Sustainable Technology for the Biological Treatment of Waste

By

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Abstract

Sustainability in waste and waste water treatment is a factor in the success and application/uptake of a technology. Initial installation costs of constructed wetland wastewater treatment systems are competitive with that of conventional wastewater treatment facilities. Reed beds are not a budget technology, because a properly engineered wetland, depending on scale, is a significant earth work. Constructed wetlands do have considerably reduced running and operational costs when compared to alternative systems such as sequencing batch reactor technology. Another positive aspect of constructed wetland treatment of wastewater is that the facility is environmentally sensitive, providing habitat for wildlife, and is environmentally sustainable in returning oxygen to the atmosphere through phytotranspiration and plant respiration.

Reed beds have a reputation as a novel biological technology and have yet to gain mainstream acceptance, despite their 50 year academic history. This may in part be due to issues of performance reliability and questions that remain in the understanding of the biologically mediated capacity of reed bed technology. What remains to be explained, and is needed, is an understanding, leading to control, of the biological, microbiological and chemical interactions within and between the substrate, flora, fauna and effluent of a reed bed treatment system.

In this study the microbiological aspects of constructed wetlands were investigated during treatment of industrial effluents, using experimental reed beds in the UK, and also during the installation of reed beds for a commercial company in Ghana. The results showed that the microbiology of a reed bed is affected by the type and concentration of effluent that it is used to treat. Observational study, effluent treatment analysis and microbiological investigation of reed bed substrate, under various loading conditions, revealed that there may be an effect exerted by certain fractions of industrial waste streams that were detrimental to reed flora and the microbiological fauna of the reed beds. Inhibition of the microbial community of the reed bed substrate occurred in the reed beds treating factory effluent which were found to have had a significantly reduced microbial community in terms of colony forming units when compared to the reed beds treating domestic effluent. Treatment efficiency for biological oxygen demand ranged from 48-93%.

In the UK, treatment of landfill leachate and tannery effluent showed that reed beds are effective in reducing effluent parameters such as chemical oxygen demand and total nitrogen. Treatment performance for landfill leachate showed total nitrogen reduction ranging from 37-97%. Chemical oxygen demand treatment efficiency ranged from 28-95%. Treatment performance in terms of total nitrogen reduction in tannery effluent ranged from 66-99%, and for chemical oxygen demand 53-87%.

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CHAPTER ONE

INTRODUCTION

1.0 Waste treatment in the context of sustainability

The concept of sustainable development was initially promoted as a strategy for addressing pressing global problems in a report by The Bruntland Commission entitled, *Our Common Future* (World Commission on Environment and Development, 1987). It is defined as:

"development that meets the needs of the present without compromising the ability of future generations to meet their own needs" (World Commission on Environment and Development, 1987).

Sustainable development is a phrase that has been coined to describe a philosophical ethic. It is a multidisciplinary way of thinking in the context of the interdependencies of the social, environmental and economic issues that confront humanity. It represents a process that gives consideration to the ramifications and consequences of human activity and gives emphasis to the promotion of social progress in which the needs of everyone are recognised; effective protection of the environment; prudent use of natural resources; and maintenance of high and stable levels of economic growth and employment. The management of waste is one of the key themes (Gertsakis and Lewis, 2003).

The overall policy context for waste management and disposal in European Union Member States was established in the EC Framework Directive on Waste (74/442/EEC as amended by 91/156/EEC) (EEC, 1974; EEC, 1991). The overriding policy objectives in Article 4 were:

"To ensure that waste is recovered or disposed of without endangering human health and without using processes or methods which could harm the environment and in particular without:

- *Risk to water, air, soil, plants or animals; or*
- *Causing nuisance through noise or odours; or*
- *Adversely affecting the countryside or places of special interest".*

In the UK the policy framework for waste management was set out in the *Waste Strategy for England and Wales* (DEFRA, 2000). The policies were based on regional factors, such as current waste management infrastructure and topography. Common

factors to all strategies included commitment to meet the *Landfill Directive*, to improve markets for waste products, and to deal with the acknowledged growth in waste.

The Landfill Directive (EC, 1999) (Council Directive 1999/31/EC) was implemented by the Landfill Regulations 2002, which came into force on 15 June 2002. The Directive aimed to prevent or to reduce as far as possible, the negative environmental effects of landfill. The regulations require that Member States establish strategies for the reduction of biodegradable waste in order to meet Directive targets. The cost of landfill is to be covered by disposal charges levied against users, and higher engineering and operating standards were to be followed. Landfill operators are required to demonstrate that their staff members are technically competent to manage the site, and adequate financial provisions are made to cover maintenance of the site after capping. Existing landfills either have to conform to the terms of the Directive, or be closed down.

One of the primary targets set by the Directive requires a staged reduction in the amount of biodegradable waste going to landfill. This meant that the 85% of municipal waste in England and Wales that went to landfill in 1995 would be reduced, so that by 2020 the volume of landfill waste would be 35% of the 1995 amount. The Directive also requires that each landfill site be given a classification as to the waste it contains: hazardous waste; non-hazardous waste; or inert waste. Certain hazardous and other wastes, including liquids, are prohibited from landfill. Pre-treatment of waste prior to landfill also becomes a requirement. In order to comply with the Landfill Directive, the *Waste Strategy for England and Wales* (DEFRA, 2000) set the following targets for the management of municipal waste:

- *To recover value from 40% of municipal waste by 2005*
- *To recover value from 45% of municipal waste by 2010*
- *To recover value from 67% of municipal waste by 2015*

To achieve the municipal waste recovery targets, through household waste recycling and composting, the Strategy set the following targets for England and Wales:

- *To recycle or compost at least 25% of household waste by 2005*
- *To recycle or compost at least 30% of household waste by 2010*
- *To recycle or compost at least 33% of household waste by 2015*

In accordance with the principles of sustainable development, a hierarchy of preferred waste management options are to provide the basis for how waste is managed and minimized. The basic premise of *waste minimisation* is that prevention of the generation of waste is preferable to disposing of waste after it has been produced (EA, 1997). This gave rise to the hierarchy of preferred waste management options known as the *Waste Management Hierarchy*. A simple descriptive summary of the environmental attributes and outcomes of the waste management hierarchy is outlined in figure 1.1.

Figure 1.1 Attributes of waste management hierarchy (Gertsakis and Lewis, 2003).

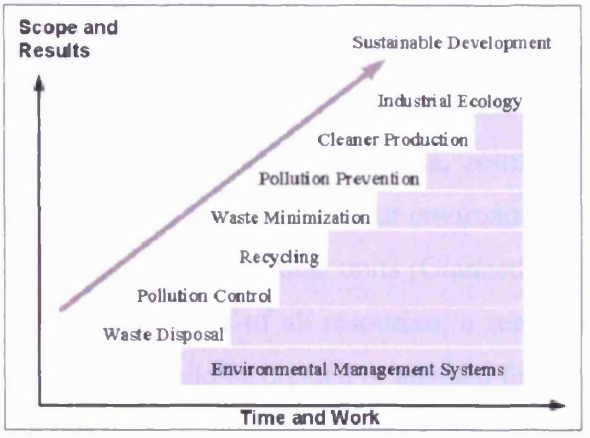
Goal	Attribute	Outcome
Reduce	Preventative	*Most desirable*
Reuse	Predominantly ameliorative, part preventative	
Recycle	Predominantly assimilative, part preventative	
Treat	Predominantly assimilative, partially ameliorative	
Dispose	Assimilative	*Least desirable*

The waste management hierarchy was officially adopted by the UK government in 1995 with the publication of *Making Waste Work* (DETR, 1995). The governing principles are that of waste reduction at source, re-use of waste materials, and waste recovery. The *Proximity Principle* (DEFRA, 1995) is considered to be an important factor in the assessment of appropriate waste disposal options. The principle states that waste should be disposed of as close to its source as possible. Used in conjunction with the waste management hierarchy, the proximity principle can be used to select and achieve the *Best Practicable Environmental Option* (BPEO) (Bond and Brooks, 1997). The best practicable environmental option was defined by the Royal Commission on Environmental Pollution (RCEP, 1988) as:

"the outcome of systematic and consultative decision making procedure which emphasises the protection and conservation of the environment across land, air and water. The BPEO procedure establishes, for a given set of objectives, the option that provides the most benefits or the least damage to the environment as a whole, at acceptable cost, in the long term as well as in the short term." (RCEP 1988)

The emerging priorities of sustainable development have given rise to many concepts created to address and explain the various aspects and principles of environmentally sustainable waste management (Schramm, 1997). Numerous terms, which have included *clean technology, cleaner production,* and *pollution prevention,* have been used to describe and interpret the essential goals of sustainable waste management. An interpretation of some of the concepts is defined in figure 1.2

Figure 1.2. Concepts in industrial environmental management.



Some authors have considered the growing numbers of conceptual environmental terms to be only the latest in a series of successive environmental management paradigms (Wang, 1999). However, the term *cleaner production* has become increasingly used, and internationally recognised, to describe the activity of reducing the environmental impact of processes, products and services by using better management strategies, methods and tools (Van Berkel, 2000; UNEP, 2001; Hilson, 2003). An accepted interpretation of cleaner production is defined as:

"preventative strategy which promotes the prevention of waste before it is systematically created, to systematically reduce pollution, and improve the efficiency of resource use" (UNEP, 2001).

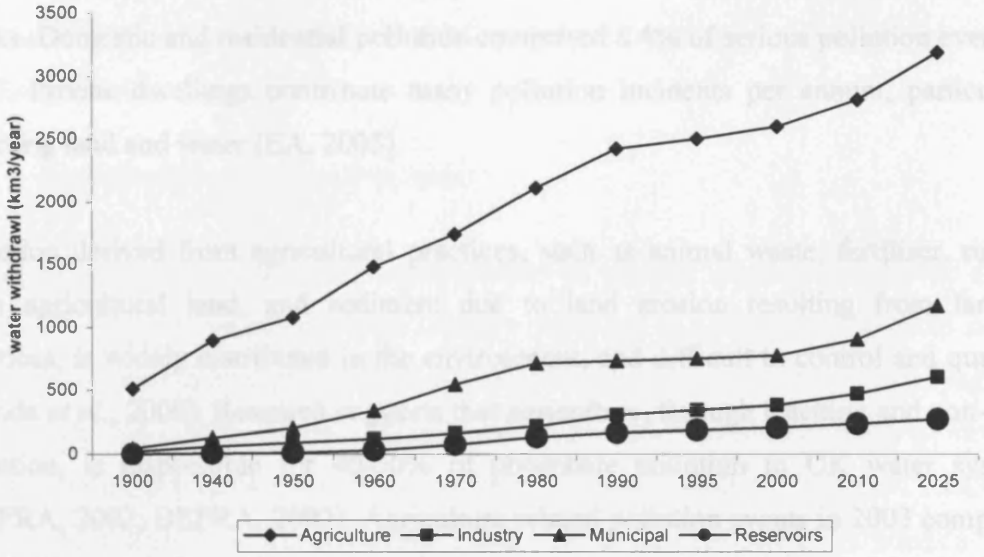
The overall aim of *cleaner production*, and *cleaner technology*, is to minimise the quantity and toxicity of the waste produced by an industrial process, so as to fall within the assimilative capacity of the biosphere. In other words to be sustainable. Properly applied it will mean that a final product will have a reduced environmental impact throughout its life cycle. *Cleaner production* involves the modernisation of manufacturing processes and the responsible use of resources. This includes consideration of the many consequences of using non-renewable materials and also consideration regarding the packaging and distribution of products (UNEP, 2001).

Cleaner Production and *Industrial Ecology* are fundamental principles that have evolved separately. However, the two principles possess a shared goal, which is to diminish the environmental impact of industrial production and products (Van Berkel, 2000). Together both principles can be applied to reduce waste and pollution (UNEP, 2001). *Industrial ecology* is the principle that, within aggregates of industrial units, a situation can theoretically be achieved where there can be reciprocal use of energy and materials, and a state of interdependence and industrial metabolism can be reached. The principle follows that the global or holistic process, resulting from several minor contributions by distinct industrial unit, has a minor environmental impact compared to the impact caused by the sum of the individual units (Giannetti *et al.*, 2004). The aim of industrial ecology is the use and reuse of all resources; a zero waste objective. Within this framework new technology could be created or adapted for use within the economic and geographical conditions of each individual country and company.

1.1 Sources of water pollution

Modern estimates claim that the Earth's hydrosphere contains 1,386 million cubic kilometres of water in various forms. This figure includes fresh water, saline water, and subsurface water, water locked in ice caps and glacier, and in the atmosphere. This huge amount of water is not evenly distributed over the populated areas of the globe and in addition much is inaccessible or too polluted for human use (Shiklomanov, 1999). Each year enormous amounts of water are required for domestic and industrial purposes. Figure 1.3 shows an assessment of water use by sector, and a forecast of the exponential rise in water use predicted by the year 2025.

Figure 1.3 Evolution in world water use per sector (Shiklomanov, 1999).



All types of pollution, natural, domestic, and industrial water pollution, atmospheric pollution, and land pollution, ultimately find their way into watercourses. The intensity of water pollution is often dependent upon the level of industrialisation and the population density of the surrounding land. In the UK industrial activity comprises one of the largest sources of water pollution (DEFRA, 2002). Although industrial pollution occurs in large amounts, one positive aspect, and perhaps an advantage of this category of water pollution in terms of treatability, is that it usually occurs in a particular location, making collection and treatment relatively simple.

Water pollution from waste management sites or landfill sites is recognised as a considerable source of industrial pollution. Landfill leachate is a complex de-oxygenating industrial effluent, containing both organic and inorganic pollution. It is pumped or passively drained from landfill sites (Robinson and Maris, 1983; Robinson, 1987). Continuous treatment and disposal of landfill leachate is a requirement under the Landfill Directive (EC, 1999), until the landfill is considered to no longer pose an environmental hazard. 8.8% of all pollution incidents with an environmental impact in 2003 were attributed to waste management sites (EA, 2005). Most of these were related to waste transfer stations and non-inert landfills.

The polluted water that comes from homes, termed domestic wastewater, is more widely distributed in the environment than industrial effluent, but not to the point that it cannot be collected and transmitted by sewers to centralised wastewater treatment works. Domestic and residential pollution comprised 8.4% of serious pollution events in 2003. Private dwellings contribute many pollution incidents per annum, particularly effecting land and water (EA, 2005).

Pollution derived from agricultural practices, such as animal waste, fertiliser, run-off from agricultural land, and sediment due to land erosion resulting from farming practices, is widely distributed in the environment, and difficult to control and quantify (Hooda *et al.*, 2000). Research suggests that agriculture, through leaching and non-point pollution, is responsible for 40-50% of phosphate pollution in UK water systems (DEFRA, 2002; DEFRA, 2003). Agriculture related pollution events in 2003 comprised 5.6% of the total. Dairy farming remained the greatest source of agricultural pollution to water. Most agricultural pollution effects water and land (EA, 2005).

1.2 Waste water treatment technologies

Conventional wastewater treatment methods include chemical, physical and biological treatment options. Often combinations of these processes are employed to take advantage of the positive properties of each technique. The characteristics of the wastewater are used to determine the combination of treatment processes required to achieve satisfactory treatment of a specific effluent. Conventional integrated treatment systems normally comprise preliminary and primary treatment by physical processes. The principal techniques employed for industrial wastewater treatment included options such as; balancing tanks, gravity settling, precipitation and/or coagulation and flocculation, neutralization, oil and grease removal and biological treatment (Hammer and Hammer, 1986).

Advanced chemical oxidation processes are also available, in which chemical oxidants are used to remove oxidisable organic and inorganic components of the effluent. A variety of these processes are used, such as chemical oxidation with hydrogen peroxide, ozone, or ozone and peroxide combined (Beltran *et al.*, 1997). Ultra-violet enhanced oxidation such as UV/ozone, UV/hydrogen peroxide, UV/air and also wet air oxidation

and catalytic wet air oxidation have also been employed (Koh *et al.*, 2004). Adsorption methods using granulated activated carbon for effluent polishing can be used as a final stage of industrial effluent treatment. These techniques remove recalcitrant or toxic material that would have a detrimental effect on receiving waters (Hammer and Hammer, 2004).

1.3 Biological waste water treatment

Biological treatment reduces the concentration of organic material in effluent and is used as a tertiary treatment step after primary treatment by chemical or physical means. Available biological treatment technologies include stabilisation ponds, aerated lagoons, percolation filters and oxidation ditches, activated-sludge treatment methods, rotating disc treatment, anaerobic digestion, and land treatment such as irrigation fields and constructed wetlands. Waste treatment and environmental protection are integral to the maintenance of a healthy society and ecosystem. This project sought to investigate the biological treatment of industrial effluent through controlled experimentation using reed beds. Investigation of the microbial flora of experimental reed beds reiterated the expectation that this aspect of biologically active waste treatment systems is integral to the achievement of treatment objectives.

CHAPTER TWO

LITERATURE REVIEW

2.0 Historical development of constructed wetlands

Constructed wetlands are biological waste water treatment systems that are designed to mimic natural wetlands in their biologically mediated assimilative capacity. The use of wetland in water pollution control has ancient origins. For as long as humans have deposited waste and wastewater onto land, biological processes in wetland sinks have reduced the pollutant load. Early written reference to this type of effluent treatment in the UK is seen in a hand written note dating from 1904 concerning the use of constructed wetlands for waste water treatment (Brix, 1994a:b).

Current historical development of constructed wetland, or reed bed technology, was traced to Seidel. In a report from the Max-Planck Institute Seidel discussed the possibility of using macrophytes to remediate contaminated water, suggesting the common bullrush *(Schoenoplectus lacustris)* for this purpose (Seidel, 1964; Seidel, 1966; Seidel *et al.*, 1978; Seidel and Happel, 1981). Through her experimental research conducted in the 1950's, Seidel showed that *Schoenoplectus* spp. enriched soils and exuded antibiotics. Coliforms, *Salmonella* and *Enterococci* spp. disappeared from polluted waters by passage through bulrushes (Seidel, 1964; Seidel, 1966). The pioneering work of Seidel resulted in the development of the Max-Planck-Institute-Process or the Krefeld System (Seidel and Happel, 1981). This wastewater treatment system consisted of four to five stages, in cascades, each with several basins laid out in parallel and planted with emergent macrophytes in gravel. In 1967, the Ijsselmeerpolders Development Authority in Holland developed large scale treatment systems, known as the *Lelystad Process* (Jong, 1976). The work of Seidel also stimulated other institutions in Germany to be involved in the study of constructed wetland treatment of wastewater. In the mid 1960s, based on Seidel's ideas, Kickuth from the Institute für Brodenkund at the University of Göttingen, Germany developed the concept of the *Root Zone Method* (Kickuth, 1970; Kickuth, 1980; Kickuth, 1981; Kickuth, 1982). The Root Zone Method wastewater treatment system design typically consisted of a rectangular bed planted with reeds (*Phragmites australis*) in selected soils. In the hydrological design of the system, water moved horizontally through the root zone, (termed the *rhizosphere*) of the reeds, in a sub-surface horizontal flow path.

Later researchers, who built upon the work of Kickuth (Kickuth, 1970; Kickuth, 1980; Kickuth, 1981; Kickuth, 1982), investigated the performance of the root zone method and showed that the wastewater treatment performance claims that had been made were generally too optimistic (Brix, 1987a; Brix, 1987b; Brix, 1990a,b; Brix, 1993a; Brix and Schierup, 1989a; Brix and Schierup, 1989b; Brix *et al.*, 1989). The primary failure in the predictive performance was thought to be attributable to Kickuth's assumption of an increase in soil permeability with time. The increased soil permeability was thought to result in water flow predominately occurring as surface flow over most of the reed bed (Brix and Schierup, 1989b).

Early European work on constructed wetlands influenced the development of wetland technology in the United States. In the late 1960s, considerable research was conducted in the NASA National Space Technology laboratories (Wolverton *et al.*, 1976; Wolverton, 1982) where gravel-based systems were developed. Other pioneering work conducted in America resulted in key projects in the mid-1980s (Spangler *et al.*, 1976; Gearheart *et al.*, 1989; Watson *et al.*, 1990; Watson, 1992; Bastian and Hammer, 1993).

2.1 Constructed wetlands for waste water treatment in the UK

The UK water industry first became interested in macrophyte systems for sewage treatment in 1985 (Cooper *et al.*, 1989; Cooper *et al.*, 1990; Parr, 1990). Reed beds have subsequently been employed in the tertiary treatment of secondary effluents and in the treatment of sewage from sites not connected to the municipal sewerage system (Cooper and Green, 1995). Reed beds have been extensively used in the UK in the treatment of landfill leachate (Robinson and Maris, 1983; Robinson, 1987; Robinson, 1990; Robinson *et al.*, 1991; Robinson *et al.*, 1992; Robinson, 1998; Barr and Robinson, 1999; Robinson and Harris, 2000; Robinson and Knox, 2004). They are a particularly applicable technology because once installed they can be operated long-term with relatively little continued investment and supervision.

Changes that have occurred in the nature of UK farming, such as the increase in intensive livestock farming, have affected the quantity and point of deposition of animal wastes. The point of deposition has been moved from the field environment, where it was naturally decomposed by soil processes, to the farmyard, where large numbers of

animals are often reared in closed conditions. These non-point sources of water pollution have been identified as potentially detrimental to human health because they are often not treated prior to discharge to surface water (Hooda *et al.*, 2000). Constructed wetlands are proposed as an invaluable solution in processing both agricultural and urban storm water (Bastian and Hammer, 1993) although research has shown that the efficient nitrification of agricultural effluent is not achieved with reed bed treatment alone, so that resultant waste is not suitable for direct discharge into surface water (Parkes *et al.*, 1998). Primary wastewater treatment, prior to tertiary treatment with reed beds, or recirculation of effluent through the reed bed is suggested, but has not been widely adopted.

2.2 Reed bed design

Reed beds constitute a simple technology consisting of a soil or gravel planting substrate, with emergent macrophyte plants such as *Phragmites australis* (common reed). The hydraulic design of the reed bed determines the direction of passage of effluent through the system which can be vertical flow or horizontal flow, surface, subsurface or free standing. Subsurface flow reed beds have received a measure of popularity in Northern Europe and the United States. The popularity of these systems, when compared to surface flow and free standing water systems, has been in part due to the perception of decreased risk of nuisances from flies and odour and the greater efficiency in land usage (Reed *et al.*, 1995); although it is clear that no design consensus exists.

The reed bed substrate media is an important factor in the construction of engineered wetland systems. Early reed bed designs often employed soil as the growth medium. The most significant limiting factor regarding this type of system is the hydraulic conductivity in a porous medium. Soil beds tend to have a relatively restricted fluid conductivity, which leads to the necessity for a 4% slope in the bed design to enable water flow (Robinson, 1998). However, the use of a flat bed is considered essential to some workers to enable flooding of the reed bed, which in turn is necessary to discourage wildlife from disturbing the plants. An alternative growth medium to soil often used is moderate size (5-20mm) gravel which is selected for its high hydraulic conductivity, ease of maintenance and consistency of specification. Gravel is thought to

provide a greater predictability of performance than alternate soil media (Robinson, 1998). There are however conflicting views regarding the success of microbial growth on gravel media (Sands, 1997; Garcia *et al.*, 2003). Overall, it is considered that the relatively slower microbial growth on the gravel media compared with other soils has little adverse affect on reed bed performance (CIRIA, 1997) and that the use of highly permeable gravels allow greater simplicity in the design of the bases of reed bed systems (Robinson, 1998). It is broadly accepted that a mean depth of 600mm and generally flat base are optimal for bed design (EWPCA, 1990; CIRIA, 1997; Robinson, 1998). The sidewalls of the reed beds should be vertical, following observations that reeds do not grow near to shallow sloped edges (Parr, 1990; Robinson, 1998). Above all it is considered important that the design of the reed bed should be simple and distribute the influent evenly across the width of the bed. For trouble free operation of the reed bed the facility for an effluent outlet to control water levels should also be simple and efficient (Robinson, 1998). Water levels should be regularly lowered to aerate the reed beds and encourage rhizome growth (Brix, 1993b).

2.3 Plants in constructed wetlands

Several plant species have been used in constructed wetland treatment systems. These include *Phragmites, Scirpus, Typha, Carex* spp. and water hyacinth (CIRIA, 1997). The most commonly used plant in reed beds in the UK is *Phragmites australis* (Haslam, 1973; Rodewald-Rudescu, 1974; Hartog *et al.*, 1989); a widespread wetland grass species, indigenous in the UK and morphologically adapted to grow in waterlogged sediment (Brix, 1993a; Brix, 1993b). *Phragmites australis* is appropriate to be used in constructed wetland design because it is easily established, resistant to occasional frosts and provides an aesthetically pleasing habitat for wild life (Robinson, 1998). Tanner (Tanner, 2001) discussed the importance of plant species in treatment wetlands and described the plants as engineers of the physical environment.

The role of macrophytes in constructed wetlands has been discussed extensively and their function continually assessed and re-evaluated. These roles include stabilisation of the surface of the bed, preventing vertical-flow systems from clogging, providing insulation from frosts during winter, a suitable environment for attached sub-surface microbial growth and also providing passageway for water and oxygen transport (Sun *et*

al., 1998a; Sun *et al.*, 1998b). The diffusion of oxygen from the plant roots is reported to encourage the growth of nitrifying bacteria in the rhizosphere (Kickuth, 1981; Armstrong and Armstrong, 1988). Oxygen diffusion is thought to be a factor in regulating the microbial population of the substrate (Brix, 1987b). However the capacity of reeds to transfer oxygen to the rhizosphere is generally considered to be much less than was originally claimed by Kickuth (Brix, 1990a; Brix, 1990b; Brix, 1993a; Brix, 1993b). Oxygen translocation to the roots and rhizomes of reeds, by the large internal aerial spaces of the plants (Brix, 1988), means that the plant is thought to have the ability to transport oxygen down the leaf and stem structure, into the rhizosphere and out through the roots (Brix, 1990b). This is reported to be possible by diffusion or by convective flow of air (Brix, 1993a; Brix, 1993b). Humidity induced convection and venturi-induced convection have been identified as mechanisms through which oxygen diffuses into *Phragmites* (Armstrong and Armstrong, 1988; Armstrong and Armstrong, 1990; Brix, 1990a; Brix, 1990b; Armstrong and Armstrong, 1991; Brix, 1993a). The humidity induced mechanism is dependent upon the difference in humidity within the internal space of the reed (sub-stomatal gas-space) and the drier air outside. The through-flow results in increased oxygen diffusion to the plant root and rhizosphere (Armstrong *et al.*, 1996a:b). Venturi-induced convection is a suction pressure induced by wind speed across the reed stands (Armstrong, 1990; Armstrong, 1992; Armstrong *et al.*, 1992; Armstrong *et al.*, 1996a; Armstrong *et al.*, 1996c; Armstrong *et al.*, 1996d). The quantified magnitude of oxygen release under *in situ* conditions remains a matter of controversy (Sorrell and Armstrong, 1994; Beckett *et al.*, 2001).

It has been suggested that the greater importance of the reed is the way that its large, distinctive root mass has physical effects on the reed bed substrate, for example in reducing the current velocities of the waste water (Pettecrew and Kalff, 1992; Somes *et al.*, 1996) and in helping develop the hydraulic conductivity of the substrate (Kickuth, 1981). However, research has failed to prove this alternative mechanism (Schierup *et al.*, 1990). Contrary to earlier belief, the growth of macrophytes is thought not to increase the hydraulic conductivity of the substrate in soil-based subsurface flow constructed wetlands. In fact the hydraulic conductivity has been reported to decrease and stabilise with time (Brix, 1994a). The metabolism of the plants may affect the treatment process of the wetland depending upon the design. Plant uptake of wastewater

contaminants is only thought to be of quantitative importance in low-loaded surface flow systems.

2.4 The rhizosphere environment

The area of enhanced bacterial population around the underground roots of the reeds is termed the *rhizosphere.* In this area populations of aerobic and anaerobic bacteria have been shown to proliferate (Brix, 1987b; Nichols, 1997; Urbanc Bercic and Gaberscik, 2001; Hinsinger *et al.*, 2003; Stottmeister *et al.*, 2003). Stimulation of microbial growth in the rhizosphere has been shown to be largely due to the leaky nature of plant roots in nutrient and oxygen release, reflected by the varied distribution of microbial populations. Organisms aggregate in association with cell junctions and moribund cells, reflecting the supply of nutrient substrate from the roots (Wood, 1989). A wide range of organic compounds is also released by the plant roots (Rovira, 1965; Rovira, 1969). The release of organic carbon exuded by the roots may act as a carbon source for denitrifying microorganisms and thus increase nitrate removal in some types of treatment wetland (Platzer and Netter, 1994; Platzer, 1996).

The microbial species present in the soil or growth media of a constructed wetland are considered a hierarchical metabolic system. Ecosystems dominated by aquatic plants are amongst the most productive in the world (Westlake, 1963), and the high productivity of these systems results in high microbial activity and therefore a high capacity to decompose organic matter and other substances (Brix, 1993a). Wetland systems remove or reduce contaminants; reduction is said to be accomplished by diverse treatment mechanisms including filtration, sedimentation, chemical precipitation and adsorption, microbial interactions and uptake by vegetation (Watson *el al.*, 1989). Constructed wetland technology takes advantage of many of the natural processes that occur in natural wetland systems and harnesses that ability in a controlled environment (Bastian and Hammer, 1993). Microorganisms play a central role in the biochemical transformation of nutrients (Ottova *et al.*, 1997). The processes of synthesis and decomposition by microorganisms are important components of the natural cycling that occurs within reed beds (Wood, 1989). Unique enzymatic capabilities, which enable bioconversion processes, are thought ultimately to be the important factor in the reed beds biotechnological processes (Portier and Palmer, 1989). Studies suggest that the

ability to successfully manage the treatment of wastewaters in constructed wetland is dependent upon understanding the relationship between the microorganisms, wastewater, substrate and vegetation (Terry III, 1993; Stottmeister *et al.*, 2003).

2.5 Pollutant removal processes

Constructed wetland systems have the tolerance for processing a broad range of wastewaters (Biddlestone *et al.*, 1991; Perfler and Haberl, 1993; Bavor *et al.*, 1995; Vrhovsek *et al.*, 1996; Parkes *et al.*, 1998; Benham and Mote, 1999; Bavor *et al.*, 2001; Begg *et al.*, 2001). The treatment of wastewater requires adequate reduction of biological oxygen demand (BOD_5) and organic and inorganic forms of nitrogen (Reddy and De Busk, 1985). Filtration and sedimentation remove the organic form of nitrogen, however it may be mineralised and released over time as ammonia. Under alkaline conditions ammonia is directly lost by volatilisation and ionises in water. Ammonium ion nitrification typically requires the activity of nitrifying autotrophic bacteria under aerobic conditions, with pH values in the range of 6.5 to 8 (Parkes *et al.*, 1998).

It has been shown that nitrifying bacterial populations are sensitive to heavy metal ions and temperature (Moshiri, 1993). Their nitrification activity can be accelerated through periodic drying of the treatment surface. Nitrogen removal is approximately 20 to 30% of the total N (Cooper, 1993). Seasonal fluctuations in efficiency were also noted. Ammonia conversion, nitrification and denitrification are variable and dependent upon system design, retention time and oxygen supply (Platzer, 1999).

The elimination of phosphorus in constructed wetlands occurs by chemical precipitation through combination with ions naturally present within the soil, for example calcium$^{(II)}$, iron$^{(III)}$ and aluminium$^{(III)}$ (Nichols, 1983; Suzuki *et al.*, 1989; Davies and Cottingham, 1993; Mann and Bavor, 1993; Reddy *et al.*, 1999; Serodes and Normand, 1999; Tanner *et al.*, 1999; Arias *et al.*, 2001; Del Bubba *et al.*, 2003). Phosphorus removal is variable and dependent upon the size of the reed bed and the substrate media involved in construction.

Biological oxygen demand (BOD_5) is a frequently measured parameter in wastewater treatment plants and their discharges (Knight *et al.*, 1993). Biological oxygen demand

(BOD_5) and chemical oxygen demand (COD) are used to determine or define water quality. Many of the pollutants in effluent are organic compounds. As these substances oxidize or stabilize, they combine with dissolved oxygen in the water. The amount of oxygen that is used is therefore an indicator of the amount of organic waste present in the sample. The BOD_5 and COD values indicate the amount of oxygen (in milligrams per litre) needed to oxidize or stabilize the sample. Although regulatory agencies, such as the Environment Agency, require the monitoring and reporting of BOD_5 levels, the COD test has several advantages for operational level assessment. Time taken (speed of analysis) is the major advantage of COD over BOD_5. A 2-hour COD reflux test is standard. The BOD_5 test is time consuming, requiring a standard 5-day incubation period. The rapid test results of the COD procedure provide an advantage in regular monitoring of wastewater discharge. Another advantage of the COD test is that the strong oxidizing conditions created within the test are independent of variations in experimental conditions and procedures. This is not true for the BOD_5 test, which is sensitive to test conditions. In addition, the BOD_5 test, unlike the COD test, does not measure biologically resistant compounds.

BOD_5 integrates the organic and chemical oxidation processes which occur in water containing solid and dissolved pollutants. Under appropriate conditions high removal efficiencies for BOD_5, suspended solids and other nutrients can be achieved within a treatment wetland, as well as 'natural die-off' of pathogens. Constructed wetland treatment can bring about a marked reduction of the pathogenic agents present in wastewater, for example in helminith egg numbers (Mandi *et al.*, 1996). A considerable decrease in parasitic load (95 -100% reduction) was achieved. It was reported that this may have been due to sedimentation of the eggs within the reed bed substrate. Once accumulated in the soil, elimination of the eggs may have occurred by numerous factors including pH, the presence of antagonistic organisms and temperature (OSM, 1982). The destruction of pathogenic agents in waste water treatment is a significant factor against potential health hazards (WHO, 1989). The removal efficiencies of the organic components of wastewater reported in constructed wetland is shown in table 2.1.

Table 2.1 Pollutant removal efficiencies of constructed wetland systems (Bastian and Hammer, 1993).

Parameter	Removal (%)
BOD_5	50 – 90
Total Suspended Solids	40 – 94
Nitrogen	30 – 98
Phosphorus	20 – 90

2.6 Hydraulic flow rate and retention time

Hydraulic factors are important in constructed wetland design and have been shown to influence both the treatment potential and performance of a wetland (Worman and Kronnas, 2005). Understanding the movement of water and fate of pollutants within the wetland should include, at some level, hydrologic parameters. These include loading volume, retention time, and hydraulic conductivity, all of which are almost inextricably linked (Hobson, 1989; Fisher, 1990; Waters *et al.*, 1993; King *et al.*, 1997; Machate *et al.*, 1998a; Machate *et al.*, 1998b; Persson *et al.*, 1999; Chazarenc *et al.*, 2003; Garcia *et al.*, 2003). The method of recirculation of effluent through the reed bed increases the residence time of the reed bed, and is therefore thought to increase the concentrations of nitrifying bacteria (Tchobanoglous, 1993), leading to improved water treatment quality (Sun *et al.*, 1998a).

Considerable investigation into the assessment of sizing criteria and the treatment performance of various constructed wetland design has been carried out in the USA. The need for passive treatment of acid mine drainage has spurred research in establishing basic design criteria. Several different methods have been used to assess treatment performance. These have included: treatment efficiency, area adjusted removal, and first-order removal, all of which have been used in modelling the effects of concentration and flow rate in determining the wetland area required for successful treatment of polluted effluent to discharge standards. The sizing of reed beds has been in part dependent upon treatment objectives, and many workers have endeavoured to formulate equations to predict the performance of reed bed systems (Kadlec and

Hammer, 1988; Balkema *et al.*, 1998; Polprasert, 1998; Tanner *et al.*, 1998; Kadlec, 2000; Werner and Kadlec, 2000; Dahab *et al.*, 2001; Giraldo and Zarate, 2001; Karunaratne and Asaeda, 2002; Kadlec, 2003; Kincanon and McAnally, 2004; Rousseau *et al.*, 2004; Worman and Kronnas, 2005).

2.7 Treatment performance

Effluent concentration at the point of discharge is a regulatory basis of compliance. Treatment efficiency is a measure based on effluent concentration (Girts *et al.*, 1987; Girts and Knight, 1989; Wieder *et al.*, 1989). The formula used to calculate treatment efficiency is;

$$\text{Treatment efficiency} = [C \text{ in} - C \text{ out}] / C \text{ in} \times 100$$

where C = concentration

However, the problem with the use of treatment performance as a measure in determining appropriate sizing criteria of wetland treatment systems is that it is a relative measure of performance, and not an absolute measure. It provides a percentage treatment that is relative to influent concentration, and therefore cannot be used alone to provide wetland size criteria. A formula based on area adjusted removal which is based on influent concentration, wetland flow (hydraulics) and wetland area was developed (Hedin and Nairn, 1990). This method has the advantage of providing wetland sizing information (Tarutis *et al.*, 1999) and variations in this method have been developed to establish guidelines for the wetland treatment area required for a specific effluent (Brodie *et al.*, 1988; Hellier, 1989; Stark *et al.*, 1990; Wildeman *et al.*, 1990; Hellier *et al.*, 1994). In the USA this method has been adopted by the US Bureau of Mines in evaluating wetland performance in consideration of sizing (Hedin *et al.*, 1994). Other researchers have questioned this model and concluded that under some circumstances treatment efficiency is more important (Stark *et al.*, 1994; Stark and Williams, 1995).

Treatment efficiency can be derived from both area adjusted removal and first-order removal with knowledge of hydraulic loading rate. First-order removal is an alternative measure of wetland treatment performance. The pollutants in question which are said to follow a first-order removal pattern are suspended solids, biological oxygen demand,

nutrients and pathogens (Dortch, 1996; Kadlec and Knight, 1996). Like area-adjusted removal this assessment also provides sizing criteria, but its application has not been explored in relation to acid mine drainage. Mathematical relationships between these measures have been derived from simple kinetic equations based on understanding the biochemical mechanisms by which wetlands remove pollutants (Tarutis *et al.*, 1999). Hydraulic loading rates have been summarised for several types of systems and design considerations have been established for certain effluents. Hydraulic loading factors are available for municipal and mining wastewaters.

Design procedures for subsurface flow constructed wetlands are based upon the simple assumptions of plug-flow and first-order decay of pollutants (Watson *et al.*, 1989; USEPA, 1993). Plug flow describes the simplest flow pattern that could be described in which there is a constant velocity of flow in every part of the system. The plug-flow assumption is widely used, despite the fact that several researchers have demonstrated that plug-flow does not adequately describe the flow of water through sub-surface flow (SSF) wetlands (Kadlec *et al.*, 1993). This is perhaps due to variation in effluent dispersion with distance travelled through the reed bed (Pilgrim *et al.*, 1992; Waters *et al.*, 1993). Short-cuts and dead zones in the substrate matrices of reed beds have been identified and shown to reduce the effectiveness of biologically mediated treatment. The effectiveness of treatment is dependent upon the contact time of effluent with biologically active surfaces within the wetland, such as attached microbial populations on the macrophyte roots and the surface of soil or gravel particles. Empirical data relating to large numbers of wetland have demonstrated an exponential decrease in pollutant concentrations with distance travelled through the wetland from the inlet to the outlet. This is consistent with a first-order removal model, which describes the relationship between rates of removal being proportional to pollutant concentration (Kadlec 2003). Multiparameter models of flow through SSF wetlands have also been described (Kadlec and Knight, 1996). Although these models are more closely aligned to tracer experiments modelling flow behaviour, there is still limited information to define spatial and temporal variations in flow patterns and to develop existing models (King *et al.*, 1997). Tracer studies have indicated that water flow through a reed bed is not uniformly distributed. It cannot be described by a single parameter plug-flow dispersion model, and a multi-parameter plug-flow model would be more appropriate. Tracer studies using effluents augmented with fluorescent chemicals, such as lithium

chloride and bromide have been used to investigate hydraulic characteristics and flow patterns in constructed wetlands (King *et al.*, 1997; Machate *et al.*, 1998a; Machate *et al.*, 1998b).

Achievement of maximum treatment potential in the design of constructed wetlands is necessarily based upon understanding the mechanisms by which wetlands function. Using these and other factors it is feasible to predict the wetland area necessary to achieve treatment objectives. However, refinement of the technology is complicated by the variability of the treatment regimes, loading conditions, effluent type and concentration, climatic conditions, and the variability and diversity of system design. Valid comparison of the technology under different operational conditions is difficult.

