Design, optimisation and costing of a novel forced-upflow bioreactor for bioremediation of leachates from selected landfill sites in KwaZulu-Natal

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by

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ABSTRACT

Most waste generated in South Africa is sent to landfills for disposal, and although it is confined in specific areas, it can potentially affect both above and below ground water resources, impacting environmental and public health. This is particularly relevant in a country where water supplies are limited and groundwater resources are prone to pollution. The primary objective of this study was to assess the performance of an upflow packed-bed bioreactor purpose-designed for the treatment of leachates produced by landfills in the Durban Metropolitan Area (DMA). The effect of parameters such as the nature of the biofilm support matrix, aeration rate and recycle rate on the efficacy of the system were investigated. Another major aim of the project was to develop a low maintenance technology that could, nonetheless, bioremediate leachate effectively at minimum cost. This aspect of process design is a crucial factor in areas where there is a shortage of both funds and skilled labour.

The glass 132 *l* packed-bed upflow bioreactor was evaluated by measuring its efficiency in terms of chemical oxygen demand (COD) and biological oxygen demand (BOD) reduction and ammonia removal. The bioreactor could be configured as a batch-type system, which was useful for comparing operating conditions; or as a continuous cascade system, which was used to assess its overall performance. Different biofilm support matrices viz. various grades of pine bark, plastic bioballs and ceramic noodles were evaluated in 22 *l* batch-type reactors.

Leachates from five landfill sites were remediated during the course of the study, and only the leachate from Shongweni landfill, which had a remarkably low BOD:COD ratio (0.05), was intractable and could not be successfully treated; even in flask trials designed to test strategies such as augmentation of microflora and biostimulation. The other leachates investigated were from the Umlazi,

Marianhill, Bisarsar Road (all general sites) and Bul-Bul Drive (a semi-hazardous site) landfills, all of which were remediated to some degree. Originally, leachate from the Umlazi landfill site was used, but it became unavailable when the site closed enforcing the use of other leachates for the remainder of the investigation. Leachates from Marianhill, Bisarsar Road and Bul-Bul Drive were treated simultaneously in duplicate operating the six-chambered bioreactor in the batch-type configuration. The highest COD removal efficiency (49 %) was obtained in the chambers treating the Bul-Bul Drive leachate, which was therefore used for further investigations. This leachate had the highest BOD:COD ratio and was therefore expected to be the most suited to biological remediation.

The bioreactor performed best when plastic bioballs were used as biofilm support matrix with a relatively low level of aeration, although the uncomposted form of pine bark was used initially as the support matrix because it is inexpensive and readily available in South Africa. However, although satisfactory COD reduction (30 – 61 %) and ammonia removal (87 – 98 %) was achieved when the Umlazi leachate was treated, the possibility of compounds leaching out of the bark and affecting the quality of the treated leachate was a concern. Also, pine bark would be prone to mechanical degradation in a full scale operation. Of the other solid support matrices tested using the Bul-Bul leachate, COD removal efficiencies were superior with plastic bioballs (60 %) than with pine bark chips (29 %). The former therefore became the preferred biofilm support matrix.

Aeration level did influence bioremediation of the Umlazi landfill leachate since those chambers aerated with an aquarium pump (0.05 – 0.1 litres air/litre leachate/min; 60 % COD removal) performed better than those aerated with a blower (0.6 -0.7 litres air/litre leachate/min; 42 % COD removal) and those that remained unaerated (44 % COD removal).

Recycle rate did not significantly affect bioremediation, but the performance of the system was higher when operated in batch mode (up to 60 % influent COD

removal), rather than in continuous flow-through (cascade) mode when only 37 % of the influent COD in the Bul-Bul leachate was removed. Under the latter conditions, most of the reduction occurred in the first four chambers and very little biodegradation occurred in the final two chambers. The cascade-mode will require some refinement to enhance the COD removal efficiencies achieved. However, it did eliminate 89 % of the BOD present in the raw leachate, producing a treated effluent with a consistent BOD:COD ratio of 0.05.

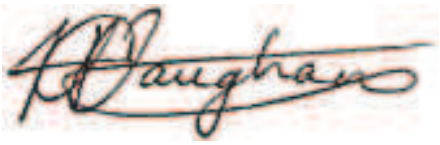
The COD removal efficiencies achieved covered a wide range from a minimum of 23 % with Marianhill leachate to a maximum of 63 % with leachate from Bul-Bul Drive. These results are comparable with many of those reported by other authors treating landfill leachate. Up to 98 % of the ammonia was removed when the Umlazi leachate was treated. However, ammonia removal from the other leachates tested was erratic.

Although the treated leachate from this system could not be released into the environment without further remediation, the reduction in concentration of pollutants would allow its return to the local water supply via a wastewater treatment plant. This was achieved without temperature and pH regulation or addition of extraneous nutrient sources. A cost-effective, low maintenance technology such as this one would be a useful tool for the treatment of effluents such as landfill leachate in countries like South Africa where although water conservation is urgently required, resources for highly sophisticated effluent remediation are often not readily available.

DECLARATION

This thesis is submitted in fulfilment of the requirements for the degree of Doctor of Philosophy in the discipline of Microbiology, University of KwaZulu-Natal, Pietermaritzburg, South Africa.

I declare that this thesis is my own unaided work. All references have been duly acknowledged. The present work has not been previously submitted at this, or at any other, institution.



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We hereby certify that this statement is correct.

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LIST OF ABBREVIATIONS

ANAMMOX	Anaerobic ammonium oxidation
AOB	Ammonium oxidising bacteria
AOP	Advanced oxidation process
AOX	Absorbable organic halogens
BCWA	Biofilm-cell wall assembly
BOD	Biological oxygen demand
BOD_7	Biological oxygen demand (seven day measurement)
BTEX	Benzene, toluene, ethylbenzene and xylene
DEHP	Di-(2-ethylhexyl)phthalate
DMA	Durban Metropolitan Area
DOC	Dissolved organic carbon
DWAF	Department of Water Affairs and Forestry
CANON	Completely autotrophic nitrogen removal over nitrite
CASP	Conventional activated sludge process

$CBOD_5$	Carbonaceous biological oxygen demand
COD	Chemical oxygen demand
CSEM	Conventional scanning electron microscopy
CW	Constructed wetland
EB	Electron beam
EPS	Extracellular polymeric substance
ESEM	Environmental scanning electron microscopy
FA	Free ammonia
GAC	Granulated activated carbon
HAIB	Horizontal-flow anaerobic immobilised biomass
HRT	Hydraulic retention time
HTOC	High temperature catalytic oxidation
ICP	Inductively coupled plasma
ICP-OES	Inductively coupled plasma optical emission spectroscopy
LC_{50}	Lethal concentration (for 50 % of a sample population in a specified time period)
MAACFB	Microorganism attached activated carbon fluidised bed
MBR	Membrane reactor
MF	Microfiltration
MSW	Municipal solid waste
NA	Natural attenuation
NEDD	N-1-napthylethylenediamine di-HCl
NF	Nanofiltration
NOB	Nitrite oxidising bacteria
OUR	Oxygen utilisation rate
PAC	Powdered activated carbon
PAF	Pretreatment aerobic degradation and flushing
PAH	Polycyclic aromatic hydrocarbon
PCB	Polychlorinated biphenyl
PFC	Perfluorinated compound
RBC	Rotating biological contactor

RO	Reverse osmosis
SBR	Sequencing batch reactor
SD	Standard deviation
SEM	Scanning electron microscopy
SOUR	Specific oxygen uptake rate
SNAD	Simultaneous partial nitrification, anaerobic ammonium oxidisation and denitrification
SRT	Sludge retention time
STP	Sewage treatment plant
TC	Total carbon
TCE	Trichloroethane
TKN	Total Kjeldahl nitrogen
TOC	Total organic carbon
TSS	Total suspended solids
UF	Ultrafiltration
UF-BAC	Ultrafiltration-biologically active carbon
VFA	Volatile fatty acid
VSS	Volatile suspended solids

INTRODUCTION

Modern society generates large volumes of waste which need to be appropriately treated; this includes an ever increasing variety of manmade or modified chemicals that may require specialised disposal techniques. Although landfilling is a cost-effective method of confining pollution to a designated area, it does very little to eliminate waste. Despite the media coverage associated with large spills and accidental release of contaminants, a much greater threat is posed by the consistent disposal of smaller quantities of hazardous materials. Much effort has been put into the development of technologies to improve waste disposal techniques; biological treatment or bioremediation, where microorganisms are used to degrade, transform or detoxify wastes, is one of the approaches that has received attention.

In 1998, more than 95 % of the waste produced in South Africa was taken to landfill sites. Approximately 43 million cubic metres of general waste and over 5 million cubic metres of hazardous waste are disposed to landfill annually. Kwazulu-Natal is responsible for a high proportion of this hazardous waste due to mining activity and fertiliser production (South African Waste Information Centre, 2006). General waste is defined as waste that does not pose a significant risk to the environment or public health if properly managed. This category includes domestic, commercial, and some industrial wastes as well as builders' rubble. Trace amounts of hazardous substances such as batteries, insecticides, weed-killers and medical waste that has been discarded on domestic or commercial premises may be present in wastes of this nature. Such materials can generate a leachate that can cause unacceptable levels of pollution due to waste decomposition and the presence of moisture; it is for this reason that landfills are categorised not only in terms of their size, but also in terms of their leachate production potential (Langmore, 1998b).

In contrast, hazardous wastes can have significant adverse affects on the environment and public health, even in low concentrations, due to their physical and chemical properties. Wastes from a diversity of industries fall into this category and include inorganic wastes (acids and alkalis, cyanide waste, heavy metal sludges and solutions, and wastes containing a significant proportion of fibrous asbestos), oily wastes (generated due to the processing, storage and use of mineral oils), organic wastes (solvent residues, phenolics, PCBs, paint and resin wastes, biocide waste, and any other organic chemical residues), putrescible organic wastes (from the production of animal and vegetable products such as edible oils, and wastes from slaughterhouses, tanneries and other such industries), and miscellaneous wastes (infectious/medical waste, redundant chemicals/medicines, laboratory waste, explosive waste and redundant munitions). Where small quantities of hazardous substances are dispersed in other wastes such as harbour dredge spoils, sewage sludge or

builders' rubble, this may need to be disposed as a hazardous waste (Langmore, 1998b).

Aquifer protection is particularly important in South Africa as many communities and almost all farmers are wholly dependent on groundwater for their water supply. In other areas, it is an essential supplementary source and the utilisation of this resource will increase as surface water diminishes. However, South African aquifers are extremely vulnerable to pollution for several reasons; one of the most obvious being that nearly all the usable groundwater occurs within 60 m of the surface. Most South African aquifers are fracture flow systems which transport pollutants at much faster rates than porous flow systems, and recharge to these systems occurs freely via infiltration from rainfalls, ponds and seepage from dumps. The flow of pollutants within these aquifers also tends to follow flow-paths which create streams. Groundwater quality has deteriorated at many waste management facilities, making the implementation of a comprehensive, standardised monitoring programme necessary to protect water resources (Langmore, 1998a).

In the Durban Metropolitan Area (DMA), poor waste disposal has impacted negatively on the environment, and has also affected the health of surrounding communities. Both soil and freshwater resources are vulnerable to pollution from landfilling, wastewater treatment and illegal dumping of waste. Although recently designed landfill sites have complex liner systems that contain the leachate generated until it can be treated and disposed, older landfills as well as older cells within landfills that are still operational have no liners or are inadequately lined because the installation of a liner was not previously required by law. Poorly managed sites may release air pollutants in the form of volatile gases formed during waste decomposition; this problem has been mitigated by landfill gas recovery at some local landfills. Landfill gas can also create odour problems over a wide area, and, in some cases, deodorising chemicals are used if the wind moves towards residential areas as these odours lower quality of life and

decrease the market value of land in the affected suburbs. Dust is often created at landfill sites due to the movement of large vehicles and during waste compaction operations; this can be overcome by wetting and/or tarring roads. One of the major issues associated with landfill sites in this area is the effect that landfill leachate and gas may have on community health; pests such as flies and rats are also attracted by the organic waste found in landfills. One of the sites (Umlazi) from which leachate was obtained for this study had to close due to public pressure amid health concerns (Cities Environment Report On the Internet, 1999).

This study was motivated by the need for a low-cost, low-maintenance technology for the treatment of the leachates produced at various landfills in the DMA.

