAS can cause negative effects on the growth of plants (Fytianos *et al.* 1998a). Marschner (1992) (in Jensen 1999) reported effects of LAS on plants as destruction of root-cell-membrane, changes in membrane permeability, changes in fine structure, and effect on physiological processes, e.g. photosynthesis. Evidence suggests that generally LAS is non-toxic to plants at concentrations of 5-10mg L^{-1} but toxic at concentrations of 10-40,000 mg L^{-1} (Sharma *et al.* 1985, Mieure *et al.* 1990). Mieure *et al.* (1990) summarised studies on the effects of LAS on terrestrial plants. In general when toxic or growth inhibition observed was at LAS conc of 10-1000mg L^{-1}.

Hence at the concentrations expected in constructed wetlands no inhibition to *Phragmites* plant growth is expected. No detrimental effects to the plants were seen in any pervious experiments reported in this thesis involving planted systems. Chapter 4 highlighted the importance of plant mechanisms in LAS removal and hence if the development and functioning of the plant is impaired then this may consequently inhibit LAS removal. Although seed germination was more sensitive at lower LAS concentrations, usually pot grown *Phragmites* are planted in new constructed wetlands so as to ensure growth and equal planting density. Hence LAS, at the concentrations observed in the inflow in chapter 2, should not affect the sustainability of operational constructed wetlands in terms of plant growth.

6.5 LAS EFFECT ON GREENHOUSE GAS EMISSIONS

6.5.1 Introduction

Critical wetland processes in the carbon and nitrogen cycles result in the emission of carbon dioxide (CO_2), methane (CH_4) and nitrous oxide (N_2O) (Kadlec & Knight 1996). These are greenhouse gases and have been extensively researched in recent years in relation to human activities and possible resulting scenarios on global warming. CH_4 and N_2O have warming potentials respectively of 20 and 200 times that of CO_2, on a molar basis (Rodhe 1990).

Natural wetlands can act as sinks for atmospheric CO_2 emissions and contain 20-30% of the world's carbon stock (Gorham 1991). However, wetlands can also act as sources of greenhouse gases. CO_2 is produced via transformation and mineralization of organic matter. CH_4 is produced via anaerobic decomposition processes where certain bacteria, i.e. methanogens, use CO_2, or a low weight organic compound, as an electron acceptor for the formation of gaseous methane which is released into the atmosphere (Kadlec & Knight 1996). Wetlands account for approximately 22% of atmospheric CH_4 production (Cicerone & Ormland 1988). N_2O is produced mainly as a result of denitrification processes in wetlands (Mitsch & Gosselink 1993).

Factors identified as affecting rates of emission include pH, redox potential, water table height, temperature and available organic materials (Bollag & Czlonkowski 1973, Freeman *et al* 1993, Brix *et al.* 2001). However, there have been few attempts to quantify the effect of pollutants such as surfactants on emissions. Effect on emissions may be an indicator of an adverse effect of the pollutant on nutrient cycling in the system that may also have implications for climate change. Hence this study monitors the impact of LAS addition at various concentrations on natural greenhouse gas production in microcosm planted constructed wetlands.

6.5.2 Methods

Phragmites australis were planted with gravel collected from the Rosset wetland (section 2.1.2.1) in 1.2L containers (11.5 x 13cm, diameter x depth) and inserted with filters packed with glass wool. LAS concentrations of 0, 5, 10 and 100mg L^{-1} dissolved in artificial sewage media (Appendix D) were added in quadruplicate (400ml). Gas samples ($10cm^3$) were taken by placing chambers, incorporating a

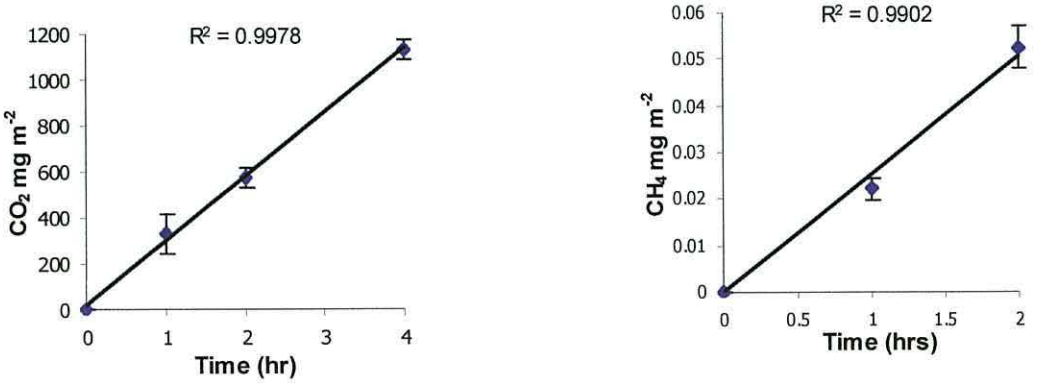
subseal septum for headspace gas sampling, after 1 hour (after checking for linearity) and quantified via GC analysis (section 3.2.1.b) for CO_2, CH_4 and N_2O content over a 7 day period. Water samples for anion analysis was also analysed (section 2.1.2). Comparisons between treatments were assessed with Repeated Measures ANOVA tests for the data that conformed to the normal distribution and had homogenous variance using Minitab^{TM} version 13.1 (Minitab Inc. 2000).

6.5.3 Results

Measurement of accumulated gas production at regular intervals over a 4-hour period confirmed that accumulation remained linear for CO_2 (R^2=0.990) but only for 2 hours for CH_4 (R^2=0.998) and N_2O (R^2=0.998) as shown in figure 6.9 for the control treatment as an example. The addition of solution generally initially increased emissions followed by a decline with time. CO_2 emissions were highest followed by N_2O and CH_4 respectively. Generally LAS present at 10mg L^{-1} or higher suppressed greenhouse gas emissions by >20% for CO_2, >30% for CH_4 and >40% for N_2O. However, LAS at 5mg L^{-1} concentration caused a stimulative effect in CO_2 and CH_4 emissions by >20% as shown in figure 6.10. Statistical analysis indicated significant differences between treatments for CO_2 ($F=11.287$, $p<0.001$), CH_4 ($F=7.693$, $p<0.05$) and N_2O ($F=15.072$, $p<0.001$). However, Tukey post hoc tests revealed that significant differences were only observed between N_2O and CH_4 emissions for the replicates exposed to 100mg L^{-1} LAS.

Figure 6.9: Linear emissions over time for the control treatment for:

(a) CO_2 (b) CH_4



(c) N_2O

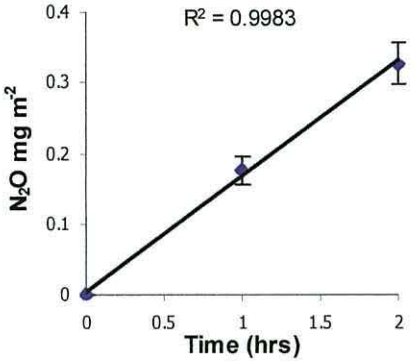
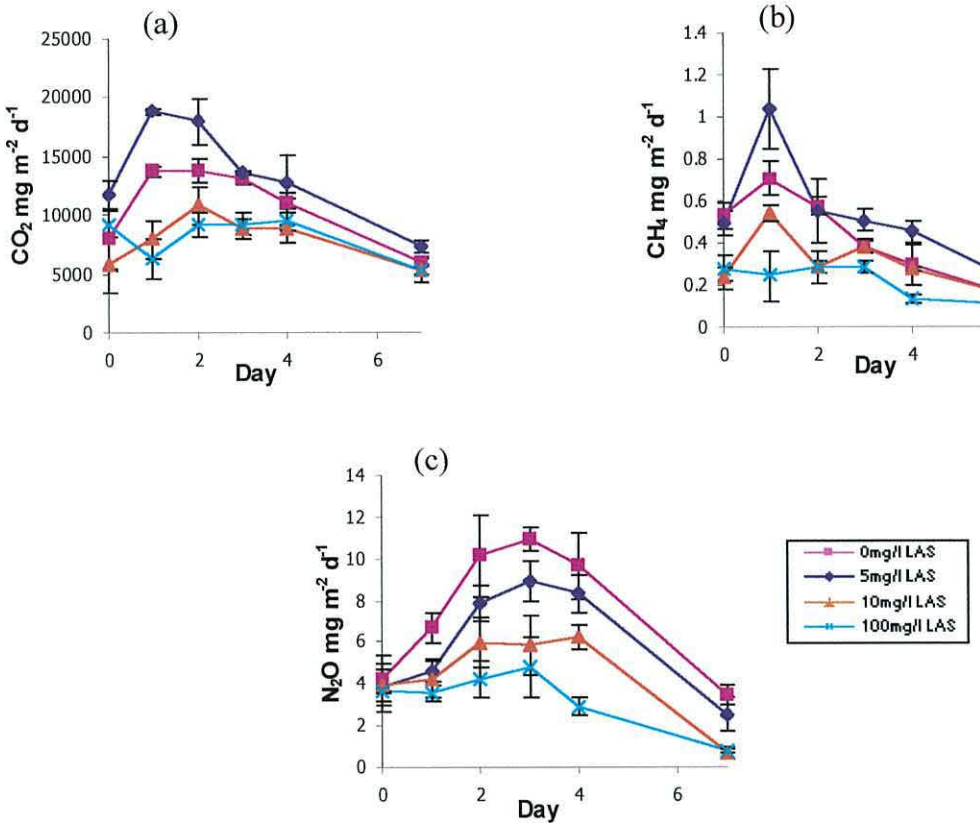


Figure 6.10: Gas emissions measured with LAS present at 0, 5, 10 and 100mg L^{-1} for (a) CO_2, (b) CH_4 and (c) N_2O.



6.5.4 Discussion

Greenhouse gas emissions in constructed wetlands can be useful indicators of biological and chemical changes due to the presence of pollutants such as LAS. Production and emissions of greenhouse gases have mainly been investigated in

natural rather than constructed wetlands (e.g. Freeman *et al.* 1993). However, Tai *et al.* (2002) reported that fluxes of greenhouse gas emissions from a constructed wetland were a significant source of methane with a daily mean of 5.22g CH_4 m^2 d, which is 250 times as much as measured in a natural wetland at the same latitude.

Freeman *et al.* (1993) reported linearity of CO_2 production for over 12 hours in peatland cores. In this study emissions were only assessed over 4 hours and proved to be linear. Similarly Freeman *et al* (1993) reported linearity over 4 hours for N_2O and 2 hours for CH_4. However, linearity was observed over a shorter time frame (2 hrs) in this study for N_2O reaching saturation after 4 hours but equal for CH_4.

LAS at concentrations higher than expected in constructed wetlands (10mg L^{-1}) suppressed greenhouse gas emissions in this study. Although wetlands can act as CO_2 sinks via accretion of organic matter into soils and photosynthetic assimilation of atmospheric CO_2 (Brix *et al.* 2001), the greenhouse gas can be released via microbial transformations and mineralization of organic matter. As discussed previously, LAS mineralization also produces CO_2. The decline in CO_2 emissions with LAS >10mg L^{-1} may be explained via reduced microbial respiration due to possible inhibition as observed in section 6.3 above and elsewhere (Malkomes & Wöhler 1983, Jensen 1999). The observed reduction (min. 20%) would be beneficial in terms of greenhouse gas emissions and subsequent predictions on climate change. However, those benefits must be balanced against the reduction in microbial and enzyme activities (sections 6.3 and 6.2), plant toxicity (section 6.4) and PO_4 adsorption (section 6.1).