2.8 Constructed wetlands and environmental conditions

The successful operation of constructed wetlands has been reported in temperate, sub-tropical and tropical climate zones. There are estimated to be more than 650 natural and constructed wetlands in North America and more than 5000 subsurface flow constructed wetlands in Europe (Kadlec and Knight, 1996). Similarly throughout the world the adoption and application of reed bed technology has increased. Wetlands have been used and researched, in Germany, Holland, Colombia, Asia, Africa and in Scandinavian countries . Extensive research on structure, function, potential application and the various designs of treatment systems has been shown to not be directly transferable from temperate to tropical environments. For example, the issue of plant species selection and suitability for Europe and North America is not consistent with tropical environments. The limited available information on tropical plant species suitable for sustainable constructed wetland development requires identification and characterisation of appropriate tropical plant candidates in terms of their tolerance to high nutrient uptake.

The use of constructed wetlands with *Eichhorinia crassipes* (water hyacinth) for wastewater treatment has been well documented (Reddy and De Busk, 1985; Vymazal *et al.*, 1998). Treatment systems with the water hyacinth are sufficiently developed to be successfully applied in the tropics and sub-tropics, where climatic conditions are favourable to continuous growth of the macrophyte. One of the reasons that the

enormous potential of water hyacinth for wastewater treatment has not been fully integrated in the Northern Hemisphere is perhaps the low winter temperatures, given their optimum growing conditions of 20-30°C. The water hyacinth plant has an extensive root system which provides a large surface area for attached microbial growth thus increasing the potential for decomposition of organic matter. Plant uptake is the major process for nutrient removal from wastewater containing water hyacinth. An additional factor is the production of a large excess of biomass which characterises the rapid growth rate of the plant. In order to achieve and maintain a successful and effective treatment system based on water hyacinth, the management plan must include provision for harvesting and secondary use of the excess plant material. The plant doubles its biomass in six days (Reddy and De Busk, 1985), this has resulted, in some instances, in the plant invading areas of natural habitat and disrupting the indigenous ecosystem.

Although the use of water hyacinth has been recommended by some workers in the tropics and sub-tropics (Vymazal *et al.*, 1998), the attitude toward this type of wastewater treatment system, and indeed the plant itself, is negative (Gopal *et al.*, 1984; Masifwa *et al.*, 2001). The concept of using hyacinths for wastewater treatment in developing countries has been persistently controversial (Kivaisi, 2001). The exotic water hyacinth plant is considered to invade natural water bodies causing economic and ecological problems. This has led to a situation of negative perception in areas such as the Lake Victoria region (waters shared by the countries Kenya, Uganda and Tanzania) where water hyacinth has caused severe ecological problems. Success of integrated rural wastewater treatment systems with water hyacinth has been demonstrated, for both small communities and industrial effluent (Gopal *et al.*, 1984; Prasad *et al.*, 1991; Gopal and Zutshi, 1998; Masifwa *et al.*, 2001).

The potential for the application of constructed wetland for wastewater treatment has grown in strength throughout the world. Research has been carried out in Africa, USA, Asia, Australia and Europe. Experiences of successful effluent treatment with constructed wetland have been reported from Nepal, Mexico, South Africa, Thailand, and Brazil, to mention but a few. The application of constructed wetland operation in Morocco was reported, in which semi-arid conditions supported reed beds planted with *Phragmites australis* (Mandi *et al.*, 1998). The finding showed successful treatment of

effluent with hydraulic loading rates of 0.86-1.44m^3 d^{-1}, organic removal 48-62%, total suspended solids removal 58-67%, and parasitic removal 71-95%.

Recommended hydraulic loading rates differ from European and American experience. For example the recommended loading rates for subsurface flow wetlands are 2-30mm/day for hydraulic loading and 135 kg/ha per day for organic loading, respectively (USEPA, 1988; USEPA, 1993). Higher organic loading rates of 12 kg/ha per day and 130 mm/day respectively were applied which offset the higher cost of wetlands when compared to activated sludge systems. However, this led to clogging of the system. The suspended solid load is said to be the limiting factor in increasing organic loading of constructed wetlands.

2.9 Economic analysis

Constructed wetlands have been shown to be an effective, environmentally benign way to treat wastewater and effluents with relatively low capital and operating costs and minimal maintenance (Robinson, 1998). Conventional water treatment methods, involving the use of chemicals are more expensive and have been shown to be unable to tolerate fluctuating loading conditions (Hammer and Hammer, 1986; Hammer and Hammer, 2004). Using cost analysis to compare construction costs, and unit treatment costs for subsurface flow wetland systems compared to the cost of activated sludge treatment systems, or other conventional means, treating a comparative volume of effluent, the unit treatment cost of the former system was lower.

2.10 Waste water treatment in countries with developing economies

The number of people lacking access to safe drinking water, mainly in developing countries, was predicted to increase to between two and three billion in the year 2000 (Strikker, 1998). In addition to the natural scarcity of water in these regions the quality of fresh water is deteriorating rapidly due to pollution. Liquid wastes from industry and untreated sewage are the major sources of pollution because the unregulated discharge of contaminated water is a common practice (Strikker, 1998). With the progress of rapid urbanisation, lack of proper sanitation in urban areas, and the contamination of surface and ground water through surface run-off from pit-latrines, which is considerable during rainy seasons (Denny, 1997), the situation continues to deteriorate. Unregulated

discharge of liquid wastes, such as untreated sewage or industrial waste, is a major source of water pollution in many countries. Municipal sewage and industrial effluent, which contain readily biodegradable organic matter, inorganic and organic chemicals, toxic substances and disease causing agents, are often present in drinking water supplies. The increased use of fertiliser in agriculture also contributes significantly to non-point source pollution through run-off. The discharge of untreated effluent pollutes natural water bodies and leads to the transmission of human pathogens and the consumption of contaminated food and water. In most countries with developing economies there are few municipal wastewater treatment facilities (Kivaisi, 2001). This is mainly due to the high costs of treatment processes and lack of effective environmental pollution control laws or law enforcement.

The impact of the discharge of urban and industrial wastewater is a matter of great concern in most countries. Important to the protection of the environment and human health is the establishment of adequate legislation and legislative control for the protection of water resources. Developed nations have already surpassed the basic stages of the establishment and enforcement of legislative control. However, in countries with developing economies there is increasing pressure, on one hand to meet continually lower international wastewater treatment objectives and, on the other, to attempt to reverse the process of continual environmental degradation (von Sperling and Augusto de Lemos Chernicharo, 2002). In this respect, inadequate treatment of wastewater in countries with developing economies is a serious problem that has considerable implications for human health and the environment.

In the European Union the phasing in of tighter environmental legislation has been used to control wastewater discharge and to improve water quality. Industry is faced with ever increasing prices for the purchase of potable water and the disposal of wastewater, and is subject to statutory limitations for the extraction of groundwater. These legislative standards have been effective in protecting receiving water body quality. However, the effect of this control, especially in the EU, has resulted in changes in production methods and in some cases the movement of certain production activities abroad (Stoop, 2003). The transfer of the most labour intensive and polluting production processes to countries with weaker environmental legislation is at present causing environmental problems in third world countries (Hage, 1994; Stoop, 2003). For some

industries, it is cost effective to opt to buy semi-manufactured goods from countries with developing economies, where labour is cheaper and environmental pollution control regulations are less stringent. These countries often have little influence over the long term problems associated with this trend, or awareness of the environmental consequences, partly due to a lack of insight into the basic principles of chemical engineering and insight into process control (Stoop, 2003).

A system of step-wise reduction of discharge consents has been proposed (von Sperling and Augusto de Lemos Chernicharo, 2002). It has been suggested that if countries with developing economies were allowed to develop their own environmental protection schedules, discharge consents and timescales for compliance with environmental and social protection standards, they would be better able to introduce sustainable technology such as constructed wetlands. However, if the countries with development issues were forced to comply with stringent discharge consents imposed by western governments, they would be drawn to using advanced technologies for waste treatment in order to achieve the discharge consents. The fact that those technologies are expensive, highly technological, and perhaps not appropriate for developing nations would be overlooked. It should not be forgotten that the so called "developed countries" took many decades/centuries to reach present levels of civilisation and during that period of development many other countries were exploited for resources and labour. The imposition of stringent treatment objectives for wastewater discharge in those struggling countries, many of whom are still recovering from past injury and injustices, has the effect of restricting progress towards economic and social development.

The United Nations Conference on Environment and Development in Rio in 1992 dealt with the issue of water in Chapter 18 of Agenda 21, called *Protecting and Managing Freshwater* (UNCED, 1992). Goals were set in 2000 for universal water supply by 2025. However, it was considered that, due to World Bank budget and the prevailing short term priorities to be financed, it was unlikely that projected goals would be achieved in the projected time frame (Strikker, 1998). It was therefore left to the individual countries to take initiatives, within the framework of Agenda 21, to reduce the depletion of water reserves, through water saving, recycling, reuse and pollution control.

Water reuse has been an established practice in many countries where there has been historical water scarcity. Examples can be taken from Morocco, Tunisia, Egypt, Sudan, Namibia, India and China, where sewage is used to irrigate vegetables, and other short-term crops, and to support fish stocks (Shuval, 1986). Health implications associated with the irrigation of crops and fish with sewage has been well documented (Stott *et al.*, 1997). This risk might be mitigated if the water is first treated through a constructed wetland to reduce the pathogen load.

Developing countries can be divided into two distinct groups in terms of the capability of the country to adopt western technology, those with adequate financial resources due to oil, such as Middle Eastern locations and those of economic poverty such as many African nations (Schertenleib, 1981). This financial division is reflected in the uptake and application of highly technologically complex western wastewater treatment, and other technologies. There are very few wastewater treatment facilities in countries with developing economies. This is mainly due to the high cost of such facilities, and to the lack of effective environmental pollution laws or law enforcement. Instead, a wide range of treatment methods have been used which include stabilisation ponds, septic tanks, activated sludge treatment, trickling filters, anaerobic treatment systems and land application (Schertenleib, 1981). The most widely used technology is stabilisation ponds, this is due to the low cost of installation and maintenance, and the optimum climatic conditions for this type of technology in tropical areas, where many developing countries are located (Kivaisi, 2001). Being low-cost and low-technology systems, wetlands are potentially an alternative or supplementary system for wastewater treatment in those countries.

2.11 Constructed wetlands in countries with developing economies

Information regarding the level of development of wetland technology in developing countries is limited. In some countries, basic research has been carried out, while in others the technology has reached pilot and full scale levels for various applications. Experimentation on the suitability of local and indigenous wetland emergent macrophytes for removal of nutrients and heavy metals has been ongoing in several countries (Kivaisi, 2001). The warm subtropical climate is conducive to higher biological activity and productivity and there is availability of a rich diversity of

indigenous biota that could be used in wetlands. Despite the obvious advantages of wetland technology for developing countries the rate of adoption of the technology for waste water treatment in these regions has been slow. The implementation of wetland technology in countries with developing economies has been critically discussed (Denny, 1997). Denny found that aid programmes from developed countries tended "to favour the more overt technologies that had commercial spin-off for the donor country". Additionally, developed world "advisors" were "entrenched in technologies appropriate for their own countries and were unable to transfer their conceptual thinking to the realities and cultures of the third world". Thus, rather than assisting developing countries to develop their own constructed wetland technologies, the tendency was the translocation of 'northern' designs to tropical environments. Depending on the countries political policy and financial situation, other reasons have also been considered.

Points of existing limitation to widespread adoption of constructed wetland technology in countries with developing economies have been investigated (Gopal, 1999). Factors included; large land requirement; lack of knowledge of tropical wetland ecology and native wetland species; prevalence of mixed domestic/industrial wastewaters; limited knowledge and experience with constructed wetland design and management in these locals. Areas of research need were identified which include the development of appropriate strategies based on local parameters (Gopal, 1999). Clear understanding of the biological, hydraulic and chemical processes involved in constructed wetland is considered essential (Denny, 1997).

Although many advantages to constructed wetland technology adoption in countries with developing economies have been identified there are also a number of disadvantages to the technology, some of which are of special concern in tropical climates. The necessity for a co-ordinated multidisciplinary approach, involving cooperation between environmental and social scientists, engineers and policy makers has been highlighted. In determining whether the technology is an appropriate option, factors for consideration include the cost of development and maintenance, land ownership, availability and cost. The relatively flat topography in some of the countries, where low cost sustainable water treatment is vitally needed is a concern, as is the importance of appropriate operation and maintenance. The necessity and importance of adequate training for operation staff has been highlighted (Denny, 1997), who reported

that in-house expertise and the exchange of local and specialised knowledge with external institutions was needed. Well-qualified scientists and engineers are needed to implement strategies for overall environmental protection through appropriate technological application.

Important considerations such as the characteristics of the wastewater and the operational environment are important to consider in the design of wetlands. Phytotoxic pollutants inhibit microbial activity and there should be an awareness of the potential transfer of toxicity of pollutants within the system. Adequate water availability throughout the year is necessary to sustain constructed wetland treatment systems and therefore extreme drought conditions are perhaps inappropriate. Selection and management of indigenous macrophyte species and knowledge and control of disease vectors, such as invading animal pests with control strategies, are vital for successful operation.

2.12 Concluding remarks

Constructed wetlands have been found to be used throughout much of world where they have provided effective low cost wastewater treatment with minimal environmental impact. Strategies are critically needed to integrate constructed wetland technology into mainstream wastewater remediation and for it to be a cost effective option. Constructed wetlands are considered to no longer represent a novel emergent technology. Application of reed bed systems throughout the world has proved that biologically mediated wastewater treatment is successful and environmentally sustainable. What is clear is that the widespread acceptance and application of the technology is in some instances dependant upon legislative changes to stringent waste water discharge consents.

2.13 Experimental aims and objectives

2.13.1 Aims

- To treat landfill leachate and other industrial effluent with reed bed technology in the UK.

- To monitor the installation and operation of two distinct reed bed systems to treat factory effluent and domestic effluent from a fruit processing factory in Ghana.

- In each case, to measure the effectiveness of the treatment of the effluent.

- To monitor the microbial consortia of the reed bed under conditions of effluent loading.

- To determine the reactions of the microbiological community of the reed beds to the addition of various effluents.

2.13.2 Objectives

- To compare the operation and functioning of reed bed technologies in temperate and sub-Saharan climates.

- To relate the degradation of effluent to the changing physical state and microbiology of the reed bed.

- To define the operational constraints and barriers to the introduction of reed bed technologies for different treatment purposes.

CHAPTER THREE

METHODS

3.0 Reed beds

The experimental reed beds at University College Northampton were built in 1997. Each reed bed was constructed with the dimensions 5.4m x 2.0m x 1.0m with a 2% gradient. The supporting substrate for each of the reed beds was a sandy clay loam mixed with peat. Six millimetre diameter gravel, at both ends of the beds, was used to allow horizontal sub-surface flow of wastewater. The reed beds were planted in May 1998 with *Phragmites australis* (common reed) at a planting density of two plants per m^2. *Phragmites australis* was chosen because it is a widespread wetland grass species, indigenous to the UK and morphologically adapted to grow in waterlogged sediment (Brix 1993). By July 1999 the plants were well established and had grown approximately 2m in height.

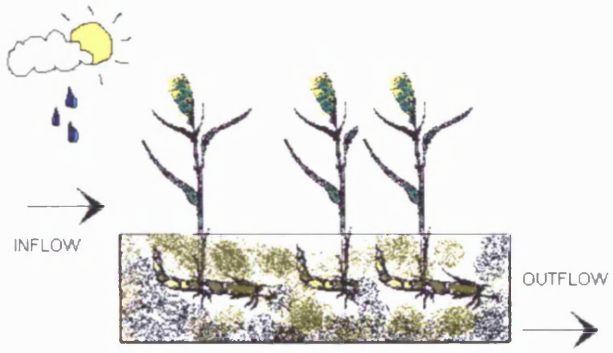
Figure 3.1 UCN experimental reed beds with individual loading tanks.

3.1 Experimental design

The UCN reed beds, of which there are nine, were used to conduct controlled experiments in treating industrial effluent. Landfill leachate used throughout the investigation was delivered to UCN from a number of co-disposal landfill sites in Shropshire, UK. Leachate was stored on-site until it was pumped into the 700 litre influent loading tanks (figure 3.1). Tannery effluent was obtained from the British School of Leather Technology (BSLT) tannery located on-site at UCN. Reed bed loading was regulated manually. A batch flow loading regime was used to load the reed beds. Wastewater samples were collected from the inflow and outflow of each of the reed beds (figure 3.2). The sampling container was held under the flow of wastewater

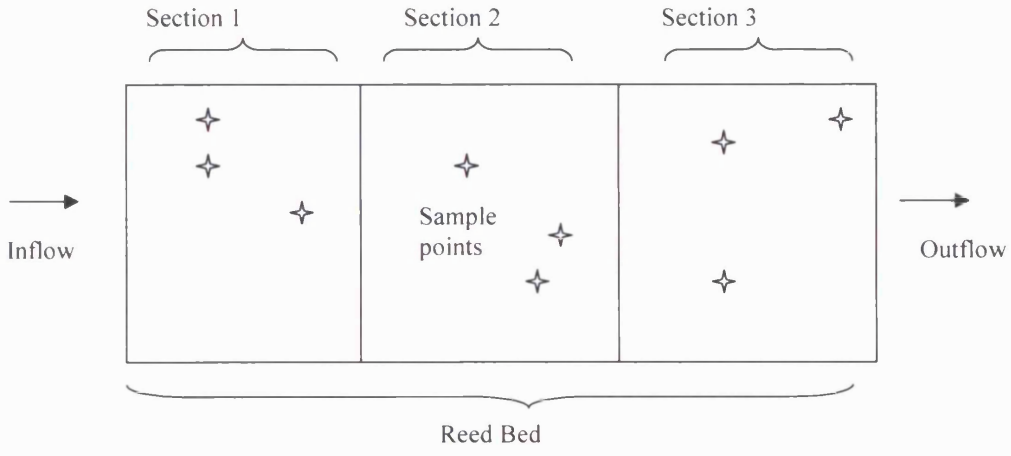
until the sample container was full. The container was then sealed. Samples were stored at 8°C for a minimum time period, no longer than 48 hours, before chemical analysis was carried out.

Figure 3.2 Basic experimental design.



Reed bed substrate samples were collected using a soil auger. Samples of the sediment were taken at the specific depths described in the individual experiments. The soil sampling strategy used involved dividing each reed bed into three equal sections. Three soil samples were taken from each section, a total of nine samples were collected from each individual reed bed. Random number generation was used to select three grid references within each of the three sections of each reed bed, on each sampling occasion, so as to avoid sampling errors and to vary the position of soil cores more than by random chance. The substrate sampling strategy is illustrated in figure 3.3. Soil substrate samples were stored in air-tight sealed bags at 8°C for a minimum time period, no longer than 3 hours, before microbiological and biochemical analyses were carried out.

Figure 3.3 Sampling strategy for one individual reed bed.



3.2 Analytical methods

Analyses were conducted according to standard scientific methods. All chemicals were of analytical grade and were obtained from Sigma-Aldrich (Poole, UK), unless otherwise stated.

3.2.1 Chemical oxygen demand (COD)

Potassium hydrogen phthalate standards were prepared by dissolving 0.1452g of potassium hydrogen phthalate in 1L of distilled water. The stock solution had a theoretical COD of 500μgO^2/mL (500ppm). Using a burette, 3mL, 10mL, 30mL, and 50mL of stock solution were transferred into 50mL volumetric flasks and diluted to the mark with distilled water. This made a series of standards containing 30, 100, 300, 400, and 500ugO_2/mL, respectively.

Digestion solution

5.1080g of potassium dichromate (previously dried at 103°C for 2 hrs), 83.5mL of concentrated sulphuric acid and 16.65g of mercury$_{(II)}$ were added to 250mL of distilled water. The solution was cooled to room temperature then diluted to 500mL.

Sulphuric acid reagent

5.05g of silver sulphate (Ag_2SO_4) was added to 500mL of concentrated sulphuric acid. This was allowed to stand until dissolved, which took approximately 48 hours.

Into a COD tube containing 1.5mL of *digestion solution* and 3.5mL of *sulphuric acid reagent*, 2.5cm of distilled water was added. This was used as a blank when calibrating the spectrophotometer (Spectronic 501, Milton Roy Company, UK). For each sample to be tested, 0.5mL of effluent sample and 2.0mL distilled water was dispensed into a COD tube containing *digestion solution* and *sulphuric reagent*. The caps of the COD tubes were replaced tightly and the solution was vortex mixed. The COD tubes were placed into a previously heated COD reactor (HACH, Camlab Ltd., Cambridge, UK), at 150°C and heated for 2 hours. The tubes were removed from the COD digester, shaken well, and allowed to cool before reading in the spectrophotometer (Spectronic 501, Milton Roy Company UK). The absorbance of each sample was read at 600nm.

Calculation

COD = (absorbance x 5)-0.0054)/0.0003

When wastewater contains only readily oxidisable organic matter and is free from toxins, the results of a COD test provide a good estimate of the BOD. This is because when dichromate is used to oxidise organic matter in aqueous solution, the half reaction is:

$Cr_2O_7^{2-} + 14H^+ + 6e^- \rightarrow 2Cr^{3+} + 7H_2O$

When oxygen is used for the same purpose, the half reaction is:

$O_2 + 4H^+ + 4e^- \rightarrow 2H_2O$

Thus, it takes 6:4 or 50% more oxygen to carry out an oxidation as it would if dichromate was used (on a molar basis). This reaction was used to relate the COD and BOD analysis results.

3.2.2 Chloride potentiometric determination

25mL of sodium chloride solution and 50mL of waste water sample were pipetted into a 100mL beaker. 1mL of potassium chromate was added and the solution was stirred magnetically. A burette was filled with 0.05 mol/L silver nitrate. Electrodes were placed into the solution and the pH meter (Jenway Ltd UK.,) was set to read millivolts. The reading on the meter was recorded. 5mL of silver nitrate was added to the beaker from the burette and the meter reading was again recorded. Silver nitrate was added 5mL at a time and the meter reading recorded after each addition, until a total of 20mL had been added. The silver nitrate was then added in steps of 0.5mL until a total of 30mL had been added, again meter readings were recorded. Finally two additions of 5mL of silver nitrate were added noting the meter readings.

A graph was plotted of the results with volume of silver nitrate on the horizontal axis and millivolts on the vertical axis. The graph was used to determine the end-point of the titration and to calculate the concentration of sodium chloride in the sample.

Reaction

$Cl^- + AgNO_3 \rightarrow AgCl^- + NO_3^-$

3.2.3 Chromium determination using flame photometry

2.828g potassium dichromate was dissolved in 200mL of deionised water. 1.5mL of concentrated nitric acid (HNO_3) was added and the solution diluted to 1L with deionised water. (Sodium standards were prepared by dissolving 22.997g sodium in 1L of distilled water). Using a pipette 2mL, 4mL, 6mL, 8mL, and 10mL of the stock solution were transferred to 100mL volumetric flasks and diluted to the mark with deionised water. This made a series of standards containing 20, 40, 60, 80, and 100µg/mL potassium dichromate, respectively.

Sample solution

The effluent samples were filtered through Grade 45, Whatman filter paper (slow).

Measurements

The photometer was set to zero using standard commercial grade deionised water. The standard solutions were aspirated in turn through the atomic absorption flame photometer and the readings were recorded.

Results

A graph was plotted of concentration against emission for the standard solutions. The concentrations of the samples were read from the graph.

1.00mL = 1.00mg Cr^{3+}.

3.2.4 Dissolved solids

15cm Grade 54, Whatman filter paper (fast) was oven dried at 105°C for 1 hour. The paper was cooled in a desiccator and then weighed. Using the dried, weighed filter paper and a Buchner funnel connected to a vacuum line, 500mL of effluent was filtered. The filter paper was removed and dried in an oven at 105°C. The filter paper plus the solids were reweighed and the concentration of suspended solids in the sample was calculated and expressed in mg/L. The filtered solution was used for the determination of dissolved solids in liquor.

50mL of the filtered effluent was transferred to a previously heated, cooled and weighed silica basin. The silica basin is necessary if the dissolved inorganic solids are later to be determined. The silica basins of effluent were placed on a hot water bath until the sample water had evaporated. The samples were then oven dried at 105°C for two hours and then cooled in desiccators. The basins were weighed and the concentration of dissolved solids in the sample was determined and expressed in mg/L.

3.2.5 Dissolved organic and inorganic solids

The weighed silica basin from the previous experiment for the determination of dissolved solids was placed in a furnace at 600°C for two hours. The samples were allowed to cool in a desiccator and were weighed accurately. The concentration of dissolved organic and inorganic solids in the sample were calculated and expressed in mg/L.

3.2.6 Electrolytic conductivity (EC) of effluent

Conductivity is the numerical expression of the ability of a water sample to carry an electrical current. Conductivity depends on: the total concentration of ionised substances dissolved in the water sample, the actual and relative concentration of each ion, the valencies of the ions dissolved, and the temperature at which the measurements were made. Effluent samples were shaken well in the sample containers to homogenise the sample before analysis. 100mL of each sample to be tested was dispensed into a beaker. The conductivity electrode and temperature probe were placed into the solution and the conductivity meter (Jenway, UK) was set to read electrical conductivity. The reading on the meter was recorded for each sample.

3.2.7 Electrolytic conductivity (EC) of substrate

10g of each substrate sample to be tested was homogenised in a beaker with 10mL of deionised water. The electrolytic conductivity of the substrate was measured in the same way as for the effluent. The conductivity electrode and temperature probe were placed in the solution and the conductivity meter (Jenway, UK) was set to read electrical conductivity. The reading on the meter was recorded.

3.2.8 pH of effluent

Effluent samples were shaken well in the sample containers to homogenise the sample before analysis. 100mL of each sample to be tested was dispensed into a beaker. A temperature probe and a pH electrode were placed into the solution and the pH meter (Jenway, UK) was set to read pH. The reading on the meter was recorded.

3.2.9 pH of soil

10g of each substrate sample to be tested was homogenised in a beaker with 100mL of deionised water. A temperature probe and a pH electrode were placed into the solution and the pH meter (Jenway, UK) was set to read pH. The reading on the meter was recorded.

3.2.10 Soil moisture

Weighing tins were cleaned and dried for each sample. The weight of each tin was recorded to 0.01g (W_1). 30g of each field moist substrate sample to be tested was placed in a dry weighing tin. The tin and the contents were weighed to within 0.01g (W_2). The weight was recorded. The weighing tins with contents were placed in an oven set to 105°C to dry to a constant weight. The tins and the contents were removed from the oven and placed in desiccators to cool. The tins and contents were weighed to 0.01g (W_3)

Calculation:

$$\text{Moisture content \%} = \frac{W_2 - W_3}{W_3 - W_1} \times 100$$

Where:

W_1 = Weight of tin (g)

W_2 = Weight of moist soil + tin (g)

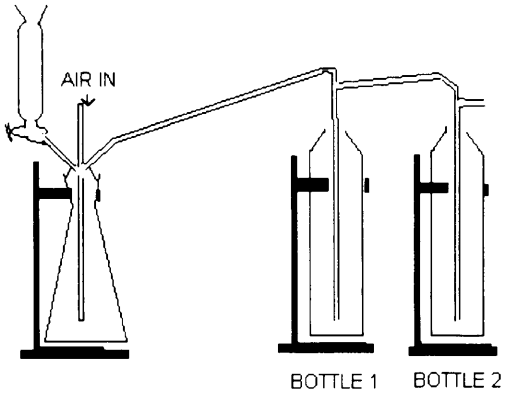
W_3 = Weight of dried soil + tin (g)

3.2.11 Sulphide

The apparatus was assembled as indicated in figure 3.4. Using measuring cylinders, 200mL of unfiltered effluent was put into in the conical flask and 80mL of 0.01 mol/L

zinc acetate solution (0.2%) was put in each of the absorption bottles as shown in the diagram. 25mL of 6M hydrochloric acid (1:1) was dispensed into the funnel. The acid was run into the flask and the tap was closed. Air was then passed through the apparatus for 30 minutes. The air was turned off and the absorption bottles were removed.

Figure 3.4 Apparatus for sulphide experiment



15mL of 0.025 mol/L iodine solution were pipetted into bottle 1, followed by a little (1 or 2mL) dilute hydrochloric acid. A few drops of starch indicator were added and the solution was titrated with 0.05 mol/L sodium thiosulphate solution.

5mL of the iodine solution were pipetted into bottle 2, followed by a little acid (1 or 2mL) and titrated in the same way. A blank titration was performed on 20mL of iodine solution plus 80mL of zinc acetate (0.2%) and 1 or 2mL of acid.

The results from the two absorption bottles were added together. The result of the blank was then subtracted. The difference was equivalent to the amount of sulphide in the effluent.

Reaction

2 moles $Na_2S_2O_3$ = 1 mole I_2 = 1 mole sulphide

3.2.12 Total Kjeldahl nitrogen (TKN)

Catalyst mixture

1000g sodium sulphate (Na_2SO_4 anhydrous) and 100g copper sulphate ($CuSO_4$ $5H_2O$) were mixed together.

9g of catalyst mixture, a few anti-bumping granules, and 50mL of effluent sample were placed into a dry Kjeldahl flask. 20mL of concentrated sulphuric acid was dispensed into each flask. The flasks were heated on Kjeldahl mantles (Electrothermal, UK) in a fume cupboard until a colour change from black/brown to blue was detected, and then for one hour longer. The solution was allowed to cool before 250mL of distilled water and a small amount (approx 3 drops) of anti-foaming agent were added to each flask. The flasks were then fixed to the distillation apparatus (Gerhardt, Germany). A conical flask containing 100mL of 1M boric acid and a few drops of screened methyl red (giving purple colour) was placed beneath the outlet of the distillation apparatus condenser. 100mL of 7.5ML sodium hydroxide (30%) was measured into the tap funnel of the distillation apparatus. This was allowed to run into the Kjeldahl flasks. The funnel taps were then closed. The condenser water was turned on and the solution was heated so that it boiled vigorously. The copper from the catalyst mixture could be seen to precipitate as a black solid. When the volume in the receiving flask was 300mL of distillate (green colour) the funnel tap was opened and the heat turned off. The whole distillate was titrated with 0.1M hydrochloric acid until a colour change was detected and the distillate returned to the purple colour.

The percentage total nitrogen of the effluent sample and effluent was calculated. The percentage ammoniacal nitrogen was calculated using a similar equation.

Calculation

2[N] (from sample) ----------> $(NH_4)_2SO_4$

$(NH_4)_2SO_4 + 2NaOH$ ----------> $(Na_2SO_4 + 2NH_3 + 2NH_3 + 2H_2O$

$2NH_3 + 2HCl$ ----------> $2NH_4Cl$

Therefore 1M of hydrochloric acid is equivalent to 14 g of nitrogen.

% leachate sample = % nitrogen x 5.62

3.2.13 Total suspended solids (TSS)

15cm Grade 54, Whatman filter paper was washed in distilled water and placed in an oven (Genlab, UK) for 6 hours at 100°C. The paper was placed in a desiccator to prevent it from reabsorbing atmospheric moisture. This process was carried out several times until the filter paper had reached a constant weight. The weight of the filter paper was then recorded.

100mL of effluent sample was filtered through the filter paper using a Buchner filter and vacuum pressure of 600mm/Hg. The filter paper was then placed in the oven (Genlab, UK) for 6 hours. It was weighed to obtain the weight of suspended solids present in the 100mL sample.

Calculation:

$$C = (A - B)$$

Where: A = Pre weight of filter paper
B = Weight of filtered paper
C = Weight of suspended solids

3.3 Biochemical tests

Analyses were conducted according to standard scientific methods. All chemicals were of analytical grade and were obtained from Sigma-Aldrich, Poole, UK, unless otherwise stated.

3.3.1 Dehydrogenase method indicator of microbial activity

The method used for the estimation of the microbial activity of the soil was developed from the work of Casida *et al.* (1964).

Day One

20g of soil and 0.2g of $CaCO_3$ were mixed thoroughly. 6g was dispensed into each of three glass centrifuge test tubes (16 x 150mm). 1mL of 3% aqueous solution 2,3,5-triphenyltetrazolium chloride (1.5g TTC made up to 50mL in volumetric flask) and

2.5mL of distilled water were added to each test tube. The liquid was sufficient to saturate the soil. The contents of each of the test tubes were mixed with a sterile glass rod. A small amount of free liquid was visible at the surface of the soil after mixing. The contents of the test tubes were mixed by vigorous mechanical shaking then corked and incubated for 24hrs at 37°C.

Day Two

The triphenylformanizan (TPF) was extracted and the spectrophotomic light absorbance of each sample was measured.

The method of Casida *et al.*, (1964) was adapted as follows. The glass test tubes were centrifuged at 1900rpm for 15 minutes. 0.35mL of the supernatant was extracted directly from the test-tube. This was made up to 10mL with methanol in a volumetric flask. Absorbency was read at 485nm, as in Casida *et al.*, (1964). This was possible because the water, methanol, TPF ratio was the same in the adapted method (Casida *et al*, 1964). Concentrations of triphenylformanizan were determined by reference to a standard curve of the formazan in methanol, and the average value of the three determinations of each soil was calculated. In instances in which no formazan, or only a small amount, was recovered in the methanolic extract, the extract was treated with metallic zinc (granular, 20 mesh) to insure that TTC was still present.

3.4 Microbiological methods

Analyses were conducted according to standard scientific methods. All chemicals were of analytical grade and were obtained from Sigma-Aldrich, Poole, UK, unless otherwise stated.

3.4.1 Total aerobic colony forming unit counts

Soil samples were taken using a core sampling tool and stored in sterile plastic bags at 8°C for not more than 3 hours. Duplicate samples of 1g of each soil sample were added to 9mL of phosphate buffered saline solution (PBS) (Oxoid, UK) (Cat. No. BR14). The PBS solution and soil sample were homogenised in a stomacher bag for approximately 2 minutes. The resulting suspension was further serially diluted with sterile PBS. A

dilution series 10^{-2} to 10^{-8} was made. 0.1mL of each dilution was dispensed and spread onto nutrient agar (NA) (Oxoid, UK) (Cat. No. 5450). Nutrient agar was selected as the growth medium to culture aerobic bacteria because it is a good general purpose agar. Plates of NA were prepared in advance. The 27 soil samples were cultured in triplicate, for the three dilutions (10^{-4}, 10^{-6}, 10^{-8}). 50µl of each sample was dispensed onto each plate with the spiral-plater (Whitley automatic spiral plater). The NA plates containing aerobic bacteria were incubated at 26°C ± 1°C for 48 hours. Colony counts were carried out after a period of 48 hours incubation or until growth was observed.

3.4.2 Total anaerobic colony forming unit counts

As above, duplicate 1g of each soil sample was added to 9mL of PBS solution (Oxoid, UK) (Cat. No. BR14). The PBS solution and soil were homogenised in a stomacher bag for approximately 2 minutes. The resulting suspension was further serially diluted with sterile PBS. A dilution series 10^{-2} to 10^{-8} was made. Fastidious agar (FA) (Lab M, UK) (Cat. No. Lab 90) was selected as the growth medium for anaerobic bacteria because it is an accepted growth medium for the culture of environmental anaerobes. Plates of FA were prepared in advance. 50µl of three of the dilutions (10^{-4}, 10^{-6}, 10^{-8}) were dispensed onto each plate with the spiral-plater. The 27 soil samples were cultured in triplicate. FA plates were placed in anaerobic jars with Merck Aerocult C (Lab M, UK). The plates were incubated for 48-72 hours at 26°C ± 1°C, or until growth was observed. Colonies were counted and the total plate count was recorded.

CHAPTER FOUR

REED BED TREATMENT OF LANDFILL LEACHATE IN THE UK

4.0 Introduction

Landfill practices in the UK have created a situation of progressive environmental damage in which encapsulated waste, degrading beneath the ground, releases toxins and pollutants to the environment at an unpredictable rate. Implementation of law and legislation has sought to improve the situation by modifying and restricting landfills. Reed bed technology has been widely used for on-site landfill leachate treatment (Robinson and Harris, 2000). Considerable variation exists in the design and operation of biological reed bed treatment systems and underperformance and failure of reed beds treating industrial effluents has occurred. This research investigated the use of experimental reed beds for landfill leachate treatment. The project aim was to determine the biological response of a reed bed when used to treat an industrial effluent. Data were collected and analysed to identify correlations between landfill leachate treatment and changes in the microbial consortia of the reed bed substrate.

4.1 Solid waste disposal to landfill

The practice of landfill waste disposal involves refuse being deposited in a suitable void and covered with material or substrate. In almost every country a major component of solid waste disposal is achieved through landfill. Although waste disposal by this route is not consistent with UK government policies for achieving sustainable waste management, it has been the most economical and common disposal route (Gettinby *et al.*, 1996). In 1996/1997 83.5% of the 26 million tonnes of municipal solid waste in the UK was landfilled (DETR, 1996). The disposal of large quantities of solid waste in this way poses severe problems in terms of environmental impact, volume and cost.

The negative environmental impact of landfill has been recognised for many years with growing concern that municipal solid waste disposal to landfill is unsustainable. Hazardous constituents of waste placed in landfills are likely to eventually migrate from the facility into the broader environment (USEPA, 1981). This potential threat has driven research to improve landfill practices and to find alternative waste treatment technologies and waste disposal routes. Legislative changes in the UK have been used to force industry towards sustainable waste management.

A variety of factors have contributed to the continuing changes in landfill practices that have occurred in the UK in recent decades. These include the Environmental Protection Act 1990, the current socio-economic climate and the landfill levy. As a result of legislative control and the increased landfill levy the costs related to landfill have increased and are likely to continue to increase. This measure aimed to reduce the proportional volume of municipal solid waste going to landfill by making it a non-viable economic option, and to create revenue for investment into waste management and research. However, as municipal solid waste volumes increase substantially on an annual basis (DEFRA, 2002), the increased cost of landfill is not enough to dramatically reduce landfill use.

A considerable proportion (over 50%) of municipal solid waste that is in landfills has been shown to be potentially biodegradable (EA, 2005). High molecular weight polymeric compounds such as cellulose, proteins, lipids, starch and lignin make up much of the material within landfill sites. Alternative waste disposal routes for this biodegradable fraction of municipal solid waste material could be used. Some of the alternatives include source separation of recyclable material, composting, and waste minimisation schemes. However, there is a persistence of social and economic barriers towards the kinds of behavioural changes required to utilise alternative waste management facilities. There is often a negative public perception of change and/or reluctance to alter established behaviour patterns for participation in novel schemes. Also attitudes of local authorities, economic viability, and the local availability of alternative waste disposal routes, mean that disposal to landfill will persist well into the foreseeable future (DEFRA, 2002).

A range of factors have had an impact on the current situation with regard to the provision of waste management services and facilities. The ability to deliver the necessary infrastructure to meet local, regional and national needs in the short, medium and long term has been a problem (EA, 2005). A paradigm shift in personal, public, commercial and governmental attitudes towards waste production and remediation would be required to have significant sway in changing the tide of predictable environmental erosion. Sustainable waste management strategies incorporating practical opportunities to minimise the ecological and financial impact of landfill are valuable to industry, society and the environment (EA, 2005).

4.2 The landfill environment

The environmental conditions created within landfill sites are dependent upon the variability of the waste within the fill, the amount of infiltrating water, and the age of the site. Dry entombment of waste inhibits microbiological degradation of waste and delays the stabilisation of the waste to an inert state (Allen, 2001). The conditions within the landfill are predominantly anaerobic. Oxygen depletion within the fill occurs within a matter of days or weeks of waste deposition. The rate of oxygen depletion is dependent upon the state of compaction of the waste and the rate at which the waste was covered. Consortia of anaerobic microorganisms mediate the decomposition process of the waste through polymer hydrolysis, fermentation to organic intermediates and mineralisation by methanogenesis. Although the stages involved in the anaerobic degradation of waste in landfills are well understood, the microorganisms responsible for these processes are less well known. This is in part due to the fact that less than an estimated 1% of natural microbial species present in landfills can be cultured under laboratory conditions (Barlaz *et al.*, 2002).