CHAPTER 1

LITERATURE REVIEW

1.1 Introduction

Land disposal of waste has been practiced for hundreds of years, and, until a few decades ago, it was believed that soil and groundwater purified all leachings from this waste without causing environmental damage. However, it is now accepted that contamination of groundwater and other natural resources by landfill leachate does occur to various extents, depending on the type of waste at a particular site. This realisation led to a revised definition of waste that subdivided it into two categories: hazardous and non-hazardous, resulting in the development of two different designs for landfill sites. Natural attenuation (NA) landfills are those where any leachate generated is allowed to percolate into the groundwater and are only viable if the waste disposed is non-hazardous. However, leachate produced by these landfills is not completely attenuated by the soil. Additional restrictions have therefore been imposed on this type of

landfill, and they are now completely banned in some places. Most modern landfills are therefore containment type systems, constructed with a low permeability liner and a leachate collection system (Bagchi, 1994). Leachate collection systems have thus become a critical feature of landfill design, and the retention of this effluent means that its treatment and disposal has become a necessity (Britz, 1995). Although other methods have been investigated, landfilling is likely to remain, at least for the foreseeable future, the most popular option for solid waste disposal due to its cost-effective nature, as well as its controllable impact on the surrounding environment during the stabilisation of waste materials (Renou, Givaudan, Poulain, Dirassouyan and Moulin, 2008). A recent review of current trends in landfill leachate treatment emphasises the continuing relevance of research in this field due to both the global increases in municipal solid waste (MSW) production and the more stringent legislation concerning waste discharge that is becoming the norm in many countries (Renou *et al*., 2008).

The need for legislation and treatment arises because leachate is a serious environmental pollutant, and the protection of both surface and ground waters must be ensured as water resources become more and more scarce. Landfill leachate can cause rapid deoxygenation in any receiving waters due to its high strength and labile nature. Ammonia present in leachate from older landfills may contribute to this effect, and cause fish kills; while growth of sewage fungus is often promoted if leachate is released into surface waters. Other effects include odours associated with reduced sulphur compounds and aesthetic problems caused by the precipitation of iron compounds. Potentially pathogenic microorganisms present in landfill leachate may also contaminate drinking water resources (Britz, 1995).

1.2 Landfills and leachate

Landfills can be defined as large areas of ground that are designated for waste disposal (Robinson, 2005). A contaminated liquid is formed when water, or any other fluid, comes into contact with solid waste. Precipitation that falls on a landfill percolates through the waste and reacts both physically and chemically with it. In addition, compaction of the waste due to its own weight also causes some liquid to be released. Thus, even if no water is allowed to percolate through the waste, a small volume of the wastewater defined as landfill leachate will still be produced. Percolating water aids the formation of leachate and increases the quantity produced, but it also dilutes any contaminants that may be present (Bagchi, 1994).

The biological processes that occur in a landfill will continue for a considerable number of years after the site has been closed and capped, so leachate will also continue to be produced for a length of time largely depending on the rainfall, waste composition and operational practices used (Robinson, 2005). Leachate generation may be minimized by constructing a final cover on a landfill as soon as possible after the waste reaches the designated final grade. However, although this method reduces leachate quantity, the leachate will still require treatment for a relatively long time after landfill closure (Bagchi, 1994). In some cases, leachate can remain a liability for as long as 30 years after the site is closed (Britz, 1995). If final cover construction is delayed, contaminants may be flushed out sooner; but landfill gas cannot be efficiently collected and odour may also pose a problem (Bagchi, 1994). However, it has also been reported that the use of a low permeability cover could inhibit the biodegradation of organic contaminants due to the reduced water infiltration. Dilek Sanin, Knappe and Barlaz (2000) monitored toluene degradation in a laboratory-scale simulated landfill and found that mineralisation rates were much improved with water addition as opposed to without, suggesting that different approaches may be required depending on the characteristics of a particular site.

1.3 Leachate composition

Leachate is likely to contain a high concentration of contaminants, with both suspended and dissolved components. Chemical reactions as well as microbiological activity within the waste mass contribute to the substances in a particular leachate, and the latter produces gases that may dissolve in the liquid and react with other components (Bagchi, 1994). All organic materials in the waste mass will be subject to partial or complete microbial decomposition. This means that all leachates will contain intermediate products combined with high concentrations of toxic organic substances, heavy metals and other xenobiotics (Britz, 1995). Eggen, Moeder and Arukwe (2010) have also shown that landfill leachates may be a significant source of new and emerging pollutants such as perfluorinated compounds (PFCs).

The composition of landfill leachate is highly variable, and extremely site-specific. Attempts to define a typical leachate include such broad concentration ranges of potential contaminants that they are virtually useless (Britz, 1995). Large variations in leachate quality may occur not only at different sites, but also at different locations within a single site as well as over the lifetime of a landfill (Robinson and Luo, 1991). For example, the chemical oxygen demand (COD) of the leachate from the Chicopee sanitary landfill (Massachusetts, U.S.A.) varied from 800 – 10000 $mg.l^{-1}$ over a nine month period, while the biological oxygen demand (BOD) to COD ratio also fluctuated from a low value of 0.1 to an extremely high value of 0.9, indicating that leachate from the same site may be much more biodegradable at certain times in the lifespan of the landfill (Keenan, Iza and Switzenbaum, 1993). Accurate risk assessments of potential contamination arising from leachate that reaches ground water therefore requires information on the transport and fate of the xenobiotic compounds it contains (Beeman and Suflita, 1987).

The most important contaminants in leachate are likely to be organics. Nitrogen, usually in the form of ammonia, is also conspicuous, while metal concentrations

are often relatively low (Kulikowska and Klimiuk, 2008). A study focussing on the occurrence of various organic pollutants found that the main compounds present in leachates were phthalates, especially bis(2-ethylhexyl) phthalate (DEHP). This substance is easily sorbed to suspended solids and dissolved organic matter, which may make the pollutant less accessible to microorganisms, thereby hindering the biodegradation process. The polycyclic aromatic hydrocarbon (PAH) concentrations in landfill leachate were similar to those in sewage samples, and these compounds were not present in the leachate produced by closed landfills. Many leachates also contained 2,6-dinitrotoluene; however most of these specific pollutants were detected only at low $\mu g.l^{-1}$ ranges (Martinnen, Kettunen and Rintala, 2003). Many emerging organic pollutants, including PFCs, pharmaceuticals and personal care products (PPCPs), and insect repellents such as N,N-diethyl toluamide (DEET) were recently detected in leachate from several municipal landfills. Although they tend to occur in trace amounts, they may pose challenges during leachate treatment as they are both mobile and persistent (Eggen *et al*., 2010). It is important to know which substances are present in leachate, particularly when it is discharged into sewage treatment plants, as the functioning of these facilities may be affected (Martinnen *et al*., 2003). Ammonia is usually released due to the anaerobic decomposition of proteins that are present in both vegetable and animal wastes. It also arises from industrial wastes containing ammonia or ammonium compounds; these include fertilisers, artificial rubber, plastics and food preservatives. Ammonia often remains trapped within the waste mass, with insignificant volatilisation and is, therefore, present in high concentrations in most leachates, making them difficult to treat using conventional processes (Pivato and Gaspari, 2006).

Due to the complex nature of landfill leachate, it is impossible to establish the exact contribution of any specific contaminant to the toxicity of the liquid. Although potentially toxic compounds can be identified using chemical analyses, a variable fraction of pollutants (especially products of polar degradation) are overlooked in many cases and complex interaction phenomena are not revealed

(Pivato and Gaspari, 2006). Olivero-Verbel, Padilla-Bottet and De la Rosa (2008) correlated the physico-chemical characteristics of a MSW landfill with its toxicity against *Artemisia franciscana* using multivariate analyses. A negative relationship between median lethal concentration (LC_{50}) and COD suggested that increases in organic content generate a more toxic leachate. This could be due to the organic compounds themselves inducing toxicity, or it could indicate that substances which are responsible for toxicity may need to form complexes with organic matter in order to become biologically active. Higher cadmium levels also result in increased leachate toxicity, while the opposite is true for conductivity (Olivero-Verbel *et al.*, 2008). Another study using luminescent bacteria to determine toxicity of leachate also identified COD and particularly ammonia as key contaminants in this type of waste. It was also noted that the toxicity of the leachate was considerably lower in sustainable landfills where the ammonia was degraded, using a new model involving pre-treatment, aerobic degradation and flushing (PAF) (Pivato and Gaspari, 2006).

1.4 Factors influencing leachate quality and quantity

Several factors influence the quality and composition of a leachate produced by a specific landfill, and it is clear that the composition of a waste will determine the types of contaminants present. Municipal waste is generally more variable than industrial waste, and the quality of municipal leachate will therefore vary widely. In addition, putrescible wastes exhibit more quality variation than non-putrescible wastes (Bagchi, 1994). Landfill category, which determines the type of waste that a site is permitted to accept, will have a significant effect on the leachate generated and thus on the biotreatability of the waste liquid. Generally, leachates from landfills containing domestic refuse have a dark colour due to the presence of humic substances, an alkaline pH, and a mineral profile in which chloride and ammonia tend to dominate. They may have a variable organic load, but biological oxygen demand (BOD) values are generally comparatively low indicating an advanced state of degradation. Leachates from landfills containing both domestic

and hazardous industrial wastes are more likely to have a lower pH, as well as higher ammonia content and conductivity with significantly greater COD concentrations (Tatsi and Zouboulis, 2002). In developing countries, high organic content and ammonia concentrations are common characteristics of landfill leachate (Borzacconi, Ottonello, Castelló, Pelaez, Gazzola and Viñas, 1999). The COD concentration of a leachate also appears to be related to the amounts of heavy metals that it contains; any treatment system therefore needs to consider the effect of increased heavy metal loading, even if it is designed primarily to deal with the carbonaceous component of the waste stream (Keenan *et al*., 1993). Tränkler, Visvanathan, Kuruparan and Tubtimthai (2005) noted that pre-treated waste showed dramatic decreases in total Kjeldahl nitrogen (TKN) present in the leachate produced when compared with untreated domestic waste and organic market waste. In this case, pre-treated waste consisted of composted, sorted and mechanically shredded waste from a vegetable market.

Landfill age is a critical factor influencing the quality of a specific leachate (Bagchi, 1994; Kulikowska and Klimiuk, 2008). Young landfills are defined as those that have been in operation for less than five years, while medium age sites are between five and ten years old. Mature landfills have usually been in use for at least ten years (Renou *et al.*, 2008). These guidelines concerning landfill age will be used throughout this thesis. The leachate generated by a young landfill is usually relatively weak. Maximum strength is reached after a few years and subsequently declines gradually. However, the concentration of all contaminants may not peak at the same time (Bagchi, 1994). Younger landfills also tend to produce leachate with a high COD due to the presence of fatty acids (Britz, 1995). Volatile fatty acids (VFAs) are the main products of the anaerobic fermentation that occurs in new landfills; this process is enhanced if moisture content within the waste mass is high (Renou *et al*., 2008). For example, in a study performed by Kulikowska and Klimiuk (2008) the concentration of organics, expressed as COD, decreased from over 1800 mg.l^{-1} at the start of landfill operation to an average of 610 mg.l^{-1} after four years. In contrast, the ammonia

levels increased as a function of landfill age. However, the pH was relatively constant over the four year monitoring period. Other parameters such as phosphorus, chlorides, calcium, magnesium, sulphates and dissolved, as well as suspended, solids seemed to vary more with the season rather than with the age of the landfill (Kulikowska and Klimiuk, 2008).

In mature landfills, VFAs are converted into biogas composed largely of methane and carbon dioxide; this is the so-called methanogenic phase (Renou *et al*., 2008) It has been noted that some landfills enter this phase relatively quickly, producing leachates with low COD concentrations, even after short periods of operation (Lo, 1996). Monitoring of leachate produced over a period of ten years by a landfill in northern Italy showed that BOD decreased significantly initially, but thereafter the concentration remained fairly stable. Likewise, the COD levels decreased at the beginning of the experimental period, but subsequently increased. It was shown that these changes were only partly accounted for by fluctuations in leachate flow, suggesting that some of the observed trends were possibly correlated with landfill age (Frascari, Bronzini, Giordano, Tedioli and Nocentini, 2004).