According to Brix *et al.* (2001) annually up to 15% of the net fixed carbon may be released to the atmosphere as CH_4. However, this study suggests that inhibition of CH_4 emissions may occur at high LAS concentrations. This may possibly be due to the release of SO_4 during LAS mineralization. Sulphate is known to have an inhibitory feedback on CH_4 emissions with C-S interactions in wetlands well reported (Mitsch & Gosselink 1993, Freeman *et al.* 1994, Kadlec & Knight 1996). Generally when SO_4 is high CH_4 production is low (Mitsch & Gosselink 1993). This may be attributed to competition for substrates between S and CH_4 bacteria, inhibitory effects of SO_4 or SO_3 on methanogenic bacteria or dependence of CH_4 producing bacteria on

the products of sulphur reducing bacteria (Mitsch & Gosselink 1993). Hence due to the SO_4 release as a by-product during LAS breakdown this may be having a detrimental effect on CH_4 production. Wagener & Schink (1987) investigated degradation of C_{12}-LAS under anaerobic conditions and reported inhibitory effect on methanogenesis at >100mg L^{-1} in an anionic digester sludge and >50mg L^{-1} in an anoxic creek sludge. Shcherbakova *et al.* (1999) found that LAS had an intermediate methanogenesis inhibitory effect with 292mg L^{-1} causing 20% inhibition. However, these tests were conducted at high concentrations that do not reflect realistic environmental concentrations. Hence evidence suggests that the methanogenic bacteria are very sensitive to toxic materials and that surfactants inhibit methane production (van der Merwe 1969, Swanwick & Shurben 1969). However, in this study only the 100mg L^{-1} LAS treatment was statistically lower than the control. Hence at environmentally realistic LAS concentrations encountered in constructed wetlands no inhibitory effect on CH_4 emissions is anticipated.

N_2O emission via denitrification is the major N-elimination mechanism in wetlands and may be affected by nitrate supply, carbon availability and hydrology (Groffman 1991). Data from this study suggest that N_2O production is more sensitive to LAS than CO_2 or CH_4, with a 20% reduction in emission with LAS present at 5mg L^{-1}, increasing to 55% reduction with 100mg L^{-1} LAS. This may suggest that the surfactant is more toxic to microbes outside of the carbon cycle. However, Painter & Zabel (1989) reported that LAS has little or no effect on nitrification in wastewater treatment even at high concentrations of the surfactant. No inhibition of nitrification at 20mg L^{-1} LAS was observed (Baillod & Boyle 1968 as reported in Painter & Zabel 1989), although inhibition at >20mg L^{-1} is reported (Janicke *et al.* 1973 as reported in Painter & Zabel 1989). Hence similar effects on denitirification may be possible. Alternatively possible manipulation of the N and C availability due to the surface-active properties of the surfactant may have caused the reduction.

CO_2 and CH_4 emissions were stimulated with LAS present at 5mg L^{-1}. This was also observed in section 6.3 and attributed to possible combined response of inhibition and stimulated compartments of microbial community with the latter due to increased microbial activity and mobilisation of nutrients and organic matter (Malkomes & Wohler 1983). Hence it is likely that similar mechanisms govern CO_2 and CH_4

emissions in this study. Alternatively as LAS is mineralized to CO_2, at low concentration whereby the surfactant is not toxic to the microbes, the stimulation in CO_2 emissions may be reflective of LAS breakdown. However, this cannot be confirmed from the data available as it is impossible to distinguish the source of the CO_2 emissions. In addition it is recognised that in the field other factors may also influence the net greenhouse gas emissions, including water table height, vegetation, climatological influences (Brix *et al.* 2001) and temperature (Gersberg *et al.* 1983).

6.6 CONCLUSION

This chapter suggests that at environmentally realistic concentrations encountered in wetlands LAS has no detrimental effects on the parameters assessed. This supports evidence by Painter & Zabel (1989) who reported that LAS has little or no effect on wastewater treatment. Hence the following conclusions may be drawn:

1. Low P sorption capacity of the gravel substrate was confirmed. LAS at 10mg L^{-1} increased PO_4 adsorption, but reduced PO_4 sorption at higher surfactant concentrations.
2. Adverse effects on enzyme activities were exhibited only at high LAS concentrations with β-glucosidase identified as being more sensitive at lower concentration than phosphatase and sulphatase.
3. A 2-fold stimulation of microbial activity at 0.1mg L^{-1} LAS in comparison to the control was observed. This was followed by progressive inhibition with increasing LAS concentration to >90% at 1000mg L^{-1} LAS.
4. Decline in seed germination with exposure to increasing LAS concentrations. However, small seedlings were less sensitive with no considerable adverse effects observed with LAS exposure <10mg L^{-1}.
5. Greenhouse gas emissions are reduced at >10mg L^{-1} LAS by a minimum of 20%, with N_2O most sensitive (>40%). In fact CO_2 and CH_4 emissions were stimulated with 5mg L^{-1} LAS concentration resulting in >20%.

CHAPTER 7: Concluding Discussion

This study has investigated removal processes of anionic surfactant LAS in wetlands treating domestic wastewater. Wetlands can act as sources, sinks and transformers of nutrients and pollutants with the dominant process depending upon several factors including vegetation presence, climatological influences, hydrology and, in constructed wetlands, design and wastewater source.

7.1 APPLICATION OF LAS METHODOLOGY TO WETLANDS

In this study, LAS measurements were undertaken via two main methods, i.e. HPLC analysis and radiochemical methods. The HPLC method, coupled with sample SPE pre-concentration, was employed for the detection of primary LAS biodegradation in field and laboratory experiments. The method was found to be sensitive and reproducible for wetland samples as reported for other wastewater treatment studies (Matthijs & De Henau 1987, Kikuchi *et al.* 1986). Successful separation of LAS from the other components and interferences in both natural and artificial sewage wastewater was observed. This method was comparable to that adopted by Inaba (1992) and Del Bubba *et al.* (2000) for LAS monitoring in wetlands with low LAS concentrations (<0.05mg L^{-1}) detected in this study. However, it is noted that this method is expensive and time consuming.

LAS biodegradation as determined by sensitive radiochemical methods used in chapters 3, 4 and 5 was used to distinguish between initial primary biodegradation and complete mineralization. Measurement of LAS biodegradation as $^{14}CO_2$ evolved provided unequivocal information on the extent of LAS mineralization as reported in other studies (Swisher 1987, Federle & Schwab 1992, Dorfler *et al.* 1996, Branner *et al.* 1999). Similarly the measurement of ^{14}C-LAS for adsorption (chapters 3 and 5) and for quantification of plant uptake (chapter 4) at extremely low LAS concentrations was possible using radiochemical techniques. However, shortcomings of this method include expensive radiochemical compounds and hazards of handling radioactivity.

7.2 LAS REMOVAL

In this study LAS removal was investigated in various laboratory experiments, small-scale field systems and in operational constructed wetlands. In chapter 2 the major LAS removal processes in wetlands were confirmed as biodegradation and adsorption. These were further studied in detailed laboratory studies in chapter 3 and were supported by evidence from other publications in various environmental compartments and alternative sewage treatment processes (Matthijs & De Henau 1985, Matthijs & De Henau 1987, Jensen 1999, Scott & Jones 2000). The balance between biodegradation and adsorption will determine the overall reversible and irreversible LAS removal in wetlands, with the data presented in chapters 2-5 confirming that wetlands are sinks for LAS.

The high LAS removal observed in the various field and laboratory experiments in chapters 3-5 (>85-90%) and on a latudinal gradient (80%) in section 2.3 highlights the potential for high LAS treatment efficiency in constructed wetland systems. However, comparison with the removal observed in the operational constructed wetlands in section 2.1 (55-60%) may suggest that limiting factors may have affected LAS removal. Similarly, Inaba (1992) reported that a mean of 60% of inflow LAS was annually removed in a wetland treating wastewater in Japan. This led to several experiments reported in this thesis investigating factors that may have led to the treatment inefficiencies. LAS removal was found to be dependent upon four main regulating factors. These were wastewater source, wetland characteristics, environmental factors and surfactant properties. These factors are discussed below.

(a) Wastewater Source

In section 2.1 mean wetland inflow LAS concentration was measured at 1.1mg L^{-1}. This was as expected after comparison with 3.25mg L^{-1} quoted by Holt *et al.* (1998) in UK raw sewage (range 1.10-5.58mg L^{-1}). Observations from fieldwork and further detailed laboratory studies found that the composition of the wastewater source affected LAS removal in constructed wetlands. Some of the factors identified were pH, water hardness, nutrient availability and presence of other surfactants.

In experiments conducted in chapter 5, investigating extreme changes in wastewater source, pH was found to affect biodegradation and adsorption. Extreme acidic or

alkaline conditions were found to detrimentally affect LAS biodegradation in comparison to the neutral control. Greatest LAS mineralization at neutral pH is reported elsewhere (Dorfler *et al.* 1996). However, greatest adsorption was exhibited under acidic conditions (section 5.2) as reported in other studies (Küchler & Schnaak 1997, Fytianos *et al.* 1998b) possibly due to changes in surface charges resulting in greater attraction to the anionic surfactant.

Water hardness was also found to affect LAS mineralization in chapter 5 with lower net mineralization observed at high $CaCO_3$ concentration. This was supported by Berna *et al.* (1989) who reported that the higher the water hardness then the higher the LAS elimination through precipitation of LAS Ca-salts, and hence greater the observed removal. Thus elimination of LAS via precipitation lowers the bioavailability of the surfactant resulting in a reduction in the in situ toxicity, as reported for *Daphnia* (Verge *et al.* 2001).

Nutrient availability was also influential with nitrate found to negatively correlate with LAS in sections 2.1, 2.2 and 4.3.1. This perhaps suggests N-limitation on LAS removal efficiency. In addition prior exposure of the biofilm microbial community to nutrient rich solution resulted in an increase net yield in LAS mineralization in chapter 3. However, prior exposure resulted in a longer initial lag phase and the net 4% increase was found to be not significant. Hence further investigations are required on the effect of nutrient availability on LAS biodegradation.

In chapter 5 the effect of cationic or non-ionic surfactants presence in wastewater was investigated on LAS mineralization and desorption. A slight decrease in LAS mineralization was observed with the cationic surfactant exhibiting the strongest effect (section 5.5). This was attributed to the lowering of LAS bioavailability due to precipitation (Berna *et al.* 1989, Ferrer *et al.* 2000) with the positively charged cationic surfactant. However, in section 5.6 the non-ionic surfactant had a greater effect of promoting LAS desorption. Hence this thesis has highlighted that it is important to consider other components and parameters of the wastewater source when assessing LAS removal as several factors may impact upon treatment efficiency.

(a) Wetland Characteristics

In section 2.3 wetland design and characteristics were identified as being influential in determining LAS removal on a latitudinal gradient. Section 2.1 also highlighted the importance of wetland design with only the Brynsiencyn site maintaining constant LAS removal during extreme rainfall events due to its in-built stormwater overflow capacity. Constructed wetlands are mainly characterised by the presence of gravel substrate, established microbial communities and the presence of plants. Hence this section discusses the role of the wetland components in LAS removal

(i) Gravel Substrate

In chapter 3 the biofilm microbial community attached to the gravel substrate was identified as a major LAS irreversible removal mechanism in constructed wetlands. This was supported by the negligible degradation observed with biofilm absence, the observed <1% mineralization in the presence of only free bacteria and the work of other researchers (Takada *et al.* 1994, Boeije *et al.* 2000). Also in chapter 3, SEM analysis of the attached biofilm indicated no visual differences between the bacterial populations. This suggests that the lack of statistically significant differences in percentage LAS removal in section 2.1 and for degradation rates reported in section 3.3.1 may be accounted for by the similar composition of the biofilms.