4.3 Landfill leachate

The process of waste degradation within a landfill site generates gaseous and liquid by-products which are enduring sources of environmental contamination. Considerable research attention has been devoted to the final stage of the waste decomposition process, in which landfill gas is generated. This is in part due to the potential of methane as a fuel source, and also because of the environmental impact of landfill gas, which typically has a carbon dioxide : methane ratio content of 60:40 (Huber-Humer, 2004; Meraz *et al.*, 2004). In addition to the production of landfill gas, municipal solid waste degradation in landfill generates liquid waste known as landfill leachate (Lisk, 1991; Allen, 2001; Slack *et al.*, 2004). Landfill leachate is described as;

"a highly polluting toxic wastewater with de-oxygenating properties generated as a consequence of the complex chemical and biological processes that occurred within the landfill system" (Robinson *et al.*, 1991; Robinson *et al.*, 1992; Robinson, 1995; Robinson and Harris, 2000).

Landfill leachate is produced as a consequence of the inherent liquid content of the waste and the infiltration of rainwater into the waste mass as the refuse decomposes. It is formed through the dissolution of pollutants as precipitation percolates down through the waste, as a result of microbial degradation processes, and by the exudation of water from the waste material (Bendz *et al.*, 1997). Leachate composition is waste- and site-specific depending on the landfill age and amount of infiltrating water. The composition of landfill leachate has been found to vary considerably from one landfill site to another and even from one area within a landfill to another area within the same site, having also been shown to fluctuate seasonally within an individual landfill (Robinson, 1995). The key pollutants common to most leachate are soluble degradable organics, normally expressed in terms of (BOD_5 in mgO_2/L), ammonium (NH_4^+-N in mg/L) and chloride (Cl^- in mg/L). The levels of these pollutants can be one or two orders of magnitude greater than raw sewage (Robinson, 1995) and consequently landfill leachate has been recognised as a potential source of environmental pollution (Kylefors, 2003; Kylefors *et al.*, 2003).

4.4 Landfill legislation

Legislation surrounding landfill practices in the UK has been evolving for many years. The *Landfill Directive* is a significant piece of legislation that became law in England and Wales, to limit the amount of biodegradable municipal waste going to landfill (EC, 1999). EU landfill regulations were enacted into law by all member states. The objective of the EU Landfill Directive is to;

"prevent or reduce as far as possible negative effects on the environment" (EC, 1999).

The Landfill Directive is particularly concerned with preventing pollution of surface and ground waters, of soil and of air. It banned the use of co-disposal landfills sites, which are landfill sites containing both industrial hazardous waste and non-hazardous waste. Certain hazardous fractions of the waste stream were banned entirely from disposal to landfill. The installation of artificial lining systems and impermeable cap systems also became mandatory for all landfills, except for sites possessing a suitable low permeability ($<10^{-9}ms^{-1}$) natural liner, that could also ensure complete containment of landfill emissions. Implementation of the landfill regulations continued and enhanced

the trend away from dilute and disperse landfill sites, known as attenuation sites, towards containment landfills. In attenuation sites leachate would be naturally treated by soaking away through the ground beneath the landfill. Containment of landfill waste was in response to increasing fears that the attenuation of pollutants in the unsaturated zones below landfills was insufficient to prevent ground water pollution (Robinson, 1995). Containment was made the only permissible landfill strategy and so all new landfills were lined with impermeable materials, such as clay and synthetic liners that prevented leachate escaping from the site (Allen, 2001).

The use of impermeable high density polyethylene (HDPE) liners (or similar) for landfills became law in the UK, even though there is no long term evidence that HDPE liners remain intact after years of exposure to degrading landfill waste. Ultimately all landfill liners will fail, and the delay in the release of pollutants to the environment may only be in the order of several decades (USEPA, 1988). Leachate that penetrates the seal of the landfill may take up to a further 40 years to percolate through to underground water supplies, which may pose a potential risk to the availability of potable water for future generations (Christensen *et al.*, 1994).

The inevitable effect of landfill site containment is for landfill leachate levels to build up within landfill sites. This necessitates the periodic or continuous removal of leachate, through pumping or passive drainage, to storage lagoons, prior to disposal. The Landfill Directive imposed post-closure responsibility for landfill sites to landfill operators (EC, 1999). The legislation was important because it meant that the landfill operator was responsible for the pollution coming from the landfill site during and after the period when the landfill was filled with waste. The activity of landfill leachate disposal became the continuous long-term responsibility of the landfill operator after the landfill site had been capped and closed. It has been estimated that this operator responsibility could be in the order of a century, or until the landfill no longer poses an environmental risk (Allen, 2001).

4.5 Sustainability of landfill

The concept of sustainability with respect to landfill waste disposal has long been argued and debated (Derham, 1995; Driessen *et al.*, 1995; Allen, 2001). The effects of legislative measures to make landfill more sustainable have been considered by some to be wholly inadvisable and fundamentally flawed (Allen, 2001). Encapsulation of the waste has the effect of inhibiting its degradation rate in the short term, and is thought to considerably prolong the activity of the waste in the long term (Joseph and Mather, 1993). This necessitates indefinite monitoring of landfills (Carter, 1993; Stegmann, 1995). Furthermore the long-term durability of the HDPE liners is unknown (USEPA, 1988) and although landfill liners are engineered to endure in the order of a century, there is no evidence for such longevity (Joseph and Mather, 1993; Joseph and Mather, 1995). Landfill sites have historically been built in locations where the natural geology and geography of the earth could offer containment for leachate and protection from pollution, such as in areas with impermeable bedrock and clay. When landfills are built with HDPE liners, often in old quarry sites and on the edge of urban conurbations, the HDPE linings are often the only protection in place, except for a thin layer of sand, to prevent leachate from leaching. The landfill site and the environment have become completely dependent upon the integrity of the liner.

There is a similar lack of scientific evidence behind the ban on co-disposal landfill. Co-disposal landfills, another target of the Landfill Directive, were banned despite the considerable research conducted in the 1980s which showed that waste degradation in co-disposal sites posed no more significant environmental risks than non-co-disposal sites (Knox, 1989). Perhaps the aim of the legislation in this case was to place industry under the financial pressure necessary to move towards sustainable methods of waste disposal, and in doing so to generate revenue for investment in waste management through exponentially increasing landfill levies. The revenue generated however is not invested in waste management, or in providing readily available alternative sustainable waste disposal routes other than landfill (Morris *et al.*, 1998).

4.6 Landfill leachate treatment and disposal

The way that landfills are designed and built has been modified, to an extent, to mitigate resultant and associated environmental damage. Most modern landfills are now engineered to control and minimise many of the environmental risks, such as the liquid and gaseous discharges (Allen, 2001). In the UK in recent years, the most common means of landfill leachate disposal has been transportation to a wastewater treatment facility. There the leachate is treated with other municipal wastewaters. However, due to the location of landfill sites, often situated on the periphery of urbanisations, transportation to wastewater treatment facilities involves the use of underground pipe lines, to carry the leachate for many miles, or transportation overland. There is an inherent potential for leaks from pipe lines to pollute farmland and waterways as the leachate is transported underground. This type of pollution is difficult to either pin-point or quantify. Similarly the alternative option of transportation overland involves the risk of spillage, and of illegal dumping of the leachate by disreputable haulage companies.

The treatment of landfill leachate in wastewater treatment facilities, even after it has arrived, is not without problems. Leachate containing various hazardous substances had been shown to interfere with the secondary biological processes at sewage treatment plants (Kylefors *et al.*, 2003). There is also sometimes resistance by wastewater treatment plants to take leachate for treatment without considerable charge. The alternative to conventional municipal wastewater treatment of landfill leachate is on-site treatment with subsequent discharge to surface water. The consent to discharge from the local water authority determines the amount of treatment that is required. On-site treatment of landfill leachate is a preferable option to municipal wastewater treatment facilities, because transportation is costly, and presents its own environmental contamination risks (Robinson *et al.*, 1992).

A variety of physical, chemical and biological wastewater treatment methods and systems have been shown to be capable of on-site landfill leachate treatment. These include activated sludge treatment, anaerobic and aerobic sequence bioreactors and low technology treatment systems such as constructed wetlands (Robinson and Maris, 1983; Urbanc Bercic, 1994; Maehlum, 1995; Barr and Robinson, 1999; Zouboulis *et al.*, 2001; Heavey, 2003; Agdag and Sponza, 2005). Large landfill sites often have a significant

amount of land available for an extensive wastewater treatment system which makes the application of reed bed technology particularly appropriate.

4.7 Reed beds and landfill leachate treatment

The key problems associated with landfill leachate treatment involve the compositional variability, excessive volume, and the long-term on-site generation of the effluent. To combat these factors, the waste treatment solution has to be low-cost, low-maintenance, robust and technically reliable. The treatment of industrial and domestic wastewater by passage through beds planted with reeds has been widely practiced in recent years, with varying degrees of success (Bulc, 1997; Barr and Robinson, 1999). Many installations have demonstrated good removal of organic components of leachate and suspended solids, although poor removal of ammoniacal nitrogen has been commonly reported (Connolly *et al.*, 2004). This has limited the use of reed beds for primary treatment of raw landfill leachate, but the technology has been widely used for secondary polishing (Barr and Robinson, 1999). Understanding the technological reliability issues associated with constructed wetlands has been described as an important step in increasing the commercial acceptance and public perception of reed beds and constructed wetlands.

4.8 Experimental investigations

Table 4.1 shows an index of the experimental investigations giving the title and experiment code of each study. Experiments were designed to test specific hypotheses (Appendix 4). Weather conditions were monitored over the experimental period. Data were obtained from the Meteorological Office from the station at Moulton Park (station No. DCNN 4364).

Table 4.1 Index of experimental investigations

Code	Title	Season
DH-1	Dehydrogenase activity of the 30cm depth horizon of the sections of three reed beds during-loading in UK spring.	Spring
DH-2	Dehydrogenase activity of the 30, 60 and 90cm depth horizons of three reed beds during- and post-loading in UK summer.	Summer
CFU-1	Aerobic counts of three reed beds at the 30cm depth horizon during- and post-loading in UK spring/ summer.	Spring/ Summer
CFU-2	Aerobic counts of three reed beds at the 30cm depth horizon during- and post-loading in UK autumn/ winter.	Autumn/ Winter
CFU-3	Aerobic and anaerobic counts of two reed beds at the 30cm horizon without loading in UK spring/ summer.	Spring/ Summer
CFU-4	Aerobic and anaerobic counts of three reed beds at the 30, 60 and 90cm horizon during- loading in UK summer/ autumn.	Summer/ Autumn
CFU-5	Aerobic and anaerobic counts of three reed beds at the 30cm depth horizon pre- during- and post-loading in UK winter.	Winter
CFU-6	Aerobic counts of three reed beds at the 30cm depth horizon pre- during- and post-loading in UK spring/ summer.	Spring/ Summer

4.9 Dehydrogenase activity of the 30cm depth horizon of sections of three reed beds during-loading in UK spring (DH-1).

4.9.1 Introduction

Dehydrogenase is an enzyme involved in aerobic respiration. Dehydrogenase activity (DHA) in a soil sample can be used as a biochemical measure of the activity of the aerobic bacteria present (Casida *et al.*, 1964; Gil-Sotres *et al.*, 2005). The substrate from the reed beds was sampled under leachate loading conditions to determine if there was a perceptible difference in the dehydrogenase activity. Detected changes in the microbial consortia of the reed bed substrate may represent a change in the activity, size, health or composition of the microbial community, in response to leachate loading (Chander, 1991; Rossell, 1991; Bardgett *et al.*, 1995; Li and Zhao, 1999; Taylor *et al.*, 2002; Benitez *et al.*, 2004).

4.9.2 Methods

Reed bed substrate samples (section 3.1) were collected from the 30cm depth horizon of the reed beds. This was anticipated to be the substrate horizon with the *greatest* aerobic microbial activity. Bacterial numbers have been shown to decrease with depth (Taylor *et al.*, 2002) The reed beds were sampled in three sections (figure 3.3). Table 4.2 summarises the experimental parameters of the investigation. Table 4.3 shows the mean weather conditions over the experimental period.

Table 4.2 Experimental parameters (DH-1)

Experimental period Loading period Post loading period	Day 1 - Day 44 Day 1 – Day 35 Day 36 – Day 44
Effluent type	Reed bed 1 (Control) Reed bed 2 (Leachate A) Reed bed 3 (Leachate B)
Loading	200 litres/day
Effluent sampling Soil sampling	Day 1 - Day 43 (once per week) Day 5 – Day 44 (twice per week) (section 3.1)
Effluent analysis	TKN, NH_3 (section 3.2.12) COD (section 3.2.1)
Soil analysis	dehydrogenase activity (section 3.3.1)

Table 4.3 Weather conditions over experimental period

Weather (SPRING)	Average (± SD)	
Max temp (°C)	Experimental period	9.9 ± 3.3
Min temp (°C)	Experimental period	2.9 ± 2.0
Sunshine (hrs)	Experimental period	3.2 ± 2.9
Rainfall (mm)	Experimental period	4.0 ± 3.6
Relative Humidity (RH)	Experimental period	85
Soil temp 30cm (°C)	Experimental period	9.1 ± 10.8

The environmental conditions for the duration of the experimental period were UK spring time. The mean maximum temperature was 9.9°C and the mean minimum temperature was 2.9°C. There was an average of 3 hours of sunshine and 4mm of rain per day. The mean soil temperature at the 30cm horizon was 9°C.

4.9.3 Experimental aims

- Determine the efficacy of the reed beds in reducing waste water parameters

- Determine if there is a significant difference in the mean DHA of sections 1, 2, and 3 of each reed bed.

- Determine if there is a significant difference in the mean DHA of the three reed beds.

4.9.4 Effluent chemistry results

Inflow and outflow analysis of the effluent from the reed beds for TKN (section 3.2.12) and NH_3 (section 3.2.12) are presented in table 4.4. The results are expressed in terms of the treatment efficiency (section 2.6) of the reed beds as a percentage reduction of the specified pollutants. The table shows that the TKN treatment performance of reed bed 3 was 66% compared with that of reed bed 2 which was 36%. An independent samples t-test was used to compare the mean outflow from reed bed 2 and the mean outflow from reed bed 3. The difference was not found to be significant. The same pattern was true for NH_3 reduction in the reed beds. Reed bed 2 was found to have 37% treatment of NH_3 and reed bed 3 68% treatment. The difference in NH_3 of the outflow from the two reed beds treating landfill leachate was not significant.

Table 4.4 TKN and NH_3 treatment efficiency (± SEM).

Reed bed	TKN (mg/L)			NH_3 (mg/L)		
	Mean IN	Mean OUT	% treatment	Mean IN	Mean OUT	% treatment
1 (control)	0.00 ± 0.00	0.01 ± 0.00	*ND*	0.00 ± 0.00	0.00 ± 0.00	*ND*
2 (leachate A)	0.55 ± 0.01	0.35 ± 0.24	36	0.02 ± 0.01	0.01 ± 0.01	37
3 (leachate B)	0.82 ± 0.01	0.28 ± 0.27	66	0.03 ± 0.01	0.01 ± 0.01	68

(ND= not determined)

The mean inflow and outflow COD of the effluent and the treatment efficiency of the reed beds, over the duration of the experimental period are presented in table 4.5.

Table 4.5 COD treatment efficiency (± SD).

Reed bed	COD (mgO_2/L)		
	Mean IN	**Mean OUT**	**% treatment**
1 (control)	19 ± 24	13 ± 13	*ND*
2 (leachate A)	1191 ± 45	607 ± 223	49
3 (leachate B)	1285 ± 79	582 ± 442	55

(ND= not determined)

The COD percentage treatment of reed bed 3 was 55%, reed bed 2 was 49%. The difference between the reed beds was not significant (t=0.14;df=12;p=0.9;NS).

4.9.5 Dehydrogenase activity results

The dehydrogenase activity of the 30cm depth horizon of the three reed beds was measured throughout the experimental period. Each figure representing dehydrogenase activity (DHA) shown in the tables represents a mean value calculated from three triplicate samples from each section of each reed bed (figure 3.3). The mean dehydrogenase activity for each reed bed at each sampling section is shown in table 4.6.

Table 4.6 DHA (TPFμg/g^{-1}dry weight) of 30cm depth horizon (± SD)

Reed bed	Section 1	Section 2	Section 3
1 (control)	30 ± 10	36 ± 28	30 ± 24
2 (leachate A)	38 ± 25	52 ± 28	34 ± 24
3 (leachate B)	38 ± 21	33 ± 25	31 ± 16

Comparison of the DHA (TPFμg/g^{-1}dry weight) of the 3 sections of the 3 reed beds

Independent samples t-tests were used to compare the dehydrogenase activity for the three reed beds and the three sections of the three reed beds. When section one (first third of reed bed from inflow) of reed bed 1 was compared with section two (middle third) of reed bed 1, and section three (final third closest to outflow) of reed bed 1, no significant difference in microbial activity was found (t=-0.4;df=5;p=0.7;NS). Similarly no significant difference was found between sections 2 and section 3 (t=0.3;df=7;p=0.8;NS). There was also no significant difference when section 1 was compared with section 3 (t=0.01;df=3.9;p=0.99;NS). There was a similar pattern found for all of the three experimental reed beds. No significant difference was found in the microbial activity of the substrate of the three sections of the other two (leachate) reed beds.

Comparison of the DHA (TPFμg/g^{-1}dry weight) of the three reed beds

There was no significant difference when the dehydrogenase activity of the 30cm horizon of reed bed 1 (control) was compared to the 30cm horizon of reed bed 2, treating landfill leachate (t=-1;df=25;p=0.3;NS). There was no significant difference when the control reed bed was compared to reed bed 3, also treating landfill leachate (t=-0.2;df=26;p=0.81;NS). There was also no significant difference when reed bed 2 was compared with reed bed 3 (t=0.86;df=25;p=0.39;NS).

4.9.6 Summary of (DH-1) results

- Both reed beds treating landfill leachate were effective in reducing specified wastewater parameters.

- TKN, NH_3 and COD treatment of the landfill leachate was not significantly different in the two reed beds.

- There was no significant difference in the dehydrogenase activity of the sections of the individual reed beds.

- There was no significant difference in the dehydrogenase activity of reed bed 1 (control) compared to reed beds 2 and 3, both treating landfill leachate.

- The reed beds can be considered homogenous biological systems. The DHA of the substrate is not dependent upon the distance of the substrate sample from the inflow. This therefore suggests that the DHA of the aerobic microorganisms in the substrate is not adversely effected (inhibited) by leachate loading.

- Low soil temperatures may have an overall inhibitory effect on the microbial activity of the microbial consortia of the reed bed.

- The amount of rainfall over the experimental period may have had the effect of decreasing the hydraulic retention time (HRT) of the reed bed.

- Rainfall has a dilution effect on the effluent.

- Decreasing HRT decreases the contact time of the effluent with the substrate of the reed bed and so may reduce the effectiveness of effluent treatment because there is less time for microbiological (and other) transformations and processes to occur within the reed bed.

4.10 Dehydrogenase activity of the 30, 60 and 90cm depth horizons of three reed beds during-loading and post-loading in UK summer (DH-2).

4.10.1 Methods

Three reed beds were loaded with leachate (or control) for the duration of the experimental loading period. Effluent and substrate samples were collected for analysis (section 3.1). Table 4.7 summarises the experimental parameters of the investigation. Table 4.8 shows the mean weather conditions over the experimental period.

Table 4.7 Experimental parameters (DH-2)

	DH-2 Experimental parameters
Experimental period **Loading period** **Post loading period**	Day 1 - Day 44 Day 1 – Day 35 Day 36 – Day 44
Effluent type	Reed bed 1 (Control) Reed bed 2 (Leachate A), Reed bed 3 (Leachate B),
Loading	200 litres/day
Effluent sampling **Soil sampling**	Day 1 - Day 43 (once per week) Day 5 – Day 44 (twice per week) (section 3.1)
Effluent analysis	EC (section 3.2.6), pH (section 3.2.8), TKN, NH_3 (section 3.2.12)
Soil analysis	Dehydrogenase activity (section 3.3.1), soil pH (section 3.2.9), EC of soil (section 3.2.7), soil moisture (section 3.2.10).

4.10.2 Experimental aims

- Determine the efficacy of the reed beds in reducing waste water parameters.
- Determine the substrate horizon (30, 60 or 90cm) with the greatest DHA.
- Determine the reed bed with the greatest DHA.

Table 4.8 Weather conditions over experimental period

Weather (SUMMER)	Average (± SD)	
Max temp (°C)	Experimental period	19.3 ± 3.1
	Loading period	18.5 ± 2.9
	Post loading period	22.4 ± 1.7
Min temp (°C)	Experimental period	11.1 ± 1.9
	Loading period	10.7 ± 1.8
	Post-loading period	12.7 ± 1.2
Sunshine (hrs)	Experimental period	4.5 ± 4.2
	Loading period	3.7 ± 4.1
	Post-loading period	7.8 ± 2.9
Rainfall (mm)	Experimental period	1.3 ± 2.7
	Loading period	1.2 ± 2.7
	Post-loading period	1.4 ± 2.8
Relative Humidity (RH)	Experimental period	79
	Loading period	80
	Post-loading period	75
Soil temp 30cm (°C)	Experimental period	17.5 ± 0.7
	Loading period	17.3 ± 0.6
	Post-loading period	18.6 ± 0.3
Soil temp 50cm (°C)	Experimental period	17.1 ± 0.6
	Loading period	16.9 ± 0.4
	Post-loading period	17.9 ± 0.2
Soil temp 100cm (°C)	Experimental period	15.7 ± 0.5
	Loading period	15.5 ± 0.3
	Post-loading period	16.4 ± 0.2

4.10.3 Effluent chemistry results

Inflow and outflow analysis was used to calculate the treatment efficiency (section 2.6) of the reed beds in terms of the percentage reduction of specified pollutants. The results for TKN and NH_3 (section 3.2.12) can be seen in table 4.9.

Table 4.9 TKN and NH_3 treatment efficiency (± SEM).

Reed bed	TKN (mg/L)			NH_3 (mg/L)		
	Mean IN	Mean OUT	% treatment	Mean IN	Mean OUT	% treatment
1 (control)	0.00 ± 0	0.01 ± 0.0	*ND*	0.00 ± 0	0.0 ± 0.00	*ND*
2 (leachate A)	1.7 ± 0.01	0.1 ± 0.0	91	2.1 ± 0.01	0.2 ± 0.01	91
3 (leachate B)	1.7 ± 0.01	0.4 ± 0.04	79	2.0 ± 0.01	0.4 ± 0.04	79

Table 4.9 shows the treatment performance of the three reed beds. Reed bed 2 was 91% treatment efficient for TKN compared with reed bed 3 which was 79% treatment efficient. Independent samples t-test were used for statistical analysis of the outflow from the reed beds and it was found that reed bed 2 and bed 3 were significantly different in TKN mean outflow (t=5.2;df=37;p=0.00;S). The mean outflow NH_3 for reed bed 2 and reed bed 3 showed that the reed beds were also significantly different in NH_3 treatment (t=5.16;df=37;p=0.00;S).

In DH-1 the outflow from the reed beds was not significantly different. However in DH-1 both beds were loaded with the same leachate from site B. In this experiment leachate from site A was loaded onto reed bed 2 and leachate from site B leachate was loaded to reed bed 3.

The effluent treatment performance of the reed beds in terms of pH and conductivity is shown in table 4.10. Reed bed 3 (62%) was more effective in reducing the conductivity of the leachate than reed bed 2 (42%). The pH was an acceptable level for discharge to surface water from all three reed beds.

Table 4.10 pH and conductivity treatment efficiency (± SD).

Reed bed	pH		Conductivity (µS/mL)		
	Mean IN	Mean OUT	Mean IN	Mean OUT	% treatment
1 (control)	6.9 ± 1.3	8.1 ± 0.2	470 ± 18	803 ± 177	*ND*
2 (leachate A)	7.9 ± 0.3	8.0 ± 0.1	14150 ± 861	8187 ± 600	42
3 (leachate B)	7.8 ± 0.1	8.1 ± 0.1	15166 ± 64	5697 ± 1640	62

4.10.4 Dehydrogenase activity results

Substrate from three depth horizons (30, 60, 90cm) were tested for dehydrogenase activity (section 3.3.1) and the results are shown in tables 4.11, 4.12 and 4.13.

Table 4.11 DHA (TPFµg/g^{-1}dry weight) of 30cm depth horizon (± SD)

Day of experiment	Reed bed 1 (control)	Reed bed 2 (leachate A)	Reed bed 3 (leachate B)
Day 8 (loading)	44 ± 29	175 ± 69	113 ± 69
Day 12	69 ± 24	138 ± 34	178 ± 61
Day 16	72 ± 38	93 ± 63	85 ± 28
Day 20	59 ± 8	80 ± 8	90 ± 16
Day 23	67 ± 13	86 ± 16	94 ± 13
Day 27	64 ± 19	97 ± 27	91 ± 22
Day 30	144 ± 153	140 ± 46	82 ± 23
Day 34	115 ± 71	72 ± 17	93 ± 42
Day 37 (post-loading)	106 ± 37	111 ± 17	111 ± 20
Day 41	80 ± 30	102 ± 21	88 ± 13
Day 44	157 ± 54	123 ± 29	117 ± 28

Comparison of DHA (TPFμg/g^{-1}dry weight) of the 30cm depth horizon

Dehydrogenase activity of the reed beds during-loading period and post-loading were compared using an independent samples t-test. It was found that the dehydrogenase activity of the reed bed substrate of the control reed bed at the 30cm horizon was greater in the post-loading period than during-loading. The during-loading period mean was 79.64 (± 65.55 SD or ± 7.15 SEM), compared to the post-loading period mean of 118.09 (± 57.82 SD or ± 13.63 SEM). Independent samples t-test showed that the difference in dehydrogenase activity was significant (t=-2.302;df=100;p=0.023;S).

It was found that the mean DHA of the substrate of reed bed 2 (leachate A) at the 30cm horizon in the post-loading period and in the during-loading period was not significantly different. The mean DHA was 109.95 (± 49.23 SD or ± 5.50 SEM) compared to 112.41 (± 26.48 SD or ± 6.24 SEM). Independent samples t-test showed that the difference was not significant (t=-0.205;df=96;p=0.838;NS).

It was found that there was no significant difference (t=0.107;df=96;p=0.915;NS) between the DHA of the during-loading and post-loading periods in reed bed 3 (leachate B). During-loading (mean 103.80 ± 45.80 SD or ± 5.12 SEM) compared to the post-loading period (mean 102.60 ± 25.84 SD or ± 6.09 SEM).

Comparison of DHA (TPFμg/g^{-1}dry weight) of the 60cm depth horizon

Table 4.12 shows the mean dehydrogenase activity of the substrate of the three reed beds at the 60cm depth horizon. The dehydrogenase activity of the substrate from the 60cm depth horizon for the loading-period and post-loading period was compared using independent samples t-test. It was found that there was a significant increase in the dehydrogenase activity of the reed bed substrate in the control reed bed at the 60cm horizon in the post-loading period. The during-loading period mean was 60.79 (± 41.62 SD or ± 4.65 SEM) compared to the post-loading period mean which was 81.44 (± 20.75 SD or ± 4.89 SEM). Independent samples t-test showed that the increase in the dehydrogenase activity was significant (t=-2.042;df=96;p=0.044;S).

Table 4.12 DHA (TPFμg/g^{-1}dry weight) of 60cm depth horizon (± SD)

Day of experiment	Reed bed 1 (control)	Reed bed 2 (leachate A)	Reed bed 3 (leachate B)
8 (loading)	36 ± 22	68 ± 26	142 ± 81
12	67 ± 30	126 ± 46	159 ± 158
16	43 ± 6	62 ± 7	75 ± 23
20	45 ± 7	54 ± 12	69 ± 30
23	47 ± 6	72 ± 14	73 ± 8
27	48 ± 7	59 ± 7	70 ± 7
30	74 ± 48	120 ± 96	66 ± 7
34	92 ± 53	64 ± 11	71 ± 20
37 (post-loading)	93 ± 79	82 ± 7	74 ± 5
41	86 ± 26	75 ± 17	75 ± 10
44	77 ± 14	96 ± 30	77 ± 13

There was no significant difference in the DHA of the substrate of reed bed 2 (leachate A) from the 60cm horizon when the during-loading DHA (mean 78 ± 44 SD or ± 4.9 SEM) was compared to the post-loading period DHA (mean 85.40 ± 25.89 SD or ± 6.10 SEM). Independent samples t-test showed that the difference was not significant (t=-0.677;df=96;p=0.500;NS).

It was found that there was no significant difference in the DHA of the substrate of reed bed 3 (leachate B) at the 60cm horizon when the during-loading period DHA (mean 89.38 ± 67.30 SD or ± 7.52 SEM) was compared to the post loading period DHA (mean 76.03 ± 11.16 SD or ± 2.63 SEM). Independent samples t-test showed that the difference was not significant (t=0.84;df=96;p=0.405;NS).

Comparison of DHA (TPFμg/g^{-1}dry weight) at the 90cm depth horizon

Table 4.13 shows the mean dehydrogenase activity of the substrate of the three reed beds at the 90cm depth horizon. There was a significant increase in the dehydrogenase

activity of the reed bed substrate in the control reed bed at the 90cm horizon when the during-loading DHA (mean 61.60 ± 32.94 SD or ± 3.68 SEM) was compared to the post-loading period DHA (mean 80.68 ± 30.77 SD or ± 7.25 SEM). Independent samples t-test showed that the increase in DHA was significant (t = -2.246; df = 96; p = 0.027 ;S).

Table 4.13 DHA (TPFμg/g^{-1}dry weight) of 90cm depth horizon (± SD)

Day of experiment	Reed bed 1 (control)	Reed bed 2 (leachate A)	Reed bed 3 (leachate B)
8 (loading)	48 ± 46	56 ± 58	82 ± 64
12	54 ± 19	67 ± 39	69 ± 27
16	48 ± 12	58 ± 13	52 ± 7
20	56 ± 35	52 ± 16	54 ± 5
23	52 ± 11	63 ± 7	46 ± 17
27	53 ± 13	66 ± 11	68 ± 7
30	75 ± 50	70 ± 17	88 ± 65
34	65 ± 7	55 ± 7	56 ± 5
37 (post-loading)	106 ± 29	74 ± 9	73 ± 10
41	65 ± 13	79 ± 10	72 ± 11
44	97 ± 36	82 ± 18	96 ± 27

There was a significant increase in the DHA of the substrate in reed bed 2 (leachate A) from the 90cm horizon when the during-loading DHA (mean 62.65 ± 25.39 SD or ± 2.84 SEM) was compared to the post-loading period DHA (mean 80.27 ± 14.18 SD or ± 3.34 SEM). Independent samples t-test showed that the increase was significant (t=-2.84;df=96;p=0.006;S).

It was found that there was also a significant increase in the DHA of the substrate in reed bed 3 (leachate B) from the 90cm horizon when the during-loading DHA (mean 65.48 ± 33.94 SD or ± 3.80 SEM) was compared to the post-loading period (mean 84.03

± 23.57 SD or ± 5.56 SEM). Independent samples t-test showed that the increase was significant (t=-2.198;df=96;p=0.030;S).

Comparison of DHA (TPFμg/g^{-1}dry weight) of the horizons of the control reed bed

The DHA of the three horizons of each of the three reed beds were compared to determine whether there was a substrate horizon with significantly more DHA than the others. The mean DHA of the 30cm horizon was 100 (± 29 SD). The mean DHA of the 60cm horizon was 75 (± 25 SD) and the 90cm horizon was 67 (± 15 SD). Independent samples t-tests were used for comparison. There was a significant difference in the dehydrogenase activity of the 30cm and the 60cm depth horizons of the control reed bed (t=2.529;df=160;p=0.012;S). There was also a significant difference in the 30cm and the 90cm depth horizons (t=2.503;df=160;p=0.013;S). There was no significant difference in the DHA of the 60cm and the 90cm depth horizons (t=-2.53;df=160;p= 0.812;NS).

Comparison of DHA (TPFμg/g^{-1}dry weight) of the horizons of reed bed 2

A significant difference was found when the dehydrogenase activity of the 30cm and the 60cm depth horizons were compared (t=4.264;df=156.18;p=0.000;S). There was a significant difference when the 30cm and the 90cm depth horizons were compared (t=7.70;df=118;p=0.000;S). There was a significant difference when the 60cm and the 90cm depth horizons were compared (t=2.93;df=160;p=0.004;S).

Comparison of DHA (TPFμg/g^{-1}dry weight) of the horizons of reed bed 3

There was no significant difference when the DHA of the 30cm and the 60cm depth horizons were compared (t=1.68;df=160;p=0.095;NS). There was a significant difference when the 30cm and the 90cm depth horizons were compared (t=6.13;df=160;p=0.000;S). There was also a significant difference when the 60cm and the 90cm depth horizons were compared (t=2.814;df=118;p=0.006;S).

Comparison of the DHA (TPFμg/g^{-1}dry weight) of the 30cm horizons of the reed beds

The dehydrogenase activity of the 30cm horizon of the control reed bed was compared with the 30cm horizon of the two reed beds treating landfill leachate. There was a significant difference in the mean dehydrogenase activity of the substrate from reed bed 1 (control) compared to reed bed 2 (t=-3.038;df=159;p=0.003;S). There was a significant difference in the dehydrogenase activity of the soil from Reed bed 1 (control) compared to reed bed 3 (t=-2.462;df=160;p=0.015;S). There was no significant difference in the dehydrogenase activity of the soil from reed bed 2 compared to reed bed 3 (t=0.79;df=159;p=0.429;NS).

4.10.5 Summary of (DH-2) results

- The treatment efficiency for TKN and NH_3 in reed bed 2 (91%) and reed bed 3 (79%) was significantly different. Leachate A was loaded to bed 2 and leachate B was loaded to bed 3.

- The substrate of reed bed 1 (control) at the 30cm horizon was found to have greater DHA (TPFμg/g^{-1}dry weight) in the post-loading period than in the during-loading period.

- There was no significant difference in the DHA (TPFμg/g^{-1}dry weight) of the substrate of reed bed 2 at the 30cm horizon during-loading and post-loading.

- There was no significant difference in the DHA (TPFμg/g^{-1}dry weight) of the substrate of reed bed 3 at the 30cm horizon during-loading and post-loading.

- The results suggest that reed bed 2 and reed bed 3, the reed beds treating landfill leachate A and B, were operating similarly and the same pattern was found in the dehydrogenase results. The control reed bed was different.

- There was a significant increase in the DHA (TPFμg/g^{-1}dry weight) of the control reed bed at the 60cm horizon post-loading compared to during-loading.

- There was no significant difference in the DHA (TPFμg/g^{-1}dry weight) of the substrate of reed bed 2 at the 60cm horizon during-loading and post-loading.

- There was no significant difference in the DHA (TPFμg/g^{-1}dry weight) of the 60cm horizon of reed bed 3 during-loading and post-loading.

- The 90cm horizon of reed bed 1 (control) showed a significant increase in DHA (TPFμg/g^{-1}dry weight) post-loading compared to during-loading. The same significant post-loading increase in DHA (TPFμg/g^{-1}dry weight) was found at the 30, 60 and 90cm horizon of the control reed bed suggesting a similar biological response throughout the substrate matrix.

- There was a significant increase in the post-loading DHA (TPFμg/g^{-1}dry weight) at the 90cm horizon of reed bed 2 compared with during-loading DHA (TPFμg/g^{-1}dry weight).

- There was a significant increase in the post-loading DHA (TPFμg/g^{-1}dry weight) at the 90cm horizon of reed bed 3 compared with during-loading DHA (TPFμg/g^{-1}dry weight).

- The post-loading decrease in DHA in reed bed 1 (control) is not the same in the reed beds treating landfill leachate.

- The reed beds treating landfill leachate had significantly higher DHA than the control reed bed.

- The DHA measured in (DH-1) was less than in (DH-2) which suggests that temperature has an effect on DHA because the soil temperature in the (DH-2) experimental period was higher than in (DH-1), by approximately 10°C.

4.11 Aerobic counts of three reed beds at the 30cm depth horizon during-loading and post-loading in UK spring/ summer (CFU-1).

4.11.1 Methods

Table 4.14 summarises the experimental parameters of the investigation. Table 4.15 summarises the mean weather conditions over the experimental period.

Table 4.14 Experimental parameters (CFU-1)

	CFU-1 Experimental parameters
Experimental period	Day 1 – Day 21
Loading period	Day 1 – Day 14
Effluent type	Reed bed 1 (Control) Reed bed 2 (Leachate B) Reed bed 3 (Leachate B)
Loading volume	200 litres/day
Effluent sampling	Day 1 – Day 21 (once per week)
Soil sampling	Day 14
Effluent analysis	EC (section 3.2.6), pH (section 3.2.8), TKN, NH_3 (section 3.2.12), COD (section 3.2.1)
Soil analysis	Total aerobic colony forming unit counts (section 3.4.1)

4.11.2 Experimental aims

- Determine the efficacy of the reed beds in reducing waste water parameters.
- Determine if there is a significant difference in the CFU/g of the 30cm substrate horizon of reed bed 1, 2 and 3.
- Determine if there is a significant difference in the CFU/g of the 30cm substrate horizon of each reed bed during-loading and post-loading conditions.

Table 4.15 Weather conditions over experimental period

Weather (SPRING/SUMMER)	Average (± SD)	
Max temp (°C)	Experimental period	16.4 ± 3.2
	Loading period	16.4 ± 3.2
Min temp (°C)	Experimental period	6.4 ± 3.0
	Loading period	6.4 ± 3.0
Sunshine (hrs)	Experimental period	5.3 ± 3.9
	Loading period	5.3 ± 3.9
Rainfall (mm)	Experimental period	2.9 ± 3.8
	Loading period	2.9 ± 3.8
Relative Humidity (RH)	Experimental period	76
	Loading period	76
Soil temp 30cm (°C)	Experimental period	12.9 ± 1.3
	Loading period	12.9 ± 1.3

4.11.3 Effluent treatment results

Inflow and outflow effluent analysis of the reed beds for TKN and NH_3 (section 3.2.12) are presented in table 4.16.

Table 4.16 TKN and NH_3 treatment efficiency (± SEM)

Reed bed	TKN (mg/L)			NH_3 (mg/L)		
	Mean IN	Mean OUT	% treatment	Mean IN	Mean OUT	% treatment
1 (control)	0.01 ± 0.00	0.01 ± 0.01	*ND*	0.02 ± 0.00	0.01 ± 0.01	*ND*
2 (leachate B)	1.9 ± 0.01	0.7 ± 0.6	64	2.3 ± 0.0	0.8 ± 0.8	63
3 (leachate B)	1.8 ± 0.1	0.2 ± 0.3	97	0.2 ± 0.3	0.1 ± 0.1	97

Reed bed 3 was as effective in reducing the TKN and NH_3 of the effluent as reed bed 2. Independent samples t-test were used for statistical analysis to find if there was a significant difference in TKN mean outflow from bed 2 and bed 3. Analysis showed that the outflow was not significantly different ($t = 1.760$; $df = 4$; $p = 0.153$; NS).

Table 4.17 shows the mean outflow pH and COD treatment efficiency over the duration of the experimental period. Reed bed 3 was more effective than reed bed 2 in reducing the COD of the leachate. All three reed beds balanced the pH of the effluent to a level acceptable for discharge to surface waters.

Table 4.17 pH and COD treatment efficiency (± SD)

Reed bed	pH		COD (mgO_2/L)		
	Mean IN	Mean OUT	Mean IN	Mean OUT	% treatment
1 (control)	6.0 ± 0.0	6.8 ± 0.1	57 ± 0.0	111 ± 55	*ND*
2 (leachate B)	7.9 ± 0.0	7.4 ± 0.1	2107 ± 0.0	657.33 ± 483	69
3 (leachate B)	8.2 ± 0.2	7.0 ± 0.1	1951 ± 62	231.67 ± 110	88

4.11.4 Microbiology (CFU) results

The aerobic CFU/g of the substrate of the three reed beds was determined for the duration of the experimental period both during-loading and post-loading (table 4.18).