Landfills initially tend to generate leachates with a high BOD:COD ratio making them more easily biodegradable than leachates from older landfills (Britz, 1995; Ozkaya, Demir and Bilgili, 2006). At the Wysieka landfill site in Poland, this ratio decreased from an initial value of 0.4 to 0.13 after four years, indicating the increase in the amount of biologically inert material present (Kulikowska and Klimiuk, 2008). A similar situation occurred at the abovementioned Italian site with a reduction from 0.50 to 0.18 over ten years (Frascari *et al*., 2004). However, the BOD:COD ratio is extremely variable, and may be as low as 0.04 or greater than 0.7, dependant not only on landfill age, but also on other factors such as waste composition (Renou *et al*., 2008). Ozkaya *et al*. (2006) found in two field scale MSW test cells that not only total soluble COD, but also the proportion of inert soluble COD in leachate increased with refuse age as

stabilisation occurred whether or not recirculation was used. However, the concentration of inert soluble COD was lower in the cell in which recirculation had been applied (Ozkaya *et al.*, 2006). BOD also tends to decrease over a landfill's lifetime, thus negatively affecting biodegradability. In addition, the ratio of volatile to total solids in leachate decreases as the site ages; which is yet another indication of reduced biodegradability (Tatsi and Zouboulis, 2002).

Relatively high pH (mainly resulting from the anaerobic consumption of free VFA's) and low heavy metal concentrations are also characteristic of leachates from older landfills (Britz, 1995; Tatsi and Zouboulis, 2002). These two characteristics are related: the lower initial pH of leachate allows for a higher degree of metal solubilisation, which is subsequently reduced by the increase in pH (Tatsi and Zouboulis, 2002). This lower initial pH is caused by the production of organic acids that occurs when a site is first used (Kulikowska and Klimiuk, 2008). Apart from this, precipitation and adsorption reactions that occur within the waste mass also contribute to the lower metal concentrations in more stabilised leachates (Lo, 1996). As leachate stabilises, the oxidation-reduction potential tends to increase from negative to positive values, which is due to oxidation of biologically available organic compounds (Tatsi and Zouboulis, 2002).

Although the concentration of most organic pollutants clearly tends to decrease as the landfill becomes older, the opposite is true for ammonia-nitrogen; this means that this substance is one of the most important contaminants in leachate from stabilised landfill sites (Tatsi and Zouboulis, 2002). This is illustrated by the results from the Polish landfill site (Wysieka) in which ammonia concentrations increased from 98 to 364 $mg.l^{-1}$ over a period of four years (Kulikowska and Klimiuk, 2008). Nitrate concentrations are often correspondingly low. Low phosphorus content is also a critical limiting factor for the aerobic biological treatment of older leachates (Tatsi and Zouboulis, 2002).

Considered together, these factors explain why biological treatment processes often become less effective as the landfill ages and the proportion of labile organic compounds in the leachate decreases (Britz, 1995). In other words, the BOD:COD ratio (which is often a useful measure of biological treatability) decreases over time, suggesting that higher proportions of organic contaminants are biodegradable in young leachates than in mature leachates (Tatsi and Zouboulis, 2002).

Other indices such as changes in phosphorus, chlorides, magnesium, calcium, sulphates, dissolved solids and suspended solids levels did not seem to be correlated with age at the Wysieka site, but fluctuations could be more easily correlated with season and consequent changes in environmental conditions (Kulikowska and Klimiuk, 2008). Landfills still in operation tend to produce leachates containing higher concentrations of pollutants such as phthalates than landfills that have been closed, although toxic compounds continue to be discharged long after sites are no longer accepting waste (Marttinen *et al*., 2003).

Ambient temperature at a landfill site also affects leachate quality as both microbial and chemical reactions are affected by this environmental factor. For example, sub-zero temperatures may freeze some of the waste mass, reducing leachable mass and inhibiting some chemical reactions as well as microbial activity. Available moisture and available oxygen also influence the quality of leachate produced. Water plays an important role in biodegradation as well as in the leaching of chemicals out of waste, and large variations in leachate quality can therefore be caused by changes in available moisture within the waste mass. The effect of available oxygen is significant, especially for putrescible waste because contaminants released during aerobic decomposition differ from those released due to anaerobic decomposition. Anaerobic conditions often develop in this environment due to the frequent covering of the landfill with soil or fresh waste (Bagchi, 1994).

Leachate quantity is mainly affected by weather and operational practice. Precipitation, which depends on geographical location, is the most influential factor affecting leachate volume, which will clearly increase during the wet periods of the year (Bagchi, 1994). Leachate quality is also affected by temporal variations in rainfall as the concentrations of some pollutants will be higher during dry periods of the year. This is due to reduced percolation, and increased evaporation. A more dilute leachate will necessarily be generated in wetter seasons (Tatsi and Zouboulis, 2002). However, Tränkler *et al.* (2005) commented that although COD concentrations were significantly affected by seasonal variations in rainfall, TKN was only marginally related to these climatic events. They also noted that the highest degradation took place during the rainy season, suggesting that lower degradation of waste during the dry season may be due to a lack of moisture which, like temperature, would affect the leachate constituents and microbial activity within the waste mass. They recommend that, especially in tropical climes, excess leachate generated during the wet season should be stored and re-circulated through the landfill in drier periods in order to provide the moisture necessary for degradation to occur. Importantly, these results were obtained in lysimeter studies, and not in full-scale landfills (Tränkler *et al.*, 2005).

Groundwater intrusion and moisture content of waste can also cause variations in the quantity of leachate produced. Groundwater intrusion occurs when the landfill base is constructed below the groundwater table, and increases leachate volume. Unsaturated waste will reduce leachate formation. However, the amount of water actually absorbed by dry waste is often much less than predicted values due to channelling, which causes water to flow through waste without being absorbed. Conversely, if waste releases pore water when compacted, leachate quantity will increase. Codisposal of sludge or liquid waste in municipal landfills has the same effect. Leachate generation is significantly reduced after landfill closure and final cover because the low permeability surface layer reduces

percolation. Evapotranspiration by vegetation grown in the topsoil also reduces the amount of water that reaches the waste (Bagchi, 1994).

Cover design, which is a vital aspect of landfill construction and management, also has a profound effect on both leachate quantity and quality. Tränkler *et al.* (2005) suggest that uncovered waste characterised by lower compaction density, together with higher organic content, allows the system to remain partially aerobic thus enhancing stability of inorganic compounds which are otherwise quickly leached through the waste mass when it is covered and percolation is restricted.

Operational practices, such as the recirculation of leachate through the landfill, can greatly affect leachate generation at a site both in terms of volume and strength. Indeed, recirculation is sometimes used as a method of leachate treatment, although it is hotly debated whether this has any significant advantages as a management option. Even though BOD and COD values may be reduced by recirculation, the concentration of metals and chloride increases. Leachate volume can also be reduced by evaporation and absorption within the waste mass. However, problems such as reduction in cover permeability, leachate perching and odour have been reported. This technique may achieve some results in the short-term, but it is unlikely to provide a long-term treatment solution (Bagchi, 1994).

Storage of landfill leachate on site can affect the quality of the effluent; lower pollutant concentrations were measured in leachate stored in basins for three months at the Iisalmi landfill when compared to fresh leachate. This may have been due to sedimentation of hydrophobic pollutants sorbed to particulate matter, or to biodegradation by indigenous microorganisms (Martinnen *et al*., 2003).

1.5 Microorganisms and the landfill environment

A landfill provides a heterogeneous environment in which a diversity of microorganisms can coexist in mixed populations. The conditions within the waste mass and the substrate specificity of the microorganisms will determine the type of biological reactions that occur (Onay and Pohland, 1998). Refuse typically has high organic carbon content, and thus sorption may render hydrophobic contaminants in the waste mass unavailable for biological metabolism. Higher moisture content may counteract this effect by stimulating desorption and increasing bioavailability of these substances (Dilek Sanin *et al*., 2000). The microbial ecology of landfills is well documented and notable for the dynamic, complex interactions that occur. As previously mentioned, these biological processes will influence the composition of the waste liquid produced by a site. This section deals with the microorganisms that are able to survive, and even grow, in the presence of the contaminants occurring in landfill leachates.

Landfill leachate also affects the microbial populations that exist in the vicinity of a site, causing a particularly obvious effect if the contaminated liquid is able to reach ground water (Ludvigsen, Albrechtsen, Ringelberg, Ekelund and Christensen, 1999). Historically, aquifers were considered lifeless habitats, but it has now been shown that subsurface environments are rich in prokaryotic life forms. This means that the influence such microorganisms have on the fate of pollutants from landfill sites is an important aspect of studies on the biodegradation of these contaminants. For example, two spatially distinct microbial ecologies were identified in a shallow anoxic aquifer below a municipal landfill site in Oklahoma, U.S.A, illustrating that leachate from a single site varies at different locations across the affected area and this in turn affects the microbial population. The dominant metabolic processes differed between the two sampling areas; methanogenesis being predominant at one site, while sulphate reduction was primarily responsible for carbon dissimilation at the other. The organisms present in the leachate-affected areas showed no signs of being nutritionally stressed, in contrast to the populations isolated from pristine areas of

the aquifer where the nutrient supply was much poorer (Beeman and Suflita, 1987).

Both microbial biomass and community composition in a habitat can be affected significantly by the introduction of leachate, which elevates the concentrations of dissolved organic carbon (DOC), a number of other substances, including methane, ammonium, chloride and manganese, and electrical conductivity often making the environment more conducive for microbial growth (Ludvigsen *et al*., 1999). Other researchers have also observed that an increase in electrical conductivity, as well as high concentrations of chloride, DOC and organics such as benzene, toluene, ethylbenzene and xylene (BTEX) in groundwater indicate contamination by landfill leachate (Röling, van Breukelen, Braster, Goeltom, Groen and van Verseveld, 2000). These compounds probably provide sources of nutrition for microorganisms in the surrounding environment. In a study performed on the microbial community of a shallow aquifer in Grindsted, Denmark, total bacterial cell numbers were relatively consistent with increasing distance from the landfill plume; however, total viable biomass decreased, indicating that a higher proportion of microorganisms were active close to the landfill border. The community nearer the landfill border was also more diverse than the community that existed further away. Certain types of microorganism, such as methanogens (Archaea) and microfungi or algae, occurred only in the presence of leachate while organisms such as the sulphate-reducers were more prolific closer to the point of origin of the leachate plume. In contrast, iron, manganese and nitrate reducers were evenly dispersed, even where their activities were not apparent, suggesting that these organisms may have been facultative anaerobes able to use more than one electron acceptor. No protozoa, either aerobic or anaerobic, were detected (Ludvigsen *et al.*, 1999). Even in stable, final disposal sites filled with so-called inert items such as plastics, gums, metals, glass and building materials where the concentration of chemical compounds is relatively low, microbial community structure is still affected

(Uchida, Hatayoshi, Syuku-nobe, Shimoyama, Nakayama, Okuwaki, Nishino and Hemmi, 2009).

Another study also showed that polluted groundwater underneath a landfill harboured a very different microbial population from the upstream and downstream communities, which were very similar to one another. In addition, the microbial community in the contaminated part of the aquifer was heterogeneous, and varied over a small spatial scale, thus increasing the chance that the leachate would encounter microorganisms with the capacity to biodegrade individual pollutants within it when it passed through the affected zone (Röling *et al.*, 2000). The fate of some organic pollutants in a leachate plume was studied by Bjerg, Rügge, Cortsen, Nielsen and Christensen (1999); this work showed that some aromatic and chlorinated aliphatic hydrocarbons, such as toluene, some forms of xylene and trichloroethane (TCE) were biologically degraded by the naturally occurring bacteria at the site. However, benzene, ethylbenzene and naphthalene were not degraded (Bjerg *et al*., 1999).

Landfill leachate plumes may also harbour iron-reducing bacteria; in a study on a polluted aquifer in Vejen, Denmark, the concentration of dissolved iron increased with distance from the landfill before decreasing further downstream to reach background levels, creating a ferrogenic zone. This was attributed to the reduction of dissolved organic matter in the leachate, leading to the formation of iron oxides in the sediment, which would then have been reduced and solubilised. There was strong evidence that a substantial part of this iron reduction was microbiologically mediated (Albrechtsen and Christensen, 1994).

Adrian, Robinson and Suflita (1994) examined spatial variability in biodegradation rates in an aquifer contaminated by landfill leachate using methane production as an indication of biological activity. They found that hot spots of high biodegradative activity occurred; these accounted for a substantial portion of the methane production measured. Cold spots, or areas in which

biological activity was relatively low, were also identified. In addition, methane production varied temporally being highest in the summer months, followed by autumn and spring. Biodegradation is influenced by pH, temperature and sulphate concentrations, although many other factors may also interact to cause and control the spatial and temporal variation observed in the microbial environments around a landfill site (Adrian *et al*., 1994).