The fate of LAS in wetlands can also be significantly affected by adsorption onto gravel substrate as demonstrated in chapters 2, 3 and 5. A mean of 37% adsorption was exhibited for the wetland gravel types assessed (section 3.3.2) with 23% being irreversible (section 5.6). Hence indicating that the gravel is a sink for LAS. Adsorption was quantified to be greatest for the Rosset gravel and lowest for the Brynsiencyn gravel. The higher Ca-content of the Rosset gravel was suggested to possibly promote LAS adsorption, with Fe and Al less important under the test conditions adopted. However, further tests with gravel substrates of different geology and mineralogy would be required for confirmation. Biofilm presence resulted in slightly greater adsorption suggesting multiple interactions between surfactant, biofilm bacteria and gravel-liquid interfaces. Sorption can also affect degradation kinetics as it is directly related to the residence time (McAvoy *et al.* 1994) and hence bioavailability of LAS. This was evident in the greater LAS adsorption reported in the winter in section 2.1. A similar trend reported by Inaba *et al.* (1988) was

attributed to the ability of bacteria to degrade LAS during the summer resulting in the adsorbed LAS being reduced. However in winter, when activity of bacteria becomes lower, LAS was only reduced by adsorption.

(ii) Microbial activity

In chapter 3 LAS biodegradation by biofilm microbial communities LAS was found to be controlled, in part, by previous exposure history. Accelerated initial LAS degradation due to adaptation after previous exposure is reported elsewhere (Larson & Payne 1981, Palmisno *et al.* 1991, Federle & Pastwa 1988, Branner *et al.* 1999). Evidence of adaptation in response to prolonged LAS exposure was also suggested spatially in the Brynsiencyn wetland in chapter 3. More rapid LAS mineralization closest to the inlet with a decreasing capacity with increasing distance was observed. Similarly evidence of microbial adaptation to LAS near sewage effluent sources in rivers is reported (Larson & Payne 1991, Shimp 1989). Adaptation was also indicated in terms of pH. The Brynsiencyn wetland usually received wastewater with pH of 6.6±0.3 (section 2.2). The decrease in degradation with extreme changes in pH reported in section 5.2 may suggest that the microbial community is adapted to the neutral conditions.

Oxygen availability also affected LAS biodegradation with greater removal evident when the gravel biofilm (section 5.3) and plants (section 4.3.5) were exposed to oxygen. In addition LAS mineralization was found to decrease with increasing depth in section 3.3.1, as reported elsewhere (Federle & Pastwa 1988, Branner *et al.* 1999), corresponding with a decrease in oxygen concentration with depth. This confirmed earlier work of rapid biodegradation under oxygenated conditions (Swisher 1987, Steber & Berger 1995, Scott & Jones 2000). Greater LAS degradation rate with increased dissolved oxygen concentration is reported (Krueger *et al.* 1998). However, biodegradation under oxygen-poor conditions was also evident, although this was significantly lower than that observed under oxygenated conditions. LAS removal under oxygen-poor conditions is reported elsewhere (Federle & Schwab 1992, Denger & Cook 1999). In addition significant LAS removal was observed in wetlands throughout this thesis even though oxygen-poor conditions can prevail in parts due to the presence of standing water resulting in a reducing substrate environment.

In terms of the longevity of the LAS-utilizing bacteria no detrimental impacts in LAS removal were observed with time. Long-term monitoring in section 2.2 revealed that LAS removal improved with time in the Brynsiencyn wetland by 14% in the latter 18-months. In addition an increase in LAS removal with time was observed for the small-scale wetlands in section 4.4.1. Adaptation of the microbial community may again be a key influencing factor. However, it is recognised that environmental conditions may also have had an effect.

(iii) Plants

In sections 2.1 and 2.2 seasonal variations in LAS removal was observed with a peak in the spring months. This peak coincides with the growth season of the *Phragmites* in the wetlands. Further investigations in chapter 4 revealed that significantly higher LAS removal was observed for planted than unplanted systems and is supported by evidence from other publications (Federle & Schwab 1989). This led to further investigations into the role of plant mechanisms in facilitating LAS removal.

The role of rhizosphere and roots as attachment sites for bacterial growth was highlighted to be significant in section 4.3.7. Rhizosphere biofilm was found to facilitate greater initial LAS mineralization, with a less distinctive lag phase, in comparison to gravel biofilm (section 3.3.1) and to washed roots (section 4.3.7). Greater and more active microbial biomass associated with the rhizosphere than the surrounding bulk soil is reported (Knaebel & Vestal 1994, May *et al.* 1990, Whipps & Lynch 1986). This may be an alternative explanation to adaptation for the greater removal of LAS observed near the inflow at the Brynsiencyn wetland discussed above. A higher planting biomass was measured near the inflow in section 3.3.1 and found by other authors in constructed wetlands (Coleman *et al.* 2001, Dušek & Kvet 2001). This would result in increased rhizosphere surface area and hence greater attachment sites for the LAS-utilizing bacteria.

Oxygen transport capacity of the plants into the root zone was also identified as and important factor. Exposure of the *Phragmites* to oxygen was found to significantly increase LAS removal in comparison to nitrogen exposure (section 4.3.5). Similarly, in a study on LAS in a wastewater pond, Federle & Schwab (1989) reported that

oxygen levels were higher and surfactant concentration lower in sections of the pond colonised by macrophytes.

Other plant-associated functions were also investigated. However, supplemental plant-derived DOC or glucose was found not enhance greater LAS degradation (section 4.3.6). In addition plant uptake was found not to be a significant removal mechanism in this study (section 4.3.4) as reported elsewhere (Knaebel & Vestal 1992).

(b) Environmental Factors

Environmental factors were also found to be an influencing factor on LAS removal. Observed changes in LAS degradation with season was discussed in relation to plant mechanisms above. Air temperature was also identified to affect both LAS degradation and adsorption in chapters 2, 4 and 5. In general the higher the temperature then greater the LAS biodegradation as suggested elsewhere (Inaba 1992, Takada *et al.* 1992, Dorfler *et al.* 1996, Palmisano *et al.* 1991). In section 5.1 an increase in temperature from 5 to 30°C induced 30% greater net LAS mineralization. This was more evident in the 3-fold higher $t_{1/2}$ values at 5°C in comparison to 30°C. Hence temperature may also be a limiting factor on LAS removal observed in section 2.1 with low mean temperatures observed in comparison to laboratory experiments.

However, the increase in LAS mineralization observed with a 6-fold increase in temperature in section 5.1 was much lower than expected. In addition in section 2.3 the highest LAS removal (96%) was observed at the Swedish wetland even though this site exhibited the lowest temperature. Also high LAS removal during winter was reported in sections 2.1 and 2.2. In addition no statistical trend was observed between LAS removal and air or bed temperature in chapters 2 and 4. Hence this may suggest that temperature alone cannot be a substantial limiting factor.

LAS removal via adsorption, either directly or indirectly, was also affected by temperature. In terms of direct effects, greater LAS adsorption was unexpectedly exhibited at 5 than 20°C in section 5.1. This was in contrast to other studies reporting that adsorption is not altered by temperature changes (Inaba *et al.* 1988). However, due to the lack of research in this area no firm conclusions can be drawn further

investigation is required. In terms of indirect effects, a significantly greater amount of LAS adsorption was measured during the colder winter months in section 2.2. This supported the suggestion by Inaba *et al.* (1988) that adsorption may play a more important role when biodegradation is low due to, for example, low temperatures.

Another environmental factor that was also found to detrimentally affect LAS removal in section 2.1 was excessive rainfall. Individual rainfall events are reported to detrimentally affect water quality in wetlands elsewhere, especially for small wetlands (Stark *et al.* 1994, Kadlec 1999, Spieles & Mitsch 2000). Possible explanations include simple dilution resulting in lower percentage removal efficiencies. No attempt was made in the field studies in chapter 2 or 4 to quantify the effect that rainfall dilution had on the LAS removal percentages calculated. The negative correlations reported for removal rates of various parameters with rainfall may suggest that dilution effects are important. Hence it is recognised that a limitation of the work is that the percentage removal rates may be lower due to dilution effects. In addition, the faster hydraulic residence time during high flows may result in a reduction in the contact time between surfactant and biofilm microbial community consequently reducing LAS removal. In addition the high flows may result in the washing down of the biofilm hence reducing removal efficiency as reported elsewhere (Takada *et al.* 1994).

(c) Surfactant Properties

Structural effects of the surfactant were found to affect biodegradation in the majority of experiments conducted and attributed to hydrophobic effects. In chapter 3, LAS structure was found to affect both yield degradation and kinetics. Two main compound structure effects were evident, i.e. isomer structure and chain length. In chapter 3, removal of LAS isomers with the sulphophenyl group attached more towards the centre of the alkyl chain was slower, confirming previous work for biodegradation and adsorption (Swisher 1987, Hand & Williams 1987, Marcomini & Giger 1987, Terzic *et al.* 1992). In addition faster biodegradation rates with increasing chain length were reported in chapters 2 and 3 and is in accord with previously published data (Swisher 1987, Kikuchi *et al.* 1986, Terzic *et al.* 1992). Hydrophobic structural effects on adsorption were also evident in chapters 2 and 3 with longer chain homologues exhibiting greater adsorption. Similar structural effects

on adsorption are reported elsewhere for wetlands (Del Bubba *et al.* 2000) and other sediment types (Hand & Williams, 1987, Swisher 1987, Küchler & Schnaak 1997, de Wolfe & Feijtel 1998). The longer chain homologues exhibit the greatest toxicity. Hence implications for constructed wetlands are that the overall toxicity of LAS to the wetland system is reduced.

7.3 NATURAL WETLAND PROCESSES

7.3.1 Nutrients

Nutrient removal was variable in the operational wetland monitored in chapter 2 with nitrate and sulphate removal more consistent over time than phosphate (see section 7.3.2 below). Although greater N removal is reported with warmer temperatures (Frankenback & Meyer 1999) no seasonal trends were recognised in this thesis. However, seasonal variation distinctive in sulphate removal with greater removal observed in the summer (section 2.1). Although in the literature there is evidence of poor wetland treatment performance for nutrients in some cases, a substantial proportion can be related to the over optimistic or inappropriate design and poor management. As observed in chapter 2, often wetlands are subjected to loadings much higher than anticipated and designed for, e.g. nitrate runoff from agricultural land, and are not always well designed for stormwater flows. Hence better design and management of wetlands is required for sustained nutrient removal.

The relationship between LAS and nutrient availability was discussed above with N limitation suggested in chapters 2 and 4. The effect of LAS on nutrient cycling assessed via greenhouse gas emissions was assessed in chapter 6. The results suggested that the surfactant was not inhibitory to this type of wetland biogeochemical process at environmentally realistic concentrations (<10mg L^{-1}), although production of N_2O was sensitive to LAS presence indicating important interactions occurring between the surfactant and the N cycle. However, in other wastewater treatment studies LAS is reported to have little or no effect on nitrification (Painter & Zabel 1989).

Plant uptake has been identified as an important nutrient removal mechanism in wetlands (Breen 1990, Brix 1997). In section 6.4 LAS, at environmentally realistic

concentrations, was found not to be toxic to plant seedlings growth. Hence presence of the surfactant should not impair on nutrient uptake in constructed wetlands.

The research on different climates in section 2.3 and temperature in section 5.1 suggests that constructed wetlands are a feasible and effective form of wastewater treatment for developing countries as reported elsewhere (Billore *et al.* 1999, Haberl 1999). Although temperature was not recognised as a reliable descriptor of LAS removal or nutrient cycling in the wetlands monitored, the general faster removal rates observed at higher temperatures suggests the potential for wastewater treatment in these countries. The warmer climate conditions are ideal to promote rapid biological growth within the wetland throughout the year resulting in high treatment efficiency (Billore *et al.* 1999). Conventional treatment technologies are unaffordable to many of these countries and hence natural treatment by constructed wetlands is an attractive cost effective alternative (Haberl 1999).