Table 4.18 Aerobic CFU/g ($log_{(10)}$ ± SD) of 30cm depth horizon

Day of experiment	Reed bed 1 (control)	Reed bed 2 (leachate B)	Reed bed 3 (leachate B)
1 Loading-period	9 ± 2.0	10 ± 0.0	10 ± 0.6
4	8 ± 1.9	11 ± 0.0	10 ± 0.0
6	9 ± 1.9	11 ± 1.5	10 ± 0.0
9	9 ± 1.2	10 ± 0.7	11 ± 1.2
12	8 ± 1.3	11 ±0.0	11 ± 0.0
14	9 ± 0.8	9 ± 1.2	10 ± 0.0
15 Post-loading period	9 ± 0.8	8 ± 2.4	11 ± 1.4
16	9 ± 2.3	9 ± 1.4	11 ± 1.2
17	9 ± 0.4	9 ± 2.0	11 ± 0.3
18	10 ±0.9	8 ± 1.7	11 ± 0.8
19	9 ± 0.9	9 ± 1.5	11 ± 0.2
21	9 ± 0.1	8 ±1.2	10 ± 1.3

Independent sample t-tests were used to compare the aerobic CFU/g of the three reed beds. Reed bed 1 and reed bed 2 were significantly different (t = -3.155; df = 28 ; p= 0.004; S). Reed bed 1 and reed bed 3 were significantly different (t = -9.297; df = 23; p = 0.00; S). Reed bed 2 and bed 3 were significantly different (t = -2.076; df = 27; p = 0.048; S).

Independent samples t-test were used to compare the during-loading CFU/g and the post-loading CFU/g of the three reed beds. The tests revealed that the aerobic CFU/g in the control reed bed during-loading and post-loading was significantly different. The mean log aerobic CFU/g of the during-loading period in reed bed 1 (control) was 8.57 (± 0.45 SD ± 0.18 SEM) and in the post-loading period the mean log aerobic CFU/g was 9.14 (± 0.31 SD ± 0.12 SEM). There was a significant difference between the two loading conditions, which was a post-loading increase in log aerobic CFU/g (t=-2.55;df=10;p=0.029;S).

Reed bed 2 aerobic CFU/g data for the during-loading period (10.40 mean log aerobic CFU/g ±0.68 SD ± 0.28 SEM) were compared to the post-loading period (8.62 mean log aerobic CFU/g ± 0.48 SD ± 0.20 SEM) using independent samples t-tests. There was a significant decrease in aerobic CFU/g in the post-loading period compared to the during-loading period. The difference was significant (t=5.193;df=9;p=0.001;S).

In reed bed 3 comparison of the mean log aerobic CFU/g of the substrate sampled in the during-loading period (10.29 mean log aerobic CFU/g ± 0.46 SD ± 0.19 SEM) and in the post-loading period (10.76 ± 0.31 SD ± 0.13 SEM) found that there was no significant difference in the mean log CFU/g (t=-2.052;df=10;p=0.067;NS).

4.11.5 Summary of (CFU-1) results

- The treatment performance of reed bed 2 and reed bed 3 was significantly different in terms of TKN, (reed bed 2 was 64% and reed bed 3 was 97%). The treatment performance of reed bed 2 and reed bed 3 was also significantly different in terms of COD, (reed bed 2 was 69% and reed bed 3 was 88%).

- The aerobic CFU/g of reed bed 1, 2 and 3 showed significant difference between all three reed beds.

- In the control reed bed there was a significant post-loading increase in aerobic CFU/g from 8.6 to 9.1.

- In reed bed 2 there was a significant post-loading decrease in aerobic CFU/g from 10.4 to 8.6. In reed bed 3 there was no significant change in the aerobic CFU/g that could be interpreted as a response to the post-loading period, 10.3 to 10.8.

- There was a greater percentage reduction of TKN and NH_3 in reed bed 3 but there was no difference in the during-loading and post-loading aerobic CFU/g.

- Reed bed 2 was less efficient in treatment and there was a significant decrease in CFU/g in the post-loading period.

4.12 Aerobic counts of three reed beds at the 30cm depth horizon during- and post-loading in UK autumn/ winter (CFU-2).

4.12.1 Methods

Reed bed 1 was loaded with water from the Anglia region domestic supply; reed bed 2 and reed bed 3 were loaded with landfill leachate. Table 4.19 summarises the experimental parameters of the investigation. Table 4.20 summarises the mean weather conditions over the experimental period.

Table 4.19 Experimental parameters (CFU-2)

	CFU-2 Experimental parameters
Experimental period **Loading period** **Post-loading period**	Day 1 - Day 44 Day 1 – Day 35 Day 36 – Day 44
Effluent type	Reed bed 1 (control) Reed bed 2 (Leachate B) Reed bed 3 (Leachate B)
Loading	200 litres/daily
Effluent sampling **Soil sampling**	Day 1 - Day 43 (once per week) Day 35 – Day 44 (twice per week at 30cm) (section 3.1)
Effluent analysis	EC (section 3.2.6), pH (section 3.2.8), TKN, NH_3 (section 3.2.12), COD (section 3.2.1)
Soil analysis	Total aerobic colony forming unit counts (section 3.4.1)

4.12.2 Experimental aims

- Determine the efficacy of the reed beds in reducing waste water parameters
- Determine if there is a significant difference in the CFU/g of the 30cm substrate horizon of reed bed 1, 2 and 3.
- Determine if there is a significant difference in the CFU/g of the 30cm substrate horizon of each reed bed during-loading and post-loading conditions.

Table 4.20 Weather conditions over experimental period

Weather (AUTUMN/WINTER)	Average (± SD)	
Max temp (°C)	Experimental period	13.4 ± 2.8
	Loading period	13.7 ± 3.0
	Post-loading period	12.2 ± 1.6
Min temp (°C)	Experimental period	5.2 ± 2.4
	Loading period	5.2 ± 2.6
	Post-loading period	4.9 ± 1.8
Sunshine (hrs)	Experimental period	2.8 ± 2.9
	Loading period	3.0 ± 2.9
	Post-loading period	1.9 ± 2.6
Rainfall (mm)	Experimental period	6.8 ± 9.0
	Loading period	6.6 ± 10.6
	Post-loading period	7.2 ± 4.1
Relative Humidity (RH)	Experimental period	89
	Loading period	87
	Post-loading period	95
Soil temp 30cm (°C)	Experimental period	12.1 ± 1.8
	Loading period	12.6 ± 1.8
	Post-loading period	10.5 ± 0.5

4.12.3 Effluent treatment results

Inflow and outflow effluent analysis of the reed beds for TKN, NH_3 are presented in table 4.21.

Table 4.21 TKN and NH_3 treatment efficiency (± SEM)

Reed bed	TKN (mg/L)			NH_3 (mg/L)		
	Mean IN	Mean OUT	% treatment	Mean IN	Mean OUT	% treatment
1 (control)	0.01 ± 0.00	0.01 ± 0.00	*ND*	0.01 ± 0.00	0.01 ± 0.01	*ND*
2 (leachate B)	0.62 ± 0.06	0.02 ± 0.01	97	0.75 ± 0.07	0.03 ± 0.02	96
3 (leachate B)	0.69 ± 0.06	0.02 ± 0.00	97	0.83 ± 0.07	0.03 ± 0.01	96

Reed bed 3 was as effective in reducing TKN and NH_3 of the effluent as reed bed 2 (table 4.21). The difference in treatment performance was within 1%. Table 4.22 shows the mean outflow pH and COD over the duration of the experimental period. Reed bed 2 and reed bed 3 were effective in reducing the COD of the leachate 83% and 79% respectively. All three reed beds balanced the pH of the effluent to a level acceptable for discharge.

Table 4.22 pH and COD treatment efficiency (± SD)

Reed bed	pH		COD (mgO_2/L)		
	Mean IN	Mean OUT	Mean IN	Mean OUT	% treatment
1 (control)	6.8 ± 0.3	7.0 ± 0.3	31 ± 38	89 ± 120	*ND*
2 (leachate B)	8.2 ± 0.2	7.2 ± 0.3	948 ± 818	160 ± 125	83
3 (leachate B)	8.3 ± 0.2	7.4 ± 0.3	756 ± 330	161 ± 58	79

4.12.4 Microbiology (CFU) results

The aerobic CFU/g of the substrate of the three reed beds was determined for the duration of the experimental period, both during-loading and post-loading. The mean aerobic CFU/g for each bed on the sample date (day 35 of the loading period) is shown in table 4.23.

Table 4.23 Aerobic CFU/g ($log_{(10)}$ ± SD) of 30cm depth horizon.

Day of experiment	Reed bed 1 (control)	Reed bed 2 (leachate B)	Reed bed 3 (leachate B)
35 (loading period)	7 ± 0.2	7 ± 1.2	8 ± 1.5
	9 ± 0.7	8 ± 1.0	8 ± 1.3
	8 ± 0.0	9 ± 0.0	7 ± 0.0
	8 ± 0.9	7 ± 0.0	8 ± 1.2
	9 ± 1.5	7 ± 0.1	9 ± 2.7
	9 ± 1.9	8 ± 1.5	8 ± 3.0
	8 ± 0.0	9 ± 0.4	9 ± 0.1
	7± 0.0	8 ± 1.4	9 ± 1.3
	9 ± 0.0	8 ± 1.3	9 ± 2.5
42 (post-loading)	8 ± 1.5	8 ± 1.6	7 ± 0.9
	7 ± 0.4	9 ± 1.8	7 ± 0.0
	8 ± 1.6	8 ± 1.0	9 ± 0.0
	8 ± 2.3	7 ± 0.8	7 ± 0.8
	8 ± 1.4	8 ± 1.3	9 ± 2.8
	7 ± 0.0	8 ± 1.1	9 ± 2.3
	9 ± 2.2	9 ± 2.2	6 ± 0.0
	7 ± 0.4	8 ± 0.8	9 ± 4.6
	8 ± 2.2	7 ± 0.5	9 ± 2.1

Independent samples t-tests showed that there was no significant difference in the aerobic CFU/g counts of the reed bed substrate during-loading in reed bed 1 and reed bed 2 (t=0.545;df=16;p=0.593;NS). There was no significant difference between reed bed 1 and 3 (t=-0.055;df=16;p=0.957; NS). And no significant difference between reed bed 2 and reed bed 3 (t=-0,660;df=16;p=0.519;NS).

The same was true in the post-loading period. There was no significant difference between reed bed 1 (control) and reed bed 2 (t=-0.958;df=16;p=0.352;NS). There was no significant difference between reed bed 2 and reed bed 3 (t=-0,027;df=16;p=0.978;NS). There was no significant difference between reed bed 1 and reed bed 3 (t=-0.699;df=16;p=0.495;NS).

There was no significant difference in the mean log aerobic CFU/g of reed bed 1 (control) during-loading and post-loading (t=1.102;df=33;p=0.278;NS). There was no significant difference in the mean aerobic CFU/g of reed bed 2 during-loading and post-loading (t=-0.148;df=38;p=0.883;NS). There was no significant difference in the mean log aerobic CFU/g of reed bed 3 during-loading and post-loading (t=-0.205;df=37;p=0.839;NS).

4.12.5 Summary of (CFU-2) results

- There was no significant difference in the aerobic CFU/g of any of the reed beds during-loading or post-loading.

- The aerobic CFU/g of the substrate in all three reed beds in this experiment was lower than in the previous experiment (CFU-1). This could have been because the time of year for CFU-2 was autumn/ winter, and there were lower ambient temperatures than in the CFU-1 experiment, which was conducted in spring/ summer. The soil temperature was lower in CFU-2 and the minimum and maximum ambient temperature was lower in CFU-2. Rainfall was also considerably higher in CFU-2 (6.6mm ± 10.6) than in CFU-1 (2.9mm ± 3.8) which may have saturated the substrate and effected oxygen availability, thus limiting aerobic microorganisms.

- Rainfall was higher than in (CFU-2) and this may have masked the post-loading change in CFU/g that was evident in (CFU-1)

- In this experimental period the weather conditions were different. In (CFU-2) there were less hours of sunshine. The reeds were also dying back.

- In (CFU-1) reed bed 2 was much less efficient than reed bed 3 in effluent treatment. In this experiment (CFU-2) reed bed 2 and reed bed 3 were as effective in TKN reduction. The results show that treatment performance was not reduced by autumn/winter weather conditions.

- In (CFU-2) reed bed 2 and reed bed 3 produced the same efficiency in TKN treatment and no significant decrease post-loading of aerobic CFU/g. Perhaps the

lower efficiency in (CFU-1) was related to the post-loading decrease in CFU/g, which reflected the microbial fauna not being sufficiently adapted to the leachate B. Reed bed 3 was always loaded with leachate B, where as reed bed 2 was previously loaded with leachate A for a prolonged period.

4.13 Aerobic and anaerobic counts of three reed beds at 30, 60 and 90cm depth horizons without effluent loading in UK spring/ summer (CFU-3)

4.13.1 Methods

Effluent was not loaded at any time during the experimental period. Table 4.24 summarises the experimental parameters of the investigation. Table 4.25 summarises the mean weather conditions over the experimental period.

Table 4.24 Experimental parameters (CFU-3)

	CFU-3 Experimental parameters
Experimental period	Day 1 – Day 5
Effluent type	No loading
Loading volume	No loading
Effluent sampling **Soil sampling**	N/A Day 1 and Day 5
Effluent analysis	N/A
Soil analysis	Total aerobic and anaerobic CFU (section 3.4.1 and 3.4.2)

Table 4.25 Weather conditions over experimental period

Weather (SPRING/SUMMER)	**Average (± SD)**	
Max temp (°C)	Experimental period	16.2 ± 2.9
Min temp (°C)	Experimental period	7.3 ± 1.4
Sunshine (hrs)	Experimental period	7.4 ± 3.8
Rainfall (mm)	Experimental period	1.9 ± 3.1
Relative Humidity (RH)	Experimental period	73
Soil temp 30cm (°C)	Experimental period	11.9 ± 0.3

4.13.2 Experimental aims

- Determine if there is a significant difference in the aerobic CFU/g of the 30, 60 and 90cm substrate horizons of reed bed 2 and 3.

- Determine if there is a significant difference in the anaerobic CFU/g of the 30, 60 and 90cm substrate horizon of reed bed 2 and 3.

4.13.3 Microbiology (CFU) results

DAY 1

The aerobic CFU/g of 30 and 60cm depth horizons of reed bed 2 (DAY 1) are shown in table 4.26. The anaerobic CFU/g of the substrate of the 30 and 60cm depth horizons of reed bed 2 (DAY 1) are shown in table 4.27.

Table 4.26 DAY 1 reed bed 2 aerobic CFU/g ($log_{(10)}$ ± SD)

Depth (cm)	Section 1	Section 2	Section 3
30	6.3 ± 0.6	6.3 ± 0.4	6.4 ± 0.5
60	6.2 ± 0.4	6.2 ± 0.4	6.2 ± 0.4
90	*ND*	*ND*	*ND*

ND=not determined

Table 4.27 DAY 1 reed bed 2 anaerobic CFU/g ($log_{(10)}$ ± SD).

Depth (cm)	Section 1	Section 2	Section 3
30cm	4.9 ± 0.2	5.0 ± 0.8	5.6 ± 0.5
60cm	5.5 ± 1.0	4.9 ± 0.2	5.3 ± 0.4
90cm	*ND*	*ND*	*ND*

ND=not determined

Comparison of the CFU/g of reed bed 2 on DAY 1

Independent samples t-test to compare the aerobic CFU/g of the 30cm and 60cm horizons of reed bed 2 found no significant difference (t=1.209;df=52;p=0.232:NS). Independent samples t-test to compare the anaerobic CFU/g of the 30cm and 60cm horizons found no significant difference (t=-0.303;df=40;p=0.763;NS).

DAY 5

The aerobic and anaerobic CFU/g of the substrate of the 30 and 60cm depth horizons of reed bed 2 and reed bed 3 is shown in table 4.28, 4.29, 4.30 and 4.31.

Table 4.28 DAY 5 reed bed 2 aerobic CFU/g ($log_{(10)}$ ± SD)

Depth (cm)	Section 1	Section 2	Section 3
30	6.6 ± 0.7	6.3 ± 0.4	6.5 ± 0.8
60	6.3 ± 0.5	6.4 ± 0.5	6.4 ± 0.8
90	6.4 ± 0.5	6.5 ± 0.5	6.7 ± 0.2

Table 4.29 DAY 5 reed bed 2 anaerobic CFU/g ($log_{(10)}$ ± SD)

Depth (cm)	Section 1	Section 2	Section 3
30	5.0 ± 1.3	5.4 ± 0.6	5.5 ± 0.8
60	4.5 ± 0.4	5.2 ± 0.5	5.0 ± 1.3
90	5.4 ± 0.6	5.7 ± 0.5	6.0 ± 0.6

Comparison of the CFU/g of reed bed 2 on DAY 5

Independent samples t-tests were used to determine whether there were significant differences in CFU/g of the substrate from the 30, 60 and 90cm horizons of reed bed 2 on DAY 5. No significant difference was found in the aerobic CFU/g of reed bed 2 at the 30 and 60cm horizon (t=0.423;df=34;p=0.675;NS). No significant difference was found in the aerobic CFU/g of reed bed 2 at the 30 and 90cm horizon (t = -0.331; df=31;

p=0.743;NS). No significant difference was found in the aerobic CFU/g of reed bed 2 at the 60 and 90cm horizon (t=-0.808;df=31;p=0.425;NS). No significant difference was found in the anaerobic CFU/g of reed bed 2 at the 30 and 60cm horizon (t=-1.698;df=25;p=0.102;NS). No significant difference was found in the anaerobic CFU/g of reed bed 2 at the 30 and 90cm horizon (t=-1.167;df=25;p=0.254;NS). No significant difference was found in the anaerobic CFU/g of reed bed 2 at the 60 and 90cm horizon (t=0.202;df=20;p=0.842;NS).

Table 4.30 DAY 5 reed bed 3 aerobic CFU/g ($log_{(10)}$ ± SD)

Depth (cm)	Section 1	Section 2	Section 3
30	6.5 ± 0.3	6.5 ± 0.6	6.3 ± 0.6
60	6.3 ± 0.5	6.2 ± 0.5	6.9 ± 0.8
90	6.1 ± 0.4	6.5 ± 0.3	6.7 ± 0.4

Table 4.31 DAY 5 reed bed 3 anaerobic CFU/g ($log_{(10)}$ ± SD)

Depth (cm)	Section 1	Section 2	Section 3
30	5.8 ± 0.8	5.4 ± 0.7	6.1 ± 0.5
60	4.8 ± 1.7	5.0 ± 0.8	5.2 ± 0.4
90	5.7 ± 0.4	5.9 ± 0.7	5.5 ± 0.8

Comparison of the CFU/g of reed bed 3 on DAY 5

No significant difference was found in the aerobic CFU/g of reed bed 3 at the 30 and 60cm horizon (t=0.277;df=27;p=0.784;NS). No significant difference was found in the aerobic CFU/g of reed bed 3 at the 30 and 90cm horizon (t=0.185;df=27;p=0.855;NS). No significant difference was found in the aerobic CFU/g of reed bed 3 at the 60 and 90cm horizon (t=0.412;df=24;p=0.684;NS).

A significant difference was found in the anaerobic CFU/g of reed bed 3 at the 30 and 60cm horizon (t=2.395;df=23;p=0.025;S). No significant difference was found in the anaerobic CFU/g of reed bed 3 at the 30 and 90cm horizon (t=0.380;df=20;p=0.708;NS). No significant difference was found in the anaerobic CFU/g of reed bed 3 at the 60 and 90cm horizon (t=-1.972;df=21;p=0.062;NS).

Comparison of the CFU/g of the substrate of reed beds 2 and 3 on DAY 5

Independent samples t-tests were used for comparison of the aerobic and anaerobic CFU/g of the two reed beds. The results showed that the CFU/g of the substrate of reed bed 2 and reed bed 3 on DAY 5 was not significantly different. No significant difference was found in the aerobic CFU/g of the 30cm horizon of reed bed 2 and reed bed 3 (t=0.124;df=32;p=0.902;NS). No significant difference was found in the aerobic CFU/g of the 60cm horizon of reed bed 2 and reed bed 3 (t=-0.533;df=29;p=0.598;NS). And no significant difference was found in the aerobic CFU/g of the 90cm horizon of reed bed 2 and reed bed 3 (t=0.711;df=26;p=0.483;NS).

A significant difference was found in the anaerobic CFU/g of reed bed 2 and reed bed 3 at the 30cm horizon on DAY 5 (t=-2.719;df=26;p=0.012;S). No significant difference was found in the anaerobic CFU/g of reed bed 2 and reed bed 3 at the 60cm horizon (t=1.406;df=22;p=0.174;NS). No significant difference was found in the anaerobic CFU/g of reed bed 2 and reed bed 3 at the 90cm horizon (t=-0.761;df=19;p=0.456;NS).

Comparison of the CFU/g of reed beds 2 and 3 on DAY 1 and DAY 5

Independent samples t-tests were used to analyse the two sets of data collected on DAY 1 and DAY5. No significant difference was found in the anaerobic CFU/g of the 30cm horizon of the reed beds on day 1 and day 5 (t=0.206;df=46;p=0.837;NS). No significant difference was found in the anaerobic CFU/g of the 60cm horizon of reed bed 2 and 3 on day 1 and day 5 (t=0.423;df=44;p=0.675;NS).

4.13.4 Summary of (CFU-3) results

- No significant difference was found in the aerobic or anaerobic CFU/g of the substrate horizons of reed bed 2 on DAY 1.

- The data suggest that during this experimental period there was a remarkably consistent aerobic and anaerobic CFU/g in the reed bed substrate of both reed beds.

- A significant difference was found in the anaerobic CFU/g of reed bed 3 at the 30 and 60cm horizons on DAY 5 ($t=2.395;df=23;p=0.025;S$).

- A significant difference was found in the anaerobic CFU/g of reed bed 2 and reed bed 3 at the 30cm horizon on DAY 5 ($t=-2.719;df=26;p=0.012;S$).

- The experiment shows that even without effluent loading changes occur in the CFU/g of the reed beds.

- However, overall there was no significant difference in most of the comparisons that were made between the three substrate horizons of the two reed beds.

- Aerobic CFU/g was generally lower in (CFU-3) without effluent loading than in (CFU-2) and (CFU-1).

4.14 Aerobic and anaerobic counts of the substrate of three reed beds at three depth horizons during-loading in UK summer/ autumn (CFU-4)

4.14.1 Methods

Table 4.32 summarises the experimental parameters of the investigation. Table 4.33 summarises the mean weather conditions over the experimental period.

Table 4.32 Experimental parameters (CFU-4)

	CFU-4 Experimental parameters
Experimental period	Day 1 - Day 14
Loading period	Day 1 – Day 14
Effluent type	Reed bed 1 (Control) Reed bed 2 (Leachate C), Reed bed 3 (Leachate C),
Loading	200 litres/day
Effluent sampling	Day 1 - 14 (twice per week)
Soil sampling	Day 13 (30, 60, 90cm) (section 3.1)
Effluent analysis	EC (section 3.2.6), pH (section 3.2.8), TKN, NH_3 (section 3.2.12)
Soil analysis	Aerobic and anaerobic colony forming unit counts (section 3.4.1 and 3.4.2)

Table 4.33 Weather conditions over experimental period

Weather (SUMMER/AUTUMN)	Average (± SD)	
Max temp (°C)	Experimental period	20.9 ± 2.4
Min temp (°C)	Experimental period	9.3 ± 1.8
Sunshine (hrs)	Experimental period	5.2 ± 3.3
Rainfall (mm)	Experimental period	0.7 ± 1.7
Relative Humidity (RH)	Experimental period	85
Soil temp 30cm (°C)	Experimental period	17.3 ± 0.4

4.14.2 Experimental aims

- ❖ Determine if there is a significant difference in the aerobic CFU/g of the 30cm substrate horizon of the reed beds during-loading conditions.

- ❖ Determine if there is a significant difference in the anaerobic CFU/g of the 30cm substrate horizon of the reed beds during-loading conditions.

- ❖ Determine effluent treatment performance.

- ❖ Determine if there is a similar pattern in effluent treatment performance as was identified in previous investigations when a "different" leachate was treated. In this case leachate C.

4.14.3 Effluent treatment results

Inflow and outflow effluent analysis of the reed beds for TKN, NH_3 (section 3.2.12) are presented in table 4.34.

Table 4.34 TKN and NH_3 treatment efficiency (± SEM)

Reed bed	TKN (mg/L)			NH_3 (mg/L)		
	Mean IN	Mean OUT	% treatment	Mean IN	Mean OUT	% treatment
1 (control)	0.00 ±	0.01 ± 0.00	*ND*	0.00 ±	0.02 ± 0.00	*ND*
2 (leachate C)	1.7 ± 0.1	0.9 ± 0.0	45	2.0 ± 0.1	1.1 ± 0.0	45
3 (leachate C)	1.7 ± 0.0	0.6 ± 0.1	66	2.0 ± 0.1	0.7 ± 0.1	66

ND=not determined

The reed beds were effective in reducing the TKN and NH_3 of the effluent. Independent samples t-tests were used for statistical analysis to determine if there was a significant

difference in TKN mean outflow from bed 2 and bed 3. Analysis showed that the beds were not significantly different (t=1.760;df=4;p=0.153;NS).

Table 4.35 shows the mean pH of the outflow from the reed beds and the treatment efficiency for COD reduction over the duration of the experimental period. Reed bed 3 was more effective than reed bed 2 in reducing the COD of the leachate. All three reed beds balanced the pH of the effluent to a level acceptable for discharge.

Table 4.35 pH and COD treatment efficiency (± SD)

Reed bed	pH		COD (mgO_2/L)		
	Mean IN	Mean OUT	Mean IN	Mean OUT	% treatment
1 (control)	6.9 ± 1.3	7.8 ± 0.2	23 ± 10	46 ± 32	*ND*
2 (leachate C)	8.1 ± 0.1	7.4 ± 0.0	1012 ± 61	730 ± 66	28
3 (leachate C)	8.2 ± 0.1	7.2 ± 0.1	1000 ± 56	658 ± 108	34

ND=not determined

4.14.4 Microbiology (CFU) results

The aerobic and anaerobic CFU/g of the substrate of the three reed beds was determined for the 30cm depth horizon for the duration of the experimental period during-loading conditions (table 4.36 and 4.37).

Table 4.36 Aerobic CFU/g ($log_{(10)}$ ± SD) of reed beds during-loading

Day of experiment	Reed bed 1 (control)	Reed bed 2 (leachate C)	Reed bed 3 (leachate C)
3	10.0 ± 0.1	10.4 ± 0.1	9.0 ± 0.6
4	10.6 ± 0.0	10.1 ± 0.0	9.8 ± 0.1
5	9.8 ± 1.0	9.5 ± 0.9	10.2 ± 0.0
6	9.0 ± 1.0	9.5 ± 0.8	10.1 ± 0.1
7	10.5 ± 0.1	10.1 ± 0.0	9.5 ± 0.8
8	10.6 ± 0.0	10.4 ± 0.2	10.0 ± 0.2
10	9.6 ± 1.0	9.6 ± 0.9	10.2 ± 0.0
12	10.1 0.0	9.9 ± 0.0	10.6 ± 0.0
14	10.6 ± 0.0	10.1 ± 0.1	*ND*

Independent samples t-tests showed that there was no significant difference in the aerobic CFU/g of the reed bed substrate during-loading in reed bed 1 (mean 9.93 ± 0.80 SD 1.84 SEM) and reed bed 2 (mean 9.9 ± 0.61 SD ± 0.14 SEM) (t=0.118;df=37;p=0.91;NS). There was no significant difference between reed bed 1 (mean 9.93 ± 0.80 SD 1.84 SEM) and reed bed 3 (mean 9.87 ±0.58 SD ± 0.14 SEM) (t=0.248;df=33;p=0.806;NS). There was no significant difference between reed bed 2 and reed bed 3 (t=0,164;df=34;p=0.871;NS).

Table 4.37 Anaerobic CFU/g ($log_{(10)}$ ± SD) of reed beds during- loading

Day of experiment	Reed bed 1 (control)	Reed bed 2 (leachate C)	Reed bed 3 (leachate C)
3	8.5 ± 1.2	8.2 ± 1.7	9.7 ± 0.1
4	8.4 ± 1.2	7.4 ± 0.0	8.9 ± 1.7
5	8.7 ± 0.9	8.7 ± 0.7	9.9 ± 0.1
6	8.4 ± 1.2	8.5 ± 0.7	9.0 ± 0.7
7	8.8 ± 1.1	8.2 ± 0.7	*ND*
8	8.9 ± 1.0	8.6 ± 1.1	9.1 ± 0.7
10	8.8 ± 1.1	7.0 ± 0.6	8.2 ± 1.2
12	7.6 ± 0.0	8.7 ± 1.5	8.3 ± 0.8
14	8.4 ± 0.9	8.9 ± 1.7	8.9 ± 0.9

Independent samples t-tests showed that there was no significant difference in the anaerobic CFU/g of the reed bed substrate during-loading, when reed bed 1 (mean 8.59 ± 0.97 SD 1.73 SEM) was compared with reed bed 2 (mean 8.34 ± 0.97 SD ± 0.20 SEM) (t=0.95;df=52;p=0.35;NS). There was no significant difference between reed bed 1 and reed bed 3 (mean 8.95 ± 0.81 SD ± 0.17 SEM) (t=-1.396;df=50;p=0.169;NS). A significant difference was found between reed bed 2 (mean 8.34 ± 0.97 SD ± 0.20 SEM) and reed bed 3 (mean 8.95 ± 0.81 SD ± 0.17 SEM) (t=-2.257;df=42;p=0.029;S).

4.14.5 Summary of (CFU-4) results

- There was no significant difference in the aerobic CFU/g of the reed beds during-loading.

- A significant difference was found in the anaerobic CFU/g between reed bed 2 (mean 8.34 ± 0.97 SD ± 0.20 SEM) and reed bed 3 (mean 8.95 ± 0.81 SD ± 0.17 SEM) (t=-2.257;df=42;p=0.029;S).

- Leachate treatment was more effective in reed bed 3 than in reed bed 2.

- There were more viable anaerobic CFU/g in reed bed 3 than in reed bed 2.

- The experiment was carried out in the autumn of 2003. This was after the particularly hot summer of 2003, which was very sunny and dry. The environmental conditions may have affected the performance of the reed beds or the physical state.

- Leachate treatment performance was not as effective as in previous experiments such as (CFU-1) and (CFU-2) where there was 60-96% reduction of TKN and 70-80% reduction TKN. In this experiment (CFU-4) TKN treatment performance was 45-66%. This may be due to the fact that this was the first experiment in which both of the reed beds were loaded with leachate C. The microbial consortia of the reed beds may not have been accustomed to the effluent, and therefore would require a period of adaptation to attain equal treatment efficiency comparable to previous treatment performance of leachate B.

4.15 Aerobic counts of three reed beds at the 30cm depth horizon pre- during- and post-loading condition in UK winter (CFU-5)

4.15.1 Introduction

Three UCN experimental reed beds were used to investigate spatial and temporal changes in the microbiology of the substrate of a reed bed during an extended experimental period that included pre- during and post-loading. Numbers of aerobic microorganisms were counted using standard techniques (section 3.4.1).

4.15.2 Methods

Table 4.38 summarises the experimental parameters of the investigation. Table 4.39 summarises the mean weather conditions over the experimental period.

Table 4.38 Experimental parameters (CFU-5)

	CFU-5 Experimental parameters
Experimental period	Day 1 – Day 21
Loading period	Day 2 – Day 16
Post loading period	Day 17 – Day 21
Effluent type	Reed bed 1 (Control) Reed bed 2 (Leachate B) Reed bed 3 (Leachate B)
Loading volume	200 litres/day
Effluent sampling	Day 2 – Day 21 (twice per week)
Soil sampling	Day 1; Day 10; Day 21
Effluent analysis	pH (section 3.2.8), TKN, NH_3 (section 3.2.12), COD (section 3.2.1)
Soil analysis	Total aerobic colony forming unit counts (section 3.4.1)

Table 4.39 Weather conditions over experimental period

Weather (WINTER)	Average (± SD)
Max temp (°C)	Experimental period 6.5 ± 2.1 Loading period 6.1 ± 2.0 Post loading period.....6.9 ± 2.5
Min temp (°C)	Experimental period 2.9 ± 1.6 Loading period 2.7 ± 1.7 Post loading period.....2.8 ± 0.9
Sunshine (hrs)	Experimental period 3.7 ± 3.4 Loading period 3.6 ± 3.4 Post loading period.....4.8 ± 3.1
Rainfall (mm)	Experimental period 0.3 ± 0.7 Loading period 0.4 ± 0.8 Post loading period.....0.1 ± 0.3
Relative Humidity (RH)	Experimental period 92 Loading period 83 Post loading period.....90
Soil temp 30cm (°C)	Experimental period 5.6 ± 1.3 Loading period 6.1 ± 1.1 Post loading period.....4.0 ± 0.2

4.15.3 Experimental aims

- Monitor waste water treatment performance for the duration of the experimental period.

- Determine the efficacy of the reed beds in reducing effluent parameters.

- Determine if there is a significant difference in the aerobic CFU/g of the 30cm substrate horizon of reed bed 1, 2 and 3.

- Determine if there is a significant difference in the aerobic CFU/g of the 30cm substrate horizon of reed bed 1, 2 and 3 pre-loading, during-loading and post-loading.

4.15.4 Effluent treatment results

Inflow and outflow effluent analysis of the reed beds for TKN and NH_3 (section 3.2.12) are presented in table 4.40. Effluent analysis for COD and pH are presented in table 4.41.

Table 4.40 TKN and NH_3 treatment efficiency (± SD)

Reed bed	TKN (mg/L)			NH_3 (mg/L)		
	Mean IN	Mean OUT	% treatment	Mean IN	Mean OUT	% treatment
1 (control)	0.01 ± 0.00	0.00 ± 0.00	*ND*	0.01 ± 0.00	0.00 ± 0.00	*ND*
2 (leachate B)	0.5 ± 0.5	0.1 ± 0.0	80	0.6 ± 0.7	0.1 ± 0.0	80
3 (leachate B)	0.6 ± 0.5	0.1 ± 0.0	92	0.7 ± 0.6	0.1 ± 0.0	91

Table 4.41 pH and COD treatment efficiency (± SD)

Reed bed	pH		COD (mgO_2/L)		
	Mean IN	Mean OUT	Mean IN	Mean OUT	% treatment
1 (control)	7.0 ± 0.1	6.5 ± 0.3	56 ± 49	79 ± 43	*ND*
2 (leachate B)	7.7 ± 0.1	6.9 ± 0.1	1490 ± 259	453 ± 178	70
3 (leachate B)	7.7 ± 0.2	6.8 ± 0.3	1624 ± 467	442 ± 189	73

4.15.5 Microbiology (CFU) results

The aerobic CFU/g of the substrate of the three reed beds was determined for the duration of the experimental period, pre- during- and post-loading. The mean aerobic CFU/g for each bed on the sample date (DAY 1; DAY 10 and DAY 21) is shown in table 4.42.

Table 4.42 Aerobic CFU/g ($log_{(10)}$ ± SD) of reed beds

Day of experiment	Reed bed 1 (control)	Reed bed 2 (leachate B)	Reed bed 3 (leachate B)
1 (Pre-loading)	9.9 ± 1.0	9.1 ± 1.1	9.5 ± 1.0
10 (During-loading)	10.1 ± 0.7	9.7 ± 0.6	9.9 ± 0.6
21 (Post-loading)	9.5 ± 0.3	9.5 ± 0.4	9.5 ± 0.2

Comparison of pre-loading and during-loading CFU/g

There was no significant difference in the pre-loading and during-loading CFU/g of the substrate of reed bed 1 (control) and reed bed 3. A significant difference was identified in reed bed 2 between the pre-loading CFU/g (mean 9.09 ± 0.19 SEM ± 1.05 SD) and during-loading CFU/g (mean 9.69 ± 0.56 SD ± 0.12 SEM).

Comparison of during-loading and post-loading

There was no significant difference in the during-loading and post-loading CFU/g of the substrate of reed bed 2. A significant difference was identified in reed bed 1 (control), between the during-loading CFU/g (mean 10.11 ± 0.20 SEM ± 0.67 SD) and post-loading CFU/g (mean 9.49 ± 0.07 SEM ± 0.29 SEM). A significant difference was also identified in reed bed 3, between the during-loading CFU/g (mean 9.88 ± 0.15 SEM ± 0.57 SD) and post-loading CFU/g (mean 9.53 ± 0.05 SEM ± 0.22 SEM).

Comparison of pre-loading and post-loading

Comparison of the pre-loading and the post-loading substrate CFU/g for the three reed beds found no significant difference in the substrate over the two experimental conditions (which were both with no loading).

4.15.6 Summary of (CFU-5) results

- The effluent data show that the treatment performance of the reed beds was more effective in this case, than in the previous experiment (CFU-4). CFU-4 was conducted in summer/ autumn using leachate C. CFU-5 was conducted in winter time using leachate B.

- There was a significant difference in the aerobic CFU/g of the reed beds during-loading.

- Treatment performance in terms of TKN was better in reed bed 3 (92%) than in reed bed 2 (80%), and there was a post-loading decrease in CFU/g in reed bed 3.

- The overall pattern identified in CFU/g is that there is usually an increase in the CFU/g during-loading from a steady state pre-loading.

- A post-loading decrease has been identified in some experiments.

- Post-loading CFU/g is similar to pre-loading CFU/g.

4.16 Aerobic counts of three reed beds at the 30cm depth horizon pre- during- and post-loading in UK spring/ summer (CFU-6)

4.16.1 Methods

Table 4.43 summarises the experimental parameters of the investigation. Table 4.44 summarises the mean weather conditions over the experimental period.

Table 4.43 Experimental parameters (CFU-6)

	CFU-6 Experimental parameters
Experimental period **Loading period** **Post loading period**	Day 1 – Day 25 Day 2 – Day 20 Day 21 – Day 25
Effluent type	Reed bed 1 (Control) Reed bed 2 (Leachate B) Reed bed 3 (Leachate B)
Loading volume	200 litres/day
Effluent sampling **Soil sampling**	Day 2 – Day 25 (twice per week) Day 1; Day 5; Day 10; Day 15; Day 20; Day 25
Effluent analysis	pH (section 3.2.8), TKN, NH_3 (section 2.12), COD (section 3.2.1)
Soil analysis	Total aerobic colony forming unit counts (section 3.4.1)

4.16.2 Experimental aims

- Determine the efficacy of the reed beds in reducing effluent parameters.
- Determine if there is a significant difference in the aerobic CFU/g of the 30cm substrate horizon of reed bed 1, 2 and 3 pre- during- and post-loading.
- Identify points of significant correlation between effluent treatment and the aerobic CFU/g.

Table 4.44 Weather conditions over experimental period

Weather (SPRING/SUMMER)	Average (± SD)	
Max temp (°C)	Experimental period	14.5 ± 3.0
	Loading period	14.4 ± 3.3
	Post loading period	15.0 ± 2.3
Min temp (°C)	Experimental period	6.3 ± 2.2
	Loading period	5.8 ± 2.1
	Post loading period	8.1 ± 2.0
Sunshine (hrs)	Experimental period	4.0 ± 3.9
	Loading period	4.3 ± 4.2
	Post loading period	2.5 ± 3.2
Rainfall (mm)	Experimental period	3.1 ± 4.4
	Loading period	3.5 ± 4.9
	Post loading period	1.1 ± 1.6
Relative Humidity (RH)	Experimental period	79
	Loading period	78
	Post loading period	88
Soil temp 30cm (°C)	Experimental period	11.7 ± 0.9
	Loading period	11.6 ± 0.9
	Post loading period	12.4 ± 0.4

4.16.3 Effluent treatment results

Inflow and outflow effluent analysis of the reed beds for TKN and NH_3 (section 3.2.12), and COD and pH are presented in table 4.45 and 4.46.