This type of information is relevant to studies involving the treatment of landfill leachate because it provides information on the interactions that occur between microorganisms and the pollutants found in the contaminated water, thus allowing for more effective remediative action (Ludvigsen *et al.*, 1999). Analysis of the composition of microbial communities in leachate polluted aquifers is also a useful tool to determine whether intrinsic bioremediation (bioremediation using the autochthonous population) is an appropriate treatment option (Röling *et al.*, 2000). This is illustrated by a study performed on the leachate plume of the Grindsted landfill in Denmark where all compounds that were degradable in the in situ microcosms were also degradable in laboratory batch experiments (Bjerg *et al*., 1999). This shows that indigenous microbial populations can potentially be used to bioremediate leachates using bioreactor technology.

1.6 Leachate treatment

Leachate transport and treatment are critical issues that have to be factored into the construction and management of most landfills (Britz, 1995), but leachate composition is so variable and heterogeneous that it is difficult to design and implement a standard treatment method. However, a desirable system is one able to remediate as many different waste streams as possible. According to Tatsi and Zouboulis (2002) leachate treatment plants cannot be designed to cope with the average leachate quality because this would mean occasional overloading due to high pollutant concentrations during peak periods. Therefore any leachate treatment technology should attempt to cater for the maximum

concentration of pollutants, or the worst-case scenario. However, the difficulty associated with identifying and quantifying the typical characteristics of a specific leachate is a major constraint. This is especially because leachate composition varies so widely depending largely on the degree of stabilisation of the landfill and seasonal production, or the effect of different climatic conditions. This is not only true for different landfills, but also for different locations within the same landfill (Tatsi and Zouboulis, 2002). Moreover, leachate characteristics change over time as the volume produced decreases causing a corresponding increase in strength so any treatment and disposal system must therefore be highly flexible (Britz, 1995). However, a general approach can be used for landfills that are located in similar climatic regions and handle similar waste types (Bagchi, 1994). One of the greatest constraints in the design of a leachate treatment system is the cost associated with managing the daily and seasonal variation in the nature and volume of the leachate generated. It is therefore important to focus on treatment options that have the required robustness and flexibility, but which also minimise labour and operating expenses (Britz, 1995).

Leachate may be disposed of in a number of ways. It can be recycled between different cells within a landfill site, flushed into a river or watercourse, fed into the local sewer system, treated in an on-site plant or taken to an alternative treatment site by road (Robinson, 2005). Landfill leachate is highly contaminated liquid that is rarely suitable for discharge directly into surface water bodies because the raw effluent usually has a negative impact on aquatic life and decreases water quality (Bagchi, 1994). It is unlikely that the concentrations of leachate constituents such as ammonia, methane, BOD, COD, suspended solids, chloride, nitrate and other potentially toxic compounds will fall within regulatory limits imposed on substances that are permitted to drain into a river or watercourse. The last option, transporting leachate to be treated at an alternative site, is only used if none of the other options are available and is severely limited by cost (Robinson, 2005). This emphasises the desirability of treating leachates on site if at all possible (Bulc, Vrhovšek and Kukanja, 1997).

Leachate can be treated using several traditional wastewater techniques, which can be divided into two categories: biological treatment and physical/chemical treatment. In general, a biological approach is most viable for leachate with high organic content, while a physical/chemical process is more appropriate for those leachates with low organic content (Bagchi, 1994). A combination of biological and physicochemical methods is often required for effective treatment and the appropriate treatment method for a specific site may also change over time. Biological treatment may need to be replaced with a combination of biological and chemical methods as the waste mass ages. The goal of these treatment processes is either to reduce the concentration of contaminants so that the effluent can be discharged into surface waters, or to reduce pollutant concentrations to acceptable levels for transfer to an off-site treatment plant (Britz, 1995). On-site treatment plants are suitable for very large municipal landfills and hazardous waste landfills, while use of an existing, nearby wastewater treatment facility is preferred for most non-hazardous wastes.

1.6.1 Leachate channelling

1.6.1.1 Off-site

Leachate from a landfill site that is constructed near a municipal wastewater treatment plant with available capacity may be piped there for combined treatment with domestic sewage (Britz, 1995). If this approach is used, the leachate should be introduced into the treatment stream gradually to avoid shock to the microbial populations in the existing plant, which means that storage facilities will probably be required. The operator and/or designer of such a multifunctional treatment plant needs to be aware of the potential variability in leachate quality and quantity over time (Bagchi, 1994).

This is one of the simplest approaches to leachate treatment, and it is usually favoured where possible due to low maintenance requirements and minimal operating costs (Renou *et al*., 2008). Although impressive results have been reported, leachate addition can reduce overall COD removal efficiency and the plant may need to be expanded in order to cope with the increased organic load (Britz, 1995). This is especially relevant where recalcitrant inhibitory compounds or heavy metals are present in the leachate, but it is also true that nitrogen may not need to be added at the plant if sewage and leachate treatment are combined (Renou *et al*., 2008).

The fate of individual compounds from landfill leachates in sewage treatment plants (STPs) depends greatly on the physical and chemical properties of each compound, as well as the process design and operational practices at the treatment facility (Byrns, 2001; Martinnen *et al*., 2003). If xenobiotic chemicals are not effectively mineralised within the treatment system, some fraction will be released into the environment either in the form of liquid effluent or in the sludges produced. Some substances may be volatilised and enter the atmosphere directly. Complete mineralisation is rare, and more often some form of biotransformation occurs, changing the composition and/or structure of the original contaminant. As it is often difficult to determine the exact nature of xenobiotic compounds introduced into an STP, it is important to establish a generalised model for the fate of such chemicals (Byrns, 2001). Some compounds may be transported through a treatment plant unaffected because dissolved organic matter can act as a carrier and protect these substances from biological attack (Bauer and Herrmann, 1998). Biotransformations may also increase toxicity relative to the parent compound, and the likelihood of this occurrence must also be assessed. However, some compounds such as di-(2-ethylhexyl) phthalate (DEHP) and polycyclic aromatic hydrocarbons (PAH) have been successfully removed by sorption onto excess sludge from leachate co-treated with sewage where the total contribution of leachate to DEHP load at the sewage treatment plant was usually less than 1 % (Martinnen *et al.*, 2003).

Dignac, Ginestat, Bruchet, Audic, Derenne and Largeau (2001) studied the changes that occur in organic matter during the biological treatment of wastewater, and found that long chain aliphatic carbons were transformed into highly branched structures. Aromatic substances were present only in low abundance both in the influent and effluent liquid. They therefore suggested that residual organic matter is more likely to be composed of products of condensation of simple biological molecules, and that it is resistant to microbial degradation because of the higher complexity of the structures, rather than being due to any specific chemical functions associated with these substances. Nitrogen-containing organic matter in treated water may be resistant to biological treatment because of the incorporation of nitrogen into heterocyclic structures, and many non-proteinaceous nitrogen-containing compounds are also generated by the microorganisms themselves (Dignac *et al*., 2001).

All the above considerations apply to any liquid treatment system, including the bioreactor technology specifically developed for handling landfill leachates that is discussed in this thesis. However, it was outside the scope of this study to examine the fate of individual pollutants in the system, and this type of investigation would necessarily relate only to a particular site because of the highly variable nature of this category of effluent. However, it is still worth mentioning some of the pathways by which compounds may be removed from a wastewater.

The partitioning or sorption behaviour of a specific organic chemical is of great importance in determining the mechanisms that may be responsible for its elimination. For example, volatilisation of a chemical from the aqueous phase is only relevant for that portion in the dissolved state; the fraction that is sorbed to particulate matter will not be available for mass transfer across the water/air interface. Advection, where a chemical is removed via association with suspended matter or due to sorption to settling solids, may also occur from the dissolved phase. If a compound has a significant negative charge at pH values

close to neutral (for instance, some organic bases or complex metal anions) then partitioning behaviour between the negatively charged activated sludge biomass and solution may be altered and advection would become the primary fate pathway, leading to higher concentrations of the substance in the final effluent than would be expected according to its molecular size and physico-chemical characteristics. Compounds with a strongly hydrophobic nature are removed predominantly by sorption to sludge particles, and not by biochemical reactions (Byrns, 2001).

Biodegradation is usually considered to be a function of the concentration of active biomass present, and the equilibrium concentration of the contaminant in the reactor. However, this does not account for compounds that are strongly sorbed to biomass solids; thus the hydraulic retention time (HRT) is used to calculate the average reaction rate for biodecay of the proportion of compound in the dissolved phase, but for the sorbed portion the operating solids retention time of the bioreactor must be used. In reality, biotransformation rates of pollutants will vary considerably depending on their structure and intrinsic biodegradability (Byrns, 2001). Using a cascade system such as the one described in this study is therefore advantageous **(Section 2.1.2)** because different compounds will be removed at different points in the system. So the combined effects of volatilisation, advection, sorption and biotransformation will influence the overall level and rate of removal of any specific organic compound from a liquid waste treatment system (Byrns, 2001).

Many leachates require pre-treatment before they can be released to sewer. Constructing and maintaining a pre-treatment plant can sometimes be more economical than paying sewer fees for organic waste loading, as little financial benefit is gained by direct discharge unless the leachate contains less than 2000mg.l^{-1} COD (Britz, 1995). The increased organic load makes it more difficult to maintain aerobic conditions within the aeration basin, and the oxygen transfer capacity must therefore be sufficient to allow for this. It is also important that

nutrients (especially phosphorus) are present in non-limiting concentrations so that the required operating conditions can be preserved. Other properties of leachates such as low acidity and high concentrations of both organic and inorganic compounds can cause problems in municipal wastewater works. The presence of toxic organic compounds that may not be removed by a conventional process must also be considered; high ammonia concentrations, toxicity to sewage-digesting microorganisms, diminished sludge settling, precipitation of iron oxides and corrosion can also adversely affect the functioning of a sewage treatment works. A high ratio of sewage to leachate is normally required to make this process successful (Bulc *et al*., 1997; Renou *et al*., 2008), and a leachate volume greater than five percent of the total sewage plant influent volume is normally unacceptable for municipal treatment works. However, the amount of leachate that is acceptable is highly variable and must be determined on a site-specific basis (Britz, 1995). Transport is also risky and expensive unless a treatment plant is available close to the landfill site and the leachate can be piped directly into the local system (Bulc *et al*., 1997).

1.6.1.2 Recycling

There is much debate surrounding the use of recirculation for treatment of municipal leachate (Bagchi, 1994; Britz, 1995). This approach is one of the least expensive options and could reduce long-term environmental impacts, while effecting leachate treatment requirements significantly (Britz, 1995). Recirculation may decrease leachate volume due to increased evaporation and absorption of liquid by the waste mass (Bagchi, 1994). Contaminant concentrations in the leachate may also be reduced via biological pathways in the landfill, thus low-strength leachate is produced and the need for leachate treatment is minimised (Britz, 1995). However, while BOD and COD concentrations in leachate may be reduced, increases in metal and chloride concentrations have been reported (Bagchi, 1994), and ammoniacal nitrogen is not significantly removed (Britz, 1995). Other problems such as reduced permeability of the cover, leachate

perching, acidic conditions and odour have also been noted (Bagchi, 1994). Benefits of recycling include faster chemical and biological stabilisation of the waste mass, as well as enhanced gas generation due to increased moisture content (Britz, 1995). Another advantage is the distribution of nutrients and enzymes that occurs between methanogenic bacteria and the solid-liquid interface, although methanogens may be inhibited if recirculation exposes them to high concentrations of organic acids (Renou *et al*., 2008).

Leachate recycling has been tested at both pilot and field scale. These experiments have shown that this method can be used successfully as part of a leachate management strategy. However, other treatment options often need to be used for final liquid disposal. The pH of the leachate also needs to be controlled because pH changes caused by leachate recycling can diminish the anaerobic microbial population, hindering biological stabilisation and reducing treatment efficacy. A lime treatment is therefore preferred for partial neutralisation and metal removal (Britz, 1995).

The hydrological characteristics of a landfill site also determine whether leachate recycling can be used effectively. Traditional methods of construction and operation do not necessarily favour optimal conditions for the correct distribution of recycled leachate and channel formation or lateral movement into surrounding water resources may result. Furrowing may alleviate surface ponding and improve leachate distribution; however, recirculation may not be viable if the water balance indicates an accumulation of liquid in the site. It may also be necessary to exclude liquid from the site if leachate potential is to be meaningfully reduced (Britz, 1995).

If landfill leachate with high ammonia concentrations is treated in a separate system, a combination of physicochemical and biological technologies may be required resulting in a high cost process with operational difficulties (Onay and Pohland, 1998). Leachate recirculation within the landfill increases refuse stability

and gas production, and Onay and Pohland (1998) have proposed that controlled landfills with leachate recycle could be adapted to achieve *in situ* nitrogen removal. Simulated landfill bioreactors with anoxic, anaerobic and aerobic zones were used to investigate this possibility and nitrogen conversion of up to 95 % was recorded. The authors therefore recommended that capped landfill sites be modified to allow this process to occur (Onay and Pohland, 1998).