7.3.2 PO_4 adsorption

Throughout this thesis phosphate removal was highly variable with reported outflow concentrations exceeding that of the inflow on several occasions in chapter 2 and reported elsewhere (Greenway & Woolley 1999, Davison *et al.* 2001). In addition PO_4 removal capacity declined with time and is frequently reported by other researchers (Tanner *et al.* 1998, Davison *et al.* 2001). This was supported by greater removal observed for the Rosset wetland, the youngest wetland, and lowest for the oldest wetland (Clutton). The decline was attributed to saturation of adsorption capacities of the gravel substrate media.

This was supported in section 6.1 whereby PO_4 sorption was investigated in the laboratory. These data suggest that the adsorption capacity for the Brynsiencyn gravel was approximately 8.3mg kg^{-1}. From this data it is possible to work out the maximum PO_4 sorption capacity of a wetland. If it is assumed that approximately 2500kg of gravel was initially added to the wetland during construction, that the wetland received 113mg P per week (as estimated by Gray *et al.* (2000) for a similar wetland) and that all P is removed via adsorption then the sorption sites will be saturated after 183 weeks (3.5 years). This corresponds to a P inflow concentration of 7.5mg L^{-1}. Gray *et al.* (2000) reported calculated saturation after 4 years for their investigated

wetland. However, this does not take into account plant uptake or sedimentation and hence may be an underestimate of actual working life of wetland for P removal (Gray *et al.* 2000). At environmentally realistic concentrations LAS did not impair on PO_4 sorption (section 6.1). In fact, in comparison to the control, LAS presence (10mg L^{-1}) promoted PO_4 adsorption. However, a reduction in PO_4 adsorption was exhibited with high LAS concentration (100mg L^{-1}) which may suggest saturation and subsequent competition of available sorption sites.

7.3.3 DOC and Phenolics

LAS concentration as a percentage of DOC was low at <5% in section 2.1 and <1% in section 2.2 but higher when compared on a latitudinal gradient in section 2.3 (10%). Berna *et al.* (1993) reported LAS contribution to DOC was low (<1%). Although LAS mineralization is quoted in terms of DOC decrease over time in other studies (Zhang *et al.* 1999), it was not an appropriate parameter in this context due to the natural and anthroprogenic compounds that cannot be distinguished from the breakdown product in wetlands.

A similar temporal pattern in DOC and phenolics were observed in chapter 2 and relationships with enzyme activity identified. The data in relation to the inhibitory effect of phenolics on enzyme activity (Wetzel 1992, Wetzel 1993) was inconclusive in this study. The relationship between DOC and enzyme activity may be related to the temperature response. Elevated temperatures correlated inversely with DOC removal efficiency possibly due to increased evapotranspiration raising DOC levels (Quanrud *et al.* 2001) or greater leaching of plant derived DOC (Pinney *et al.* 2000).

7.3.4 Microbial and Enzyme Activities

The MUF-substrate method employed for the investigation of enzyme activity on the gravel in constructed wetland samples proved suitable even though the majority of such studies have been applied to only soil. As expected the order of greatest activity for the enzymes monitored were phosphatase>β-glucosidase>sulphatase throughout this thesis. A drop in enzyme activity in the spring (May) was also observed as reported elsewhere (Kang 1999, Kang & Freeman 1999, Shackle *et al.* 2000a) possibly due to competition between plant root systems and microorganisms for

nutrients (Kang 1999). However, although presence and species of plants can have a marked effect on enzyme activity (Khan 1970, Kiss *et al.* 1974 as quoted in Speir & Ross 1978), the results in chapter 4 did not support this hypothesis.

Despite the known importance of temperature as a controlling regulator of enzyme activity reported by other authors (Kang & Freeman 1998, Kang & Freeman 1999, Shackle *et al.* 2000a) few correlations were established with air temperature. This may suggest that interactions with other factors are paramount, e.g. vegetation exudates (Shackle *et al.* 2000), substrate type (McClaugherty & Linkuns 1990), hydraulic loading and flow rate, or that inhibition is occurring. Enzyme activity was also affected by waterlogging (Kang & Freeman 1999, Pulford & Tabatabai 1988). However, LAS at environmentally realistic concentrations was not found to adversely impair on enzyme activity in section 6.2.

In addition LAS presence at the low concentrations encountered in wetlands (max. of 3.2mg L^{-1} reported in section 2.1) was not inhibitory to the microbial population activity measured as CO_2 respiration in section 6.3. Hence LAS toxicity to the microbial community would not be expected to occur in constructed wetlands at the concentrations observed in the inflow and hence cannot account for the sub-optimal conditions for LAS removal observed in the operational wetlands in section 2.1. Other researchers also support the proposed absence of inhibition to microbial activity at low LAS concentrations (Litz *et al.* 1987, Elsgaard *et al.* 2001a, Elsgaard *et al.* 2001b).

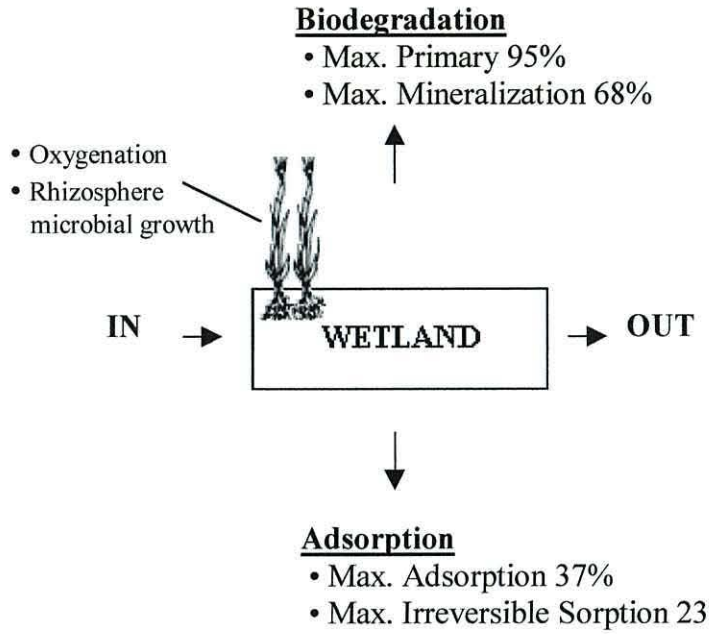
7.4 CONCLUSION

In this thesis biodegradation and adsorption were found to be the major LAS removal processes in constructed wetlands. Various experiments highlighted the potentially high (85-90%) LAS treatment capacity of wetlands. Although only a mean of 55% LAS removal was observed in the operational constructed wetlands (section 2.1), peak removal of 84% was recorded. The sampling period coincided with extreme weather events that notably contributed to the low annual LAS removal observed. This was reflected in the improvement in LAS removal at the Brynsiencyn wetland with time (section 2.2) and the high removal in international wetlands (section 2.3). In addition effluent LAS concentrations discharging from the wetlands were still low (min. 0.02mg L^{-1}) and comparable to other sewage treatment studies.

Possible factors influencing LAS removal in wetlands were identified as wastewater source, wetland characteristics, environmental factors and surfactant properties. However, many of the possible detrimental impacts of these factors on LAS removal could be overcome by the incorporation of simple design modifications. For example, increases in temperature resulted in greater LAS removal (section 5.1) which could be achieved by better insulation by straw (Mæhlum *et al.* 1995) or the introduction of more plants. In addition greater oxygenation also resulted in greater LAS treatment (sections 4.3.5 and 5.3). Aeration of wastewater by physical means, greater plant biomass per unit area (section 4.4.2) or the incorporation of pipes (Cooper 2001) in the wetland to promote oxygenation may result in greater LAS treatment. However, any modifications will be dependent upon LAS inflow concentration, current removal rates, a cost/benefit assessment and the effects on other wetland processes.

The results of the various experiments conducted in this thesis demonstrate that the interactions in constructed wetlands between inflow wastewater, biofilm microbial community, substrate and plants in determining LAS removal are complex. However, figure 7.1 simplifies the data obtained in chapters 3 and 5 illustrating that wetlands are a sink for LAS.

Figure 7.1: LAS removal in constructed wetlands.



7.5 FURTHER WORK

In this thesis LAS removal in wetlands has been thoroughly investigated and several factors affecting the rate of removal identified. It is recognised that during this research more questions have arisen and further work is suggested in order to address these issues.

Sub-optimal conditions for LAS Removal

Despite the high LAS removal observed in various laboratory (chapter 3, 4 and 5) and field (chapter 4) studies in this thesis, the removal observed in the operational constructed wetlands monitored in chapter 2 are relatively low in comparison. Possible factors identified as contributing to the sub-optimal conditions include climatological influences, mainly temperature and precipitation, previous exposure and plant effects. Hence long term monitoring of LAS removal in wetlands receiving various inflow concentrations and sources is recommended.

Springtime peak in LAS removal

The springtime peak in LAS removal observed in Chapter 2 was attributed to plant growth mechanisms. Further investigation in chapter 4 confirmed that plants do have

a significant effect on LAS removal in terms of oxygen transport, rhizosphere microbial community and species but not through uptake or release of DOC exudates. Further investigations would be most useful if focused on interactions between plant growth and microbial population activity and/or changes to determine if favourable conditions could be manipulated to promote LAS removal in wetlands.

Identification of LAS-utilizing biofilm bacteria

Although several authors have suggested that a consortium of bacteria is required for complete LAS mineralization, no attempt was conducted to identify the microbial community responsible in a wetland in this thesis. Identification of the forms and functions of the microbial community responsible for LAS degradation in wetlands may give indication to the optimal conditions required for removal and any limitations on their activity. Comparison of gravel and rhizosphere biofilm microbial communities could possibly provide an understanding of the enhanced removal of LAS observed in planted then unplanted wetlands.

Climate change

Section 6.5 suggested that LAS may have an effect on the natural biogeochemical processes occurring in wetlands that result in the emissions of greenhouse gases. Although the reverse was investigated in section 5.1 in terms of temperature changes, a possible consequence of global warming, other climatological influences were not researched. In section 2.1 extreme rainfall was identified as detrimental for LAS removal via flow rate affects. Takada *et al.* (1994) reported high LAS removal during low flow in a river due to an increased residence time and subsequent contact of LAS with the biofilm. However, high flows would wash down the biofilm and reduce the removal efficiency (Takada *et al.* 1994). In addition Knaebel *et al.* (1990) in a laboratory experiment involving wetting and drying of soils indicated that both the rate and extent of LAS mineralization may be effected by climatological events. Hence possible effects of climate change could be investigated in terms of consequences on LAS removal processes.

Intermediates

This thesis has concentrated on the biodegradation of LAS via primary biodegradation or mineralization. However, the Sulpho Phenyl Carboxylates (SPCs) intermediates

formed can vary from the parent compound in bioaccumulation and toxicity. Hence further research on the fate of SPCs in wetlands is recommended via complex analytical methods for detection, e.g. LC-MS (Gonzales-Mazo *et al.* 1997).

Investigation of other surfactants

Limitations on machinery and costs did not enable other surfactants to be monitored in this study. However, although LAS represents a large proportion of the surfactants currently available it is also important to monitor the fate of other surfactants in wetlands, e.g. Alkyl Phenol Ethoxylates (APE) and Nonyl Phenol (NP).

Long-term phosphate removal

The work conducted on the effect of LAS on PO_4 adsorption highlighted the low adsorption capacity of gravel and how competition between anions in wetlands can possibly result in even lower adsorption occurring. Hence there is a requirement for further research into either the introduction of units purposely for P removal prior to the wastewater entering the wetland or mixing of a high P-sorption capacity substrate with the gravel in the wetland. However, as discussed in chapter 6, the research would also require an investigation into the effect of artificial substrates on other biogeochemical processes in the wetland, e.g. nutrient cycling, plant growth, etc.