Table 4.45 TKN and NH_3 treatment efficiency (± SD)

Reed bed	TKN (mg/L)			NH_3 (mg/L)		
	Mean IN	Mean OUT	% treatment	Mean IN	Mean OUT	% treatment
1 (control)	0.00 ± 0.00	0.01 ± 0.01	*ND*	0.00 ± 0.00	0.01 ± 0.00	*ND*
2 (leachate B)	1.0 ± 0.1	0.1 ± 0.0	94	1.2 ± 0.1	0.1 ± 0.0	94
3 (leachate B)	1.0 ± 0.1	0.0 ± 0.0	97	1.2 ± 0.1	0.0 ± 0.0	98

Table 4.46 pH and COD treatment efficiency (± SD)

Reed bed	pH		COD (mgO_2/L)		
	Mean IN	Mean OUT	Mean IN	Mean OUT	% treatment
1 (control)	7.8 ± 0.5	6.4 ± 0.9	*ND*	38 ± 67	*ND*
2 (leachate B)	7.8 ± 0.2	6.7 ± 0.6	1571 ± 560	171 ± 69	89
3 (leachate B)	7.8 ± 0.2	6.8 ± 0.4	1571 ± 560	71 ± 25	95

4.16.4 Microbiology (CFU) results

Aerobic CFU/g of substrate

The aerobic CFU/g of the substrate of the three reed beds was determined for the duration of the experimental period, pre- during- and post-loading. The mean aerobic CFU/g for each bed on the sample dates (DAY 1; DAY 5; DAY 10; DAY 20 and DAY 25) are shown in table 4.47.

Table 4.47 Aerobic CFU/g ($log_{(10)}$ ± SD) of reed beds

Day of experiment	Reed bed 1 (control)	Reed bed 2 (leachate B)	Reed bed 3 (leachate C)
1 (Pre-loading)	8.1 ± 0.8	8.0 ± 0.8	7.6 ± 0.8
5 (During-loading)	8.9 ± 1.5	8.9 ± 1.5	8.6 ± 1.6
10 (During-loading)	8.4 ± 0.9	8.7 ± 0.9	8.4 ± 0.7
15 During-loading	9.2 ± 1.7	9.2 ± 1.9	8.6 ± 1.9
20 Post-loading	7.7 ± 0.8	8.1 ± 0.6	7.8 ± 0.6
25 Post-loading	8.5 ± 1.1	7.9 ± 0.3	7.8 ± 0.3

Comparison of pre-loading and during-loading CFU/g

CFU/g data were collected under pre-loading conditions (DAY 1) and after the commencement of the leachate loading period (during-loading DAY 5; 10; 15). A significant difference was found between the pre-loading CFU/g of the reed beds and the during-loading CFU/g.

There was no significant difference in the pre-loading (DAY 1) CFU/g of reed bed 1 (control) when compared to the during-loading (DAY 5) data. There was a significant difference in the CFU/g of reed bed 2 and reed bed 3 when the pre-loading data were compared to the first set of during-loading data.

A significant difference was identified between reed bed 1 pre-loading (DAY 1) and during-loading (DAY 15). A significant difference was also found between the pre-loading and during-loading CFU/g of reed bed 2.

Comparison of all during-loading (DAY 5; 10; 15) CFU/g

All CFU data collected during-loading conditions were compared using independent samples t-tests to determine if the CFU/g of the substrate of each reed bed were consistent during-loading conditions or whether there were significant differences. The results found no significant difference in the during-loading condition for all three reed beds over the loading period.

Comparison of during- (DAY 5; 10; 15) and post-loading (DAY 20; 25) CFU/g

The during-loading aerobic CFU/g data for each reed bed were compared to the aerobic CFU/g data collected from the same reed bed under post-loading conditions. Significant differences in CFU/g were identified in all three reed beds. In the control reed bed there was a significant decrease in CFU/g post-loading. In reed bed 1 (control) between day 5 (mean 8.91 ± 1.46 SD) and day 20 (7.73 ± 0.77) there was decrease in CFU/g.

In reed bed 2 there was also significant decrease in the CFU/g of the substrate between day 5 (8.93 ± 1.52 SD) (during-loading period), and day 20 (8.07 ± 0.64) (post-loading). The difference was significant.

Similarly in reed bed 3 a post-loading decrease in the CFU/g of the reed bed substrate was identified. Between day 5 of the experimental period (8.57 ± 1.63 SD) and day 20 (7.80 ± 0.63) there was a significant decrease in CFU/g.

Between day 5 (during-loading) and day 25 (post-loading) there was no significant change in the CFU/g of the substrate of reed bed 1 (control). There were however significant changes in reed bed 2 and reed bed 3, both of which showed a significant decrease in CFU/g of the substrate. Between day 10 (during-loading) and day 20 (post-loading) there were significant change in the CFU/g of the substrate of the reed beds. In all three reed beds there were significant decreases in the CFU/g of the substrate.

In the control reed bed there was a significant decrease in CFU/g post-loading. On day 10 the mean value for the control reed bed was 8.42 (± 0.92 SD) and on day 20 the mean value was 7.73 (± 0.77 SD).

In reed bed 2 there was a significant decrease in the CFU/g of the substrate on day 10 (mean 8.65 ± 0.90 SD) of the loading period, and on day 20 (mean 8.07 ± 0.64) (post-loading). The difference was significant. Similarly in reed bed 3 a post-loading decrease in CFU/g of the substrate was identified between day 10 of the experimental period (mean 8.36 ± 0.69 SD) and day 20 (mean 7.80 ± 0.63). The decrease in CFU/g was significant. Between day 10 and day 25 there was a significant decreases in the CFU/g of reed bed 2 and reed bed 3. However, in reed bed 1 (control) there was no significant change.

Between day 15 (during-loading) and day 20 (post-loading) there was a significant decrease in the CFU/g in reed bed 1 (control) and in reed bed 2. The change in CFU/g in reed bed 3 was not significant. There was also a significant decrease in the CFU/g of the substrate of reed bed 2. The decrease in both reed 3 and reed bed 1 (control) were not significant.

Comparison of post-loading (DAY 20) and post-loading (DAY 25) CFU/g

The two sets of data collected in the post-loading period, on day 20 and day 25 of the experimental period, were compared using independent samples t-tests. No significant differences were found in the CFU/g of any of the reed beds.

Comparison of pre-loading (DAY 1) and post-loading (DAY 20; 25) CFU/g

The aerobic CFU/g of the substrate in the pre-loading period (day 1) was compared to the CFU/g on day 20 (post-loading). The experimental conditions on both samples dates were those of no effluent loading. No significant difference was found in the CFU/g of the substrate of reed bed 1 (control), reed bed 2 or reed bed 3.

The aerobic CFU/g in the pre-loading period on (day 1) of the experimental period was compared to the CFU/g on day 25 (post-loading). The experimental conditions on both samples dates were those of no effluent loading. No significant difference was found in the CFU/g of the substrate of reed bed 1 (control), reed bed 2 or reed bed 3.

Anaerobic CFU/g of substrate

Table 4.48 shows the anaerobic CFU/g of the reed beds throughout the duration of the experimental period.

Table 4.48 Anaerobic CFU/g ($log_{(10)}$ ± SD) of reed beds

Day of experiment	Reed bed 1 (control)	Reed bed 2 (leachate B)	Reed bed 3 (leachate B)
15 (During-loading)	7.6 ± 1.5	7.6 ± 1.9	8.0 ± 1.8
25 (Post-loading)	7.4 ± 1.5	7.5 ± 1.8	8.0 ± 1.9

Independent samples t-tests were used to determine whether there was a significant change in the anaerobic CFU/g of the reed bed substrate when the DAY 15 (during-loading) was compared to DAY 25 (post-loading). The difference between reed bed 1 (control) day 15 and reed bed 1 (control) on day 25 was not significant (t=0.25; df=26; p=0.806). Similarly there were no significant difference in the reed bed 2 data (t=0.201; df=24; p=0.842; NS) or in the reed bed 3 data (t=0.334; df=23; p=0.742).

4.16.5 Summary of (CFU-6) results

- There was a significant difference in the aerobic CFU/g of the reed beds during-loading and post-loading.

- No significant difference was found in the anaerobic CFU/g between the reed beds during-loading and post-loading

- In reed bed 2 and reed bed 3 there was a decrease in aerobic CFU/g in the post-loading period.

- There was not a significant decrease in aerobic CFU/g post-loading in reed bed 1 (control)

4.17 Overall analysis of seasonal data and discussion of results

Constructed wetlands are increasingly used throughout the world for the treatment of domestic and industrial waste waters. Primary, secondary and tertiary applications for the technology have been established. Constructed wetlands have been used to treat effluent from a variety of sources. They can act as physical filters providing sediment traps for suspended matter, and provide chemical and biological conditions conducive to the removal of contaminants from solution. Despite the many applications of constructed wetland technology the microbial communities associated with these systems have remained poorly characterised. It was the case until a number of years ago, that previous work on constructed wetland performance was limited to reports of inflow and outflow effluent treatment for various wastewater chemistry parameters. In

the last decade far more complex studies have sought to understand the functional processes that occur within a reed bed, and so published data have moved away from the "black-box" analysis of early reported literature.

The combination of the biochemical and microbiological assessment of the reed beds, and the chemistry analysis of the influent and effluent in the present study, were undertaken to reveal and evaluate changes and trends that occurred within the matrix of the reed bed, under effluent loading conditions, over an extended period of operation, encompassing seasonal and climatic variations.

Recent studies coming from China (Liang *et al.*, 2003), Germany, United States (Lorah and Voytek, 2004; Diemont, 2005) and India (Verma *et al.*, 2001) have recognised the importance of microbiologically mediated interactions within wetlands. They have gone further than speculating the contribution of microbial consortia to treatment performance, and have actually investigated the role of microorganisms within the wetland.

Microbial processes within a constructed wetland are the driving force in the reduction of nitrogen. Nitrogen exists mainly in the form of ammonia. The ammonia form of nitrogen is a primary product of the microbial decomposition of organic material. As microorganisms break down organic material in water, oxygen is consumed. The result can be oxygen depletion. Ammonium is the most reactive form of nitrogen present in aquatic systems and is the preferred form for algae and plant growth. Ammonium-N adheres to soil and sediment. In the presence of dissolved oxygen ammonium-N is quickly oxidised and converted to nitrate and nitrite through nitrification by nitrifying bacteria, such as *Nitrosomonas* spp. and *Nitrobacter* spp., which add oxygen to the ammonium ion. Nitrite and nitrate generated by nitrification is immediately removed by denitrification. Simultaneous nitrification and denitrification is said to occur within wetland sediment (Hammer, 1994). Denitrifying bacteria remove oxygen from nitrite (NO_2^-) and nitrate (NO_3^-) ions for their own use, releasing N_2 and/or N_2O back to the atmosphere.

Nitrifying organisms only function when free oxygen (O_2) is present. In saturated soils there is a limited availability of free oxygen, suppressing the growth of nitrifying

microorganisms. This situation is enhanced by denitrifying bacteria since they thrive in the oxygen-free environment of a saturated soil, consuming nitrate at a rapid rate. Excessive rainfall promotes nitrogen loss by nitrate leaching from the plant root zone, and also by creating wet soil conditions that favour denitrification. Evaporation has the opposite effect to excessive rainfall, by removing water from the upper layers of the soil profile. Pore space becomes available for oxygen making the environment suitable for the growth of nitrifying bacteria.

TKN reduction in constructed wetland treatment of effluent is related to nitrification and denitrification activity within the reed bed substrate matrix. Nitrogen removal in European constructed wetlands has been reported in the range of 30-40% (Brix, 1994). Ammonium (NH_4-N) and total-N are often reported parameters. Nitrogen removal efficiencies vary between reports. Data provided by Kuschk *et al.,* (2003) investigating a SSF pilot plant wetland in Germany provided perhaps the most comprehensive analysis of nitrogen processing in constructed wetland to date. The research investigated long term stable operation of a wetland with short sampling intervals. The results showed 35-52% average elimination efficiencies of ammonia–N. The 36–97% TKN reduction range reported in the present study is higher than was previously reported by Kuschk *et al.,* (2003).

In spring and autumn, in temperate zones, microbial activities are changing due to changing environmental conditions. Numbers of aerobic microorganisms may be reduced due to dissolved oxygen depletion in the substrate cause by increased rainfall. Rain may cause nitrate to be washed from the soil and leach from the wetland increasing effluent-N. Low N removal rates reported in winter conditions may be related to temperatures lower than the optimum temperature range for nitrification.

Mander *et al.,* (2000) reported 12-85% reduction of N in a wetland cell in Estonia, and stated that there was not evidence of a decrease in treatment performance during cold weather conditions. Maehlum and Stalnacke (1999) reported less than 10% removal differences between warm and cold periods. Other researchers have demonstrated 40-97% removal efficiencies and no general correlation with seasonal temperature gradients (Geller, 1997). Other reports demonstrate seasonal effects, mainly related to temperature. Significant differences between seasonal treatment performances have

been cited by Kadlec (1999) and Spieles and Mitsch (2000). Mostly research has reported treatment efficiencies for nitrogen removal independent of seasons (Hammer and Knight, 1994; Reed *et al.,* 1995). Kadlec (1999) however has called constructed wetlands solar powered ecosystems that are affected by complex annual cycles, water and soil temperature, air temperature, solar radiation (annual and diurnal), humidity, precipitation, pollutant concentration and vegetation. These parameters cause and influence changes in nutrient supply, availability, uptake or release of chemical substances and biological activities of microorganisms and plants.

Experimental work on reed beds and constructed wetlands is often carried out using model wetland cells rather than operational or pilot scale wetlands. The structure of these model wetlands is such that they often do not adequately model an active *in situ* reed bed. If constructed wetlands are solar powered ecosystems as Kadlec (1999) suggested, in which removal efficiencies are dependent upon the interaction of solar radiation, water/ soil temperature, rainfall, humidity and pollutant concentrations, model wetland cells may not be an adequate model of this type of treatment system. Many of the model wetland cell facilities are made from a plastic tank, filled with soil and plants, which is sited above the ground, and is batch loaded with effluent. Information is often not presented within published literature to describe the period of establishment of the cell in terms of the plants and the associated substrate microbiology. In the model wetland cell there is exposure to ambient temperatures on all sides of the model. When a reed bed is built into the ground exposure to the elements from all sides of the substrate does not occur. The reed bed is buffered from the elements by the surrounding land. The contribution of laboratory based research using model wetland cells is however valuable in providing laboratory scale experiments which can control the operational aspects of the constructed wetland system, allowing freedom to explore the internal functional processes of the technology. Whether the processes are analogous in the macro-scale constructed wetland system depends on the individual experimental design.

Reed beds, and the processes that occur within them, are effected by rainfall, ambient air temperature, soil temperature and the temperature of the loading influent (Brix, 1994). These factors limit and drive nutrient supply, uptake and release of chemical substances, and the activities and biological processes of plants and microorganisms.

This work sought to provide sufficient effluent chemistry analysis as to inform of landfill leachate treatment performance and to provide data relating to the aerobic and anaerobic microorganisms from an established operational pilot-scale wetland. The experimental conditions were controlled in terms of experimental design, loading rate, frequency and volume so that the factors of environmental weather conditions and the microbiological activity of substrate microbial community could be interpreted with minimal extraneous interference. The data have been used to provide seasonal mean values for all experimental parameters that can be used to explore the effects of seasonality on treatment performance. Data from reed beds 2 and 3 were combined for each season, for all years. The average treatment performance was then calculated with standard deviations shown on the graph (figure 4.1).

Figure 4.1 Seasonal means of % TKN and COD reduction

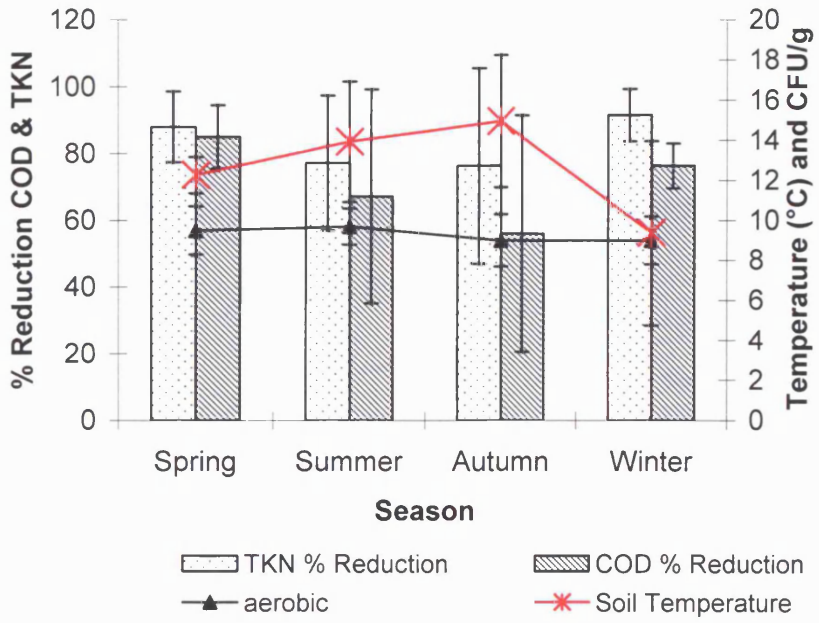
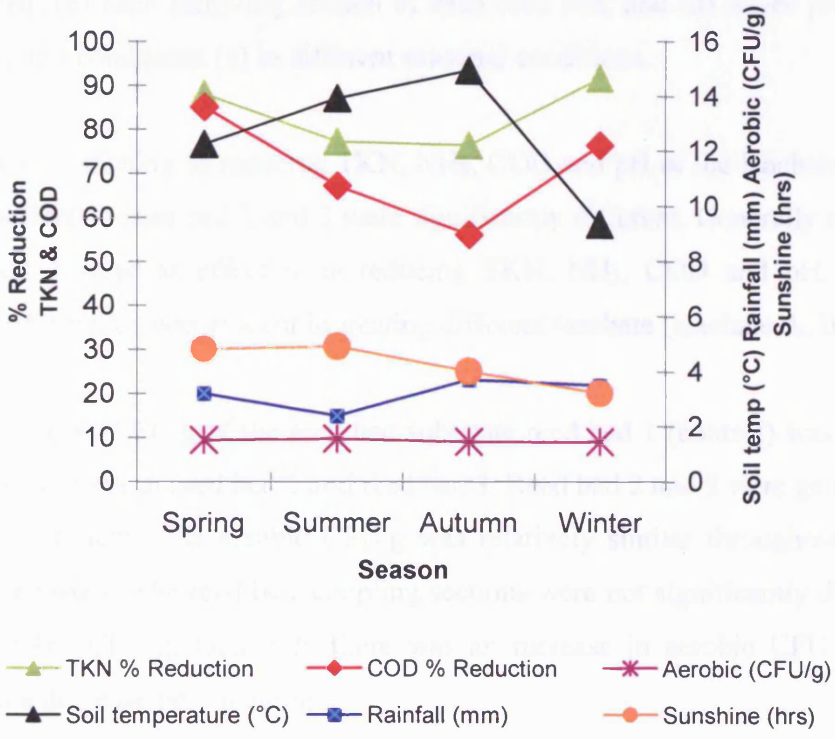


Figure 4.1 shows seasonal treatment performance averages which were calculated using all available loading period data. The results show that soil temperatures were lowest in winter (9.4°C). Soil temperatures increased through spring and summer and were highest in autumn (15.0°C). Lower COD reduction occurred in summer and autumn than in spring and winter. Greatest COD reduction occurred in spring (85%). Greater

TKN reduction occurred in spring and winter than in summer and autumn. Greatest TKN reduction occurred in winter (91.5%).

Surface *et al.*, (1993) reported a decrease in BOD and TKN removal in winter, in a temperate zone, due to decreased microbial activity in reduced temperature substrate. Kadlec and Reddy (2001) noted that microbiologically mediated reactions are most effected by temperatures below 15°C. The data collected in this study did not fully support the finding of Surface *et al.*, (1993) or Kadlec and Reddy (2001). Soil temperature showed an increase from the lowest recorded temperature in winter, to the highest recorded soil temperature in autumn. TKN reduction was highest in winter decreasing through spring and summer to a lowest recorded TKN reduction in autumn. This suggests that TKN reduction is not only effected by temperature. In the temperate climate of the UK the highest recorded seasonal rainfall was in the autumn (3.7mm), and lowest in summer (2.4mm) (figure 4.2). High rainfall may have reduced TKN removal by saturating the substrate, therefore reducing the dissolved oxygen conditions, limiting nitrification and exacerbating the conditions of reduced oxygen by enhancing conditions appropriate for denitrifying organisms.

Figure 4.2 Seasonal means of % treatment and aerobic CFU/g and weather conditions



A mid-summer restriction of NH_4 removal has been previously reported (Kuschk *et al.*, 2003). Factors interpreted by Kuschk *et al.*, (2003) to affect microbial nitrification included temperature and oxygen availability. The temperature of inflow during the summer was 17-22°C (Kuschk *et al.*, 2003) which was lower than optimum temperature for nitrification. The results of the individual experiments of this investigation generally support this finding. Mean seasonal treatment averages calculated using the present set of data were used to investigate whether long term study of wetland function would supported the assertions of Kuschk *et al.*, (2003). Figure 4.2 shows a general summer and autumn restriction in TKN removal. Trends that may have been evident in individual experiments may have been masked by many interfering factors such as high summer temperatures experienced in CFU-4 (max ambient 20.9°C) or high rainfall such as in CFU-2 (6.6mm).

4.18 Concluding remarks

The experiments in this chapter were designed to follow three lines of questioning with regard to reed bed treatment of industrial effluent. That was to investigate (1) the overall treatment of landfill leachate in terms of removal efficiency of specified pollutants; (2) the aerobic dehydrogenase activity of the reed bed substrate; and (3) the aerobic colony forming unit counts of the reed bed substrate in (a) each reed bed; (b) depth horizons of each reed bed; (c) each sampling section of each reed bed; and (d) under pre- during- and post-loading conditions (e) in different seasonal conditions.

Reed beds were effective in reducing TKN, NH_3, COD and pH of the leachate. In some experiments (DH-1) reed bed 2 and 3 were significantly different. Generally reed bed 2 and reed bed 3 were as effective in reducing TKN, NH_3, COD and pH. Different treatment performance was evident in treating different leachate (leachate A, B and C).

In terms of aerobic CFU/g of the reed bed substrate reed bed 1 (control) was generally significantly different to reed bed 2 and reed bed 3. Reed bed 2 and 3 were generally not significantly different. The aerobic CFU/g was relatively similar throughout the reed bed substrate matrix. The reed bed sampling sections were not significantly different in terms of aerobic CFU/g. Generally there was an increase in aerobic CFU/g during-loading, and a decrease post-loading.

Seasonal conditions may have effected treatment performance in the experiments. Seasonality could not however be used to predict treatment performance, as efficiency was variable, ranging from TKN 37%-97% treatment, and COD ranging from 28%-95%, throughout the years. TKN removal may have been restricted in summer/ autumn.

Pre-loading, during-loading, and post-loading were used as terms to describe the operational state of the reed bed. The reed bed section delineation was used as an indication of distance travelled from inlet to outflow. Reed bed depth horizons were used to obtain the optimum sampling region of the reed bed. The fact that reed beds have been described as having temporal and spatial differences in the matrix was both supported and refuted by the differences that were identified between the 30, 60 and 90cm substrate horizons of the reed beds.

A large multifaceted set of data was generated relating to reed bed operation and function. The interaction between the microbiological community of the reed bed and effluent treatment performance was explored. Dehydrogenase activity and total viable counts were used to quantify the reed bed microbial consortia in terms of activity and viability. Extensive analysis of reed bed treatment performance and the functional microbial consortia, and of the climatic conditions, through analysis of meteorological office data, enabled interpretation of the effects of seasonality on constructed wetland performance.

CHAPTER FIVE

REED BED TREATMENT OF TANNERY EFFLUENT IN THE UK

5.0 Introduction

Tannery effluent is highly polluting mixed wastewater emanating from the leather industry (Bajza and Vrcek, 2001). The by-products of the leather manufacturing process have the potential to cause significant environmental pollution. This has led to the adoption of legislation to minimise the environmental impact of tannery operations and associated by-products (Bosnic *et al.*, 2000). The large volume of tannery effluent produced in leather manufacture causes severe environmental problems if it is not properly treated (Song *et al.*, 2000). The waste water produced is characterised by high chemical oxygen demand (COD), chromium salts, sulphides, and chloride ions and is strongly alkaline (Bajza and Vrcek, 2001). There can also be a deep colour content which comes from the dyes used in the leather processing (Song *et al.*, 2000). Liquid and solid waste from leather processing should be disposed of safely and economically to ensure protection of the environment (UNIDO, 2000). Discharge of untreated tannery effluent causes associated environmental pollution and damage that has been widely recognised (Lancet, 1898; Hamilton and Timmons, 1980; Aislabie and Loutit, 1984; Armienta *et al.*, 1996; Song *et al.*, 2000). Reed bed technology has been used for tannery effluent treatment. In this investigation the UCN reed beds were used for tertiary treatment of primary treated tannery effluent. The seasonality of the treatment performance of reed beds was studied as were the changes in the aerobic microbial consortia of the reed bed substrate under-loading and post-loading conditions.

5.1 The leather industry

The leather industry is the processor of meat production by-products (Germann, 1999). Leather production is a complex process which requires the tanner to stabilize the properties of the hide through a number of chemical processes, turning it into a material desirable to the consumer. In processing the skin into leather there are four primary processing steps called: *beamhouse*; *tanning*; *post-tanning*; and *finishing*.

5.2 Tannery effluent

Distinct wastewater is generated in the different stages of leather tanning. This is an important factor when investigating the treatment of tannery effluent. Washing, soaking and liming of hides contributes up 31% of the total amount of wastewater volume

(Bajza and Vrcek, 2001), however this stage in processing contributes 88% of the BOD_5, 73% of COD, 83% of TSS, an overall 90% of the pollution effect of the final mixed effluent (Konrad *et al.*, 2000). By contrast tanning operations contribute 11% of the total wastewater volume (Bajza and Vrcek, 2001), this is 8% BOD_5, 13% COD and 11% TSS (Konrad *et al.*, 2000). Re-tanning contributes 25% of the total volume of wastewater. Wash-down water contributes 26% total volume and machine water a further 7% (Bajza and Vrcek, 2001). The mean water characterisation for conventional pollutants in untreated mixed tannery effluent are BOD_5 1600mg/L, COD 4600mgO_2/L, Sulphides 64mg/L, Total Chromium 76mg/L, (UNEP, 1991).

5.3 Tannery effluent treatment

Many environmental action programmes have been established to address pollution issues associated with leather manufacture. Environmental directives, regulations and legislation have been issued to define water quality standards and to set consent standards for discharge of industrial waste (Buljan and Bosnic, 1994; Bosnic *et al.*, 2000). In the UK, tanners have generally been able to meet the discharge limits set by regional water companies. However the high COD and TSS load of the wastewater still pose a problem for tanneries. COD and TSS have been employed by water companies as major parameters for effluent discharge (Song *et al.*, 1998). Lowering of the legal discharge limits can be expected in the future, providing ever more stringent targets which the tannery industry will be required to meet.

The leather industry in countries with developing economies is characterised by a number of distinctive features. Many of the countries in the regions attach particular importance to the sector in terms of the enormous potential it offers in providing employment, nurturing economic growth and increasing exports. However, at the same time, failure to appreciate the environmental impact and to enforce appropriate regulations for waste treatment and management in the leather manufacturing industries has led to land and water degradation (Makdisi, 1991). In eastern and southern Africa, where the leather industry is a key area of industrial development, most tanneries now have water treatment plants and many are experimenting with reducing solid waste volume. Leading leather manufacturers are coming to realise that there are considerable benefits to be made from the reduction of effluent and solid waste volume (UNIDO,

2000). In some leather producing countries direct discharge of untreated tannery effluent is common practice (Contreras-Ramos *et al.*, 2004).

5.4 Tannery effluent treatment methods

A number of treatment methods can be applied to tannery effluent. Primary treatment is the removal of floating and suspended solids, using physical processes. It is the first stage in the wastewater treatment process. Secondary treatment is the second major step in wastewater treatment processing where bacteria consume the organic constituents of the wastewater. It can be accomplished through trickling filters or in the activated sludge process. Tertiary treatment is the advanced cleaning of wastewater that goes beyond secondary treatment. This process removes nutrients, such as phosphorus and nitrogen, and most residual biological oxygen demand and suspended solids. It can include the removal of inorganic minerals and plant nutrients after primary and secondary treatment. Tertiary treatment is a term given to any type of advanced process that upgrades effluent quality to meet specific reuse requirements. Physio-chemical treatment processes applied to tannery effluent include coagulation, flocculation, ozonation, reverse osmosis, ion exchange, and activated carbon absorption (Song *et al.*, 2004). Coagulation (Arvanitoyammis *et al.*, 1989; Amokrane *et al.*, 1997; Vanukuru *et al.*, 1998; Duan *et al.*, 2002), is probably the most widely used. Application of biological processes to tannery effluent treatment remains complicated principally due to the presence of biologically inhibitory and/or bio-recalcitrant compounds (Carucci *et al.*, 1999). Coagulation and subsequent biological treatment of coagulated tannery effluent has been effective (Song *et al.*, 2004). After physical- chemical treatment activated sludge systems have also been used effectively to remove organic matter and nitrogen (Jochimsen and Jekel, 1997; Jochimsen *et al.*, 1997; Ros and Gantar, 1998). Table 5.1 summarises data from various aerobic systems for tannery effluent treatment.

Table 5.1 Aerobic treatment processes for tannery effluent (Vidal *et al.*, 2004).

Method	Wastewater characteristic				Control Parameter	Removal		Reference
	Type	BOD_5 mgO_2/L	COD mgO_2/L	TKN mg/L	HRT (days)	COD (%)	BOD (%)	
SBR	Total	*ND*	300-1400	50-200	6 cycles	12	*ND*	(Carucci *et al.*, 1999)
AS	unhairing	7081	24089	18	18	*ND*	67	(Menendez and Diaz, 1998)
AS	Total	855-965	1412-1454	311-369	10-38	79-81	97-98	(Ros and Gantar, 1998)
AS	Total	510	880-1212	65.8	12	72-87	*ND*	(Ahn *et al.*, 1996)
AS	Total	*ND*	1000	*ND*	8-48	80.5	*ND*	(Nandy *et al.*, 1993)

AS= activated sludge; SBR = sequencing batch reactor;
HRT=hydraulic retention time; *ND* = not determined

Aerobic treatment is particularly effective for the elimination of readily biodegradable organic matter (Vidal *et al.*, 2004). COD removal efficiencies are reported between 12 and 87%. BOD_5 removal efficiencies range from 67-98%.

Effective control of tannery wastewaters requires regular analysis of process liquors and effluent (UNEP, 1991). The accepted sulphide concentration in discharge effluent is 2mg/L. Tannery effluent containing chromium(III) salts must be treated to reduce the chromium concentration in the final effluent discharge to below the value specified in the discharge consent conditions (Song *et al.*, 2000). The chromium(III) salt concentration of tannery effluent is usually around 2-3g/L, although accepted levels are lower than 1mg/L (Buljan and Bosnic, 1994; Besserer, 1996).

5.5 Reed beds for tannery effluent treatment

Constructed wetlands have been used for tannery effluent treatment, although little published data were available. A body of information and experience has been collected and treatment performance has been reported in the range of 12–87% efficiency for COD removal (Prasad *et al.*, 1991).

5.6 Experimental aims and objectives

5.6.1 Aims

- To characterise the output of primary treated tannery effluent from the British School of Leather Technology.

- To treat primary treated tannery effluent with reed bed technology in the UK.

- To monitor the microbial consortia of the reed bed substrate under conditions of effluent loading.

- To determine the reaction of the microbiological community of the reed beds to the addition of tannery effluent.

5.6.2 Objectives

- To determine the seasonal effectiveness of the UCN experimental reed beds for tertiary treatment of primary treated tannery effluent.

- To relate the degradation of tannery effluent to the changing physical state and microbiology of the reed bed.

5.7 Methodology

The present study of leather processing effluent was conducted at the British School of Leather Technology (BSLT), University College Northampton, UK. The BSLT uses an on-site primary effluent treatment plant which operates through a series of treatment stages. A diagram of the effluent treatment plant can be seen in figure 5.1.

Figure 5.1 BSLT tannery effluent (primary) treatment system.

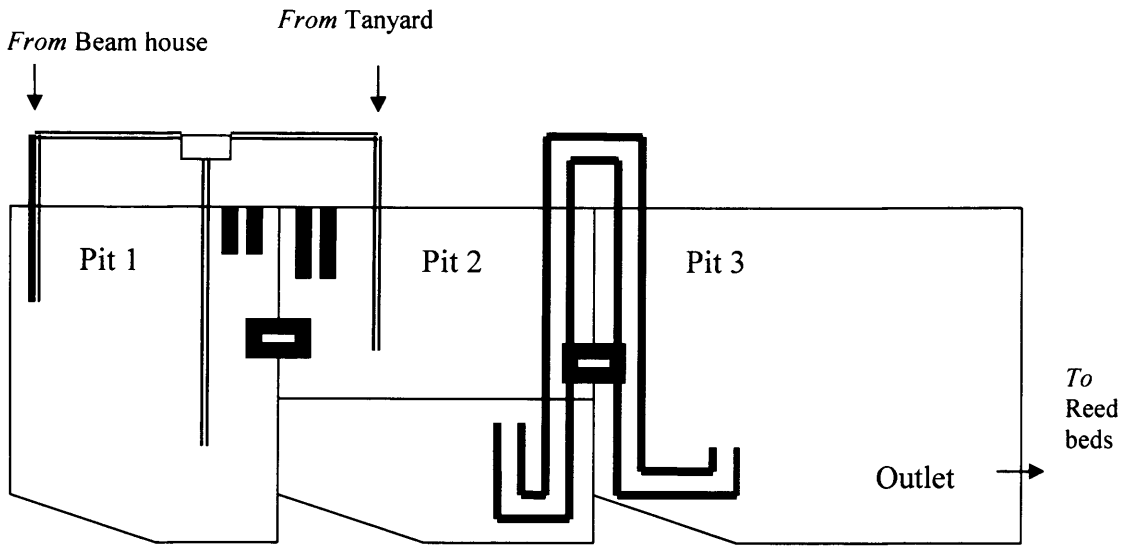


Table 5.2 shows the mean effluent values at each stage of the BSLT primary tannery effluent treatment plant. The outlet wastewater was collected for tertiary treatment with reed beds.

Table 5.2 Effluent parameter in primary effluent treatment system (± SEM)

Parameter (mg/L)	**Pit 1**	**Pit 2**	**Pit 3**	**Outlet** *(to reed beds)*
pH	7.8 ± 0.4	6.9 ± 0.4	6.2 ± 0.4	6.9 ± 0.5
DOS	8891 ± 332	1695 ± 769	1513 ±187	2575 ± 27
DIS	1210 ± 35	1190 ± 768	1227 ± 99	518 ± 51
TDS	10102 ± 363	2775 ± 896	2740 ± 282	3093 ± 261
TKN	209 ± 11	86 ± 25	84 ± 16	87 ± 14
COD(mgO_2/L)	2112 ± 149	664 ± 87	1464 ± 203	880 ± 131
Cl^-	4304 ± 193	1517 ± 184	1229 ± 125	1370 ± 110
SO_4	94 ± 4.4	272 ± 241.2	35.8 ± 5.0	32.1 ± 3.7
EC (mS/mL)	14.1 ± 0.6	5 ± 0.5	4.5 ± 0.5	5.0 ± 0.5

5.8 Experimental investigations

Primary treated tannery effluent was loaded on to the UCN experimental reed beds. Data were collected and analysed to identify points of correlation between tannery effluent treatment and changes in the microbial consortia of the reed bed substrate. The collection of experimental data was carried out over a 12 month period. Investigations were designed to test specific experimental hypotheses. Each investigation was designed to build upon the previous study. Table 5.3 shows an index of the experimental investigations giving title and experiment code for reference throughout the remainder of the chapter.

Table 5.3 Index of experimental investigations

Code	Title	Time of year
TE-1	Tertiary treatment of tannery effluent with reed beds in UK, autumn.	Autumn
TE-2	Tertiary treatment of tannery effluent with reed beds in UK, summer.	Summer
TE-3	Aerobic colony forming units (CFU/g) of the substrate of three reed beds at the 30cm depth horizon during-loading and post-loading of tannery effluent in UK, winter.	Winter

5.9 Tertiary treatment of tannery effluent with reed beds in UK autumn (TE-1)

5.9.1 Introduction

The experiment investigated the efficacy of reed beds for tertiary treatment of primary treated tannery effluent in the UK in autumn conditions. Study of the microbial community of the reed bed did not commence in the initial experiment until treatment efficiency had been established.

5.9.2 Methods

The experimental reed beds at University College Northampton (section 3.0) were used for the described experiment. Reed bed 1 (control) was loaded with water from the Anglia region domestic supply; reed bed 4 and reed bed 5 were loaded with primary treated tannery effluent (section 3.1). Table 5.4 summarises the experimental parameters of the investigation. Table 5.5 shows the mean weather conditions over the experimental period.

Table 5.4 Experimental parameters (TE-1)

	TE-1 Experimental parameters
Experimental period	Day 1 – Day 28
Loading period	Day 1 – Day 28
Effluent type	Reed bed 1 (Control) Reed bed 4 (Tannery effluent) Reed bed 5 (Tannery effluent)
Loading volume	200 litres/day
Effluent sampling	Day 1 – Day 28 (twice per week)
Effluent analysis	pH (section 3.2.8), TKN; NH_3 (section 3.2.12), COD (section 3.2.1)

Table 5.5 Weather conditions over experimental period

Weather (AUTUMN)	Average (± SD)	
Max temp (°C)	Experimental period	9.9 ± 2.4
Min temp (°C)	Experimental period	4.5 ± 2.6
Sunshine (hrs)	Experimental period	1.8 ± 2.3
Rainfall (mm)	Experimental period	2.7 ± 3.7
Relative Humidity (RH)	Experimental period	92
Soil temp 30cm (°C)	Experimental period	8.7 ± 1.0

5.9.3 Experimental aims

- Determine reed bed efficiency in reducing pH, COD, TKN and NH_3 of tannery effluent

- Determine whether the reed beds are significantly different in pollutant removal efficiencies

5.9.4 Effluent treatment results

Analysis of the TKN and NH_3 of the inflow and outflow from the reed beds is presented in table 5.6. The results are also expressed in terms of the treatment performance of each bed as a percentage reduction.

Table 5.6 TKN and NH_3 treatment efficiency (± SD)

Reed bed	TKN (mg/L)			NH_3 (mg/L)		
	Mean IN	Mean OUT	% treatment	Mean IN	Mean OUT	% treatment
1 (control)	0.00 ± 0.0	0.0 ± 0.0	*ND*	0.00± 0.0	0.00 ± 0.0	*ND*
4 (tannery)	1.0 ± 0.0	0.01 ± 0.0	97	0.1 ± 0.0	0.0 ± 0.0	99
5 (tannery)	1.0 ±0.0	0.01 ± 0.0	99	0.1 ± 0.0	0.01 ± 0.00	95

ND = not determined

Independent samples t-test was used for statistical analysis to determine if there was a significant difference in the TKN mean outflow from reed bed 4 and reed bed 5. Analysis showed that the beds were not significantly different (t=0.214;df=4;p=0.561; NS). Table 5.7 shows the mean pH and COD of the inflow and outflow effluent over the duration of the experimental period.

Table 5.7 pH and COD treatment efficiency (± SD)

Reed bed	pH		COD (mg O_2/L)		
	Mean IN	Mean OUT	Mean IN	Mean OUT	% treatment
1 (control)	7.1 ± 0.0	6.2 ± 0.0	63 ± 21	12 ± 3	*ND*
4 (tannery)	7.8 ± 0.1	6.8 ± 0.1	707 ± 153	304 ± 156	57
5 (tannery)	7.9 ± 0.1	6.7 ± 0.1	1838 ± 86	388 ± 346	79

5.9.5 Summary of (TE-1) results

- Reed bed 4 and reed bed 5 were effective in reducing the COD, TKN and NH_3 of the tannery effluent. COD treatment ranged from 57-79% reduction, TKN ranged from 97-99%, and NH_3 ranged from 95-99%

- All three reed beds balanced the pH of the effluent to a level acceptable for discharge.

- Reed bed 4 and 5 were not significantly different in treatment efficiency.

5.10 Tertiary treatment of tannery effluent with reed beds in UK summer (TE-2)

5.10.1 Methods

Reed bed 1 (control) was loaded with water from the Anglia region domestic supply; reed bed 4 and reed bed 5 were loaded with primary treated tannery effluent. Table 5.8 summarises the experimental parameters of the investigation. Table 5.9 shows the mean weather conditions over the experimental period.