Although the costs associated with leachate recycling are low compared to biological treatment methods, this approach does not offer a complete solution for leachate management and a further treatment step is usually required to ensure that the effluent meets recommended disposal standards for discharge (Britz, 1995). Recirculation may appear viable initially, but is unlikely to be a suitable option for long-term treatment of municipal landfill leachate (Bagchi, 1994).

1.6.1.3 Irrigation

Leachate irrigation involves using surrounding land as a natural medium for biological and physico-chemical remediation. However, this is often regarded as unacceptable as the high concentrations of chemicals present in most leachates could potentially impact surface and groundwater if land disposed (Britz, 1995). This means that it is only viable where a thick clay layer, either natural or man-made, exists between the soil at the site and the water table (Bollag and Bollag, 1995). Symptoms of toxicity have been observed on plants irrigated with municipal leachate (Bagchi, 1994), and it is possible that damage to the soil structure also occurs. The land may therefore become unsuitable for agricultural use (Britz, 1995).

Despite these issues, irrigation may be a viable, low-cost option if suitable areas, such as completed landfills, are available. BOD reductions of 95% have been

reported. However, land disposal is generally only effective for low-strength leachates and it cannot be used when it is likely to lead to waterlogged conditions (Britz, 1995).

Where vegetation is present, additional reductions in leachate volume are caused by plant uptake and transpiration, but it is important that leachate application rates are regulated so that they are consistent with the evapotranspirational needs of the plants in the area. If application rates give rise to reduced conditions, vegetation die-back will occur. Leachate also has a varying effect on vegetation depending on its strength; higher strength leachates may damage plants. In addition, leachate irrigation generally has an adverse effect on total microbial biomass present in the soil, and some shifts in the species composition of the microbial community may be caused (Britz, 1995).

There is strong resistance to using land spraying as a leachate treatment technique. Many factors including soil type, presence of cations, moisture content, presence of organic matter and water table need to be considered before this approach can be recommended. If high concentrations of heavy metals are present, pre-treatment may be necessary to prevent phytotoxicity. Large areas of land are required for effective treatment and there is little information on long-term effects associated with exposing soil environments to leachate. Odour production could also cause problems in the areas around the disposal site (Britz, 1995). Thus land disposal does not appear to be a viable approach to the problem of leachate treatment (Bagchi, 1994).

1.6.2 Physical and chemical treatment

Physico-chemical methods of leachate treatment offer many advantages. These include flexibility as well as fast start-up times and reaction rates which reduce the plant size required compared with biological treatment (Britz, 1995). These

methods are usually insensitive to temperature and can be automated in many instances. However, operating costs may be high and potentially hazardous sludge that must be correctly disposed is often generated. Physical and chemical methods are usually inadequate for complete leachate treatment and they are most commonly used in combination with other processes, especially biological technologies (Britz, 1995).

1.6.2.1 Chemical evaporation

Evaporation has been shown to reduce both organic and metal concentrations of leachate, when a two-stage distillation at high pH with a subsequent acid step was used. However, this method is prohibitively expensive (Britz, 1995).

1.6.2.2 Chemical oxidation

Chemical oxidation can be used to make leachate constituents insoluble, gasify them, or stabilise them as less toxic substances. Several oxidation techniques have been studied, including wet oxidation, ozonation, peroxide treatment and chemical reduction (Britz, 1995).

These methods, using substances such as chlorine, ozone, potassium permanganate and calcium hypochlorite, generally result in poor COD reduction of less than 50% even at high doses, although iron and colour removal may be very good (Britz, 1995). Advanced oxidation processes (AOP) have received attention recently and use a combination of strong oxidants (ozone and hydrogen peroxide), irradiation (ultraviolet, ultrasound or electron beam) and catalysts (transition metal ions or light) (Renou *et al*., 2008).

Chemical oxidation is a very expensive, energy intensive method requiring many special precautions, and is therefore viable only when the leachate has a low

organic concentration and BOD:COD ratio. Thus this approach could be used to treat recalcitrant leachates (Britz, 1995) and improve their biodegradability so that subsequent biological treatment can be cost-effectively used, or to achieve complete mineralisation (Renou *et al.*, 2008).

For example, Steensen (1997) used chemical oxidation to treat biologically pre-treated leachates in which complete nitrification had already occurred and only the COD or adsorbable organic halogens (AOX) exceeded permissible levels for discharge. Three processes were tested: hydrogen peroxide with UV, ozone and ozone with a fixed bed catalyst. In all cases COD was reduced sufficiently for discharge. A biological post-purification phase may reduce COD even further due to structural changes brought about during chemical oxidation. This additional phase could also be used to lessen the requirements of the oxidising process (Steensen, 1997).

1.6.2.3 Chemical precipitation, coagulation and flocculation

Compounds such as lime, alum, ferric chloride, sodium sulphide and ferrous sulphate have been used to remove heavy metals and part of the organic matter from landfill leachate (Britz, 1995; Renou *et al.*, 2008). These processes usually focus on the removal of metals such as As, Cu, Zn, Pb, Ni, Ag, Hg, Cr and Fe, which makes the effluent more suitable for downstream processing, and ensures that biological activity will not be inhibited. Suspended solids, stabilised sludge and precipitate from the leachate can be removed using sedimentation or filtration techniques, which can reduce the organic loading on a biological system (Britz, 1995). Flocculants such as alum or polyelectrolytes (Britz, 1995), or even bioflocculant (Renou *et al.*, 2008) can be used to promote particle coagulation and settling. Precipitation, coagulation and flocculation do not generally result in effective COD removal, although reductions of up to 50 % have been achieved in

some cases. These processes are therefore limited to use in combination with other treatment techniques. However, colour, suspended solids, NH_4^+, and heavy cations can be significantly reduced (Britz, 1995), and this method is often used to decrease ammonia concentrations prior to a biological treatment stage (Renou *et al*., 2008).

Fenton's treatment is a chemical flocculation method for COD removal. Bae, Kim and Chang (1997) used this technique in combination with several biological activities including nitrification, denitrification and activated sludge processes. The Fenton process was achieved by adding $FeSO_4.7H_2O$ and H_2O_2 at a ratio of 1:1, adjusting the pH to 3.5, flash mixing for three hours at 200 rpm and allowing 20 minutes for flocculation before decanting the supernatant. This was performed after the nitrification-denitrification stages, and the supernatant was fed into the activated sludge plant which was amended with sucrose (Bae *et al*., 1997).

The photoassisted Fenton reaction is a variation on this method, and involves the combination of chemical flocculation with exposure to ultraviolet (UV) light to eliminate nonbiodegradable and toxic organic compounds from waste effluents. Kim, Geissen and Vogelpohl (1997) investigated the applicability of this technique for further purification of municipal landfill leachate that had already been through a biological treatment system. They found that the photoassisted Fenton reaction was three times more effective than the dark Fenton reaction, indicating that irradiation significantly improved the efficacy of this method. However, the results are highly dependent on pH, illustrated by the 70 % COD removal recorded at pH 3 in contrast to the very poor COD removal (20 %) observed at pH 8.2. Thus the pH of most leachate streams would need to be adjusted to make this a viable option. The BOD of the leachate also increased after this procedure, meaning that an additional biological phase could be used to obtain better effluent quality (Kim *et al.*, 1997).

Fenton oxidation is rarely used on its own, and is often used as a post-biological polishing process as in both the studies described above. It can also be combined with other methods: Wang, Chen, Gu and Wang (2008) performed a pilot-scale study involving coagulation with poly-ferric sulphate, Fenton oxidation and aerobic biofiltration to reduce the COD of leachate that had already been through a sequencing batch reactor (SBR) system. The rationale behind this approach was to target different contaminants during each phase: coagulation was used to remove suspended solids and colloids, Fenton oxidation to degrade organic pollutants, while the biological aerated filter reduced the concentration of any remaining COD, BOD and ammonia. Before treatment, the waste stream had an extremely low BOD:COD ratio of approximately 0.01, with COD and colour concentrations failing to meet governmental discharge standards. However, this combination of chemical and biological methods resulted in a COD removal efficiency of about 88 % and a significant colour reduction, generating a product that satisfied regulations (Wang *et al*., 2008).

Karrer, Ryhiner and Heinzle (1997) suggest that additional chemical oxidation should be used in combination with a biological treatment to remove refractory organic compounds. However, they caution that the oxidising agent should be carefully chosen in order to avoid the creation of secondary pollutants that occurs when some chemicals, such as chlorine containing oxidants, are used. Even ozone can become an additional pollutant, and needs to be destroyed before discharge of any effluent. A combination of biological and chemical treatment is preferred to reduce costs. Karrer *et al*. (1997) used alternating fluidised bed reactors and ozonation units to treat various types of wastewater including landfill leachate. This procedure was used because ozonation converts refractory organics into more easily biodegradable compounds that can then be attacked by the microbial community in the subsequent bioreactor. The landfill leachate samples had to undergo a nitrification phase before being ozonated to prevent the reaction of ozone with the large amounts of ammonia typically found in this type of wastewater. When a synthetic wastewater containing *m*-

chloronitrobenzene was treated, a combined system was much more effective than using ozonation on its own, even though the substrate used is theoretically not biodegradable. This was due to the break-down of the initial substance by ozonation and an increase in the bioavailability of the resultant intermediate compounds. A combination process also consumed significantly less ozone, even if the substrate reacted well with this chemical oxidant (Karrer *et al*., 1997).

The use of chemical oxidants to treat biologically pre-treated leachates has also been suggested by Steensen (1997). In this case, three different forms of chemical oxidation were used: hydrogen peroxide with UV light as a catalyst, ozone with UV light and ozone with a fixed bed catalyst. The fixed bed catalyst is used in a commercially developed process that reduces ozone consumption as well as the reaction time required for oxidation. Hydrogen peroxide is most effective at low pH values, and this means that alkalinity has to be completely reduced before oxidation, if energy consumption is to be maintained at a reasonable level. Acidification using HC*l* will significantly increase the concentration of chloride ions in the wastewater, making this a major disadvantage. Ozone combined with UV light is not very effective because sparsely soluble oxalates which cause hydraulic problems in the system are generated due to the high concentration of hydrocarbonates typically found in leachates. Such effects do not occur with the fixed bed catalyst, and chemical oxidation of up to 80 % was achieved using this method. A COD elimination of 70 % was obtained with very little increase of ozone consumption, although further reduction required the addition of notably larger amounts of the oxidant. A biological post-purification stage was also investigated with the aim of reducing the requirements for chemical oxidation. This was achieved by cycling the final effluent back into the chemical oxidation stage. This resulted in a reduction in the amount of ozone required by 10 – 15 % (Steensen, 1997).

1.6.2.4 Activated carbon adsorption

Granular or activated carbon adsorption, either in columns or powder form, is the most widely used physico-chemical method of removing organics from leachate. Although good COD removal is usually obtained and the method is reportedly more effective than other chemically based treatments (Renou *et al*., 2008), concentrations of organics such as acetone and methanol are not effectively reduced (Britz, 1995). This technique is best used in combination with biological treatment methods to remove recalcitrant organics, inert COD and unacceptable colour (Renou et *al*., 2008). A disadvantage is the frequent regeneration of carbon columns required or the high consumption of carbon powder, which make this option an expensive one. Hot air stripping of adsorbed organics followed by incineration is used to regenerate spent carbon; back-washable sand filters can also be used as a pre-treatment stage to prevent clogging of carbon adsorption beds. Factors such as preparation method, storage conditions, pore size, surface area and solution pH all affect the adsorptive capacity. Due to high energy and handling costs, this method is viable only for treating residual organics where the total dissolved solids are less than 200 $mg.l^{-1}$. It is therefore suitable for leachates from old landfill sites, or as a tertiary treatment option in a biological treatment plant (Britz, 1995). Activated carbon is sometimes used in bioreactors to act as both a microbial support and an adsorptive substance (Renou at al., 2008). Despite the limitations described, such systems require minimal capital input and are simple and effective (Britz, 1995).