Springtime decline in enzyme activity

The monitoring of enzyme activity in Chapter 2 highlighted a springtime decline as previously reported by other authors (Shackle *et al.* 2000a, Kang 1999, Kang & Freeman 1999). The decline was attributed to plant growth mechanisms. However, it was not possible to confirm the decline in the subsequent spring season neither at the Brynsiencyn site nor at the mesocosms in Chapter 4 as planned due to the Foot and Mouth outbreak. Hence further research is recommended on plant-enzyme interactions. Artificially stimulating plant growth, via additional light exposure to promote photosynthesis, to investigate whether a decline in enzyme activity is observed could be conducted. Effects of climate change on plant growth would also be relevant with a late spring potentially delaying plant growth and the drop in enzyme activity.

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APPENDIX A

LINEAR ALKYLBENZENESULFONATES (LAS) REMOVAL IN A PILOT SUBMERGED HORIZONTAL FLOW CONSTREUCTED WETLAND

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ABSTRACT

An experiment was conducted at Florence, Italy, in January-March 2000 to investigate LAS removal processes in a pilot scale SF-h constructed wetland (0.65m^2 surface area and 0.6m depth) planted with *Phragmites australis*. Two separate experiments with nutrient solutions containing 35 and 350mg/l LAS were performed. Several parameters such as filtered and unfiltered COD, TSS, MBAS, dissolved LAS and those adsorbed in particulate matter were analysed. High removal percentages of LAS were observed even at temperature values as low as 5-9°C. The kinetic constants for LAS removal were calculated at two different wetland temperatures. Sulfophenylcarboxylic acids (SPC) represent the primary biodegradation products of LAS and, among these, sulfobenzoic acid is present at significant percentages.

KEYWORDS

SF-h constructed wetland; *Phragmites australis*; LAS removal.

INTRODUCTION

Horizontal submerged flow (SF-h) constructed wetlands, vegetated with *Phragmites australis* were extensively used for the treatment of domestic wastewater (Reed and Brown, 1995; Brix, 1998), while it is less frequent to find application for industrial (Wass and Fox, 1993; Davies and Cottingham, 1994; Knight *et al.*, 1999) or for the removal of selected organic pollutants (Machate *et al.*, 1996, Chong *et al.* 1998).

Among anthroprogenic organic compounds, linear alkylbenzenesulfonates have had widespread use both in domestic and industrial applications (e.g. textile). LAS are toxic to aquatic organisms (Kimerle and Swisher, 1977; Schröder, 1993) and can be transported from the water to the coast by sea-spray, giving rise to deterioration of coastal vegetation (Gellini *et*

al., 1985). Linear alkylbenzenesulfonates from a residential area in Japan were found to be purified naturally via a surface-flow wetland (Inaba, 1992).

In the present study removal of LAS on a pilot scale SF-h constructed wetland was investigated. The surfactants were dosed in the wetland at two different concentrations (35 and 350ppm). The lower concentration enabled simulation of the composition of mixed (domestic and industrial) wastewater, which is usually characterised by concentration of around 10mg/l. The highest concentration is typical for stream water origination from surfactant manufacturing and textile industries or commonly encountered in soil washing and other surfactant-based remediation technologies (Zhang *et al.*, 1999).

METHODS

Site description

The investigation was carried out during the period of January-March 2000 in a pilot system situated at Florence (Italy) receiving outlet wastewater from the activated sludge municipal treatment plant "Torre", previously treated by a settling tank (Del Bubba *et al.*, 1998).

The plant was made with sheets of Plexiglas (12mm thickness), placed above the ground level and outside protected by black polyurethane panels (40mm thickness) in order to avoid algae growth on the interior walls of the cell. The reed bed was established in 1997 using rhizomes of *Phragmites australis* at a density of 10/m^2 and it was completely occupied by the root zone during the experiments. The pilot plant (basin slope 1%) was located in the country, on the outskirts of Florence.

Wastewater temperature was measured continuously *in situ* by a thermocouple and registered every two hours. Daily values were the average of these 12 measures.

The specific area of the SF-h system and the bed depth were 0.65m^2 and 0.6m, respectively; the average flow through the wetland was 0.024m^3/d. The experimental hydraulic retention time (HRT) of wastewater in the same period was 5±0.4days (n=6).

The media was clean gravel (mean grain sze=8mm, range 6-10mm, theoretical porosity 0.35, density 1.36g/cm3) while settling and draining zones (both 10cm length) were built with clean rubble (Ø~8cm). Gravel composition was the flowing: SiO_2 (78%), Al_2O_3 (9%), Fe_2O_3 (4%), CaO (1%), K_2O (3%), MgO (1.5%), Na_2O (2%), others (1.5%). The system was covered with a nylon-tarpaulin to avoid dilution effects due to rainwater.

LAS dosing and chemical analyses

LAS, as a mixture of C_{11}-C_{14} homologues and of positional isomers resulting from the attachment of the phenyl ring to the carbon atoms (from the second to the central one), were added at different concentrations to the wastewater by a peristaltic pump. The commercially available LAS product (Fluka, Switzerland) was purified by liquid/liquid extraction with n-hexane before use.

Within a hour from collection, grab wastewater samples (250ml) taken at the inlet, outlet and at different distances (40, 80 and 120cm) from the inlet were filtered on glass fibre filter (GF/F, Whatman, porosity ~ 0.45μm, England). Total suspended solids (TSS) were quantified through the gravimetric method (IRSA-CNR, 1979). Chemical oxygen demand was determined both on filtered and unfiltered (total COD) samples according to the IRSA-CNR method (1987). Methylene Blue Active Substances (MBAS) were analysed by IRSA-CNR method (1983).

Extraction of dissolved LAS and SPC and HPLC analyses were performed according to the method of Inaba and Amano (1988) using a double-pump liquid chromatograph (LC-10ADVP, Shimadzu) equipped with diode array detector SPD M10AVP (Shimadzu). A CLASS VP 4.2 chromatography data system (Shimadzu) was used for chromatogram acquisition and handling. The samples were injected in the column by a 20-μl loop using a 50-μl syringe (Kloehn, U.S. A.). LAS and SPC adsorbed on particulate matter were extracted by the method of Küchler and Schnaak (1997).

Quantitative determination of LAS and SPC

Calibration lines or standard solutions of LAS and sulfobenzoic acid (SBA) were obtained assuming that the response factor is the same for each of LAS components. Quantitative determination of SPC was effected supposing an identical response factor for SBA and each of SPC homologues.

RESULTS AND DISCUSSION

LAS dosing with 35mg/l

The experiment was carried out in the period January-February 2000 with an average wetland temperature of 6oC (range 5-9oC). The results for filtered and unfiltered COD, TSS, MBAS, dissolved LAS and SPC and for those adsorbed on particulate matter were reported in Table 1. Such data point out very high removal percentages for all the examined parameters with the exception of SPC, which were formed during the primary step of biodegradation process of

LAS. It should be also noted that the values of outlet COD are similar to those of blank, showing a background level which cannot be further on decreased. Blank values are the mean determined during a period of one year in the effluent wastewater from the activated sludge treatment plant.

The ratio between LAS adsorbed on particulate matter ad those dissolved in water is 1.2 and such value depends on the ratio between the total concentration of LAS and that of sediment particles (Inaba *et al.*, 1988).

Since constructed wetlands do not produce sludge, removal of the inlet suspended solids promotes the removal of LAS adsorbed on particulate matter with respect to those dissolved in water.

SPC data point out that their concentration slightly increase passing from the inlet to the outlet wastewater, confirming that their origin is from the primary biodegradation of LAS. Moreover, it should be noted that the concentration of SPC is constantly much lower than that of dosed LAS, pointing out a further degradation of these intermediates.

Table 1 – Mean (mg/l), standard deviation and removal percentage for COD, TSS, MBAS, LAS and SPC on ten samples of inlet and outlet wastewater (January-February 2000), temperature range 5-9oC). Las dosing: 35mg/l. Blank is reported as comparison.

PARAMETER	BLANK	INLET	OUTLET	% REMOVAL
COD*	50±43	140±36	63±3	55.0
TOTAL COD	64±56	170±44	76±4	55.2
TSS	19±11	35±14	4±1	88.5
MBAS*	0.5±0.1	17.1±5.3	0.9±0.4	94.7
LAS*	b.d.l.***	15.6±4.9	0.9±0.5	94.2
LAS**	0.04±0.02	18.8±3.5	0.06±0.03	99.7
SPC*	0.01****	2.2±1.6	3.4±2.4	-
SPC**	0.04±0.03	0.03±0.02	0.04±0.01	-

* determined on filtered solution

** determined on particulate matter

*** b.d.l. = below detection limits

**** detection limit

On 2000/01/03 five samples, simultaneously collected at inlet, outlet and at different distances from the inlet, were analysed to study the removal ability with respect to the HRT. The results of dissolved and adsorbed LAS are shown in Figure 1. It should be underlined that a very high removal of LAS adsorbed on particulate matter was observed during the first 40cm of the reed bed (HRT=1.25 days). A similar decrease was obtained for TSS (data not reported).

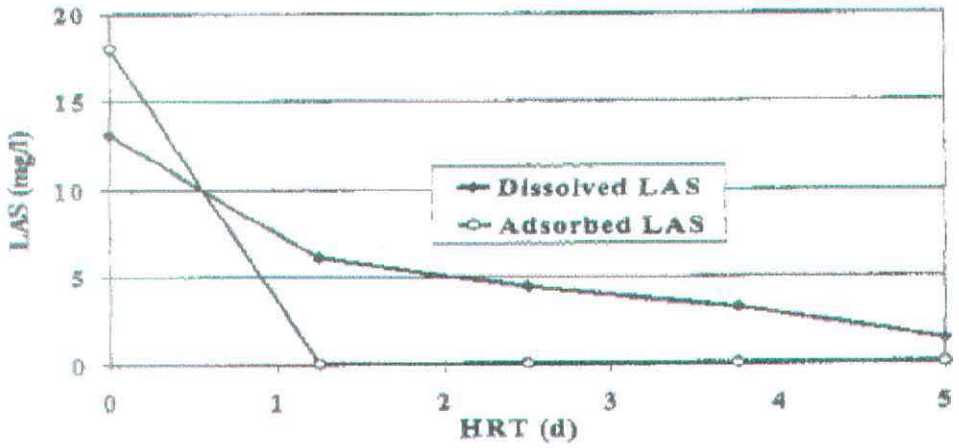

Figure 1 – Trend of dissolved and adsorbed LAS as a function of HRT. LAS dosing 35mg/l; inlet TSS=44mg/l. Wetland temperature 9°C. Sample collected on 2000/1/31.

LAS dosing with 350mg/l

This experiment was carried out in the period 2000/2/8-2000/03/16 (37 days). During this period the average temperature was 11°C (range 9-13°C). Results are reported in Table 2.

Table 2 – Mean (mg/l), standard deviation and removal percentage for COD, TSS, MBAS, LAS and SPC on fifteen samples of inlet and outlet wastewater collected during the period February-March 2000. LAS dosing: 350mg/l.

PARAMETER	INLET	OUTLET	% REMOVAL
COD*	605±250	146±22	75.8
TOTAL COD	1864±1130	197±24	89.4
TSS	1619±1382	8±3	99.5
MBAS*	71±34	1.9±0.4	97.3
LAS*	65±23	1.8±0.6	97.2
LAS**	278±62	0.18±0.05	99.9
SPC*	20±15	3.1±0.5	-
SPC**	13±11	0.36±0.19	-

* determined on filtered solution ** determined on particulate matter

It should be noted that inlet TSS strongly increased from the beginning of the experiment (reaching value up to 3g/l) because of a failure of the final settler of the activated sludge plant. Under these experimental conditions, the inlet concentration of several parameters (total COD, TSS, adsorbed LAS, dissolved and adsorbed SPC) were very high, giving rise to an unusual situation. For instance, the ratio between the mean concentration of adsorbed and dissolved LAS was 4.3, a value much higher than that obtained in the previous experiment, A similar behaviour was observed for the inlet SPC. With so large quantities of sludge in the influent of reed bed, a very high removal was observed for dissolved LAS and, particularly, for those adsorbed on particulate matter, which represent the most part of LAS present in the inlet. A typical chromatogram of the organic extract obtained from the inlet sediment particles was shown in Figure 2.