Table 5.8 Experimental parameters (TE-2)

	TE-2 Experimental parameters
Experimental period	Day 1 – Day 14
Loading period	Day 1 – Day 14
Effluent type	Reed bed 1 (Control) Reed bed 4 (Tannery effluent) Reed bed 5 (Tannery effluent)
Loading volume	200 litres/day
Effluent sampling	Day 1 – Day 28 (once per week)
Effluent analysis	pH (section 3.2.8), TKN, NH_3 (section 3.2.12), COD (section 3.2.8)

Table 5.9 Weather conditions over experimental period

Weather (SUMMER)	**Average (± SD)**	
Max temp (°C)	Experimental period	22.3 ± 3.7
Min temp (°C)	Experimental period	12.1 ± 2.3
Sunshine (hrs)	Experimental period	7.0 ± 4.6
Rainfall (mm)	Experimental period	0.2 ± 0.5
Relative Humidity (RH)	Experimental period	74
Soil temp 30cm (°C)	Experimental period	16.8 ± 1.2

5.10.2 Effluent treatment results

Inflow and outflow effluent analysis of the reed beds for TKN, NH_3 are presented in table 5.10. Table 5.11 shows the mean outflow pH and COD over the duration of the experimental period.

Table 5.10 TKN and NH_3 treatment efficiency (± SD)

Reed Bed	TKN (mg/L)			NH_3 (mg/L)		
	Mean IN	Mean OUT	% treatment	Mean IN	Mean OUT	% treatment
1 (control)	0.01 ± 0.00	0.01 ± 0.01	*ND*	0.01± 0.0	0.01 ± 0.01	*ND*
4 (tannery)	0.05 ± 0.01	0.01 ± 0.00	80	0.1 ± 0.01	0.02 ± 0.00	66
5 (tannery)	0.03 ± 0.03	0.01 ± 0.00	67	0.1 ± 0.01	0.01 ± 0.00	83

Independent samples t-tests were used to determine if there was a significant difference in the mean outflow from reed bed 4 and reed bed 5. Analysis showed that the outflow from the reed beds was not significantly different (t=-1.24;df=4;p=0.3;NS).

Table 5.11 pH and COD treatment efficiency (± SD)

Reed bed	pH		COD (mgO_2/L)		
	Mean IN	Mean OUT	Mean IN	Mean OUT	% treatment
1 (control)	7.5 ± 0.5	6.4 ± 0.9	49 ± 24	61 ± 34	*ND*
4 (tannery)	6.8 ± 0.4	6.9 ± 0.4	108 ± 36	33 ± 18	69
5 (tannery)	5.6 ± 1.0	7.1 ± 0.3	419 ± 252	54 ± 29	87

5.10.3 Summary (TE-2) results

- Both reed bed 4 and 5 were effective in reducing the COD of the tannery effluent.

- Reed bed 4 (69%) and reed bed 5 (87%) were not significantly different in COD percentage reduction.

- Reed bed 4 (80%) and reed bed 5 (67%) were not significantly different in TKN percentage reduction.

- All three reed beds balanced the pH of the effluent to a level acceptable for discharge.

- The weather conditions are different in this experiment from that in (TE-1). In (TE-2) the weather was warmer and also there was less rainfall.

- The COD of the tannery effluent loaded to reed bed 4 and 5 in experiment (TE-2) was similar to the COD load of the outflow from experiment (TE-1). In (TE-1) a 57 and 79% treatment percentage was achieved, reducing COD from 1838 to 388. The inflow for bed 5 in TE-2 was of a similar magnitude in terms of COD load as the effluent from bed 5 in TE-1. In TE-2 the COD load was reduced by another 87% reducing it to COD 54.

- The results suggest that the reed beds were effective in reducing the COD of the tannery effluent. The results do not show whether the reduction is of BOD or COD as BOD would be included in the COD measure. It is likely that the reduction seen is of readily biologically oxidisable BOD – and the perhaps a proportion of the remaining undegraded COD is recalcitrant COD.

5.11 Aerobic counts of the 30cm depth horizon of three reed beds during-loading and post-loading of tannery effluent in UK winter (TE-3)

5.11.1 Methods

Table 5.12 summarises the experimental parameters of the investigation. Table 5.13 shows the mean weather conditions over the experimental period.

Table 5.12 Experimental parameters (TE-3)

	TE-3 Experimental parameters
Experimental period	Day 1 – Day 21
Loading period	Day 1 – Day 15
Post-loading	Day 16 – Day 21
Effluent type	Reed bed 1 (Control) Reed bed 4 (Tannery effluent) Reed bed 5 (Tannery effluent)
Loading volume	200 litres/day
Effluent sampling	Day 1 – Day 21 (twice per week)
Soil sampling	Day 10 and Day 20
Effluent analysis	pH (section 3.2.8), TKN, NH3 (section 3.2.12), COD (section 3.2.1).
Soil analysis	Total aerobic colony forming unit counts (section 3.4.1)

5.11.2 Experimental aims

- Determine reed bed efficiency in reducing pH, COD, TKN and NH_3.
- Determine the CFU/g of the 30cm horizon of the reed bed substrate of the reed beds during effluent loading.
- Determine the CFU/g of the 30cm horizon of the reed bed substrate of the reed beds post-loading.

Table 5.13 Weather conditions over experimental period

Weather (WINTER)	Average (± SD)	
Max temp (°C)	Experimental period	6.5 ± 2.1
	Loading period	6.1 ± 2.0
	Post-loading period	7.4 ± 2.5
Min temp (°C)	Experimental period	2.7 ± 1.5
	Loading period	2.7 ± 1.7
	Post-loading period	2.7 ± 0.9
Sunshine (hrs)	Experimental period	3.7 ± 3.4
	Loading period	3.6 ± 3.4
	Post-loading period	4.0 ± 3.4
Rainfall (mm)	Experimental period	0.3 ± 0.7
	Loading period	0.4 ± 0.8
	Post-loading period	0.1 ± 0.2
Relative Humidity (RH)	Experimental period	92
	Loading period	92
	Post-loading period	92
Soil temp 30cm (°C)	Experimental period	5.5 ± 1.3
	Loading period	6.1 ± 1.1
	Post-loading period	4.1 ± 0.3

5.11.3 Effluent treatment results

Table 5.14 shows the TKN and NH_3 treatment performance of the reed beds. The reed beds were effective in reducing the TKN and NH_3 of the tannery effluent. Table 5.15 shows the pH and COD of the outflow from the reed beds over the duration of the experimental period. Reed bed 4 and 5 were not significantly different and were both effective in reducing the COD of the tannery effluent by 53%. The reed beds balanced the pH of the effluent to a level acceptable for discharge.

Table 5.14 TKN and NH_3 treatment efficiency (± SD)

Reed bed	TKN (mg/L)			NH_3 (mg/L)		
	Mean IN	Mean OUT	% treatment	Mean IN	Mean OUT	% treatment
1 (control)	0.0 ± 0.0	0.0 ± 0.0	*ND*	0.0± 0.0	0.0 ± 0.0	*ND*
4 (tannery)	0.1 ± 0.0	0.0 ± 0.0	67	0.1 ± 0.0	0.0 ± 0.0	86
5 (tannery)	0.1 ± 0.0	0.0 ± 0.0	67	0.1 ± 0.0	0.0 ± 0.0	86

Table 5.15 pH and COD treatment efficiency (± SD)

Reed bed	pH		COD (mgO_2/L)		
	Mean IN	Mean OUT	Mean IN	Mean OUT	% treatment
1 (control)	7.1 ± 0.0	6.2 ± 0.0	63 ± 21	12 ± 3	*ND*
4 (tannery)	7.2 ± 0.1	6.6 ± 0.4	657 ± 59	307 ± 59	53
5 (tannery)	7.5 ± 0.0	6.8 ± 0.0	1524 ± 0	724 ± 554	53

5.11.4 Microbiology (CFU) results

The aerobic CFU/g of the substrate of the three reed beds was determined for the duration of the experimental period, both during-loading and post-loading. The results are shown in table 5.16. Anaerobic assessment of the substrate microorganisms was attempted in each experimental investigation however in most assays there was a failure to successfully and reliably culture anaerobes under laboratory conditions. This was probably due to transient exposure to oxygen concentrations during the experimental sampling procedure or during sample preparation.

Table 5.16 Aerobic CFU/g ($log_{(10)}$) of 30cm depth horizon of reed beds (± SEM)

Day of experiment	Reed bed 1 (control)	Reed bed 4 (tannery)	Reed bed 5 (tannery)
10 (During-loading)	9.9 ± 0.3	10.1 ± 0.1	*ND*
20 (Post loading)	9.5 ± 0.1	9.6 ± 0.1	*ND*

ND=not determined

Independent sample t-tests were used to compare the CFU/g of the three reed beds. The results show that the CFU/g of reed bed 1 (control) (mean 9.91 ± 0.27 SEM) and reed bed 4 (mean 10.07 ± 0.12 SEM) was not significantly different in the during-loading period (t=-0.490;df=19;p=0.630;NS). Similarly in the post-loading period reed bed 1 (control) (mean 9.49 ± 0.069 SEM) and reed bed 4 (mean 9.57 ± 0.083 SEM) were not significantly different (t=-0.750;df=34;p=0.458;NS). An independent sample t-test was used to compare reed bed 1 (control) during-loading (mean 9.91 ± 0.27 SEM) and post-loading (9.49 ± 0.069 SEM). The CFU/g was not significantly different in the during-loading and post-loading period (t=1.765;df=28;p=0.088;NS). Independent samples t-test was used to compare reed bed 4 in the during-loading period (10.07 ± 0.36 SD ± 0.12 SEM) and the post-loading period (9.57 ± 0.35 SD ± 0.08 SEM). There was a significant decrease in CFU/g in the post-loading period (t=3.47;df=25;p=0.002;S).

5.11.5 Summary of (TE-3) results

- There was no significant difference in the aerobic CFU/g of the reed beds during-loading.
- There was a significant decrease in CFU/g in reed bed 4 post-loading.
- There was not a significant post-loading decrease in CFU/g in reed bed 1 (control).

5.12 Effect of seasonality on tannery effluent treatment with reed beds in UK.

5.12.1 Introduction

The experimental data from TE-1, TE-2 and TE-3 were used to investigate how seasonality in a temperate climate such as in the UK may affect the treatment performance of reed beds.

5.12.2 Methods

Reed bed 1 was loaded with water from the Anglia region domestic supply; reed bed 4 and reed bed 5 were loaded with primary treated tannery effluent (section 3.1).

5.12.3 Effluent treatment results

Figure 5.2 shows the percentage removal efficiencies for TKN, NH_3 and COD under autumn, summer and winter seasonal environmental conditions.

Figure 5.2 Effect of seasonality on tannery effluent treatment (removal efficiency %).

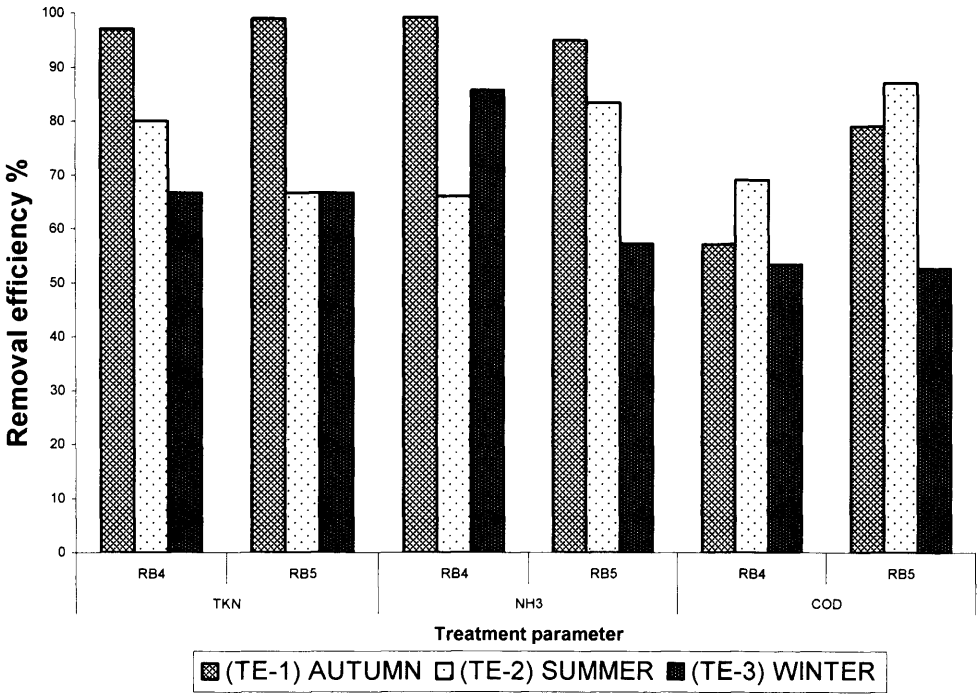


Figure 5.2 shows that COD reduction was most efficient in both reed beds 4 (69%) and 5 (87%) in the summer (TE-2). COD reduction under winter weather conditions was less effective (TE-3) than in summer.

However, considering the variability of the tannery influent that was treated, it was useful to interpret the data in terms of the amount of COD that was removed, rather than the treatment efficiency of the reed beds. Table 5.17 interprets the data to show that the treatment efficiency of the reed beds was greatest during the summer, but that the COD load decrease was actually greatest in autumn and winter. This was because the COD of the influent used in the experiments in the summer months was lower than the influent tannery wastewaters used in the autumn and winter.

Table 5.17 COD treatment of tannery effluent in reed beds in seasonal conditions

Experiment	Season	Reed bed	Influent COD (mgO_2/L)	Effluent COD (mgO_2/L)	Treatment efficiency (%)	COD load decrease (mgO_2/L)
(TE-1)	Autumn	4	707	304	57	403
(TE-1)	Autumn	5	1838	388	79	1450**
(TE-2)	Summer	4	108	33	69*	75
(TE-2)	Summer	5	419	54	87*	365
(TE-3)	Winter	4	657	307	53	350
(TE-3)	Winter	5	1524	724	53	800**

* *Treatment efficiency greatest*
* * *Greatest COD decrease*

5.12.4 Discussion

Modernisation of the leather industry and investments into environmental protection, waste reduction, recycling, and recuperation of secondary raw material is important in reducing the environmental implications of leather production. Cleaner technologies (Johnston, 1995) and process modification can reduce the contributions of conventional processes to mixed tannery wastewater so that it can be effectively treated on-site using a primary treatment process followed by a constructed wetland system.

Cleaner production (Hilson, 2003) is already used to reduce the toxicity of the wastewaters from the British School of Leather Technology. Modification to beamhouse operations, such as de-hairing by shaving before chemical un-hairing, has reduced the significant contribution that this stage in processing makes to the relative pollution of the resultant mixed tannery wastewater. Degraded keratin often constitutes the largest contribution to the BOD of mixed tannery wastewater (Bajza and Vrcek, 2001), so by removing the hair before processing, a significant contribution to the overall water pollution is avoided.

The tannery industry, with its distinct waste streams also lends itself to benefit from the application of the principles of industrial ecology. In a situation of industrial ecology waste streams generated by two polluting industries, such as mining and leather tanning, can be combined or treated in synchrony so as to lead to a lower environmental impact when compared to separate management of their wastes (Stoop, 2003; Giannetti *et al.*, 2004).

The use of constructed wetland technology in an integrated wastewater treatment system has been shown to effectively treat tannery effluent on-site. In the case of the BSLT constructed wetland treatment of the primary treated effluent might reduce the cost of conventional wastewater treatment in a municipal facility. The BSLT are investigating the use of the UCN reed beds for on-site tertiary treatment of tannery effluent, and the findings of this investigation show that the constructed wetland treatment performance could be adequate and sustainable throughout the year.

The treatment of tannery wastewater under various seasonal climate conditions investigated in the series of experiments showed that the reed beds were able to function throughout the year. The experiments found that the highest percentage decrease for TKN and NH_3 was in autumn in experiment (TE-1) ranging from 97%-99%. The lowest percentage treatment for TKN and NH_3 was in winter in (TE-3) when it was 66%. COD decrease ranged from 53% in winter in (TE-3) to 87% in summer in (TE-2). Allen *et al.*, (2002) showed that plant species selection was particularly important in oxygen transport to the substrate in cold conditions. The reason the TKN treatment efficiency of the reed bed was reduced in the winter may have been because *Phragmites* has limited

ability to transport oxygen to the roots during senescence. Species apparent ability to increase root oxygen supply.

Variations in COD treatment efficiency that were observed may have been related to the biomass of the reed bed becoming adapted to the characteristics of the tannery effluent. This would occur in terms of the successive adaptation of the microorganisms in the substrate, until the community was adapted to use the tannery effluent as a nutrient source for biomass accumulation.

Mutations arise in bacterial populations, some are induced and some are spontaneous. Bacteria have a rapid growth rate and so the pressure of selective advantage enriches for mutants within a population. There are three major natural strategies involved in the spontaneous generation of genetic variants in bacteria. These can be small changes in the nucleotide sequence of the genome; intragenomic reshuffling of segments of genome sequences; and the acquisition of DNA sequences from other organisms (Aber, 2000). In bacteria gene transfer is unidirectional, only occurring from donor to recipient, a portion of the DNA is transferred. Gene transfer can occur not only between bacteria of the same species but also between species. Factors that affect gene transfer include the competence of the recipient (*Bacillus, Streptococcus)* and the induced competence affected by the gene transfer. The three mechanisms of gene transfer in bacteria have been identified: transduction; transformation; and conjugation (Dale, 1994).

The acquisition of new genes by horizontal gene transfer gives bacteria the ability to exploit new environments and respond to selective pressure (Davison, 1999). Most mutations are detrimental and so are selected against but some survive. Most mutations are incorporated permanently with neutral effect to the organism. However, sometimes mutations occur that improve the function of a gene and/or the protein encoded by it. Sometimes mutations occur that originally appear harmful but subsequently turn out to be beneficial under new environmental conditions (Clarke and Russell, 1997).

Genetic information can be passed horizontally, for example, in the case of antibiotic resistance. This can be carried on plasmids that can be passed between unrelated types of bacteria. Genes carried on plasmids can be incorporated into the chromosome, a gene may easily move from one organism to an unrelated one. Microbial variability due to

switching of gene expression modulated by activation of silent genes as a consequence of genetic rearrangements also contributes to microbial evolution in which major changes are induced very rapidly (Hacker and Kaper, 2000). The environment can influence this speed of evolution, and bacterial species take successful advantage of this mode of high-speed evolution to gain selective advantage. The natural transfer of genetic material between bacteria in the environment is necessary for the generation of genetic diversity and provides the raw material of natural selection and evolution.

The fact that bacteria evolve over relatively short periods of time and are subject to genetic variation through mutation means that the population can quickly change and respond to changing environmental conditions. The microbial community within a constructed wetland can become adapted to the conditions of the wastewater that the wetland is used to treat.

Other factors that may have affected the results of the experiment described are that in the winter there may be an influx of nutrients and organic matter to the reed bed from the decomposition of plant litter. In summer there would have been increased plant growth, and elevated soil temperatures.

The COD of the tannery effluent loaded to reed bed 4 and 5 in experiment (TE-2) was similar to the COD load of the outflow from experiment (TE-1). In (TE-1) 57% and 79% treatment percentage was achieved. In actuality the reed beds decreased the COD of the influent from 1838mg/L to 388mg/L. The influent for bed 5 in (TE-2) was of a similar magnitude in terms of COD load as the effluent from bed 5 in (TE-1). In (TE-2) the COD load was decreased by 87% to COD 54mg/L. The results demonstrate that in the autumn experimental period (TE-1), in rb5, the COD load of effluent was decreased by 1450(mgO_2/L). In the summer experimental period in the same reed bed (TE-2), the COD load of effluent was decreased by 365(mgO_2/L) and in the winter (TE-3) by 800(mgO_2/L). The results suggest that the seasonal conditions of the autumn provided most effective COD treatment. Similarly the results for reed bed 4 showed the same pattern (Table 5.17). In the autumn experiment (TE-1) in reed bed 4 the COD load of the effluent was decreased by 403(mgO_2/L). In the summer experiment (TE-2) in reed bed 4 the COD load of the effluent was decreased by 75(mgO_2/L). In the winter

experiment in (TE-3) in reed bed 4 the COD load of the effluent was decreased by 350(mgO_2/L).

The results showed that the treatment of COD in autumn (TE-1) and winter (TE-3) was more effective in terms of the quantity of COD that was removed than in the summer (TE-2) (Table 5.17). The effects of reduced efficiency in COD reduction during the summer months may be exacerbated by high temperatures and low humidity that evaporate the effluent and concentrate the pollutants. In countries with high temperatures and relatively low humidity it is important that reed beds for tannery effluent treatment are designed with a hydraulic loading regime that will maximise treatment while minimising evapotranspiration and water loss from the system. This could be achieved with an unsaturated infiltration zone at the surface of the reed bed. The unsaturated zone should be highly permeable and sufficient to receive influent loading volume, quickly moving the wastewater away from the surface of the reed bed, into the saturated treatment horizon at depth within the substrate. Otherwise effluent that remains on the surface of the reed bed for a length of time would be susceptible to evaporation.

Constructed wetlands represent a sustainable technology for onsite tannery effluent treatment. The treatment of contaminated wastewater through wetland areas is an historical concept which has been practiced, with varying degrees of design intervention, for many centuries (Brix, 1994b). Over recent decades the understanding of the scientific basis of the complex processes operating within a constructed wetland has evolved, so that constructed wetlands can be designed and engineered for the specific purpose of the wastewater for which they are intended (Girts and Knight, 1989; Pastor *et al.,* 2003). Reed beds can be engineered to harness the desired biologically mediated treatment of natural wetlands, to effectively mimic the appropriate aspects of the natural biological and geochemically mediated assimilative capacity. Design experience has developed constructed wetland technology to a point where the wetland ecosystem can be manipulated to optimally treat specific wastewater contaminants. Reed beds can effectively be engineered so that the biological transformations and geochemical reactions that are promoted in the wetland can be skilfully managed through design and operational intervention. This allows optimisation of the basic

constructed wetland and allows the technology to be applied to numerous distinct wastewater sources.

The hydraulic flow path of wastewater as it percolates through the reed bed substrate forms the basis of the treatment method. The residence time of the wastewater within the treatment horizons of the wetland can be controlled through hydraulic control of the saturation level. Aerobic and anaerobic conditions in the sediment encourage aerobic or anaerobic microorganisms to proliferate, which promotes the associated transformation pathways and processes that those organisms facilitate. Processes that occur in the aerobic horizon of the reed bed in the presence of the plant root such as nitrification and denitrification. At depth within the sediment, beyond the infiltration of the plant roots, sulphate reduction and oxidation occurs. Distinct microbial populations regulate these processes and those populations can be encouraged by creating the appropriate conditions within horizons of the reed bed substrate. Redox potentials in the reed bed are an effect of the depth of the infiltrating plant roots, and the control of the substrate saturation depth. The treated effluent that emerges from the reed bed system has a reduced pollutant load according to the treatment process that have impacted its transit through the reed bed matrix.

The construction materials used in building a constructed wetland can be varied and fit to the purpose of the wastewater characteristics that the reed bed is designed to treat. The selection of substrate material, such as the type of soil, sand or gravel, affects the hydraulic conductivity of the reed bed, and therefore the rate at which the wastewater can infiltrate the bed and permeate through the sediment. The type of soil, the size of particulate material, and the porosity influence residence time and infiltration rate. The percentage of clay, silt and sand of the soils convey properties to the substrate matrix. This can be in supporting the microbial biomass, the potential for substrate adsorption, and anion exchange capacity, and in maintaining hydraulic conductivity in the substrate.

Hydraulic conductivity and flow velocity are important factors in reed bed design because they affect the passage of wastewater through the reed bed and therefore are instrumental in determining the contact time between the wastewater and the treatment mechanism within the reed bed that will remove or reduce the pollutant load. Such as, the time of contact between the wastewater and the nitrifying and denitrifying microbial

community in the aerobic saturated zone of the wetland. This determines the rate and extent to which nitrification and denitrification in the aerobic zone can occur. The hydraulic factors control the time that the effluent will remain in the desired treatment horizon, and ensure that that amount of time is sufficient for the necessary functional processes to occur. The substrate effectively supports the biomass, providing large surface area for attached microbial growth to support a biomass of sufficient size.

The lower depth horizons of the reed bed could be engineered to promote sulphate-reduction and metal adsorption. This can be achieved through hydraulic design of a saturation zone where anaerobic conditions are maintained. Reed beds designed for optimal sulphur-reduction can be designed deeper (2.0m) than nitrifying reed beds (0.60m). A substrate from a location with reducing saline conditions, such as river dredging, could also be used to amend the reed bed substrate during construction, so that both the plants and the microorganisms are pre-adapted to the influent conditions (King *et al.,* (2002). Chromate-tolerant bacterial strains could be seeded into the substrate such as those chromate-tolerant bacteria strains that have been isolated from tannery effluent treatment wetlands (Verma *et al.,* 2001). Seeding reed beds with microorganisms is already practiced in commercial application of the technology in some instances (Lorah and Voytek, 2004). Scientific basis for the types and proportions of microbial communities that should be used could be valid in increasing the reliability of reed beds, perhaps even reducing the length of time taken for the reed bed to establish itself and be ready for the first leachate application. Reed beds are can be loaded gradually over a period of time that allows the microbial community to become accustomed to the toxicity of the effluent (Benitez *et al.,* 2004).

The selection of macrophyte is vital to the success of the reed bed system because if the plants are to convey the oxygen releasing functions in the substrate and to enhance the permeability of the soils in the long term, through the channels in which the roots grow into the soil, then the plants must be adapted to the conditions that the effluent create. Mixed flora in a reed bed can be useful because different plant species have different abilities / qualities that are useful to the designed reed bed. However community and population succession and competition may mean that a single plant community thrives over the others, and may out-compete the less well adapted plant species for light and

space. In the case of tannery effluent treatment the plants used must be able to tolerate the salinity of the effluent, and/ or be generally robust. *Phragmites* was shown in the experiments to be sufficiently robust to tolerate the primary treated tannery effluent from the BSLT that was used. If raw tannery effluent were to have been used *Phragmites* may not have been able to tolerate the salinity and toxicity. Some macrophyte plant species have active salinity control mechanisms (Rout and Shaw, 2001) such as plasticity to changing environmental conditions and high productivity (Kern and Idler, 1999) and can survive under saline conditions (Howard and Rafferty, 2005; Naidoo and Kift, 2006). Salinity can impair the plant through osmotic stress and ionic toxicity (Lissner *et al.*, 1999). Problems associated with tannery effluent treatment in constructed wetlands in terms of high salt concentrations can be overcome with the selection of appropriate halotolerant plant species. Saline tolerant species such as *Spartina, Elytrigia, Juncus, Puccinellia* can be used. Previous research has shown that a number of halotolerant species are more salt tolerant than others, such as *Typha augustifolia* (Cattail) and *Digitaria* species (Asia crabgrass) (Klomjek and Nitisoravut, 2005).

The experiment showed that the reed beds were effective in reducing the pollutant load of the primary treated mixed tannery effluent in terms of COD. However, the results do not show whether the reduction was of BOD or COD, as BOD would be included in the COD measurement. It is probable that a large part of the measured reduction that was seen is of readily biologically oxidisable BOD, and that perhaps a proportion of the remaining un-degraded COD is recalcitrant. Recalcitrant COD possibly could not be effectively reduced by biological means within the reed bed and so a further treatment step may be necessary to improve water quality below the threshold level of recalcitrant COD that remains in the treated tannery effluent. The recalcitrant COD load of the tannery effluent would probably be related to the efficiency of the tannery operations so this could potentially be minimised by effective process modification in the beamhouse and tanyard.

Important factors to consider in designing a reed bed for tannery effluent treatment is effective control of an unsaturated zone for rapid effluent infiltration so that the tannery effluent, which likely has a strong unpleasant odour due to the BOD, is rapidly removed from the surface of the reed bed into the sediment. The substrate should have available

open pore space and flow velocity to ensure that the reed bed can cope with the volume of effluent and the required residence time for treatment can be accommodated without overflow and flooding.

An aerobic saturated zone, with well developed plant roots for oxygen transport to the sediment should be maintained for nitrification and denitrification to occur. The plants selected for the reed bed must be tolerant to the conditions or they will not thrive and will therefore not fulfil their function in the design. Plants used to treat tannery effluent must be tolerant of saline conditions or be able to actively control the salinity of their environment through adaptive mechanisms (Lissner *et al.*, 1997; Lissner *et al.*, 1999; Fogli *et al.*, 2002; Klomjek *et al.*, 2005). The reed bed should be designed to maintain an anaerobic saturated zone in which metal precipitation, and mineral adsorption / complexation can occur (Woulds and Bryne, 2004).

The experiments in this chapter showed that tannery effluent can be treated and, in this case, using the generic UCN reed beds, treatment was achieved as a percentage reduction of the influent in a range of 53-87%. The final effluent was easily and reliably treated to acceptable limits for discharge to sewer. This could reduce the cost of effluent treatment in the UK where water charges are applied per polluted volume by water companies and sewage treatment works.

In developing countries a simple vertical flow constructed wetland with an overflow controlled saturation zone could be designed. The system could use appropriately adapted and tolerant plant species (Haberl, 1999; Gopal, 1999). Aerobic, anaerobic microbial processes of treatment could be facilitated in various treatment horizons at depth within the substrate. Waste water components that could be effectively reduced with reed beds include, TKN, pH, COD and metals, to comply with discharge consent treatment objectives. Tannery effluent could be treated to comply with surface water discharge if the necessary stages of reed beds were added for sufficient nitrification and BOD and COD removal.

Reed beds also have the added benefits of being low cost, low maintenance; have minimal mechanical operations that could fail, usually being loaded with either gravity or a fixed static pump. The on-going operational and maintenance costs are reduced

compared to alternative treatment technologies. Reed beds have low energy requirements due to them not requiring chemical additives, such as is necessary in coagulation or flocculation processes, or electricity or heat such as is needed for sequencing batch reactors or electrochemical oxidation treatment. Reed beds also provide habitat for wildlife, are aesthetically pleasing and convey ecological benefits to the surrounding environment.

CHAPTER SIX
REED BED TREATMENT OF FACTORY AND DOMESTIC EFFLUENT IN GHANA

6.0 Introduction

Sustainable biological waste treatment technology was applied to a commercial company in West Africa. A reed bed wastewater treatment system was built to treat spent-fruit-processing water and human sewage. The treatment outcomes were monitored with reflection upon Environmental Protection Agency (EPA) objectives.

6.1 The project company

The project company is based in the UK and supplies fresh-cut tropical fruits and juice to a number of major European food retailers. The fruit products are processed and packaged in factories in Africa and are then air-freighted to Europe for distribution. The food and beverage industries in general are major contributors to wastewater discharge. Large volumes of spent process-water are generated in different stages of production and are then usually mixed on-site, in equalisation tanks, prior to discharge to municipal works (Noronha, 2002). However, in rural Ghana municipal wastewater treatment facilities are not available and so on-site effluent treatment is needed.

The factory under study uses a considerable amount of water on a daily basis, estimated to be in the region of 200 – 500m^3/day. This is extracted from subsurface water reserves by means of two 100m deep boreholes. The spent process-water (COD 1000–3000mgO_2/L) requires treatment prior to discharge into to a nearby water course. When the factory was first visited the existing situation for wastewater treatment was a series of stabilisation ponds that were not operating correctly, and were therefore not adequately treating the wastewater. Treatment objectives stipulated by the Ghanaian EPA discharge consent are outlined in table 6.1.

Table 6.1 Ghanaian EPA discharge consent

Parameter	EPA Discharge Consent
Biological Oxygen Demand (BOD_5)	Less than 50mg/L
Total Suspended Solids (TSS)	Less than 50mg/L
pH	6-9
Temperature	<29°C
Electrolytic Conductivity (EC)	500 – 1500µS/L

To monitor the interface of the company operations with the environment, an Environmental Management System (EMS), promoting sustainability in waste treatment and the reuse of waste products, was implemented at the factory in Ghana. The EMS highlighted the environmental aspects of the factory operations and was used to monitor environmental performance, whilst enabling control and mitigation of potential environmental problems. The waste streams from the factory were categorised (table 6.2) for the purposes of the EMS which enabled continual internal monitoring of compliance with the stipulations of the EPA, and other internationally recognized standards for waste treatment and disposal.

Table 6.2 Categorisations of waste streams

Waste category	Waste composition
Factory effluent	Spent process water
Domestic effluent	Human sewage and shower water
Organic fruit residue	Fruit skins, pineapple crowns, mango stones
Plastic (recyclable* and non-recyclable)	Aprons*, sleeves, gloves
Combustible (potential compost)	Paper, cardboard and paper towels
Non-combustible waste	Metal and engineering waste
Hazardous waste	Chemicals, engineering waste

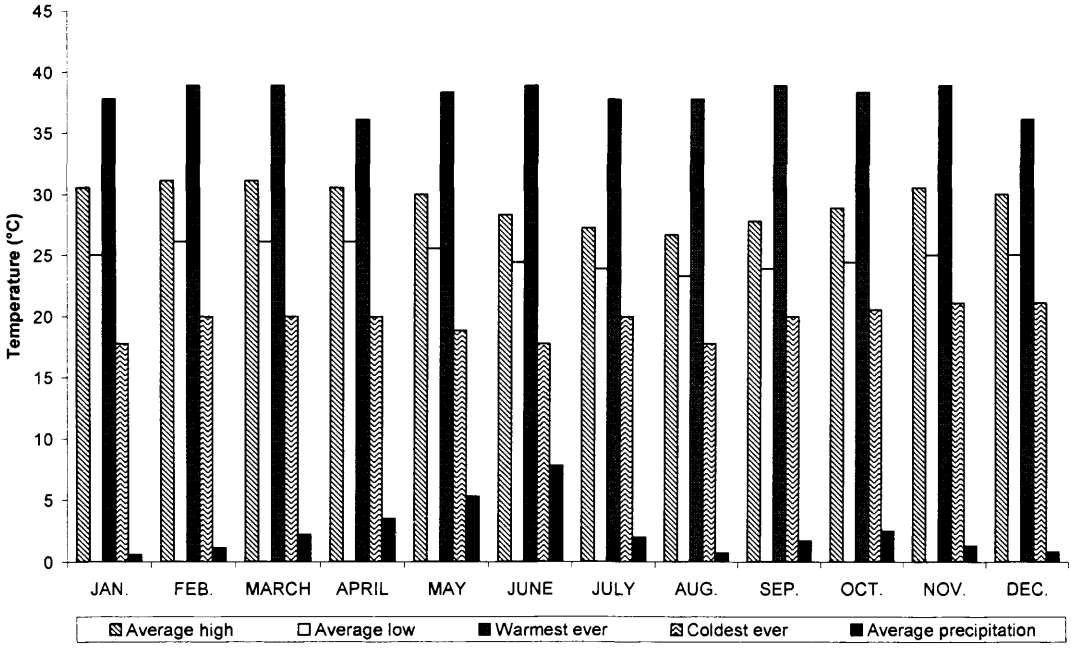
Wastewater from the factory site was of two distinct types; factory effluent and domestic effluent (table 6.2). The factory effluent from the fruit processing operations was extremely variable in terms of COD, BOD_5, TSS, EC and pH (ranging from pH 3.5 to pH 10 throughout the day). This variability in effluent composition was due to the fruit juice content, the chemicals used in processing the fruit, and the detergents, sanitizers and disinfectants used in the strict cleaning regime of the factory. The second effluent type was a mixture of human sewage and washing water from the toilets and showers. The human sewage and shower water were mixed at source are subsequently be referred to as domestic effluent.

6.2 Environmental conditions in Ghana

The country of Ghana is situated on the south coast of West Africa, bordering Togo to the east, Côte D'Ivoire to the west and Burkina Faso to the North. The project company

is located in the south of the country near the capital Accra. The weather conditions in Ghana are sub-tropical and humid for much of the year. Temperatures vary with season and elevation within the country. The south west is generally hot and humid. Two rainy seasons occur from April to July and from September to November, except in the north. The annual rainfall ranges from about 1100mm in the north to 2100mm in the southeast. The *"Harmattan"* is a dry desert wind that blows from the northeast from December to March, lowering the humidity and creating hot days and cool nights. In the south, the effects of the *Harmattan* are usually felt in January. Figure 6.1 shows average weather conditions in Accra. In most areas the highest temperatures occur in March and the lowest in August. The temperature is coolest from June to September when the main rain fall occurs.

Figure 6.1 Average weather conditions in Accra



(Source data: http://www.ghanaweb.com/GhanaHomePage/geography/climate.php.)

6.3 Methods

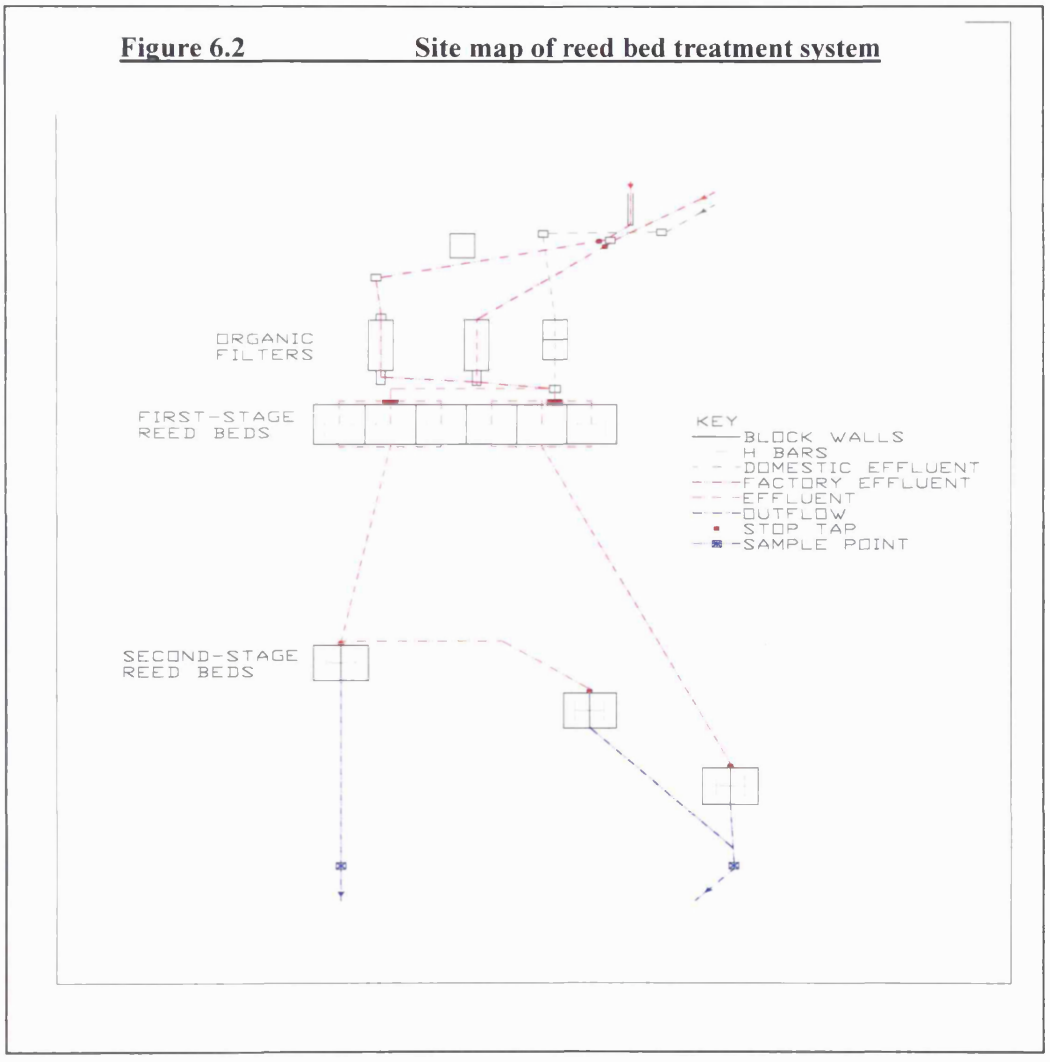
The effluent treatment performance of the project company was monitored for 4 years, between 2000 and 2004. A reed bed treatment system was built on the factory site in 2001. Reed bed effluent samples were collected from the reed beds every 30 days and analysed by an accredited laboratory in Ghana. Duplicate samples were sent to an

independent accredited laboratory to verify the accuracy of the analysis. 98% of samples were reliable to within 5%. When a discrepancy in the results occurred a mean value was recorded in four cases.