Fettig, Stapel, Steinert and Geiger (1996) used this method to purify leachate that had already been through an aerobic biological treatment phase; some of these samples were ozonated, while others were not. Pre-ozonation increased the fraction of non-adsorbable and weakly adsorbable compounds in the waste liquid although the total concentrations of dissolved organic carbon decreased due to partial mineralization. The activated carbon columns were therefore less effective when applied to these samples than when applied to samples that had

come straight from the biological phase. However, the removal of carbon was higher than predicted in this case, illustrating that biological degradation took place in the activated carbon columns, usually in the initial sections of the fixed-bed. In addition, denitrification was observed in the adsorber columns without the addition of an external carbon source (Fettig *et al*., 1996). A microorganism-attached activated carbon fluidised bed (MAACFB) process was used to remove refractory organics and nitrogen from mature landfill leachate by Imai, Iwami, Matsushige, Inamori and Sudo (1993). The system consisted of two reactors arranged in series, each with an effective bed volume of one litre, with the first being anaerobic and the second aerobic. Phosphorus and methanol were added as supplementary nutrient sources. The MAACFB process showed consistent and effective removal over the experimental period. However, the activated carbon was responsible for adsorbing a large percentage of the refractory organics removed, while only about 50 % of the influent dissolved organic carbon was biodegraded and stabilised as carbon dioxide (Imai *et al*., 1993).

1.6.2.5 Irradiation

Radiation-induced oxidation can be used to degrade refractory organics in leachate under aerobic conditions. These substances can eventually be converted into carbon dioxide, without the production of sludge that occurs when other methods are used. However, high organic concentrations require large doses of radiation which makes this an extremely expensive option (Britz, 1995). Alternatively, gamma radiation can be combined with the use of a coagulant such as ferric chloride, which will reduce costs and can be very effective (Britz, 1995).

Electron-beam (EB) radiation was used to remove refractory substances remaining in a treated leachate after a biological process (Bae, Jung, Kim and Shin, 1999). An activated sludge system was shown to be effective in reducing BOD by 98 % in a leachate with a relatively high BOD:COD ratio (0.33), but high

concentrations of COD still remained. In this case, EB radiation very successfully removed high molecular weight organic compounds such as aquatic humic substances from a synthetic humic solution, but was less successful when used on actual activated sludge effluent, possibly because landfill leachate components used up the radicals formed during the radiation process. The effects of EB radiation depended largely on the pH of the solution (a lower pH produced more satisfactory results) and the dose used (breakdown was proportional to dose). This method is not recommended for leachate that has not been biologically pre-treated because the radicals formed by EB radiation react non-selectively with both biodegradable and refractory organics, and thus would be an expensive option if used alone (Bae *et al*., 1999).

1.6.2.6 Membrane reactor technology

Membrane reactor (MBR) technology can be divided into microfiltration (MF), ultrafiltration (UF), nanofiltration (NF) and reverse osmosis (RO) based on the pore size of the membrane used. MF and UF use membranes with pore sizes of approximately 0.1 µm and 0.01 µm respectively. Neither of these methods is effective as a stand-alone treatment, although UF has been used as a pre-treatment for RO to prevent membrane fouling. Few studies discuss the use of NF (pore size of approximately 0.001 µm) for leachate treatment, but it has been used to remove refractory COD in conjunction with other methods. RO is the most promising membrane technology for the treatment of landfill leachates and employs membranes with pore sizes of approximately 0.0001 µm (Renou *et al*., 2008). This method generates a concentrate that can be recycled back onto the landfill site, and while this is often promoted as a cost-efficient aspect of the technology, it can have serious effects on the leachate that is subsequently generated and may actually increase treatment costs (Britz, 1995). Such a system was implemented at the Wischhafen landfill site in Germany, but it had to be modified because the COD and ammonia concentrations of the leachate

increased dramatically, as did the conductivity. The higher conductivity resulted in higher salinity, affecting the performance of the RO system. Thus, although RO can play an important role in leachate treatment, it is inadvisable to use this technology for primary treatment. In the Wischhafen case, a bio-oxidation stage was added with the option of incorporating a denitrification phase if required. Sludge produced by a landfill leachate MBR is much harder to filter than sludge from industrial or domestic processes. Membrane fouling occurs more easily when this type of effluent is treated and preventative action may need to be taken. For this reason, a sidestream technique is recommended in adverse conditions as opposed to a submerged MBR, an example being the BIOMEMBRAT-Loop designed by Wehrle Environmental (Robinson, 2005). Membrane fouling is a major drawback of reverse osmosis, and regular chemical cleaning of membranes is required. Elimination of suspended solids and colloidal material can prolong membrane life, and an appropriate pre-treatment method may help to prevent severe membrane fouling. Membrane type, pH, pressure and pre-treatment all contribute to the effectiveness of RO as a leachate treatment technique (Britz, 1995).

A number of researchers have recommended the use of RO for the treatment of landfill leachate, and have shown that both organic and inorganic compounds can be removed and reduced to insignificant levels using this technique (Chianese, Ranauro and Verdone, 1999). Better results are usually obtained if leachate is treated immediately after collection because any biological activity may cause hydrogen sulphide odours in the permeate and gas build-up can cause problems at the start of the process. However, biological treatment prior to the application of RO seems to be advantageous.

In a pilot-scale study performed by Chianese *et al*. (1999), COD values were significantly reduced in a RO unit, as were the concentrations of zinc, copper and cadmium (which had been added to aqueous samples of a municipal leachate). However, when initial COD concentrations were increased, copper and zinc

removal was adversely affected, perhaps because of the formation of complexes with organic matter. However the percentage removal of these metals was still satisfactory and cadmium removal was not affected. The leachate was pre-treated by MF to remove suspended solids and was also diluted to produce a range of samples with relatively low COD concentrations (the highest concentration tested was 1749 mg.l^{-1}) before the RO treatment was carried out. In addition, the pH of the leachate in the processing tank was lowered from eight to six and maintained at the latter value using hydrochloric acid. Higher initial COD concentrations reduced permeate flux and obtaining maximum removal may depend on increasing the applied pressure according to the amount of organic compounds present (Chianese *et al*., 1999).

1.6.2.7 Ammonia stripping

Many leachates have high concentrations of ammoniacal nitrogen, which must be removed before discharge. This can be accomplished using air stripping: lime is used to raise the pH of the leachate to above nine, NH_3 gas is formed, and air is bubbled through the system for atmospheric discharge. The high pH also causes metal precipitation, which allows such contaminants to be removed (Britz, 1995). Volatile organics may also be eliminated using this method (Bagchi, 1994). A typical air-stripping plant requires a lagoon, an aeration system, a base feeding unit and a pH control system. The efficacy of this method is affected by several factors including temperature, wind speed, aeration rate, lagoon configuration, pH, surface area and ammonia concentration in the leachate. Although construction of stripping towers can be expensive and operational problems such as formation of adherent scale inside the tower have been reported, lagoons are a low-cost option suitable for on-site leachate treatment. Air-stripping is therefore usually seen as a cost-effective, simple practice (Britz, 1995).

If high concentrations of ammonia are present, stripping may be applied by increasing the pH of the leachate to above 10.5 and flashing off to atmosphere under a vacuum (Robinson, 2005). The contaminated gas phase must be treated with either sulphuric or hydrochloric acid, but air pollution may still result (Renou *et al*., 2008). The process requires heating, and although capital investment is low, the high energy requirement means that operating costs will be considerable (Robinson, 2005).

1.6.3 Biological treatment

One of the most cost-effective treatment techniques for the removal of BOD, COD and ammonia is biological oxidation (Robinson, 2005), or bioremediation. Microorganisms possess enzymes that allow them to degrade environmental contaminants and use them as a nutrient source; in addition, their small size means that they readily come into contact with such contaminants (Bagchi, 1994). Bacteria are especially useful for bioremediation because of their rapid growth and metabolism, genetic plasticity, ability to adjust rapidly to a variety of environments and unparalleled metabolic flexibility (Baker and Herson, 1994b). Recent leachate treatment strategies often involve the combination of biological and physicochemical methods to ensure that the process is as efficacious as possible. Generally, it is assumed that leachates with high organic content are best treated biologically, while those with low organic content may be more suited to physiochemical methods (Bagchi, 1994).

One of the advantages of bioremediation over physicochemical methods is the relative ease with which it can be implemented. This is because it can be done on site, thereby minimising transport costs and liabilities, and also because it keeps site disruption to a minimum, allowing the site to remain operational while the biotechnology is functioning. In many cases, waste can be permanently eliminated by decomposition to carbon dioxide and water, which reduces long-

term liability. Bioremediation is often less expensive than other treatment methods, and can easily be coupled with other treatment technologies to create a treatment train (Baker and Herson, 1994a).

There are also limitations and disadvantages associated with the use of biological methods. As previously mentioned, some compounds are not readily amenable to biodegradation and in the case of some chemicals, microbial degradation may lead to the production of more toxic or mobile substances than the parent compound. Polymerisation of contaminants is undesirable because this does not deal with the problem, but merely delays it (Baker and Herson, 1994a). Bioremediation also tends to be scientifically intensive and, because it must be tailored to site-specific conditions, the initial costs for site assessment, characterisation and feasibility evaluation may be higher than costs associated with conventional technologies. Extensive monitoring is required and, in some areas, regulatory constraints may exist especially if genetically modified organisms are to be used (Baker and Herson, 1994a). Optimal growth conditions for the specific microorganisms involved must be determined, controlled and maintained, which can be difficult and often requires extensive labour. It is also important to be aware that even if indigenous or introduced microorganisms can metabolise a particular pollutant, they may prefer other more readily available nutrients that are present. Inhibitory compounds may also prevent efficient biodegradation. However, new microorganisms that are capable of utilising recalcitrant contaminants are continually being discovered (Bollag and Bollag, 1995).

The successful microbial destruction of man-made contaminants depends on three factors: the species of microorganisms present, the type of contaminant and the chemical conditions. Geological conditions also play a role where *in situ* bioremediation is used (Bagchi, 1994). Bacteria and/or fungi which have the physiological and metabolic ability to degrade the contaminants must be present; these microorganisms may either be indigenous or exogenous. They must also

be in close proximity to the contaminants: the presence of a biodegradable contaminant may have enriched the environment for organisms capable of degrading it, but if such populations are not present, exogenous microbes with the necessary metabolic pathways must be brought into contact with the substrate. Environmental conditions such as temperature, pH, and concentrations of inorganic nutrients and/or electron acceptors can also be adjusted to create conditions that optimise the growth and activity of relevant microorganisms (Baker and Herson, 1994a).

Several approaches to bioremediation can be used on a site such as a landfill. These include biostimulation, where the growth of an indigenous microbial population is stimulated; bioaugmentation, where bacterial cultures are added to the contaminated medium, and the use of bioreactors which involves biodegradation in a container or reactor (Baker and Herson, 1994a). The use of a vessel, or bioreactor, containing either suspended or attached microorganisms and incubated with contaminated wastes as liquids or slurries allows for extensive process control and the maintenance of ideal conditions. This means that nutrient availability, interaction between organisms and pollutants, moisture content and the rate of contaminant loading can be monitored and kept at optimum levels (Bollag and Bollag, 1995).

1.6.3.1 Aerobic versus anaerobic biological technologies

Aerobic microorganisms have a faster metabolic rate than anaerobes, which means that residence times are shorter, and that the same amount of waste can be treated in a smaller reactor. Start-up times can also be reduced. Aerobic systems are usually able to handle a greater range of wastewaters than anaerobic, which was an important consideration in this study, as landfill leachate is a highly variable waste stream both over time, and in different locations. It is not necessary to control temperature and pH as precisely in an aerobic treatment

plant (Armenante, 1993), a fact that complements the low-technology, low-maintenance approach that characterises the present research programme. Aerobic processes also remove BOD, nitrogen and phosphorus more efficiently (Britz, 1995). They tend to be more stable than anaerobic processes because a diverse population of microorganisms operate (to a large extent) independently of one another, whereas in anaerobic systems different classes of organisms are responsible for successive steps in the degradation of contaminants (Armenante, 1993). However, significant energy expenditure may be required to supply enough oxygen to the system due to the low solubility of oxygen in water, and the large air-water interface that must therefore be generated (Britz, 1995). Aerobic systems also tolerate lower loading rates than anaerobic systems and produce more biomass per unit of waste metabolised, which leads to the production of large volumes of sludge. Waste streams containing volatile hazardous substances cannot be treated in aerated bioreactors because of potential air pollution problems. Anaerobes also have some unique degradation capabilities that cannot be exploited in a completely aerobic system. These include the dehalogenation of highly recalcitrant compounds that could then be made suitable for an aerobic treatment stage. Anaerobic respiration can also be used to carry out specific degradation reactions, such as denitrification (Armenante, 1993).