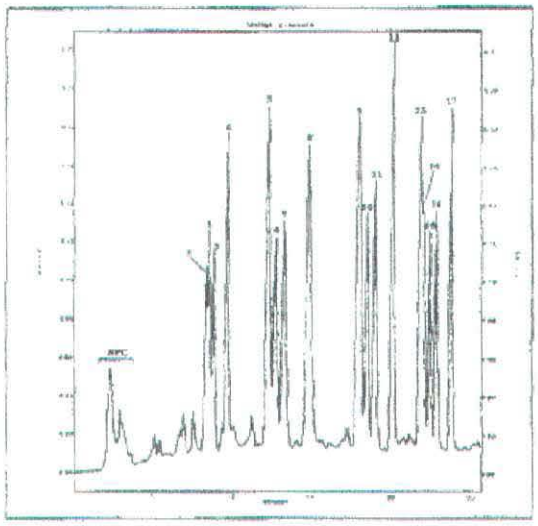

Figure 2 – HPLC chromatogram of an inlet sample of particulate matter, LAS dosing 350mg/l. Peak number: 1-4) C_{11}-LAS; 5-8) C_{12}-LAS; 9-12) C_{13}-LAS; 13-17) C_{14}-LAS.

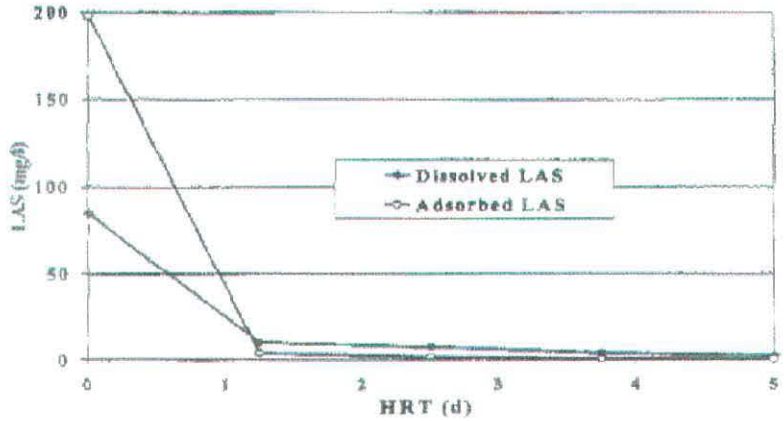

Figure 3 – Trend of dissolved and adsorbed LAS as a function of HRT. LAS dosing 350mg/l; inlet TSS = 236mg/l. Wetland temperature 12°C. Sample collected on 2000/3/8.

The trends of LAS as a function of HRT are reported in Figure 3. These trends confirmed that an inlet sludge concentration as low as 236mg/l was more than enough to promote a high LAS removal.

LAS removal rate parameters

The biodegradation availability of LAS homologues increases with increasing their carbon atom number (C_{14}>C_{13}>C_{12}>C_{11}). Average removal rate constants (KT) of soluble LAS at each experimental wastewater temperature (9 and 12°C) and at two different LAS concentrations were calculated, using the following relationship:

$$C_{OUT} = C_{IN}.\exp(-K_t.t)$$

Where:

K_t = removal rate constant for LAS (d^{-1})

T = hydraulic retention time (d)

C_{IN} = influent LAS concentration (mg/l)

C_{OUT} = effluent LAS concentration (mg/l)

T = temperature (°C)

Plot of log C_{IN}/C_{OUT} as a function of HRT at the two temperatures are shown in Figure 4. Linear regression was used as a first approximation to estimate K_t at the two different temperatures and LAS concentrations. The resulting K_T values are 0.4298 and 0.8078 for the 9 (dissolved LAS 13.1 mg/l) and 12°C (dissolved LAS 85mg/l) plots, respectively.

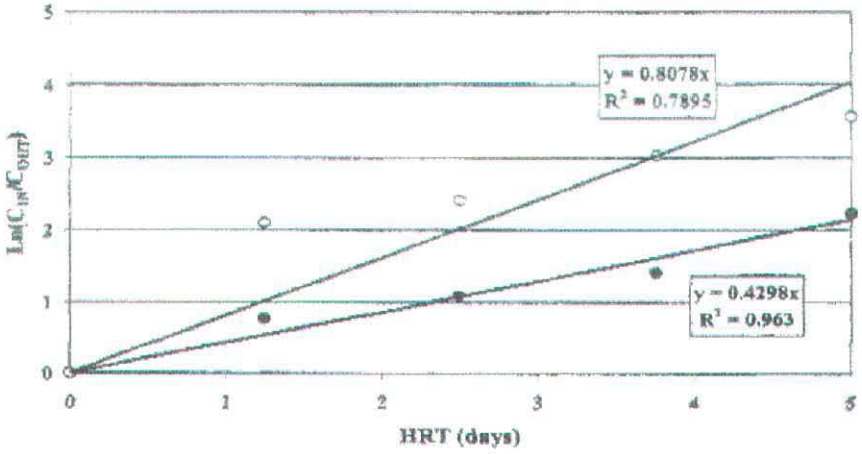

Figure 4 – LAS removal rate at 9°C (●) and at 12°C (○). R^2 is the correlation coefficient.

According to Swisher (1987), SPC are the biodegradation intermediates of LAS and SBA is the ultimate degradation product before mineralization. The percentage of dissolved and

adsorbed SBA increased with increasing HRT at both LAS concentrations, pointing out that the rate of the mineralization step is lower than the formation rate of SBA from SPC.

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APPENDIX B

Removal of Linear Alkylbenzene Sulphonate (LAS) in Constructed Wetlands

In: Vymazal J (ed.), 2003 (in print). Transformations of nutrients and pollutants in natural and constructed wetlands. Bachways Publishers, Netherlands.

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ABSTRACT

Worldwide the application of constructed wetlands in sewage treatment, usually in a secondary or tertiary capacity, has greatly expanded in recent years. Research has mainly focused on BOD, nutrient and heavy metal removal. However, removal of other pollutants, such as surfactants, has largely been ignored. This study focuses on the removal of the anionic surfactant Linear Alkylbenzene Sulphonate (LAS) in constructed wetlands used in sewage treatment in the UK. A 12-month investigation was conducted in three constructed wetland systems, planted with *Phragmites australis* in gravel media, in the UK. Using High Performance Liquid Chromatography (HPLC), coupled with an initial solid phase extraction (SPE) pre-concentration step, LAS alkyl homologues of C_{10}-C_{13} were identified at each sampling site. The average total LAS inflow concentration for the three sites was 1.1mg L^{-1} and 0.43mg L^{-1} in the outflow. Comparison of the systems showed similar LAS removal with a typical annual average of c.55%. This paper outlines the influence on three main factors of loading, seasonal variations and distribution of the alkyl chain homologues on the LAS removal efficiency. Sharp changes in loading concentrations detrimentally affected the overall efficiency. Greater LAS removal was observed in the spring coinciding with the plant growth season. The longer alkyl chain homologues were removed to a greater extent than the shorter alkyl chain homologues in the order of $C_{13} > C_{12} > C_{11} > C_{10}$. The removal of individual alkyl homologues is important as they differ in their capacity for biodegradation, adsorption and toxicity.

KEY WORDS

Constructed wetlands; Linear Alkylbenzene Sulphonate (LAS); *Phragmites australis*; Surfactants.

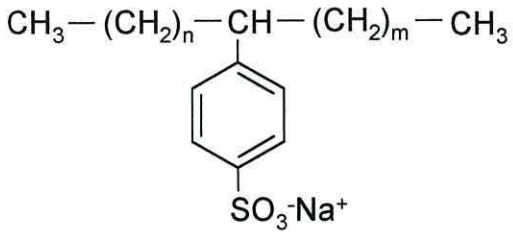
INTRODUCTION

In the 1960s rising levels and foaming problems of surfactants resulted in an increased interest in these pollutants. The major surfactant on the market at that time was Alkyl Benzene Sulphonate (ABS). The problems were caused by the poor biodegradation of the

surfactant due to its chemical structure (Swisher 1987). To reduce these problems a more degradable surfactant was produced in the early 1960s as a replacement for ABS. This was readily degradable anionic Linear Alkylbenzene Sulphonate (LAS) and since its introduction the concentration of anionic surfactants in rivers and streams has dramatically decreased (Swisher 1987). Today LAS is one of the world's major synthetic surfactants with an annual worldwide consumption estimated at 2 x 10^6 t per year (de Wolfe & Feijtel 1998).

LAS is present in many household detergent formulations, mainly in laundry and cleaning products. Commercial LAS formulations generally consists of a mixture of alkyl homologues, mainly C_{10}-C_{14}, with an average chain length of approximately $C_{11.8}$, and a benzene ring with a sulphonate group (Matthijs & De Henau 1987). It is the production of the sulphonate group when dissolved in water that gives LAS its anionic properties. The chemical structure of LAS is shown in Figure 1. The phenyl group can be positioned on different carbon atoms on the alkyl chain resulting in the formation of LAS isomers (Matthijs & De Henau 1987). These varying alkyl homologues and isomers vary in their biodegradability (Terzic *et al.* 1992), adsorptivity (Prats 1993) and toxicity (Swisher 1987).

Figure 1: LAS chemical structure.



Where n and m = 10 to 14

Research suggests that two main processes are involved in LAS removal; biodegradation and adsorption (Swisher 1987, Inaba 1992, Matthijs & De Henau 1987). From the literature it is evident that LAS is readily biodegradable under aerobic conditions and its mechanism is well documented with the mechanism of breakdown involving degradation of the straight alkyl chain, benzene ring and sulphonate group (Swisher 1987). Commercial LAS is generally readily biodegradable in aerobic conditions by c.95% primary biodegradation in the OECD screening test (Jensen 1999). Surfactants are prone to adsorption due to their surface-active properties and hence LAS adsorption onto sediment particles also plays an important removal role. The longer chain LAS homologues have higher adsorption tendencies in comparison to

shorter chain homologues (Jensen 1999). McEnvoy & Giger (1986) state that an increase in alkyl chain length increases the hydrophobic nature of LAS homologues, hence, making them more prone to adsorption. Hand & Williams (1987) found that adsorption of LAS homologues onto river sediments also increased as the phenyl position moved to the end of the alkyl chain. These two processes are in fact also interactive. For example, as a result of adsorption the time available for biodegradation to occur in a system is increased.

Due to the 'down the drain' disposal of detergents the majority of LAS eventually reaches sewage treatment plants where its removal before being discharged into the environment occurs. The concentration of LAS in raw sewage has been reported as 1.10-5.58mg L^{-1} in the UK (Holt *et al.* 1998). Due to the high usage of LAS its fate in the various stages of sewage treatment has been extensively researched. LAS removal in the sewer system has been reported at over 50% when aeration conditions and length of sewer are sufficient (Moreno *et al.* 1990). Evidence of the high removal (≥95%) of LAS during the sewage treatment process is well documented in the literature (Jensen 1999, Castles *et al.* 1989, Matthijs & De Henau 1987) with an estimate of approximately 30% being adsorbed onto suspended particles entering a sewage treatment plant (Matthijs & De Henau 1985).