6.3.1 Construction of the reed bed wastewater treatment system

The reed bed system was designed to treat the domestic and factory effluent separately. This was to enable the reuse of treated factory effluent for low risk cleaning activities such as cleaning vehicles, and the reuse of domestic effluent for irrigation of non-edible trees and shrubs. The construction of the reed bed system was carried out using locally available labour and materials. Existing masonry from the stabilisation ponds was incorporated in the design of the new reed bed system in the second-stage reed beds (figure 6.2).

Figure 6.2 Site map of reed bed treatment system



The treatment steps involved in the new reed bed system design were; organic filters to separate solid waste from liquid waste; first-stage vertical-flow reed beds for primary effluent treatment; and second-stage vertical-flow reed beds for tertiary effluent treatment (figure 6.2). When the reed beds were built and commissioned in May 2001 only one of the organic-filters, three of the first-stage reed beds and one of the second-stage reed beds were completed. The wastewater streams were therefore not separated at this time, and both factory and domestic effluent continued to be treated together as they had been in the stabilization pond system. In this way the wastewater treatment system was built piece by piece, further stages were added as financial constraints allowed.

Organic filters

The organic filters were the first stage in the wastewater treatment system. Their function was to physically separate the solid material from the effluent. Wood-chips were used to filter the effluent, and also to provide substrate for attached microbial growth. The base beneath the woodchips (figure 6.3a) was used to allow increased airflow underneath the organic solids. Wood-chips were piled onto the base of the organic filter chamber to a depth of approximately 0.5m (figure 6.3b). The aerobic conditions in the organic filters promoted microbial activity and the breakdown of organic matter.

Figure 6.3 Organic filter chambers

Figure 6.3a

Figure 6.3b

The first organic-filter chamber in the domestic effluent waste stream received effluent directly from the toilets in the factory. When the first chamber was full, then the second chamber was used. When filling the second chamber the first chamber was not in operation. This allowed time for the solid material to decompose. The time taken for the process to occur was estimated at approximately one year. Before the second chamber was full the first one was emptied and prepared for use. Neither the effluent, nor the compost from the organic filter were tested for faecal coliforms and they were therefore used exclusively to fertilize shrubs, trees and non-edible plants due to the cross-contamination dangers present to human health (Shuval, 1986).

Pumping station

The pumping station collected approximately 700 litres of pre-cleaned domestic and factory effluent from the organic-filters. When sufficient volume was reached the effluent was automatically pumped onto the first-stage reed beds. It was necessary to make a metal filter for each of the pumps in order to prevent wood-chips from the organic filter clogging the pump mechanisms.

First-stage reed beds

Figure 6.4 depicts three of the first-stage reed beds early in the construction process. The first layer of stones can be seen around the ventilation pipes, which consist of 4-inch pipes drilled at interval along their length. The pipes were used to promote aerobic conditions in the reed bed substrate and aid in hydraulic flow to the outlet.

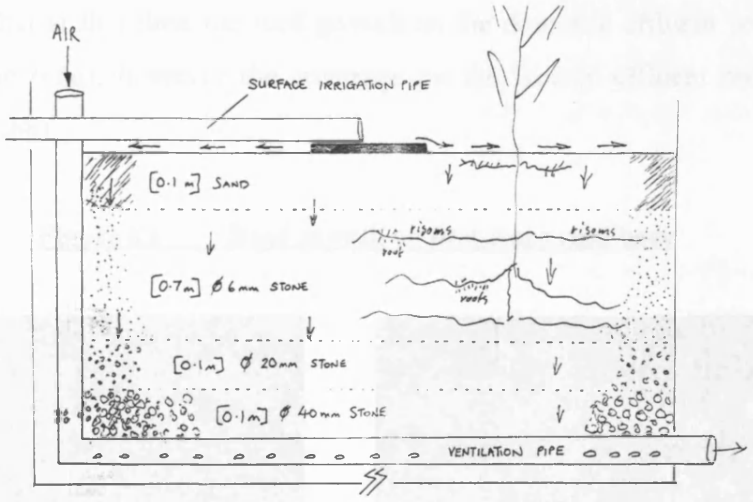
Figure 6.4 Construction of first-stage reed beds

Figure 6.4a

Figure 6.4b

Under operating conditions the effluent was pumped from the pumping station and loaded onto the surface of the first-stage reed beds. The effluent slowly seeped down through the various layers of substrate (figure 6.5). The sandy surface of the reed beds was approximately 10cm thick. Beneath the sand were layers of various grades of gravel (figure 6.5). The larger stones in the layers towards the bottom of the reed bed allowed gaps between the stones to contain air. This promoted aerobic conditions and increased the proliferation of aerobic microorganisms in the gravel matrix.

Figure 6.5 Diagram of cross section of the reed bed.



The reed beds were designed to be used either as six reed bed operating in parallel, or to be isolated, with the use of stop valves, and operated independently. This enabled each reed bed to be manually closed and drained by lowering water levels, so as to prevent the surface of the reed beds from clogging with sediment and solids. The independent operation of the reed beds ensured that maintenance could be carried out and that prolonged periods of excessive flooding, that may provide a breeding habitat for mosquitoes, could be prevented, without compromising effluent treatment performance.

By December 2001 construction of the wastewater treatment system was fully completed. The final three first-stage vertical-flow reed beds and two second-stage vertical-flow reeds had been built. The waste streams were separated so that domestic and factory effluent were treated independently. The reed beds were not planted with *Phragmites australis* at this time due to a delay in locating suitable plant stocks in

Ghana. The import of rhizomes and seedlings was forbidden by the EPA when an application to obtain a phytosanitary certificate to import *Phragmites australis* for use in the design was rejected. Eventually a local source for *Phragmites australis* was discovered and the reed beds were planted in February 2002, nine months after the first reed beds were built.

6.3.2 Reed bed plants

The photographs (figure 6.6a and b) taken in April 2002 illustrate the extent of reed grown in the two months since the initial planting with *Phragmites australis* rhizomes. It can be seen that at this time the reed growth on the domestic effluent reed beds was extensive (figure 6.6a), however the coverage on the factory effluent reed beds was patchy (figure 6.6b).

Figure 6.6 Reed growth on first-stage reed beds

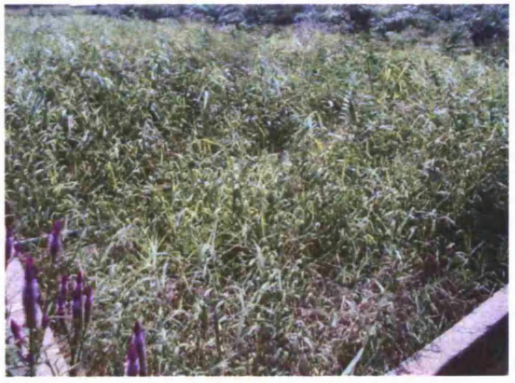


Figure 6.6a Domestic

Figure 6.6b Factory

The reeds growing on the beds receiving factory effluent beds were in thin stands and the general growth appeared extremely stunted. Visual observation of the plant symptoms suggested that the poor growth observed may be attributable to a combination of the toxicity of the factory effluent to the reeds, a lack of plant nutrients in the factory effluent, perhaps oxygen depletion in the substrate due in part to excessive periods of flooding or possibly phytotoxin accumulation induced plant damage preventing adequate plant mediated oxygen transport to the rhizosphere. Phytotoxins have been implicated in reed decline. It was suggested that higher than normal levels of volatile organic acids, mainly acetic, butyric, propionic, valeric and caproic acids may

be injurious to plants. Studies have shown that accumulation of toxins in reeds resulted in die-back of *Phragmites* stands. This was shown to produce symptoms in the plants which include a clumped habit, stunting and death of shoots and roots, premature senescence, death of buds and rhizomes (Armstrong *et al.*, 1996a; Armstrong *et al.*, 1996c; Armstrong and Armstrong, 2001).

The effects of pH on the toxicity of organic acids and of cocktails of acids have been described (Brix, 1997). These, together with the plant symptoms also induced by toxic concentrations of sulphide were discussed in relation to die-back in *Phragmites*. Toxic effect on aquatic plants used in constructed wetlands for primary treatment has also been reported due to high organic loading of influent (Gersberg *et al.*, 1986). Exposure of plants to chlorine concentrations of 0.05mg/L depressed shoots and total dry weight 30% relative to controls.

It was considered that the chemicals used in the factory may be exerting a biocidal effect on the plants and microorganisms. The smell of chlorine could often be detected around the factory outflow and so the effluent was tested for residual free-chlorine using a Merck test kit. The test was carried out every hour throughout the day for an entire week to establish whether there were short transient increases in chlorine levels. The detectable chlorine levels were found to be above 5ppm at certain times indicating excessive use in the factory. A representative from the chemical company who supplied the factory assisted in an audit of the cleaning procedure in the factory. It was reported that there was an excessive use of sanitisers and detergents in the production and cleaning process. Staff training was used to ensure that the minimum amounts of cleaning products required were used in the production areas of the factory. Broad spectrum analysis of the factory effluent would have been beneficial to characterise the effluent, and where possible to determine the exact composition. However due to the financial constraints of the project this expense could not be justified.

A number of steps were taken to improve reed coverage on the factory effluent beds. A second species of reed were introduced to the monoculture *Phragmites* reed beds. More *Phragmites australis* rhizomes were planted and also small *Glyceria maxima* plants (approximately 15-30cm plants). The *Glyceria* species was taken as portions of plant roots from an existing reed bed system that had been used to treat tannery effluent. The

plants were encouraged to grow in a nursery so that they could become accustomed to the climate and the effluent (tertiary treated effluent) before being transplanted.

Factory effluent and domestic effluent streams were combined in the pumping station to stabilise the pH of the combined effluent before application to the reed beds in an attempt to reduce the potential toxicity of the factory effluent to the plants. Previous research supported the premise that mixing sewage with spent processing water from the fruit industry improved treatment. Sewage was reported to provide necessary nutrient dosing for plants under similar wastewater condition (Brix and Schierup, 1989). However, it has been shown experimentally that the neutralisation of organic acids is unfavourable for their biodegradation (Noronha, 2002). Regardless, the combined effluent was loaded onto both domestic and factory reed beds so as to supply nutrients from the domestic effluent to the failing reeds on the factory effluent reed beds.

6.3.3 Operation and maintenance

In July 2003 the sand surface of the first stage reed beds was blocked with sludge. This had accumulated when one of the pumps in the pumping station had broken, causing a build-up of wastewater in the pumping station. As the effluent levels in the pumping station rose the effluent flooded back into the organic chamber and eventually solids from the organic filter chamber were pumped onto the reed beds when the pumps were repaired (figure 6.7a and b).

Figure 6.7 Clogging of first-stage reed beds.

Figure 6.7a

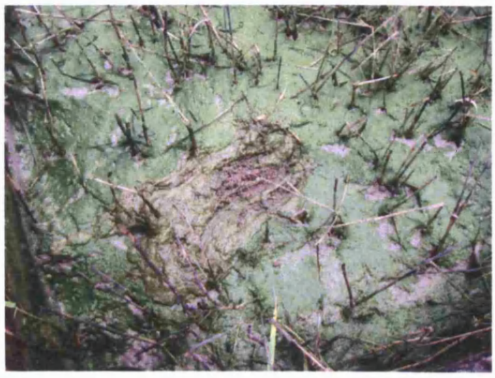


Figure 6.7b

Figure 6.7b shows the thick sludge that was revealed after the reed beds were drained. Several days were required to clear the surface of the reed beds. The reed beds had to be drained and allowed to dry so that the sludge layer would bake in the sun and could be reasonably and safely removed. Once the surface of the reed bed had been returned to its operating state it was replanted with indigenous macrophyte species taken from the wild bush area just below the factory effluent reed bed outlet. The reeds taken from this area were considered to have already been exposed to the treated effluent from the factory reed beds, and as they were thriving in this locality they were assumed to have developed some tolerance to the residual chemical constituents of the factory effluent. Planting these reeds in the reed beds also had the effect of assisting in seeding the beds with indigenous specialist microbial colonisers, which may play a part in the biological transformation of residual contaminants from the effluent.

The second-stage factory effluent reed beds showed similar poor reed growth and so were also re-planted with *Phragmites australis* rhizomes, *Glyceria maxima* and local indigenous reed species. By March 2004 the domestic effluent reed beds showed complete hybridised reed coverage. However, the initial problems encountered with establishing the reed growth on the factory effluent reed beds had not been overcome. The factory effluent reed beds were again re-planted with local reeds. Despite efforts to reduce the quantity of cleaning chemicals used in the factory, to hybridise the reed beds, and to combine the effluent flows, the vegetation on the factory effluent reed beds still did not grow well. This can be seen in the differential growth on the two reed beds illustrated in figures 6.8a and b

Figure 6.8 Reed growth on first-stage reed beds

Figure 6.8a Domestic

Figure 6.8b Factory

6.3.4 Infestations and wildlife

In 2003 / 2004 there was a marked increase in incidences of reported mosquito bites and cases of malaria in and around the factory. This fact suggested that perhaps the free-standing water on the reed beds were providing a habitat for insect breeding. This was considered detrimental to the health of the staff and so spraying with insecticide commenced in order to protect the human population. The fact that mosquitoes were so abundant in the reed beds was considered a good indication that the day to day management of the reed beds was not as effective as it might have been, and that maintenance instructions (Appendix 6) were not being followed by the staff on-site. The management of the wastewater treatment system was reviewed and the importance of periodically drying the beds was emphasised to maintenance staff in revised maintenance instruction (Appendix 6). The reed beds also provided an excellent habitat for lizards, frogs and small birds, which were welcome wildlife on the factory site. The wastewater treatment system was by this time an attractive area of the factory and a place where visitors were taken.

6.4 Chemical analysis of effluent

The chemical analyses used in this study are referenced in the methods chapter. Analysis of effluent samples carried out in Ghana followed "Standard Methods for the Examination of Water and Wastewater", American Public Health Association, 1989 edition (APHA, 1975). The test parameters included pH, BOD_5, TSS, COD and EC.

6.5 Results

6.5.1 Effluent treatment results

The treated combined effluent from the stabilization pond system was sampled and analysed on a monthly basis. The results for stabilisation pond treatment between 2000 and 2001 are shown in table 6.3

Table 6.3 Stabilisation pond outflow effluent

Date (mm/yy)	pH	TSS (mg/L)	BOD (mgO_2/L)	EC (mS/mL)
04/00	4.4	186	1545	*ND*
06/00	4.4	81	2532	910
08/00	4.6	165	1440	776
01/01	4.5	170	1625	780
03/01	4.5	90	1565	750

The results in table 6.3 show that the combined factory and domestic effluent were not adequately treated to within EPA discharge consents (table 6.1) by the stabilisation pond system. The stabilisation ponds failed particularly in terms of BOD and pH. The data presented in table 6.4 and 6.5 show the analysis of the outflow from the factory effluent and domestic effluent reed beds.

Table 6.4 Domestic effluent reed bed outflow

Date (mm-yy)	pH	TSS (mg/L)	BOD (mgO_2/L)	EC (mS/mL)
02-02	6.0	83	272	1169
03-02	*ND*	*ND*	*ND*	*ND*
04-02	4.6	82	2176	1126
05-02	4.8	110	848	987
06-02	*ND*	*ND*	*ND*	*ND*
07-02	5.8	32	160	958
08-02	5.8	38	432	980
09-02	5.9	35	296	966
10-02	6.9	68	192	1280

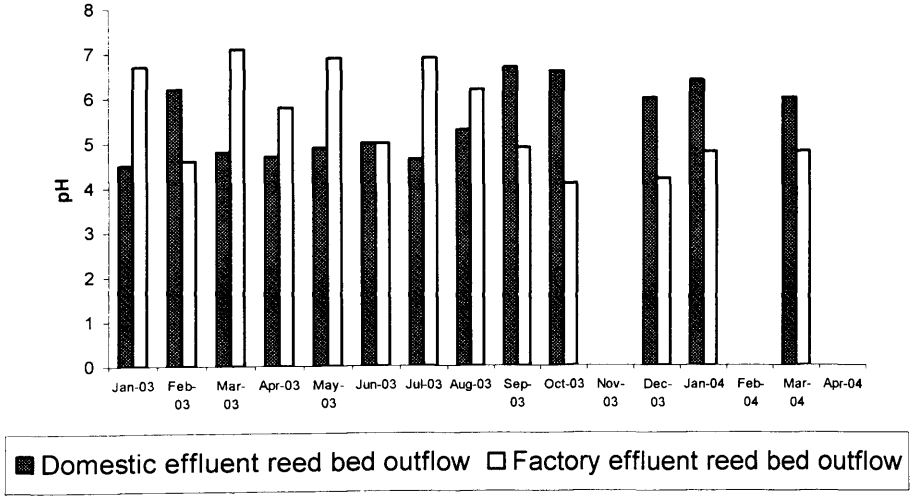
Table 6.5 Factory effluent reed bed outflow

Date (mm-yy)	pH	TSS (mg/L)	BOD (mgO_2/L)	EC (mS/mL)
02-02	4.5	67	304	956
03-02	*ND*	*ND*	*ND*	*ND*
04-02	5.4	43	1760	1314
05-02	7.2	44	640	1009
06-02	*ND*	*ND*	*ND*	*ND*
07-02	4.6	28	192	777
08-02	4.2	31	304	796
09-02	4.5	13	104	587
10-02	4.6	32	591	891

ND=not determined

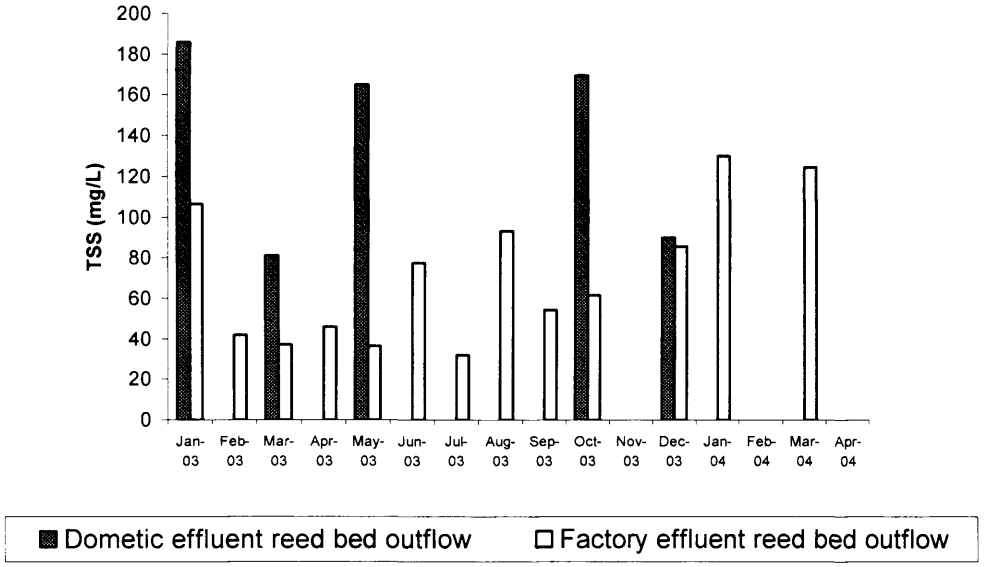
The pH of the outflow from the domestic effluent reed beds in February 2002 was pH 6.0 and the factory effluent reed bed outflow was pH 4.5 for the same month. Similarly in July, August and September 2002 the pH of the outflow from the domestic effluent reed beds was higher than the outflow from the factory effluent reed beds. In the outflow treatment results from certain months there was a reversal of this pattern. The factory effluent reed bed outflow was for example higher in pH than the domestic effluent reed bed outflow in April and May 2002. The data from tables 6.4 and 6.5 were presented in graphical form in figure 6.9a, to 6.9d. The previously described reversal in the type of effluent loaded onto the reed beds (effluent streams separated, combined or switched) should be considered in the interpretation of the data.

Figure 6.9a pH of outflow from domestic and factory effluent reed beds



The explanation for the apparent reversal in effluent treatment performance from the two reed bed systems in some of the months was that the type of effluent loaded onto the reed beds was reversed. The domestic effluent reed beds were loaded with factory effluent and the factory effluent reed beds were loaded with domestic effluent to try to encourage reed growth on he factory effluent reed beds.

Figure 6.9b TSS of outflow from domestic and factory effluent reed beds



Figures 6.9a to 6.9d show that there was a considerable difference in the quality of the outflow effluent from the domestic and factory effluent reed bed systems. However the results presented in this way do not make clear how the development and modification of the reed bed system over time effected treatment performance.

Figure 6.9c BOD_5 of outflow from domestic and factory effluent reed beds

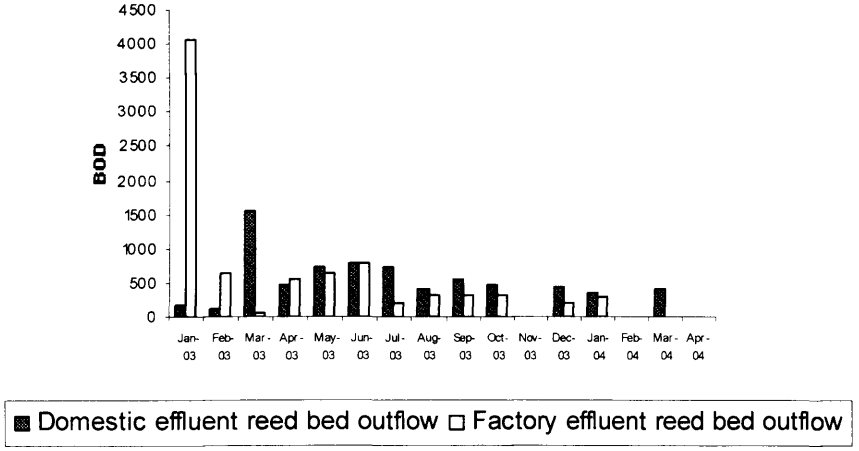
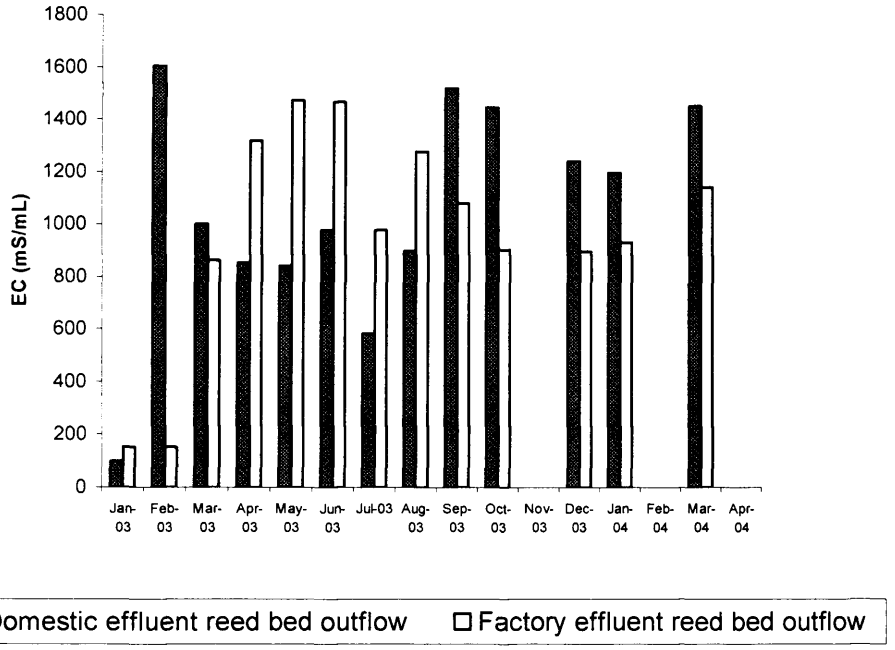


Figure 6.9d EC of outflow from domestic and factory effluent reed beds



The variations in the outflow from the reed beds may have been effected by a number of external factors. Local meteorological data were not collected and so the impact of rainfall and temperature were not fully investigated. The most significant factor to have contributed to the variations in outflow was the switching and separation of the effluent flows to the *factory* and *domestic* effluent reed bed systems. The effluent output from the factory was variable in terms of volume and concentration. The volume and concentration of both domestic and factory effluent increased. The workforce doubled over the duration of the study, and production output increased four-fold. Another factor was that the company introduced bottled fruit juices as a new product line. This added a new process-step to production, and the contribution of waste juice to the factor effluent may have increased the BOD_5 substantially.

6.5.2 Results interpreted in relation to expansion of reed bed system

The four year period under study was divided into six phases, for the presentation of wastewater treatment results, which coincided with field visits and expansion of the reed bed system (table 6.6).

Table 6.6 Time period of study divided into phases

Phase	Time Period	Events
1	April 2000 – April 2001	Stabilisation ponds in use. Malodorous environment **Effluent streams combined**
2	June 2001 – December 2001	*Field visit : May 2001* First reed beds built **Effluent streams combined**
3	January 2002 – May 2002	*Field visit : December 2001* Reed beds construction completed **Effluent streams separated** February 2002 Initial planting with *Phragmites australis* rhizomes
4	July 2002 – January 2003	*Field visit : April 2002* **Effluent streams combined.** Reed beds planted with *Glyceria maxima* and re-planted with *Phragmites australis* General maintenance including minor sludge removal. Domestic effluent organic-filter chamber emptied Staff appointed and trained in maintaining the system.
5	February 2003 – August 2003	*Field visit : January 2003* Major sludge removal Reed beds planted with local bush reeds **Effluent streams switched to other RB system**
6	September 2003 – March 2004	*Field visit : August 2003* Problems with factory effluent reed bed persisting **Effluent streams separated**

6.5.3 Effluent treatment performance

The treated discharge from the reed bed systems was monitored for biological oxygen demand (BOD_5), total suspended solids (TSS), pH, electrolytic conductivity (EC) and temperature. The water treatment results were presented in time periods, which coincided with field visits, according to the previously outlined phases (table 6.6). Table

6.7 shows the mean outflow effluent values from the domestic effluent reed beds for the period under study, from April 2000 to March 2004.

Table 6.7 Domestic effluent reed bed outflow (± SEM)

Phase	pH	TSS (mg/L)	BOD_5 (mgO_2/L)	EC (mS/mL)	COD (mgO_2/L)
1*	4.5 (± 0.0)	138 (± 22)	1741 (± 200)	804 (± 36)	*ND*
2	7.0 (± 0.2)	47 (± 8)	136 (± 100)	618 (± 262)	*ND*
3	5.1 (± 0.4)	92 (± 9)	765 (± 264)	798 (± 350)	1666
4	5.8 (± 0.4)	52 (± 10)	240 (± 64)	829 (± 254)	*ND*
5	5.1 (± 0.2)	59 (± 7)	687 (± 170)	967 (± 118)	*ND*
6	6.3 (± 0.2)	28 (± 6)	450 (± 35)	1373 (± 64)	*ND*

Phase 1=stabilization ponds; 2=combined;3=separated;4; combined; 6 separated; ND=not determined

**The first two lines of data in the two tables were the same because they relate to the pre-reed bed stabilization pond. This was a single treatment system used to treat combined effluent.*

Table 6.8 shows the mean outflow effluent values from the factory effluent reed beds from April 2000 to March 2004.

Table 6.8 Factory effluent reed bed outflow (± SEM)

Phase	pH	TSS (mg/L)	BOD_5 (mgO_2/L)	EC (mS/mL)	COD (mgO_2/L)
1*	4.5 (± 0.0)	138 (± 21.9)	1741 (± 200)	804 (± 36)	*ND*
2	7.0 (± 0.2)	47 (± 8.2)	136 (± 100)	618 (± 262)	*ND*
3	5.7 (± 0.8)	52 (± 7.8)	901 (± 440)	1093 (± 112)	2217
4	4.9 (± 0.5)	42 (± 16.5)	1054 (± 365)	626 (± 125)	*ND*
5	6.1 (± 0.4)	52 (± 9.0)	461 (± 101)	1078 (± 177)	*ND*
6	4.6 (± 0.2)	91 (± 15.7)	287 (± 27)	992 (± 51)	3071

Phase 1=stabilization ponds; 2=combined; 3=separated; 4; combined; 6 separated; ND=not determined

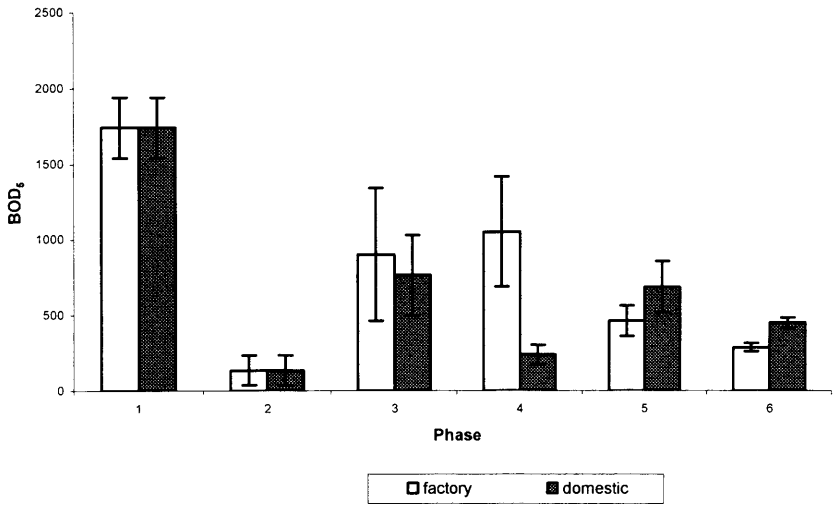
**The first two lines of data in the two tables were the same because they relate to the pre-reed bed stabilization pond. This was a single treatment system used to treat combined effluent.*

6.5.4 Biological oxygen demand (BOD_5)

The results showed that there was a significant difference (t=4.74;df=5;p=0.005;S) between the BOD_5 of the outflow effluent from both the factory and domestic reed bed systems when *Phase 1* and *Phase 2* were compared statistically. This suggested that the construction of the reed bed treatment system in *Phase 2* significantly improved the quality of the effluent, in terms of BOD_5, that was discharged from the factory.

When the BOD_5 of the outflow effluent from the domestic effluent reed beds was compared between *Phase 1* and *Phase 3* (t=2.966;df=6;p=0.025;S); *Phase 1* and *Phase 4* (t=6.428;df=7;p=0.00;S); *Phase 1* and *Phase 5* (t=4.014;df=10;p=0.002;S) and *Phase 1* and *Phase 6* (t=6.365;df=8;p=0.000;S), all were found to be significantly different. In *Phase 3* effluent streams were separate. In *Phase 4* the domestic and factory effluent streams were combined. In *Phase 5* effluent streams were switched to load the other waste stream (e.g. factory effluent was loaded to the domestic effluent reed beds and vice versa). In *Phase 6* effluent streams were separated (e.g. factory effluent was loaded onto the factory effluent reed beds, and domestic effluent was loaded onto the domestic effluent reed beds).

Figure 6.10 BOD_5 of domestic and factory effluent reed bed outflow (± SEM)



The outflow BOD_5 from the factory effluent reed beds was compared between *Phase 1* and *Phase 3*; *Phase 1* and *Phase 4*; *Phase 1* and *Phase 5*; and *Phase 1* and *Phase 6*. It

was found that the difference in BOD_5 between *Phase 1* and *Phase 3* was not significant (t=2.012;df=6;p=0.091;NS). In *Phase 3* effluent streams were separate, therefore only factory effluent was treated through the factory effluent reed beds. The results showed that although the BOD_5 of the outflow was less than in *Phase 1*, the period before the reed beds were built, the improvement in effluent quality was not significant (p=0.091). In *Phase 4* when the effluent streams were combined the improvement in outflow effluent quality in terms of BOD_5 was evident from comparison of the BOD_5 between *Phase 1* and *Phase 4*. The difference between the outflow BOD_5 results was significant (t=2.614;df=8;p=0.031;S). In *Phase 5* the effluent streams were switched over so that the domestic effluent reed beds were loaded with factory effluent and the factory effluent reed beds were loaded with domestic effluent. Comparison of the outflow BOD_5 between these two periods showed that the improvement in effluent quality was significant (t=6.236;df=10;p=0.00;S). In *Phase 5* when the flow of wastewater to the two separate reed beds systems was switched over, the BOD_5 of the effluent could be seen to be reversed, with the domestic effluent outflow BOD_5 greater than the factory effluent outflow BOD_5. Comparison of the BOD_5 of the outflow effluent between *Phase 1* and *Phase 6* was found to be significant (p=0.00). In *Phase 6* effluent streams were once again separated and factory effluent was treated through the factory effluent reed beds. The difference in the outflow BOD_5 between *Phase 1* and *Phase 6* was significant (t=6.383;df=7;p=0.000;S)

The pattern of results for BOD_5 were similar for both domestic effluent and factory effluent reed bed systems, except for in *Phase 4*. During *Phase 4* effluent flows were combined. This was for two main reasons; firstly to effectively feed the reeds on the factory effluent reed beds with the more nutritious domestic effluent, and also to add the microbiologically active domestic effluent to the factory effluent to begin biological breakdown of the effluent at the same time as stabilising the pH by effectively balancing the mixed effluent.

6.5.5 BOD_5 percentage reduction

The BOD_5 percentage reduction of both the factory effluent (table 6.9) and domestic effluent (table 6.10) wastewater treatment systems was calculated for each *Phase* of the duration of the investigation.

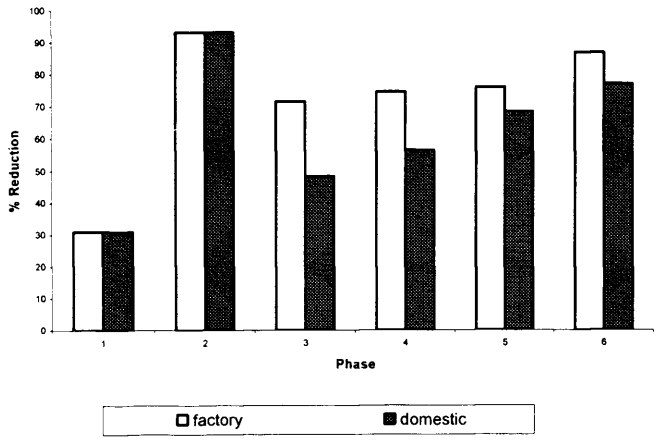
Table 6.9 BOD_5 reduction in factory effluent reed beds

Phase	Effluent	% Reduction
1 (stabilisation ponds)	Combined	31
2	Combined	93
3	Separate (factory effluent)	71
4	Combined	74
5	Switched (domestic effluent)	76
6	Separate (factory effluent)	86

Table 6.10 BOD_5 reduction in domestic effluent reed beds

Phase	Effluent treated	% Reduction
1 (stabilisation ponds)	Combined	31
2	Combined	93
3	Separate (domestic effluent)	48
4	Combined	84
5	Switched (factory effluent)	68
6	Separate (domestic effluent)	77

Figure 6.11 Percentage reduction of BOD_5 in each *phase*



Phase: 1 = stabilisation pond, 2=combined, 3=separate, 4=switched, 5=combined, 6=separate

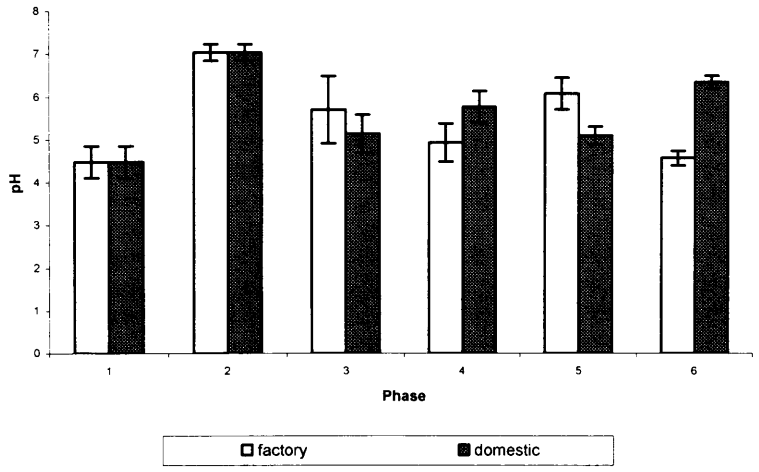
Figure 6.11 shows that both the factory effluent and domestic effluent reed bed systems reduced the BOD_5 of the effluents over the duration of the study. The percentage reduction rate for *phase 3* in the domestic effluent reed bed is low. This is perhaps due

to unauthorised maintenance of the organic filters, insufficient reeds established on the beds and because of high organic loading rates.

6.5.6 pH

In *Phase 2*, immediately after the reed beds were built, the basic substrate mediated assimilative potential of the reed beds in terms of geochemical adsorption, was at its greatest. During this time the combined effluent outflow was pH 7 for both domestic and factory effluent reed beds. Statistical analysis of the improvement in the pH between *Phase 1*, before the reed beds were built, and *Phase 2* after the first reed beds were built, was significant (t=-17.68;df=6;p=0.00). However, the difference in the pH of the outflow from the domestic effluent reed beds between *Phase 1* and *Phase 3* was not significant (t=-2.022;df=6;p=0.90). Between *Phase 1* and *Phase 4* the difference was significant (t=-3.471;df=8;p=0.008). Between *Phase 1* and *Phase 5* the pH of the domestic effluent was significantly different (t=-2.401;df=10;p=0.037;S). During *Phase 5* the effluent streams were switched. The pH of the outflow from the factory effluent reed beds was closer to the acceptable range than the domestic effluent reed beds in the same period. When comparing the outflow pH between *Phase 1* and *Phase 6* the difference was significant (t=-12.264;df=4.516;p=0.000;S). In *Phase 6* the pH of the domestic effluent reed bed outflow (when the effluent streams were separate) was closer to the desired range for discharge than it was in *Phase 4* or *Phase 5* (in which the flows were combined and subsequently switched).

Figure 6.12 pH of domestic and factory effluent outflow (± SEM)



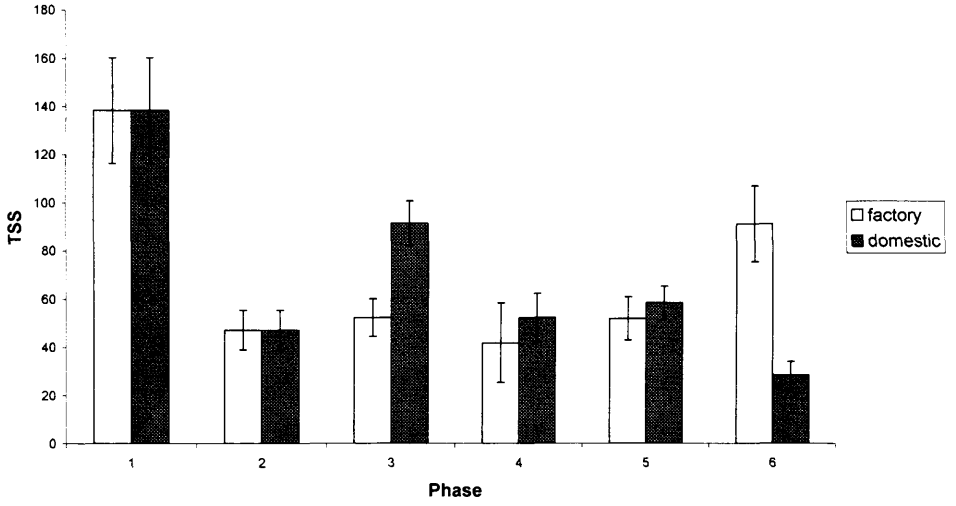
Phase: 1 = stabilisation pond, 2=combined, 3=separate, 4=switched, 5=combined, 6=separate

The difference in the pH of the outflow factory effluent over the period of investigation between *Phase 1* and *Phase 3* was not significant (t=-1.535;df=2.009;p=0.264;NS). The difference between *Phase 1* and *Phase 4* was also not significant (t=-0.972;df=8;p=0.359;NS). The difference between *Phase 1* and *Phase 5* was significant (t=-4.249;df=6.121;p=0.005;S). The difference between *Phase 1* and *Phase 6* was not significant (t=-0.462;df=8;p=0.656;NS).