Landfill leachate is often produced in anaerobic conditions, and many investigations have used anaerobic systems to treat this type of wastewater. For example, Lin (1991) used both conventional and two-phase anaerobic digestion to bioremediate a young leachate with a high BOD:COD ratio. COD and BOD removal efficiencies were high at over 90 % in semi-continuous mode, but organic nitrogen was converted into ammonia making this method unsuitable for leachates with high concentrations of ammoniacal nitrogen. In addition heat input and long digestion times are required to obtain satisfactory results from this system (Lin, 1991), which would increase operating costs substantially.

Despite the successes of anaerobic methods, aerobic bioremediation has many advantages. Bertin, Majone, Di Giola and Fava (2001) used an aerobic bioreactor with a similar design to that used in the current study to treat anaerobic effluent from olive mills, illustrating that the benefits of aerobic treatment technologies can be applied to anaerobic wastewaters. Aerobic and anaerobic pathways can also be combined in a single bioreactor. A simultaneous aerobic and anaerobic (SAA) bioreactor used to treat landfill leachate from a site in China, in which anoxic zones were created due to limited oxygen diffusion, achieved 94 % COD removal efficiency. Various organic pollutants, including highly saturated alkanes, were completely degraded (Yang and Zhou, 2008).

1.6.3.2 Biological treatment technologies

A variety of on-site processes have been used for leachate treatment, and the preferred method will depend on the nature of the leachate generated at a particular landfill. For weak leachates, aerated lagoons, sometimes followed by constructed wetlands, may be used prior to discharge into a nearby watercourse (Robinson, 2005). Lagoons are able to cope with significant variation in leachate strength; they have the ability to remove organic compounds, nitrogen, phosphorus and suspended solids as well as pathogenic microorganisms and they are easy to maintain with low associated costs (Maynard, Ouki and Williams, 1999). They are often used as a pre-treatment stage in wastewater treatment processes, but there are few studies concerning their use to treat landfill leachate. One such study evaluated the use of a five basin, non-aerated lagoon system to pre-treat leachate over a ten year period at a landfill in northern Italy. The average removal efficiencies for COD and BOD were 40 and 64 % respectively with biodegradation a major contributor to the removal of organics from the leachate. Ammonia removal was typified by an initial high rate of 95 % which fell to 60 % over the course of the study. However, the lagoons effectively reduced the burden on the municipal treatment plant by removing a significant

percentage of the leachate contaminants (Frascari *et al*., 2004). More recently, an aerated pond containing biofilm promoting mats has been used to successfully remove benzene from contaminated groundwater. Similar technologies could be used to remediate landfill leachate, although ammonium concentrations were not significantly decreased in the system (Jechalke, Vogt, Reiche, Franchini, Borsdorf, Neu and Richnow, 2010).

Constructed wetlands (CW's) can be used to treat landfill leachate with advantages such as low costs for construction and operation, as well as relatively simple installation and maintenance (Martin and Johnson, 1995). This is desirable in cases where physicochemical leachate treatment systems are inapt due to high installation costs and where a system incorporating lagoons and CWs may be appropriate, particularly if the leachate is a low-strength effluent (Mæhlum, 1995). The success of CWs depends on the vegetation, optimum water column depth, an appropriate substrate and the presence of certain microbial populations (Martin and Johnson, 1995). The efficacy of this method depends on the establishment of microbial associations in the root zone of the wetland plants used, and these plants may require a long period of adaptation to become acclimated to the toxicity of the leachate components. Wetlands are also prone to either flooding or drought, which may mean that the treated effluent varies in quality seasonally, and from year to year. In one study, phosphorus concentrations appeared to be limiting and therefore appropriate amendments may need to be added to the system (Bulc *et al*., 1997).The wetland plants and organic litter not only provide the environment for microbial growth, but also deter flow and capture suspended solids. The substrate, in turn, provides support for the vegetation and a reactive surface for the adsorption and desorption of ions (Martin and Johnson, 1995). CWs have a number of features that enable them to be used as a wastewater treatment system, including high plant productivity, large adsorptive surfaces on sediments and plants, aerobic-anaerobic interfaces and diverse microbial populations that can often metabolise contaminants in the waste stream (Mæhlum, 1995). They also reduce effluent volume due to

evapotranspiration (Martin and Johnson, 1995). However, in an integrated system that consisted of an anaerobic lagoon and an aerated lagoon followed by two CWs (a sub-surface flow design, and a subsequent surface flow design), significant biodegradation was observed only in the aerated lagoon, although it is possible that the role of the CWs may assume more importance as they become more established (Mæhlum, 1995). Another study, where an aerated lagoon followed by a series of surface-flow CWs was used to treat landfill leachate, reported much higher removal efficiencies; COD, BOD, ammonia and suspended solids were all reduced to satisfactory levels (Martin and Johnson, 1995).

Leachates can be collected and remediated in a purpose-built vessel or bioreactor which contains microorganisms in suspension or on a fixed support medium. This approach allows for extensive control over process parameters such as nutrient availability, environmental variables and influx of the wastewater. Ideal conditions for biodegradation can therefore be created (Bollag and Bollag, 1995). COD removal in biological treatment systems is influenced by reactor type, and the different fractions of COD that are readily biodegradable, slowly biodegradable and/or refractory may be eliminated more or less effectively depending on the specifications of a particular bioreactor. The plug-flow characteristics of continuous flow bioreactors seem to enhance biodegradation rates and increase the removal efficiency of slowly biodegradable COD (Dockhorn, Dichtl and Kayser, 2001).

Traditional activated sludge plants consist of an aerobic tank followed by a sedimentation chamber, where solids settle to the bottom of the vessel. The supernatant liquid can then be removed as treated leachate while the remaining solids are recycled into the aerobic tank for reuse (Robinson, 2005). The activated sludge process can be used to treat leachates from hazardous waste landfills. However, the conventional activated sludge process (CASP) requires large areas, produces a large amount of sludge and is easily affected by many operational parameters. The performance of the system can be enhanced by

replacing the sedimentation stage with an alternative technology, such as membrane separation. Although high energy requirements have hindered the application of membrane separation in wastewater treatment, several researchers have investigated ways of making this technique more cost-efficient. The combination of activated sludge treatment and membrane separation achieved poor COD removal (31.3 %) and 66 % BOD removal with an ammonium removal efficiency of 98 % when leachate from a hazardous landfill was treated (Setiadi and Fairus, 2003).

Sequencing batch reactors (SBRs) allow biodegradation and sedimentation to take place within a single chamber by aerating the wastewater for a specified length of time and then allowing the solids to settle before the “clean” leachate is removed (Robinson, 2005). A bench scale upflow sludge blanket reactor has been used to remove both carbon (as COD) and nitrogen from leachate with moderate success; nitrogen removal was significant, but COD reduction was variable. The design required temperature control and the addition of a nitrate source to correct the COD:NO_3 ratio (Borzacconi *et al*., 1999), and was thus not suitable for the rationale of the present study, which was the development of a low-cost, low-maintenance laboratory-scale system that could be easily converted into a full-scale plant.

Any biological treatment system using microorganisms that are attached or immobilised onto a solid surface, rather than freely suspended in a liquid medium, depends on the formation of a biofilm by the microbial community. A biofilm is an aggregation of bacteria (sometimes with other types of microorganisms) that exist in a hydrated exopolymeric matrix of their own synthesis. This extracellular structure fundamentally changes both the physical and chemical environment of the microorganisms involved and consequently affects their metabolic activities. In the context of wastewater treatment, the unique set of enzymes produced by this consortium of microorganisms can then be used to consume or degrade pollutants present in the liquid that is to be

treated. Environmental conditions simply need to be controlled and maintained at levels that will ensure that the biofilm remains viable and operates at peak activity (Chappell and Evangelou, 2002).

There are four steps that occur in the process of biofilm generation; firstly cells must be transported to the solid surface, after which initial adhesion occurs by formation of a weak or reversible complex with this surface. The third step is the formation of a more irreversible attachment to the solid surface, usually caused by the secretion of extracellular polymers. Finally these firmly attached cells start growing, new cells remaining attached to each other to colonise the substratum and create the biofilm. This process stimulates cellular changes, which may be phenotypic and/or behavioural (Chappell and Evangelou, 2002). Mechanically, the structure of the extracellular polymeric substance (EPS) is extremely stable. Research has primarily focussed on the polysaccharide portion of the EPS, but extracellular proteins are also present, some of which may be exoenzymes, while others fulfil different functions (Flemming and Wingender, 2001). Although the EPS consists primarily of naturally excreted biopolymers, lysis products and even macromolecules adsorbed from wastewaters may play a significant role in biofilm formation and activity (Choi, Yun, Park, Lee, Jeong, Kim, Lee, Rho and Gil, 2001). Biofilms are hydrogels, and thus essentially hydrophilic, but some hydrophobic areas must exist as well and this is responsible for versatility in adsorbing a variety of substances (Flemming and Wingender, 2001).

Biofilms are extremely heterogeneous often displaying several structural levels because a variety of microbial polysaccharides can be secreted. The structure of a biofilm appears to be preferentially designed to support its function. Microenvironments are formed which can provide protection against adverse redox, osmotic or dehydrating conditions, and even against heavy metals or antibiotic substances (Chappell and Evangelou, 2002). Indeed, when a biofilm begins to dry out, stronger cohesion between the cells as well as stronger adhesion to the substratum can be observed (Flemming and Wingender, 2001).

It has also been shown that only a limited proportion of the flocs that constitute the activated sludge in wastewater treatment plants can be eroded due to turbulence and that this proportion depends on the amount of EPSs present because they significantly increase floc strength. Floc composition also influences shear sensitivity and the dispersible fraction of the floc (Mikkelsen and Nielsen, 2001).

One of the most important functions of the EPS is the creation of an interfacial boundary; separating cells from the bulk solution while allowing them to maintain enzymatic activity, even within a mixed population. The biofilm-cell wall assembly (BCWA) surface will also have an effect on substrate availability by acting as a molecular sieve that will contribute to the passive regulation of product formation. These attached cells seem to be more effective, metabolically, than those remaining suspended in the bulk liquid (Chappell and Evangelou, 2002). Additionally, biofilms, particularly the EPS matrix, are able to sorb dissolved substances which often leads to complex effects as the microbial community seems to respond dynamically to sorption processes by varying the amount and composition of the EPS produced. Particles can also be retained by the extracellular matrix due to its sticky nature. Such particulate matter may be biodegradable, providing a source of nutrients for the cells, but inorganic particles, like sand grains, can also become attached to the biofilm because of the adhesion properties of EPS molecules (Flemming and Wingender, 2001). High molecular weight organic polymers forming the colloidal fraction in wastewaters often cannot cross the extracellular matrix formed by biofilms, but are biosorbed to the microbial aggregate and must thus be hydrolysed by exoenzymes embedded in this structure in order to be assimilated by the microbial cells (Frølund, Griebe and Nielsen, 1995; Guellil, Boualam, Quiquampoix, Ginestat, Audic and Block, 2001). It has been demonstrated that the proteinaceous fraction of the EPSs exists mostly in the colloidal state, rather than being soluble, while the inverse is true of the sugars that are present (Guellil *et al*., 2001). EPS extracts from activated sludge sourced from a municipal

treatment facility appeared to hydrolyse the colloidal protein-containing organic matter in a wastewater sample from the same plant into soluble compounds that could be taken into bacterial cells. However, these extracts did not exhibit any glycolytic activity. This illustrates both the qualitative and quantitative adaptation process that occurs in the biofilm, which ensures that enzymes capable of degrading the colloidal protein-containing fraction of the wastewater are present in the extracellular matrix. It was suggested that easily hydrolysable sugars would probably be depolymerised during transport in the sewer and it would therefore be energetically unfavourable for the microbial community to produce glycolytic enzymes for assimilation of refractory substances. Such poorly biodegradable substances may be better used to consolidate the structure of the biofilm (Guellil *et al*., 2001).

Total nitrogen removal, which is an important element of leachate treatment, has been correlated positively with the EPS content of the biofilm; in contrast total phosphorus removal did not seem to relate to the amount of EPS present (Choi *et al*., 2001). The authors suggested that a thin biofilm is preferable for nitrification, as sufficient oxygen needs to be supplied to the active cells. A thick biofilm would be better for the denitrification process, which usually requires anoxic conditions. Also an internal carbon source may be provided for the responsible microoorganisms due to cell lysis occurring deep within the biofilm. Increasing biofilm thickness corresponded with higher EPS content, and this led to increased removal of suspended solids (Choi *et al*., 2001). Controlling microbial activity is therefore an essential aspect of the operation of biofilm reactors in wastewater treatment.