With the increase in the usage of constructed wetlands in sewage treatment plants, usually in a secondary or tertiary capacity, it is essential to monitor the removal of LAS in these systems as they are often the last source of treatment before discharge into the surrounding environment. However, the fate of LAS in wetland systems has largely been ignored. As demand increases for these less energy intensive systems to replace more conventional treatments it is important to gain an understanding of their removal efficiency with regard to LAS.

In the UK the application of constructed wetlands in the sewage treatment process, usually in a secondary or tertiary capacity, has greatly expanded in recent years (>350 systems, Nuttall *et al.* 1997). These systems are also widely used in Europe (>500-1000 systems, Haberl 1995), e.g. Sweden (Whittgren & Maehlum 1997), Norway (Maehlum & Stalnacke 1999), and worldwide, e.g. India (Billore *et al.* 1999), Australia (Greenway & Woolley 1999) and US (Kadlec & Knight 1996).

Constructed wetland systems are designed to optimise the ability of natural wetland ecosystems, several of which have been receiving run-off from various urban sources and discharging water of much improved quality for years (Hammer & Bastain 1989). Treatment is achieved either by removing or transforming pollutants via various physical, chemical and

biological processes occurring through interactions between the plants, microbes and substrate. Hence these systems can act as both sources and sinks of nutrients and carbon (Mitsch & Gosselink 1993).

Research on the efficiency of constructed wetlands in sewage treatment has mainly focused on the removal of BOD, phosphate (Brix *et al.* 2001), nitrate (Hammer & Knight 1994) and metals (Dunbabin & Bowner 1992). However, there is still a gap in the knowledge of the fate and removal of LAS in constructed wetlands designed for tertiary sewage treatment which is the most common application worldwide. With the growing interest and demand for more cost-effective, sustainable and environmentally friendly forms of sewage treatment the application of constructed wetlands will almost certainly rise in the future. Thus an understanding of the removal of LAS in constructed wetlands is a key issue that must be addressed.

This study focuses on the removal of LAS in three constructed wetland systems used in sewage treatment plants at different locations in the UK over a 12-month period. A sensitive HPLC analytical procedure coupled with an initial solid phase extraction (SPE) pre-concentration step was adopted to separate and identify individual LAS alkyl homologues via reversed phase separation and fluorescence detection.

METHODS

Sampling sites

LAS was monitored at three sewage treatment plants in the UK which have constructed wetlands for tertiary treatment. The first site at Brynsiencyn, Anglesey (National Grid Ref. SH491666) had the largest total surface area (500m^2), served the largest population (2000) and received the highest loading (665 m^3 d^{-1}). The second largest (172m2) served a small community (200 people) at Clutton, Wrexham (National Grid Ref. SH460544) and had been in operation for the longest period. The third study site treated wastewater at Rosset, Chester (National Grid Ref. SH370565) receiving wastewater from a small hospital and a few nearby households (150 people) and is the most recently constructed. All systems sampled were subsurface flow constructed wetlands planted with *Phragmites australis* in gravel media. Detailed characteristics of the sites are presented in Table 1.

Table 1: Characteristics of the sampling sites.

Site	Area (m^2)	Population	Loading (m^3 d^{-1})	Construction Year
Brynsiencyn	500	2000	665	1998
Clutton	172	200	25	1996
Rosset	130	150	100	1999

Samples

Inflow and outflow water samples were taken from each constructed wetland, using methanol washed bottles, on a monthly basis from January to December 2000 (except for Clutton which commenced in March). The samples were preserved on site with 3-5% formaldehyde (37% v/v) and refrigerated (<4°C) whilst awaiting analyses for LAS content.

Analytical Procedure

Several analytical methods have been developed for the detection of LAS with the most widely used being the colorimetric methylene blue method (MBAS) (Matthijs & De Henau 1987). However, for specific determination of LAS alkyl homologues, a sensitive HPLC method was used in this study. The method adopted was based on that developed by Matthijs & De Henau (1987) but modified slightly to improve the selectivity.

The analytical procedure involved Solid Phase Extraction (SPE) to isolate and concentrate the LAS in the aqueous samples before HPLC analyses. Each sample was initially passed through a preconditioned C18 (1g/6ml) SPE column (10ml methanol followed by 10ml distilled water) and then eluted with methanol onto a preconditioned SAX (500mg/3ml) anion-exchange SPE column (3ml hexane followed by 10ml methanol). LAS was then eluted into a glass vial with 3ml of CH_3OH:HCl solution (80:20) and evaporated to dryness at 75°C under a gentle stream of nitrogen. The samples were stored in the dry state in a refrigerator (>4°C) whilst awaiting analyses. Figure 2 shows the scheme for the analytical procedure.

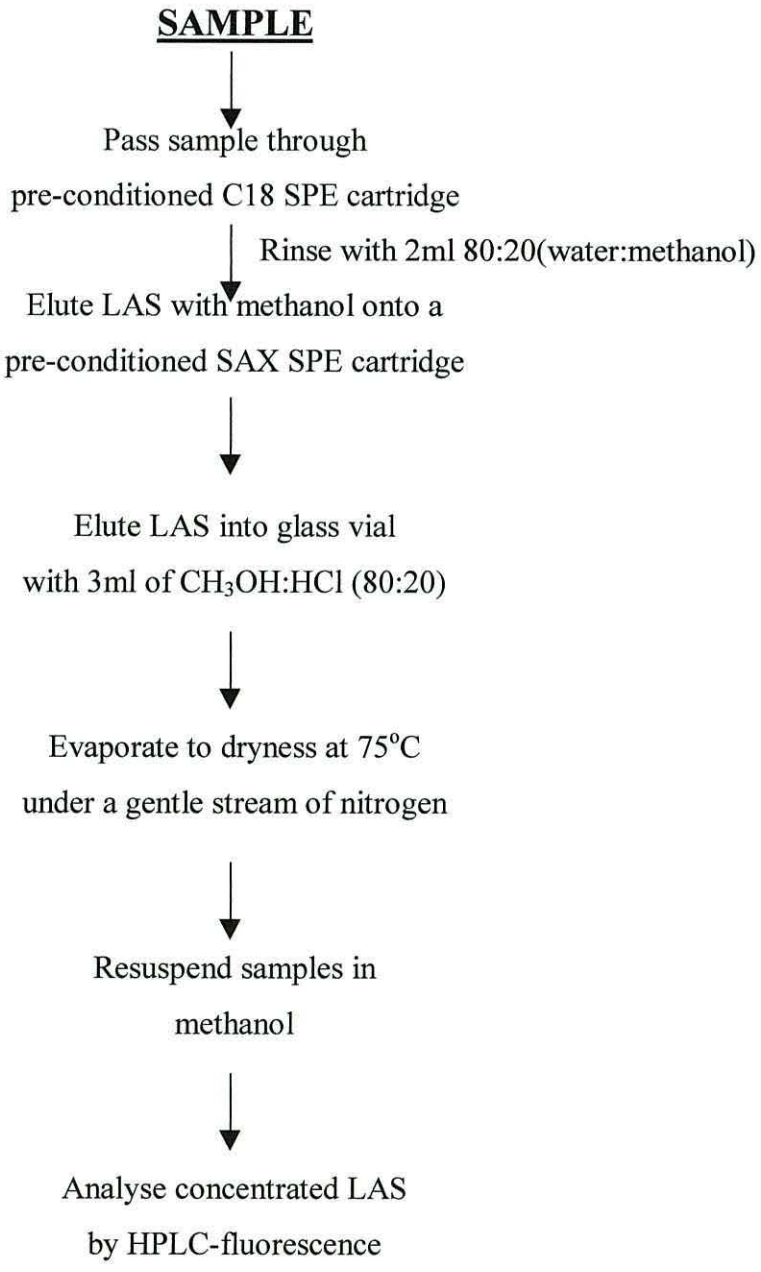
HPLC Analyses

Separation of LAS homologues was achieved by reversed phase separation using the DX-300 HPLC system, with a 10μm, 30cm x 3.9mm i.d., μ-Bodaclone C18 analytical column. The mobile phase (22:78 water:methanol), containing sodium perchlorate buffer (0.0875M), at a flow rate of 2 ml min^{-1} was used and a Perkin Elmer LS-4 fluorescence detector (Excitation wavelength = 232nm; emission wavelength = 290nm; slit width = 10nm). For calibration

Nansa® HS 80/S was used containing C_{10}-C_{13} LAS homologues (with distribution of alkyl chains of C_{10} 15.8%; C_{11} 41.5%; C_{12} 30.1%; C_{13} 12.5%).

The analytical procedure adopted was validated using both control and environmental samples spiked with various concentrations of LAS in order to assess the repeatability and accuracy of the method. In order to minimise contamination all glassware were washed in methanol before use and appropriate glassware then conditioned with LAS to reduce loss of surfactant to the glass surface.

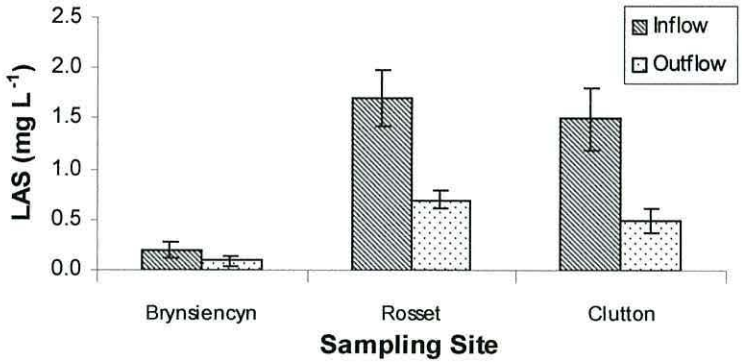
Figure 2: Scheme for the LAS analytical procedure.



RESULTS

The annual mean LAS inflow concentration for the three sites was 1.1mg L^{-1} and 0.43mg L^{-1} in the outflow. The highest total LAS concentration was measured at the Rosset site at 3.2mg L^{-1}. This site also received the highest overall annual mean LAS loading of the sites monitored at 1.7mg L^{-1} and the Brynsiencyn the lowest at 0.2mg L^{-1}. The outflow concentrations followed a similar pattern to that of the inflow with a minimum of 0.02mg L^{-1} measured at the Brynsiencyn site. Figure 3 shows the mean annual LAS concentrations measured at the sampling sites.

Figure 3: Mean annual inflow and outflow LAS concentrations.



All three sites exhibited similar average LAS removal capacities of 57% at Clutton and 55% for both Brynsiencyn and Rosset. The constructed wetland in operation longest, i.e. Clutton, exhibited the highest maximum LAS removal recorded at 84%, whereas the lowest removal recorded, 21%, was at the Rosset site where the constructed wetland had been in operation the shortest time.

In terms of monthly analysis figures 4-6 show the variations in inflow and outflow total LAS concentration at the Brynsiencyn, Rosset and Clutton sites. A sharp peak in LAS inflow concentration was observed in August at Brynsiencyn whereas, in contrast, a sharp decline is observed at the other sites. Low concentrations in the inflow in October at these two sites were also exhibited.

Figure 4: LAS concentration measured at Brynsiencyn.

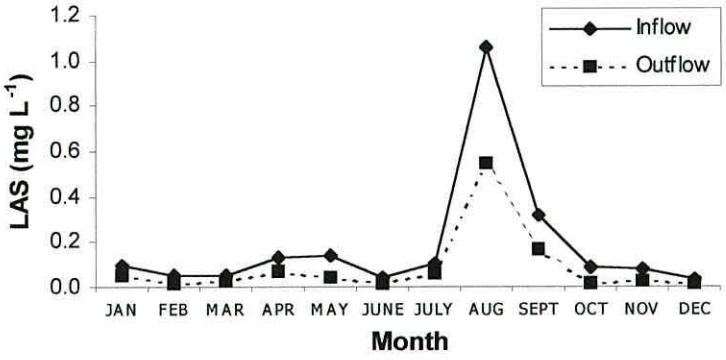


Figure 5: LAS concentration measured at Rosset.