6.5.7 Total suspended solids

Total suspended solid reduction was variable over the period of study (figure 6.13). The effective removal of solids may have been effected by problems that were encountered with the organic filters.

Figure 6.13 TSS of domestic and factory effluent outflow (± SEM)



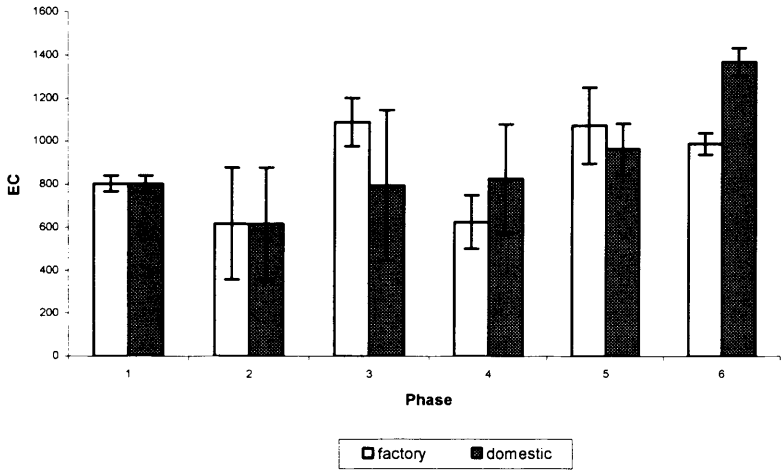
Phase: 1 = stabilisation pond, 2=combined, 3=separate, 4=switched, 5=combined, 6=separate

6.5.8 Electrolytic conductivity

Figure 6.14 shows that there was an initial decrease in the EC of the domestic effluent outflow when the reed beds were built (between *Phase 1* and *Phase 2*). In the factory effluent beds, after the initial decrease in EC there was an increase in *Phase 3*. This may have been because in *Phase 1* and *Phase 2* the effluent streams were combined. It was in *Phase 3* that the factory effluent was treated alone. There was a significant decrease

in EC between *Phase 4* and *Phase 3* (t=2.51;df=6;p=0.046;S). In *Phase 4* the waste water streams were switched, so it was domestic effluent that was treated in the factory effluent reed beds.

Figure 6.14 EC of domestic and factory effluent outflow (± SEM)



Phase: 1 = stabilisation pond, 2=combined, 3=separate, 4=switched, 5=combined, 6=separate

6.5.9 Concluding remarks

- The reed bed wastewater treatment system was effective in reducing the pollutant load of both the factory and domestic effluent in terms of BOD_5, TSS and COD.

- An improved method of pre-treatment is probably required for primary treatment of the factory and domestic effluent prior to reed bed treatment.

- Operational and technical problems that were encountered were resolved through collaboration with the workforce in Ghana.

6.6 Assessment of the microbiology of the reed bed substrate

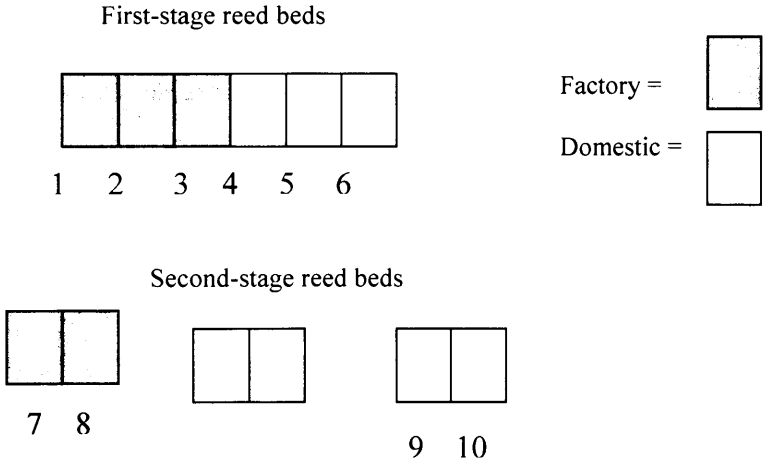
6.6.1 Introduction

The experiment investigated the potential difference in the microbiology of the domestic and factory effluent reed bed systems to see whether the treatment of two distinct types of effluent had an effect on the microbial consortia of the reed beds.

6.6.2 Methods

The reed bed substrate was sampled according to the standard methods (section 3.1). Samples were transported to UK in atmosphere controlled conditions at 8°C for microbiological analysis. Microbiological analysis was carried out within 24 hours of sample collection according to standard methods (section 3.4.1). Figure 6.15 shows the reed beds labelled from reed bed 1 to 10. The reed beds loaded with factory effluent were labelled beds 1, 2, 3, 7, 8. The reed beds loaded with domestic effluent were labelled beds 4, 5, 6, 9, 10.

Figure 6.15 Substrate sampling strategy



6.6.2 Microbiology results

The results of the microbiological analysis of the substrate of the first-stage reed beds found that there was 6.17 mean log CFU/g (SD± 0.33). The second-stage reed beds mean log CFU/g was 5.98 (SD ± 0.17). Independent samples t-tests were used to

compare the data. Comparison of the first stage reed beds (figure 6.15 labelled reed beds 1, 2, 3, 4, 5, 6,) with the second-stage reed beds (figure 6.15 labelled reed beds 7, 8, 9, 10) revealed no significant difference in the mean log CFU/g of the reed bed substrate (t=1.22;df=10;p=0.25;NS). Independent samples t-test comparison of the factory effluent reed beds with the domestic effluent reed beds revealed a significant difference in mean log CFU/g. Factory effluent reed bed were found to have 5.96 (SD ± 0.16) mean log CFU/g and domestic effluent reed beds 6.29 mean log CFU/g (SD ± 0.25). The experiment was repeated. Factory effluent reed beds mean log CFU/g 5.87 ± 0.16. Domestic effluent reed beds 6.26 mean log CFU/g (SD ± 0.25). The factory effluent reed bed data were compared to the domestic effluent reed bed data. Independent samples t-test revealed a significant difference (t=-3.19;df=8;p=0.013;S).

6.6.3 Microbiology experimental summary

The microbiology experiment showed that there was a significant difference in the microbiology of the reed bed substrate of the two reed bed systems. There were more CFU/g in the domestic effluent reed beds than in the factory effluent reed beds. This may have been due to the higher concentration of micro-organisms in the domestic effluent than in the factory effluent. The microbiological contamination may have been transferred from the effluent to the substrate. The factory and domestic effluent were not microbiologically cultured for this determination. There may have been the same numbers of microorganisms in the two reed bed systems when they were built, but long-term treatment of domestic effluent may have supplied additional nutrients to the domestic effluent reed bed substrate and increased the numbers of CFU/g. The effect of the toxicity of the factory effluent, on the reed beds, by the chemicals, specifically chlorine/chloride, used in processing the fruits in the factory, may have reduced microbial numbers in the soil. The biocides used to prevent fruit spoilage may have had a similar disinfectant effect on the reed bed substrate. There was significantly less reed coverage on the factory effluent reed beds than on the domestic effluent reed beds (figure 6.6a and b). The reeds provide surface area within the substrate for attached microbial growth, and are important in oxygen transfer to the substrate. The positive effects the reeds convey to the system in the factory effluent reed beds, compared to domestic reed beds, were significantly diminished.

6.7 Comparison of the treatment efficiency of the two reed bed systems

6.7.1 Introduction

A 14 day effluent treatment investigation of the Ghana reed beds was carried out to compare the treatment efficiency of both systems in treating the same combined effluent load. The purpose of the experiment was to determine if there was a different treatment response from the two systems (domestic and factory effluent reed bed systems) when they were both used to treat the same effluent. The previous study showed that the CFU/g of the reed beds was significantly different. This experiment was designed to look at how the independent microbial communities may effect the treatment performance of the reed beds.

6.7.2 Methods

The reed bed systems (*domestic* and *factory*) were loaded with combined effluent. Effluent samples were collected, according to the standard method, from each of the effluent sampling locations illustrated in figure 6.16 and table 6.11. The samples were stored in air-tight containers which were filled to the lid and stored under refrigeration conditions at 8°C until analysis could be carried out in UK. Analysis was within 24 hours of sample collection.

Figure 6.16 Effluent sampling locations

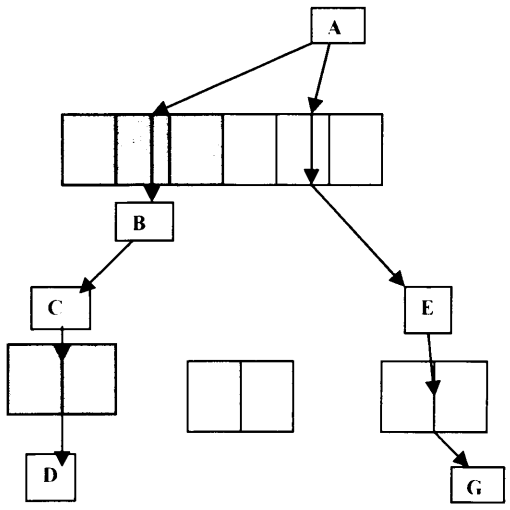


Table 6.11 Effluent sample description and location

Sample	Effluent sample description
A	Untreated combined effluent from pumping station
B	Combined effluent after first-stage treatment with factory effluent reed beds
C	Inflow for second-stage factory effluent reed beds
D	Combined treated effluent outflow from factory effluent reed beds
E	Combined effluent after first-stage domestic effluent reed bed treatment
G	Combined treated effluent outflow from domestic effluent reed beds

6.7.3 Results

The treatment of combined domestic and factory effluent through the factory (table 6.12) and domestic (table 6.13) effluent reed bed systems, in week one is shown.

Table 6.12 WEEK 1 combined effluent treatment in *factory effluent* reed beds

Sample	COD (mg O_2/L)	N (mg/L)	NH_3 (mg/L)	pH	Cl^- (moles/L)
A (inflow)	202	0.02	0.03	4.2	0.0024
C	339	0.07	0.08	4.3	0.0027
D (outflow)	217	0.06	0.08	4.8	0.0026
Treatment efficiency	-7.4	-187.5	-187.5	*ND*	-11.86

Table 6.13 WEEK 1 combined effluent treatment in *domestic effluent* reed beds

Sample	COD (mg O_2/L)	N (mg/L)	NH_3 (mg/L)	pH	Cl^- (moles/L)
A (inflow)	202	0.02	0.03	4.2	0.0024
E	244	0.05	0.05	4.5	0.0024
G (outflow)	84	0.06	0.07	6.2	0.0027
Treatment efficiency	59	-150	-150	*ND*	-14

It terms of COD reduction the *domestic* effluent reed beds were more effective than the *factory* effluent reed beds in treating the combined effluent. The *domestic* effluent reed beds attained 58% treatment reduction, while the treatment in the *factory* effluent reed beds was -7.4% (table 6.12 and 6.13).

In week two the inflow COD of the combined effluent was greater than in week one (inflow COD week one was 202 and week two was 937). The COD of the samples may have been reduced by sample transportation and storage prior to analysis. However, the reduction due to storage would have been uniform across all of the samples, so would not affect the validity of the data for comparison within this study. Table 6.14 and table 6.15 show the combined effluent treatment in the factory and domestic effluent reed beds in week two.

Table 6.14 WEEK 2 combined effluent treatment in factory effluent reed beds

Sample	COD (mg O_2/L)	N (mg/L)	NH_3 (mg/L)	pH	Cl^- (moles/L)
A (inflow)	937	0.06	0.07	3.2	0.002
B	292	0.15	0.18	5.1	0.004
C	337	0.07	0.09	4.3	0.003
D (outflow)	192	0.07	0.09	5.2	0.003
Treatment efficiency	80	*ND*	*ND*	*ND*	*ND*

ND=not determined

Table 6.15 WEEK 2 combined effluent treatment in domestic effluent reed beds

Sample	COD (mg O_2/L)	N (mg/L)	NH_3 (mg/L)	pH	Cl^- (moles/L)
A (inflow)	937	0.06	0.07	3.2	0.002
E	397	0.08	0.10	4.4	0.003
G (outflow)	215	0.06	0.07	5.3	0.003
Treatment efficiency	77	*ND*	*ND*	*ND*	*ND*

ND=not determined

In week 2 in the *factory* reed beds the percentage reduction COD in the factory reed beds was 80%. In the domestic reed beds it was 77%. In week 2 the reed beds were not significantly different in percentage reduction of COD.

6.7.4 Summary of treatment efficiency of the two reed bed system

The study revealed that the inflow wastewater was variable in composition and concentration. It varied from COD 202 in week 1 to 937 in week 2. This aspect

highlighted the need for an aerated balancing tank, at the beginning of the system as a pre-treatment step, to equalize the pH and nutrient load of the effluent before reed bed treatment. The domestic and factory effluent reed bed systems were different in their treatment performance in week 1 compared with week 2. In week 1 the domestic beds achieved 59% reduction in COD, whilst the factory effluent reed beds achieved -7.4% reductions in COD which equates to an increase in COD. In week 2 the COD removal rate of both factory and domestic systems had increase to 80% and 77% respectively. This may be attributed to a dramatic increase in nutrient loading for the factory effluent system which was habituated to receiving a low nutrient load from the factory effluent. However the variability of the combined inflow makes it difficult to be certain of this without further investigation.

The differential response of the two reed bed system, *factory* reed beds and *domestic* effluent reed beds, in treating combined effluent over a two week period revealed that the substrate of the reed beds had been altered overtime by loading different types of effluent. It appeared that in week one the microbiological composition of the two reed bed systems was significantly different so that the reed beds responded differently in treatment performance to the combined effluent loading. This was measured by observing the treatment performance in terms of COD reduction. The improved performance of the reed beds in week two suggests that the treatment of combined effluent through the reed bed systems is more effective (in terms of reducing the COD) than treatment of the domestic and factory effluents separately.

6.8 Discussion

The need for the improvement and conservation of the environment in Ghana, and other countries with developing economies, necessitates the provision of efficient on-site wastewater treatment. Economic and technological constraints to the adoption of overtly technological modern wastewater treatment methods make the application of biological wastewater treatment, with constructed wetlands, a viable and sustainable option.

A reed bed wastewater treatment system was built in Ghana to treat the effluent from a fruit processing factory. The construction, maintenance and treatment performance of the reed bed system was monitored over a four year period. Despite the fact that EPA

discharge consent was not consistently achieved the removal efficiency for the studied time frame was encouraging. The study highlighted the need for appropriate reed bed management and understanding of the biological basis of operation.

A number of factors, namely the urgency for which effluent treatment had to commence, and the undefined increase in effluent volume throughout the period of study, contributed to the underperformance of the reed bed system. Immediately after the reed beds were built, within minutes of completion, they were loaded with effluent. Previous researchers have recommended an establishment period for constructed wetlands (Fisher, 1990; Billore, 1999) and although this was acknowledged the wastewater had to be loaded on the reed beds because during construction it was diverted directly into the watercourse. Similarly, the reed beds should have been planted with reeds before loading commenced, and the reeds should have been given time to grow and for the reed beds to mature. However, difficulty encountered in finding an economical source of appropriate reed species in Ghana, or permission to import, and the limited frequency of visits to Ghana throughout the project prevented adequate reed bed maturation.

Accurate water volume data and thorough compositional analysis of the factory effluent were not available so the size of the treatment system was based on estimated figures. There was insufficient area of reed beds for the volume of waste water that was treated, and as a result the hydraulic retention time was short (1 day). The increase in the size of the factory and in production rate over the four year period exacerbated the fact that the effluent loading volume was high and hydraulic retention time was too short for adequate treatment.

Outflow effluent treatment data, collected in the long-term over the 4 years of study as monthly returns for *domestic* and *factory* effluent, were difficult to interpret. The effluent was not consistently below EPA discharge consents for all parameters. The BOD was not sufficiently reduced so as not to pose an ecological threat to the receiving water body, however the improvement in water quality was significant. The reed beds were more effective in treating domestic effluent when the effluent streams were treated separately. However, the best overall treatment performance for both effluent streams was achieved when the effluent flows were combined at source. Evidence for this was

shown in *phase 2* and in *phase 4* and was demonstrated in the short-term study which looked at the combined effluent treatment performance over a 14 day period.

The experiment compared the treatment differential of the *domestic* and *factory* effluent reed beds when used to treat combined effluent. The results showed that in the first week the domestic effluent reed beds were significantly more effective in COD treatment than the factory effluent reed beds. Microbiology assessment of the domestic and factory effluent reed bed systems in the first week showed that the reed beds were significantly different in terms of CFU/g. In week two the treatment performance of the two systems was not significantly different. The "recovery" of the microbial consortia of the factory effluent reed beds was remarkably fast to have had such a dramatic effect on week 2 treatment performance.

The results from this study can be compared with previous work which reported treatment performance data from a sub-surface horizontal flow reed bed in Dar es Salam where COD removal was 33.6%, 56% and 60% (Kaseva, 2004). In this study treatment performance exceeded those reported with 58.58%, 79.51% and 77.02%. Similar to Kaseva (2004) results the pH of the effluent in this investigation reliably increased from the inflow to the outflow of the reed bed. Total suspended solid load was reduced through wetland treatment. In *phase 3* when the suspended solids of the outflow was at its highest measured, it could be attributed to the fact that the new reed beds (3 first-stage and two second-stage) had recently been completed and loose sediment was probably flushing from the system. Or the high TSS load may have been effected by the organic filters being emptied.

Analysis of the removal efficiencies of BOD and COD from the factory effluent suggest the need for a pre-treatment step to be built into the Ghana treatment system to reliably comply with EPA discharge consent standards. Effective pre-treatment would reduce the risk of biological inhibition of the reed bed microbiology by potential phytotoxins in the factory effluent. The primary treatment step would be installed at the beginning of the reed bed system. The factory effluent would flow through membrane filters into a series of balancing and settling tanks. The COD would be reduced by aeration with submersible pumps. The combined pre-treated effluent would have balanced pH and reduced COD/BOD_5.

CHAPTER SEVEN

DISCUSSION

7.0 Reed beds: A sustainable technology for the biological treatment of waste

Constructed wetlands are effective in treating a number of distinct types of industrial effluent (e.g. landfill leachate; tannery effluent; fruit-factory spent process water; and human sewage), the uncontrolled discharge of which has been shown to cause environmental damage and pollution of water bodies. Effective pilot-scale and full-scale constructed wetland treatment of wastewater was respectively carried out in the temperate climate of the UK, and in the tropical climate of sub-Saharan Ghana.

The data collected in the UK and in Ghana were useful in determining areas of specific interest for comparison of the operation of reed bed wastewater treatment systems in temperate and sub-tropical climate zones. Throughout the investigation treatment performance was assessed in terms of the pollutants removal efficiency of each reed bed. This assessment was made for effluent treatment for the duration of the experimental period. Microbiological indicators have been used to determine information about fate processes in soils and have been used to understand organic and synthetic transformations (Casida *et al.*, 1964; Chander, 1991; Rossell, 1991; Bardgett *et al.*, 1995; Li and Zhao, 1999; Taylor *et al.*, 2002; Benitez *et al.*, 2004; Gil-Sotres *et al.*, 2005). In this research mean values for dehydrogenase activity, and mean colony forming unit counts were used to identify patterns and trends in the changes that occur in the microbial community of the substrate of reed beds, at different depth horizons, under various loading conditions, with industrial effluents from a number of industries, under different climatic conditions.

The substrate of the experimental UCN reed beds in the UK was studied over a considerable time period. Differences in the microbial dehydrogenase activity and in the colony forming unit counts of the reed bed substrate were identified. Variables that were considered in the experiments included: weather conditions, loading regime (fixed 200L/day), and effluent type (landfill leachate, tannery effluent, water control). The type/source of landfill leachate (leachate A, B or C) was found to effect reed bed treatment performance. Changes in the aerobic and anaerobic microbial community of the reed bed substrate were studied in the pre-loading, during-loading, and post-loading period of reed bed operation. Significant changes in the microbial CFU/g and dehydrogenase activity of the substrate, in response to loading, were identified in both

the treatment of tannery effluent and landfill leachate. Temporal and spatial differences in reed bed matrices have been reported in previous research (Weisner and Strand, 1996; Garcia *et al.*, 2003; Hinsinger *et al.*, 2003; Woulds and Ngwenya, 2004; Wießner *et al.*, 2005). The pattern that emerged from this research was complex, but generally there was an increase in CFU/g during loading and a decrease in CFU/g post-loading.

In Ghana the effect of combined domestic and factory effluent loading onto the specialised microbial communities of the *domestic* effluent and *factory* effluent reed bed systems showed that the treatment performance of the reed beds was dependent upon the health and magnitude of the substrate microbial community. The work in Ghana was useful in the fact that it gave insight into the possible effects of combined effluent loading onto two seemingly identical reed bed systems. The research question that was addressed was whether the treatment performance of the combined effluent was better on the factory or domestic effluent reed beds. When the effluents were combined, and the two reed bed systems were both treating combined effluent it was interesting to see that the performance of the two systems was significantly different. This was further supported experimentally when it was shown that the treatment performance of the factory and domestic effluent reed bed systems became similar in treatment performance after a time of acclimatisation. The findings are similar to previous work, which reported the ability of substrate to respond to a "toxic" waste can clearly differ after a period of exposure to the waste (Benitez *et al.*, 2004).

A factor that initially caused concern, and subsequently led to problems with treatment performance of the Ghana reed bed system was the maturation phase of the reed beds and the establishment of the plants (reeds). The reed beds were not given adequate time to mature before effluent was loaded, and so the development of the reed bed flora and microbial community was delayed. The reed beds were planted with reeds a number of months after effluent loading commenced. The reeds failed to establish on the factory effluent reed beds, which may have been due to stress or recalcitrant phytotoxic compounds in the factory effluent (Armstrong *et al.*, 1996a&c; Armstrong and Armstrong, 2001). Without established plant flora in the reed beds the availability of the rhizosphere environment was reduced (Gersberg *et al.*, 1986; Brix, 1987; Nichols, 1997; Stottmeister *et al.*, 2003). Limited development of the roots and rhizosphere would have limited the space available for microbial attachment, effectively reducing the

microbiologically mediated assimilative capacity of the reed beds. In addition, oxygen flux and active transport to the substrate by the reeds was reduced, limiting the availability of oxygen for aerobically mediated microorganisms (Armstrong *et al.*, 1992; Armstrong *et al.*, 1996; Urbanc Bercic and Gaberscik, 2001; Hinsinger *et al.*, 2003; Stottmeister *et al.*, 2003).

Reed bed design is a critical factor in creating sustainable biological wastewater treatment systems that are effective in reducing the pollutant load of effluent, and have sufficient longevity for long-term operation (EWPCA, 1990; Cooper, 1993; Cooper, 1999; Cooper *et al.*, 1999; Gschlossl and Stuible, 2000; Cooper, 2001; Davison *et al.*, 2001; Scholz and Xu, 2002). Appropriate sizing, in terms of the surface area and depth of the reed bed necessary for the volume and type of effluent, is important in obtaining desired treatment objectives (Brix *et al.*, 1989; Hobson, 1989; EWPCA, 1990; Findlater, 1990; Haberl, 1990; Schierup *et al.*, 1990; Cooper, 1993; Li *et al.*, 1995; Li and Chuncai, 1995; Markantonatos *et al.*, 1996; Mandi *et al.*, 1998; Griffin and Upton, 1999; Williams *et al.*, 1999; Bahlo, 2000; Gschlossl and Stuible, 2000; Davison *et al.*, 2001; Vymazal, 2002). Population equivalent has been used to determine wetland size, however in different countries and climatic regions different recommended loading rates exist (Kadlec, 2000; Tanner *et al.*, 2002; Kadlec, 2003).

Selection of substrate media, available pore space and particle size has an effect on wastewater treatment performance (Garcia *et al.*, 2003; Arias *et al.*, 2001; Scholz *et al.*, 2001; Scholz and Xu, 2002; Scholz and Xu, 2002; Del Bubba *et al.*, 2003). Clogging of the surface of the reed bed treatment system in Ghana with suspended solid material was experienced on occasion. When the infiltration rate of the effluent was reduced, by clogging, this resulted in pooling of the effluent on the surface of the reed beds. This created problems associated with infestations and pests, such as mosquito proliferation, and led to increased risks to human health (Knight *et al.*, 2003). Pre-treatment prior to reed bed loading was important in removing suspended solids and in reducing the BOD_5 and COD load of the effluent. Through the experience of long-term wastewater treatment with the reed beds in Ghana, this factor was found to be critical in sustaining effective operation of the treatment system. When an excess of suspended solids was loaded to the reed beds it interfered with the infiltration rate, operation and performance of the beds. The clogging experienced was probably due to the slow infiltration rate of

the sand surface layer and, most importantly inadequate pre-treatment of the effluent prior to reed bed loading. Without pre-treatment the high COD of the outflow effluent was not adequately reduced by reed bed treatment alone. The organic filters used for pre-treatment did not reduce the total suspended solid load sufficiently, so that on occasion suspended solids were pumped directly to the reed bed surface and subsequently clogged the surface of the bed. Clogging has been shown to lead to the development of anoxic conditions in the substrate of reed beds (Platzer and Mauch, 1997; Zhao *et al.*, 2004). Research on anti-sized reed bed designs have showed that clogging can be prevented, without effecting treatment performance, by layering of the substrate of the reed bed with large sized substrate media on the top layer of the reed bed. This method could be useful to employ in first-stage of the Ghana reed bed system. An anti-sized reed bed design may reduce the clogging phenomenon, however eventually even an anti-sized reed bed would clog without effective pre-treatment of the effluent (Zhao *et al.*, 2004). It has been shown that anti-sized design can also increase oxygen flux to the substrate when reed beds are pulse loaded. Attached microbial biofilm has been shown to be unaffected by varied media pore size although early research claimed that biofilm accumulation may be less (Zhao *et al.*, 2004).

Reed bed operation was a critical factor that affected the treatment performance of the Ghana reed bed system. When the reed beds were not properly managed, (e.g. loaded with effluent before the reeds were established; loaded with sludge) and were not maintained as a living ecosystem, the stability and balance of the reed bed ecology was disrupted. The reed beds then did not operate at a steady-state, and did not achieve optimum treatment capability, (e.g. high removal efficiency of pollutant parameters) instead the reed beds became simple filtration and sedimentation units. The loading regime is important for sustainable reed bed operation because it affects the retention time of the reed beds (Tanner *et al.*, 1998). Hydraulic retention time is a factor of reed bed size, substrate permeability, effluent volume per day, and loading regimen. Excessive loading may decrease the retention time to such an extent that on occasion the reed beds operated as a simple filter. If this were the case adequate contact time was not given for biologically mediated transformations to occur and therefore optimum treatment was not achieved.

Differences between tropical and temperate environments can have significant implication for treatment wetland function. The bulk of published data on constructed wetland operation came from temperate regions of Europe, The United States and Australia. There was a limited availability of published research literature on tropical treatment wetlands (Koottalep and Polprasert 1997, Williams *et al.,* 1999, Davison *et al.,* 2001, Kivaisi 2001, Lim *et al.,* 2001, Meutia 2001, Abira *et al.,* 2003, Kaseva 2004, Diemont 2005). Additionally the available wetland data did not often provide information on local weather conditions, such as humidity, rainfall, hours of sunshine and soil temperature. Information on the weather conditions would have been valuable for accurate comparison with other research work that was conducted under different environmental conditions, and for use in fully interpreting the body of data. In the tropics weather phenomenon include year round high humidity, one or two wet seasons, dry seasons, and that the temperature does not dip below freezing, as it does in the UK and in other temperate zones further from the equator. Evapotranspiration has been shown to increase treatment performance during summer months by improving hydraulic retention time (Chazarenc *et al.*, 2003).

Using the data from the four year effluent treatment profile, built up regarding the Ghana reed beds, the effect of seasonality on effluent treatment performance could be addressed. Nitrification and denitrification rates may be affected by the factor of seasonality. However seasonality was not found to be a predictive determinant of the treatment performance of the Ghana reed bed system. This was interpreted through consideration of the contradictory effects of evapotranspirtaion in the hot temperatures, which would increase the concentration of pollutants in the effluent by reducing the effluent volume, but at the same time increase the HRT of the reed beds (Chazarenc *et al.*, 2003). In the rainy seasons increased precipitation would have exerted a dilution effect on the effluent and a reduction in HRT. This may have cancelled out the effect of seasonal weather conditions on treatment performance that may otherwise have been detected.

In assessing the differences in reed bed operation in different climatic zones and under different environmental conditions it has already been established that hydraulic retention time (HRT) and hydraulic loading rates (HLR) effect treatment performance (Brix, 1994b). It is becoming increasingly clear that the effect of the environment on the

wetland is a significant factor in treatment performance. To understand the complex interaction of biology, microbiology, ecology, and environmental conditions, and to appreciate how these factors impact upon the interaction of the hydrodynamics and geochemical interaction, that may or may not be microbiologically mediated, long term study of stable operation full scale wetlands throughout the world is required. To establish design criteria for tropical and subtropical zones an appreciation of the impact of weather conditions and cycles, temperature, and of different botanical species and perhaps microbial species is important and could be evaluated and explored.

Design and installation of sustainable biological wastewater treatment systems must consider effluent volume, treatment potential, contaminant characteristics and treatment goals, in order to select the appropriate technology. The microbiology of the reed bed system and maintenance of an effective ecological balance is vital in achieving consistent and sustainable biological treatment performance. The selection of appropriate wastewater treatment technologies for countries with developing economies should consider factors such as the level of competence of the operator, level of pay, and the provision for technical supervision. The time taking in establishing a constructed wetland is ultimately important in the treatment performance, and should be a factor in planning the timescale for installation.

Public perceptions and acceptance of biological low-technology systems, such as reed beds, benefit from the demonstration of the effective operation and performance of the systems. This demonstrates the reliability and longevity of constructed wetland for sustainable industrial effluent treatment, and may be a factor in influencing the regulatory standards controlling the widespread adoption of the technology. Failure in constructed wetland treatment performance does not, in many cases, reflect the failure of the reed bed, but rather the failure of the reed bed operator, construction engineer, or the architect of the design.

Reed beds should be adopted for sustainable effluent treatment when the selection of the technology considers that the treatment objectives are most effectively achieved. Constructed wetlands are an ideal technology for onsite landfill leachate treatment where the requirements are for long-term sustainable treatment for discharge to sewer or surface water. In this case constructed wetlands can provide effective treatment, at

comparable installation cost to alternative treatment technologies such as SBR. The nature of the wetland would accommodate seasonal variability in leachate composition and volume. Constructed wetlands require only minimal maintenance and have low annual running costs. Reed beds are not always the appropriate technology for all situations, such as in servicing the wastewater treatment requirements of a large UK town or city, if there were not enough land available, or if the treatment objectives were too high. Constructed wetlands could be perhaps be used for tertiary effluent treatment or final polishing in such a scenario.

Waste management and wastewater treatment can aspire to be a closed-loop system in terms of biological interdependence and sustainability. The adoption of cleaner biological technology within industry is being driven by restricted outlets for waste disposal; limited access and availability of facilities; the cost associated with cleaner processes; and the changing public perception toward current industrial practices. Reduced environmental impact for waste can be achieved, alongside financial benefits, such as reduced cost, increased profits, and increased customer confidence, through the adoption of sustainable technology for waste treatment.

CHAPTER EIGHT

FUTURE WORK

8.0 Future work

- Investigate the proportional changes of the microbiological community in reed beds, in terms of types and numbers of aerobic and anaerobic species, under different climatic and effluent conditions, by analysis of microbial community.

- Identify the level of microbial activity in a reed bed necessary for the effective treatment of various effluents.

- Investigate the use of indigenous reed flora from countries with developing economies. Work with local communities in countries with developing economies to formulate strategies for the indigenous development of reed bed technology.

- Analysis of the microbial communities of reed beds selecting certain groups of organisms such as *Bacillus* spp. and nitrifying bacteria.

CHAPTER NINE

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APPENDIX

CHAPTER FOUR

HYPOTHESES RELATING TO DEHYDROGENASE ACTIVITY (DH-1)

H_0 The **dehydrogenase activity** of the reed bed substrate from the 30cm horizon is **the same** in the control bed as in the reed beds treating landfill leachate.

H_1 The **dehydrogenase activity** of the reed bed substrate from the 30cm horizon is **greater** in the control bed than in the reed beds treating landfill leachate.

H_2 The **dehydrogenase activity** of the reed bed substrate from the 30cm horizon is **less** in the control bed than in the reed beds treating landfill leachate.

H_3 The **dehydrogenase activity** of the reed bed substrate from the 30cm horizon of the reed beds is **the same** in sections one, two and three.

H_4 The **dehydrogenase activity** of the reed bed substrate from the 30cm horizon of the reed beds is **less** in section one of the beds than in sections two and three.

H_5 The **dehydrogenase activity** of the reed bed substrate from the 30cm horizon of the reed beds is **greater** in section one of the beds than in sections two and three.

HYPOTHESIS RELATING TO DHA OF DEPTH HORIZON DURING- & POST-LOADING (DH-2)

H_0 The dehydrogenase activity of the reed bed substrate is **not significantly different** in the 30, 60 and 90cm depth horizons.

H_1 The dehydrogenase activity of the reed bed substrate is **greater** in the 30cm depth horizon than in the 60 and 90cm horizons.

H_2 The dehydrogenase activity of the reed bed substrate is **greater** in the 60cm depth horizon than in the 30 and 90cm horizons.

H_3 The dehydrogenase activity of the reed bed substrate is **greater** in the 90cm depth horizon than in the 30 and 60cm horizons.

H_4 The dehydrogenase activity of the reed bed substrate is **not significantly different** in the **during-loading** period as in the **post-loading** period.

H_5 The dehydrogenase activity of the reed bed substrate is **greater** in the **during-loading** period than in the **post-loading** period.

H_6 The dehydrogenase activity of the reed bed substrate is **less** in the **during-loading** period than in the **post-loading** period.

HYPOTHESES RELATING TO COLONY FORMING UNIT COUNTS (CFU-1)

H_0 The **colony forming unit count** of the reed bed substrate from the 30cm horizon is **the same** in the control bed as in the reed beds treating landfill leachate.

H_1 The **colony forming unit count** of the reed bed substrate from the 30cm horizon is **greater** in the control bed than in the reed beds treating landfill leachate.

H_2 The **colony forming unit count** of the reed bed substrate from the 30cm horizon is **less** in the control bed than in the reed beds treating landfill leachate.

H_3 The **colony forming unit count** of the reed bed substrate from the 30cm horizon of the reed beds is **the same** in sections one, two and three.

H_4 The **colony forming unit count** of the reed bed substrate from the 30cm horizon of the reed beds is **less** in section one of the beds than in sections two and three.

H_5 The **colony forming unit count** of the reed bed substrate from the 30cm horizon of the reed beds is **greater** in section one of the beds than in sections two and three.

HYPOTHESES RELATING TO DURING-LOADING CONDITIONS

H_0 The **colony forming unit count** of the reed bed substrate from the 30cm horizon is **the same** in the control bed as in the reed beds treating landfill leachate **during-loading**.

H_1 The **colony forming unit count** of the reed bed substrate from the 30cm horizon is **greater** in the control bed than in the reed beds treating landfill leachate **during-loading**.

H_2 The **colony forming unit count** of the reed bed substrate from the 30cm horizon is **less** in the control bed than in the reed beds treating landfill leachate **during-loading**.

HYPOTHESES RELATING TO POST-LOADING CONDITIONS

H_0 The **colony forming unit count** of the reed bed substrate from the 30cm horizon is **the same** in the control bed as in the landfill leachate reed beds **post-loading**.

H_1 The **colony forming unit count** of the reed bed substrate from the 30cm horizon is **greater** in the control bed than in the landfill leachate reed beds **post-loading**.

H_2 The **colony forming unit count** of the reed bed substrate from the 30cm horizon is **less** in the control bed than in the landfill leachate reed beds **post-loading**.

HYPOTHESES RELATING TO PRE-LOADING CONDITIONS

H_0 The **colony forming unit count** of the reed bed substrate from the 30cm horizon is **the same** in the control bed as in the landfill leachate reed beds **pre-loading**.

H_1 The **colony forming unit count** of the reed bed substrate from the 30cm horizon is **greater** in the control bed than in the landfill leachate reed beds **pre-loading**.

H_2 The **colony forming unit count** of the reed bed substrate from the 30cm horizon is **less** in the control bed than in the landfill leachate reed beds **pre-loading**.

APPENDIX

CHAPTER SIX

Effluent Treatment System Operating Instructions

Each Morning

1. Clear obstructions from drains and ensure all water is flowing correctly to the organic filter chambers.
2. Clear the chlorinated water channel and remove debris from the mesh filter.
3. Add wood-chips to the organic filter making sure that the wood covers all the liquid, and is heaped directly beneath the flow of water. Remember to turn the pipe when needed to ensure that the chamber fills evenly. Do not add too many wood-chips or the chamber will fill up too quickly and need emptying before the compost in the other chamber is ready.
4. Check that the pump is not blocked, and is operating correctly. An indication that the pump is blocked is that the pumping station chamber will be filled with water, and the reed beds will not have been loaded automatically. Remove debris from the wire mesh filters, which should be on the end of the two inflow pipes that bring the factory effluent and sewage water into the pumping station. If necessary also remove floating wood-chips which may appear in the pumping station chamber.
5. Adjust effluent flow to the six first-stage reed beds using the red valve taps. Dry reed beds if there is standing surface water. The point of this is to ensure that the reed beds do not remain flooded for a prolonged period and clog up.
6. Adjust "H" bars if necessary to ensure even water distribution over the surface of the reed beds.
7. Rake the surface of the dry reed beds if a crust is present which is preventing the water from percolating down through the sand. Manually remove crust layer.

When Necessary

8. If the secondary reed beds appear to be clogged, and there is constant standing surface water, it may be necessary to drain and dry them. Closing the red valve taps to prevent additional water flowing onto the beds will do this. The effluent can temporarily back-up the pipes from the first-stage reed beds. To allow time for the reed beds to drain, stop the flow of effluent to the reed beds in the evening. The reed bed should have drained by the next morning. Leave the taps off, to allow the surface of the sand to bake dry in the sun all the next day. In the evening rake off the crust and remove this manually. Open the red valve taps again and the reed bed should operate normally. This procedure should not be done often. Taps to the secondary reed beds should only be closed when a crust has formed on the surface, which stops the reed bed functioning.
9. Check that the water is flowing effectively at the end of the reed bed treatment system. Clear the stream channel by digging into the bush to make the water flow freely and prevent it from stagnating.
10. Once or twice a year, empty the dried compost from the organic filter, clear the chamber and prepare for next use.
11. Ensure that the pipes from the organic filter chamber to the pumping station are free from blockages. This can be done when the chamber is emptied.

Effluent Treatment System

Operating Instructions (REVISED)

(reed bed operative responsibilities)

Each Morning

- **Clear obstructions from drains**
- **Remove debris from the mesh filter.**
- **ONE load of wood-chips** to the organic filter.
- **Check that the pump is not blocked.**
- **Remove debris from the wire mesh filters.**

Reed bed Supervisor Instructions

- Adjust effluent flow to the six **first-stage reed beds** using the red valve taps. Dry reed beds if there is standing surface water. Ensure that the reed beds do not remain flooded for a prolonged period and clog up.

- If the **second stage reed beds** appear to be clogged, and there is constant standing surface water, it may be necessary to drain and dry them. Closing the red valve taps to prevent additional water flowing onto the beds will do this. The effluent can temporarily back-up the pipes from the first-stage reed beds. To allow time for the reed beds to drain, stop the flow of effluent to the reed beds in the evening. The reed bed should have drained by the next morning. Leave the taps off, to allow the surface of the sand to bake dry in the sun all the next day. In the evening rake off the crust and remove this manually. Open the red valve taps again and the reed bed should operate normally. This procedure should not be done often. Taps to the secondary reed beds should only be closed when a crust has formed on the surface, which stops the reed bed functioning.

- Rake the surface of the dry reed beds if a crust is present which is preventing the water from percolating down through the sand. Manually remove crust layer.

• *When Necessary*

- Check that the water is flowing effectively at the end of the reed bed treatment system. Clear the stream channel by digging into the bush to make the water flow freely and prevent it from stagnating.

- Once or twice a year, empty the dried compost from the organic filter, clear the chamber and prepare for next use.

- Ensure that the pipes from the organic filter chamber to the pumping station are free from blockages. This can be done when the chamber is emptied.

The End