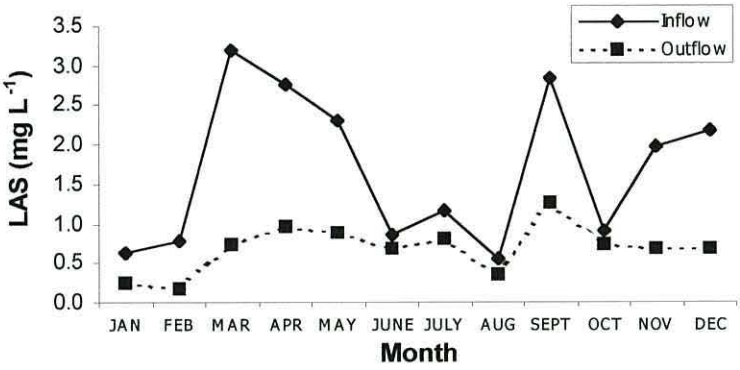
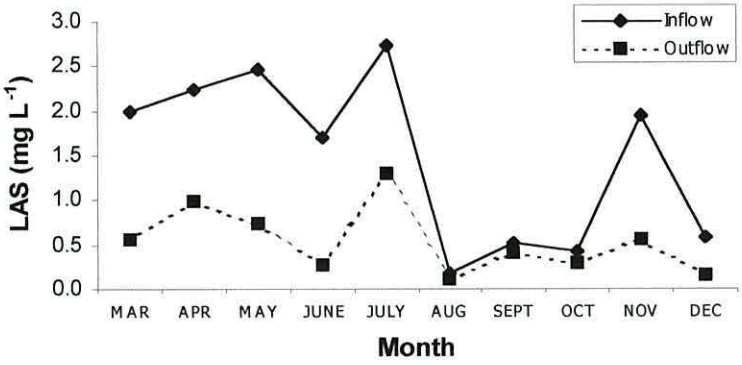


Figure 6: LAS concentration measured at Clutton.



The sensitive HPLC procedure adopted not only enabled total LAS concentrations to be measured but also individual LAS alkyl chain homologues. Four LAS homologues were identified and quantitatively measured with chain lengths of C_{10}-C_{13} in both the inflow and outflow at all sampling sites. Variation in homologue distribution was evident with an average alkyl chain length of 11.5 in the inflow for both Rosset and Clutton sites and a lower average of 11.3 in the outflow. At Brynsiencyn an average chain length of 11.7 in the inflow and 11.5 in the outflow was measured. The mean annual percentage removal distribution of

the LAS alkyl homologues show that the longer alkyl chain homologues are removed to a greater extent than the shorter alkyl chain homologues in the order of $C_{13} > C_{12} > C_{11} > C_{10}$.

DISCUSSION

The HPLC analytical procedure adopted, coupled with the initial SPE extraction step, successfully separated LAS from the many other components and interferences found in sewage wastewater. Holt *et al.* (1998) reported an average LAS concentration of 3.25mg L^{-1} in raw sewage (range 1.10-5.58mg L^{-1}). The annual average total LAS inflow concentration for the three sites investigated in this study was in the lower range at 1.1mg L^{-1}. The highest total LAS concentration was measured at the Rosset site at 3.2mg L^{-1}. The high inflow concentration may be expected as the main source is a small hospital which would be expected to have a higher consumption of detergents and cleaning agents than the average household. The mean LAS measured in the outflow from the three sites was 0.43mg L^{-1},with a minimum of 0.02mg L^{-1} measured at the Brynsiencyn site.

The overall LAS removal observed at the three sites were similar with a typical annual removal of c.55% of the inflow LAS concentration. Similarly Inaba (1992) reported that >60% of inflow LAS was annually removed in a natural wetland receiving gray water investigated in Japan. The small difference in efficiency between the sites investigated in this study is interesting as they varied in loading concentrations, loading rates, size and years in operation.

The findings of this study suggest that three main factors govern the extent of LAS removal in the constructed wetland sites monitored. These are; loading, seasonal variations and the distribution on the alkyl chain homologues. The results will be discussed in relation to these three factors.

1. Loading

Figures 4-6 show examples of high and low LAS loading. The high loading in August at the Brynsiencyn site was almost certainly due to a high tourist influx into the area as it corresponds with the main holiday season in the UK. Removal of 49% was still achieved and hence the high LAS loading did not dramatically reduce the annual performance efficiency.

In contrast a case of low LAS loading was observed in August at the Rosset and Clutton sites as shown in figures 5 and 6 respectively. Better performance of the secondary sewage treatment facilities at these sites working more efficiently at low rainfall and warmer

temperatures may explain the lower LAS inflow concentrations. The peak in LAS received at the Brynsiencyn site may have overloaded the secondary treatment during this month.

Environmental factors also affect the loading, the LAS input concentrations can be diluted by rainfall. The low loading observed in the early autumn may be explained by the extreme weather conditions in the UK with October 2000 being the wettest on record since the beginning of the century (UK Met. Office, Pers. com.). However, the Brynsiencyn site, being the largest and receiving the highest flow, coped better with the increased rainwater and the LAS removal was not as affected as the other smaller wetlands.

2. Seasonal variations

Seasonal variations were evident with greatest LAS removal observed at all three sites in the spring. This springtime peak in LAS removal coincides with the growth season of the Phragmites australis in the constructed wetlands. The maximum removal occurred in the spring/summer months, i.e. March, May and June respectively for sites Rosset, Brynsiencyn and Clutton. Inaba (1992) also reported seasonal temperature effects in LAS removal. Approximately 95% of the influent LAS was removed in the summer, but only 50% in the winter due to measured inhibition of bacterial activity (<7°C) (Inaba, 1992). However, without rainfall data for the wetland in Japan it is difficult to compare this summertime peak.

Significant removal efficiency was also observed in this study during the colder, wetter autumn and winter months. Constructed wetlands performance in lower temperature conditions has also been reported, e.g. Sweden (min. -3°C) (Whitgren & Mæhlum 1997), Norway (min. –5°C) (Mæhlum & Stålnackle 1999). However, periods of high rainfall during these seasons detrimentally affected LAS removal. The Brynsiencyn site received the highest monthly average rainfall (99.6mm), then Rosset (78.5mm) and finally Clutton (77.5mm) with an average air temperature of 11°C for the first two sites and 9.7°C for Clutton. Rainfall impacts were especially prevalent in October where low overall LAS removal due to dilution of the inflow was observed at Rosset and Clutton sites.

3. Alkyl chain homologue distribution

The distribution of the LAS alkyl chain homologues were also found to affect the fate of LAS in the constructed wetland systems monitored. The sensitive HPLC method adopted successfully separated and quantitatively measured alkyl homologues of chain lengths C_{10} to C_{13}.

In terms of the LAS chain length distribution an average of 11.5 for both Rosset and Clutton sites, and 11.7 at Brynsiencyn was measured in the inflow. A lower average alkyl chain length was measured in the outflow than in the inflow by a factor of 0.2 at each site indicating the greater removal of longer chain alkyl homologues. This decrease has been found by other authors and has been attributed to the differences in the degree to which the homologues are adsorbed onto suspended particles and different biodegradation rates (Swisher 1987, Painter & Zabel 1989).

In this study the average percentage removal of the longer chain homologues was found to be greater than of the shorter alkyl chain homologues. This is in accord with the distance principle. The distance principle states that the position of the phenyl group and length of the alkyl chain influence the rate of biodegradation of LAS (Swisher 1987). It states that the longer chain alkyl homologues will have faster degradation than shorter chain homologues (Swisher 1987). In this study the average percentage removal of the LAS alkyl chain homologues were of the following order;

$$C_{13}>C_{12}>C_{11}>C_{10}$$

The increased biodegradation rate and adsorption are related to the increased hydrophobicity due to the longer alkyl chain (Swisher 1987). Faster rate of degradation of the longer chain homologues has been confirmed for all LAS chain lengths from C_6 to C_{16} (Swisher 1987, Terzic *et al.* 1992). The faster biodegradation and greater adsorption tendencies of the longer chain alkyl homologues have important ecotoxicological implications as lower molecular weight LAS homologues have lower aquatic toxicity values (Swisher 1987).

Greater removal of the longer chain alkyl homologues in various test systems, other sewage treatment systems and different aquatic environments has been reported (Prats *et al.* 1993). Hence the findings of this study confirms that LAS is removed in constructed wetlands by similar processes to that in other more conventional sewage treatment systems.

CONCLUSION

The potential for constructed wetlands design to facilitate greater LAS removal has been highlighted in this paper. The findings of this study suggest that the following conclusions may be drawn;

1. LAS removal was evident at all three sampling sites with a typical annual mean of *c.*55% observed.

2. Three main factors were found to affect LAS removal; loading, seasonal variations and distribution of the alkyl chain homologues.
3. Sharp increase or decrease in loading detrimentally affected LAS removal.
4. Rainfall dramatically affected LAS removal suggesting design modifications to accommodate periods of high rainfall is required.
5. Alkyl chain distribution was found to behave similarly as in more conventional sewage treatment systems and followed the distance principle with longer chain homologues removed more rapidly.

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APPENDIX C

The recipe for the nutrient solution used in chapter 3 is given below (Hewitt 1966).

Long Ashton solution

Composition of full strength Long Ashton solution is as follows;

K (156µg ml-1); N (168µg ml-1); S (48µg ml-1); Ca (160µg ml^{-1}); Mg (36µg ml^{-1}); P (41µg ml^{-1}); Na (33µg ml^{-1}); Fe (6µg ml^{-1}); Cl (3.5µg ml^{-1}); Mn (0.55µg ml^{-1}); B (0.54µg ml^{-1}); Zn (0.065µg ml^{-1}); Cu (0.064µg ml^{-1}); Mo (0.044µg ml^{-1}).

To make up 1 litre of Long Ashton solution then the following proportions of the solutions 1-6 below is required;

4ml of solution 1

2 ml of solutions 2, 3, 4 and 10mg of sodium metasilicate

1ml of solutions 5 and 6.

All of the following solutions are for 500ml.

Solution 1

KNO_3	50.5g

Solution 2

$Ca(NO3)_2.4H_2O$	236g

Solution 3

$NaH_2PO_4.2H_2O$	52g

Solution 4

$MgSO_4.7H_2O$	82g

Solution 5

EDTA Fe Na salt	18.65g

Solution 6: Micronutrients

$MnSO_4.7H_2O$	1.125g
$CuSO_4.5H_2O$	0.125g
$ZnSO_4.7H_2O$	0.145g
H_3BO_3	1.55g
$Na_2MO_4.2H_2O$	0.06g
NaCl	2.93g

APPENDIX D

The recipe for the artificial sewage solution used is given below (originally adapted from Painter & Viney 1959).

Table A: Artificial sewage solution

Ingredient	Concentration (mg L^{-1})
Peptone	70
Urea	25
Sucrose	35
Soluble starch	35
Ammonium sulphate	140
Mixed acids (see below)	105
Potassium hydrogen phosphate	28
Ferrous ammonium sulphate	21
Trace metals solution (see below)	1ml

Table B: Mixed acids solution

Acid	Concentration (g L^{-1})
Sodium acetate	136
Sodium propionate	28
Sodium butyrate	12
Sodium benzoate	100
Sodium citrate	44

Table C: Trace metals solution

Metal	Concentration (mg L^{-1})
$CuCl_2.2H_2O$	0.25
$Co(NO_3)_2.6H_2O$	0.25
$Na_2B_4O_7.10H_2O$	0.25
$ZnCl_2.2H_2O$	0.25
$MnSO_4.H_2O$	1.00
K_2Mo_4	0.25
NH_4VO_3	0.10