The type of bioreactor and shear stress affect the composition of the microbial community and the structure of the biofilm. Cao and Alaerts (1995) found that filamentous microorganisms dominated both a batch-type, plug flow bioreactor and a completely mixed system; probably due to the soluble, easily available carbon substrate used. However, cocci co-existed with the filaments in the batch

system, which was not the case in the completely mixed reactor where the cell community was almost exclusively filamentous. It was also noted that cocci were hardly ever found in the freely suspended cell population, and attached preferentially to the biofilm, in contrast to the filamentous component of the community, which consisted of both free-living and attached cells. Most of the freely suspended biomass seemed to arise from erosion of the biofilm. Plug flow seemed to encourage floc formation which resulted in a more compact biofilm, while the biofilm in the completely mixed system was less dense. The batch reactor thus had the largest internal mass transfer resistance. Reactor type therefore influences both the composition of the microbial community and its structure, while shear stress influences only biofilm structure and does not seem to have an effect on microbial ecology. The relationship between microbial population activity and reactor performance is of major importance in any wastewater treatment plant, and needs to be considered when such a system is designed (Cao and Alaerts, 1995).

Various attached-growth bioreactors have been used to treat landfill leachate. Submerged biofilms grown on synthetic fibres have been used to treat undiluted Tunisian leachate at ambient temperature with COD removals of up to 92 %. The dominant genera in this system were *Bacillus, Actinomyces, Pseudomonas* and *Burkholderia* (Ismail, Tarek, Mejdi, Amira, Murano, Neyla and Naceur, 2011). A combination of a pre-denitrifying anaerobic filter and a rotating biological contactor (RBC) has been used to treat leachate with high ammonia concentrations. Although not all nitrogen was removed, the ammonia present in the influent waste stream was substantially reduced by between 80 and 95 %. A large percentage of the BOD (92 %) was eliminated, but the COD removal efficiency (49 %) was much lower, which is a commonly observed result. Despite their use of an anaerobic stage, the authors discovered that the majority of organic matter and ammonia were removed during the aerobic phase. However, it was noted that metals are unlikely to be removed from a stable landfill leachate with an alkaline pH in aerobic conditions (Henderson and Atwater, 1995). A

system with both anaerobic and aerobic phases such as the one described above was not considered for the present study because of the emphasis on a treatment technology that is cost-effective to install and maintain and simple to operate. As the focus was on COD, BOD and, to a lesser extent, ammonia removal, an aerobic set-up was selected.

Packed-bed bioreactors consist of a vessel filled with packing material on which a biofilm can grow. These reactors have been used in both aerobic and anaerobic processes. The packing material is submerged in the wastewater undergoing treatment and is designed to maximise the interface between the waste stream, the gas phase (if the system is aerobic), and the immobilised microorganisms. It is intended to provide an appropriate surface for microbial attachment and growth in order to facilitate the development of a biofilm. Loose materials such as pebbles, lava rock and plastic particles are most commonly used for this purpose. Immobilisation ensures that biomass is retained in the bioreactor and exploits the preference of microorganisms for growing on a solid surface rather than in suspension (Cannon, Gray, Biddlestone and Thayanithy, 2000). Microorganisms that are immobilised in this fashion allow the maintenance of a high concentration of biomass and also ensure that slow-growing bacteria (such as the nitrifiers) are retained without sludge recycling (Cannon *et al*., 2000; Jou and Huang, 2003). The packed-bed thus aims to supply an open matrix in which increase in biomass is simply controlled by the turbulence within the bioreactor (Cannon *et al*., 2000).

Oliveira, Moraes, Adorno, Varesche, Foresti and Zaiat (2004) used an anaerobic packed-bed reactor to treat a synthetic wastewater containing formaldehyde and ascribed the favourable results to the immobilisation of the biomass, which created a protected environment for the microbial population and prevented severe inhibitory effects. In addition, microorganisms organised in this way are believed to be more resistant to unfavourable conditions.

The type of bioreactor described above has been used as a pre-treatment for dairy dirty water; the effluent from this plant passes through a settlement tank before entering a reed bed system. At pilot-scale, this aerobic packed-bed bioreactor was found to be efficient for nitrification, but less satisfactory for BOD reduction (Cannon *et al*., 2000). These authors also commented that a multi-stage plant would be more efficient than a single-stage process. Thus the bioreactor described in **Section 2.1.2** was constructed so that it could ultimately be operated as a cascade system.

Upflow packed-bed bioreactors have been successfully used to treat other highly contaminated effluents and this design was therefore selected for the research discussed in this thesis. Bioreactors that use immobilised cells are considered the most effective for the treatment of wastewaters, especially those contaminated with organic pollutants, and it has been stated that fixed-film bioreactors, either in the form of rotating biological contactors or packed-bed bioreactors, seem to have the greatest efficiency (highest removal rates) and stability when a high degree of degradation is desired. They have advantages over activated sludge systems; for example, two major problems associated with the latter, viz. inability to settle the sludge and excess formation of scum-foam, are not experienced when a fixed-film bioreactor is used. Important advantages over the variety of other processes that are currently used include simple operation, ability to handle shock loading due to the retention of a high biomass concentration, formation of less solid sludge waste and increased energy efficiency (Jou and Huang, 2003). All these factors influenced the choice of reactor type, as they are extremely relevant to the rationale behind this study, which is the development of a cost-effective treatment technology for landfill leachate that is easy to maintain, but still retains versatility.

A packed-bed aerobic bioreactor was used as a continuous system to remove COD from food processing wastewater, and achieved removal efficiency of 82 % (Kariminiaae-Hamedaani, Kanda and Kato, 2003). Bertin *et al*. (2001) inoculated

this type of reactor with a co-culture of two specific bacteria to treat olive mill wastewaters, which contain many recalcitrant compounds. They observed that the co-culture biofilms were able to degrade two complex organic compounds that were not metabolised when the cells were free-living in shake flask batch conditions. Biodegradation rates of other compounds were also significantly higher when the co-culture was immobilised, suggesting that cells in a biofilm exhibit enhanced biodegrading activity and versatility as compared to freely suspended cells (Bertin *et al*., 2001).

Choi *et al*. (2001) used four variants of sequencing batch biofilm reactors to remove nitrogen and phosphorus from domestic sewage, an effluent which has very low total COD and ammonia levels compared to landfill leachate. Hence the high COD reduction ranging from 84 – 90 % that was achieved; nitrification varied from 70 – 97 %. However, some important information can still be gained from this study as it was noted that those bioreactors that were operated as packed-bed reactors removed suspended solids better than those in which the support medium was moving (Choi *et al*., 2001). This is relevant to the choice of bioreactor design in the research described here as landfill leachate will typically have a high concentration of suspended solids and particulate matter.

Loukidou and Zouboulis (2001) compared the remediative efficiency of two attached-growth moving-bed biological processes using sanitary landfill leachate as the influent waste stream. A moving-bed system consists of porous polymeric biofilm carriers that are suspended in continuous movement in an aeration tank. This has advantages over conventional suspended-growth processes because it allows for higher biomass concentrations (and therefore reduced biomass wastage) and the co-existence of aerobic and anoxic microbial activity within one reactor. It also eliminates the need for sludge-settling, is cost-effective and is less sensitive to adverse environmental conditions than suspended-growth systems. However, a high concentration of dissolved oxygen is necessary to promote nitrification; and, where a waste stream containing large amounts of nitrogen is

treated, inhibition of nitrification may occur. In contrast with fixed-bed bioreactors, the continuous motion in a moving-bed system eliminates the difficulties associated with clogging and dead space and so enhances efficiency. The study demonstrated that this approach can be used to remove organic carbon from sanitary landfill leachates achieving between 55 and 81 % COD and up to 90 % BOD reduction. However, although nitrification was satisfactory and 85 % of the influent ammonia was removed, complete denitrification was not realised (Loukidou and Zouboulis, 2001).

Amendments are often made to biological treatment systems in order to increase their efficacy. One of the most widely used is powdered activated carbon (PAC), which enhances treatment efficacy, removes some of the more refractory organic compounds and improves nitrification ability. It has been suggested that a synergy exists between microorganisms and PAC in these bioreactors. Aghamohammadi, bin Abdul Aziz, Isa and Zinatizadeh (2007) found that PAC increased COD and ammoniacal-nitrogen removal efficiency in continuous flow activated sludge reactors at laboratory-scale. The improvement in COD reduction was attributed to the adsorption of inhibitory leachate constituents, and/or to the removal of recalcitrant organics which were not degraded without the addition of PAC. The PAC amended bioreactor produced effluent with a lower pH as a consequence of enhanced nitrification and retention of ammoniacal-nitrogen by the PAC (Aghamohammadi *et al*., 2007). Aktas and Çeçen (2001) showed that free ammonia in highly nitrogenous leachates had a significant impact on nitrification in continuous flow activated sludge bioreactors inhibiting this process and causing a resultant nitrite build-up. Again, the addition of PAC alleviated this effect, in addition to adsorbing other inhibitory leachate components, but the effect of the amendment was much more pronounced in continuous as opposed to batch operations. However, they point out that PAC addition would increase costs, but claim that other advantages such as better sludge settling and increased sludge dewaterability offset this disadvantage (Aktas and Çeçen, 2001).

Many of the bioreactor designs discussed here required temperature control in order to achieve maximum efficiency and stability. This normally involves heating the leachate to encourage mesophilic microbial growth and maintaining this temperature throughout the treatment process; this does, however, add significantly to operating costs and is therefore not always appropriate where low-cost systems are desired. Some studies have investigated the effect of low temperatures on the efficacy of wastewater treatment systems in order to determine whether biodegradative removal could be operated without large energy input. Oil shale ash leachate was treated in both aerobic and sequential anaerobic-aerobic bioreactors operated at temperatures of 7 – 10^0C and 20^0C; in the aerobic reactor, which was the most effective, COD removal was only slightly affected by the reduction in temperature, while BOD removal efficiency remained the same under both experimental conditions (Kettunen, Pulkkinen and Rintala, 1996). Another study demonstrated that anaerobic bioreactors treating landfill leachate can also operate effectively at low temperatures. Methanogenesis occurred at temperatures as low as 5^0C, and the level of activity at low temperatures was notably improved by pre-adaptation of biomass, although this did not affect COD removal (Kettunen and Rintala, 1997).

Some of the most successful technologies combine biological treatment with physicochemical processes to maximise pollutant removal. An operational leachate treatment plant treating effluent from a landfill in Mechernich, Germany, incorporates biological pre-treatment and physical post-treatment stages. In the first phase, microbial nitrification occurs in a contactor system, where 60 % of the total nitrogen load is eliminated (presumably by aerobic deammonification), followed by denitrification in an activated sludge plant. The physical treatment occurs in a two stage RO plant, and it is clear that the biological pre-treatment allows for a greater permeate flow rate than would otherwise be possible, especially because of the reduced conductivity and ammonia concentrations.

The amount of sulphuric acid required for pH correction was reduced as was the amount of residual material that had to be discarded. However, the designer of the treatment system recommended that nanofiltration membranes replace the RO membranes because they show high retention of organic substances and are also ion selective; the relatively high operational costs of the plant would also be significantly reduced by this strategy. NF is, like RO, a pressure-driven membrane process for the treatment of diluted solutions. This modification would allow organic components, heavy metals and bivalent inorganic salts to be retained while monovalent ions such as chloride would pass through, to a large degree. Although it is sometimes useful to recirculate the concentrates produced by NF to the biological pre-treatment phase in order to increase COD removal efficiency, the authors point out that, in this case, sulphates and heavy metals would accumulate and cause problems due to calcium sulphate scaling. The extraction of these substances would not be cost-effective, and therefore this step was not considered (Baumgarten and Seyfried, 1996).

Another system combined an impinging-stream loop bioreactor with MF in order to maintain high biomass concentrations and create a high-performance aerobic treatment technology for a synthetic wastewater (Lübbecke, Vogelpohl and Dewjanin, 1995). The maximum limit of biomass concentration that can be achieved is dependent on wastewater composition; in addition, substances in wastewaters such as landfill leachate tend to have a reducing effect on the viscosity of the activated sludge-water mixture inside the bioreactor. When a similar pilot-scale unit was tested at a landfill site, COD removal efficiencies reached 80 %, while the recorded BOD removal efficiency was 99 %. Nitrogen was effectively eliminated. These results show that such a system is suitable for the treatment of landfill leachate especially where poor bacterial growth and weak sedimentation adversely affect other technologies. Advantages such as the removal of solid matter and pathogenic microorganisms from the treated liquid add to its appeal. The system is compact and closed, which also makes it desirable for this type of wastewater. However, it is associated with high

investment and operating costs (Lübbecke *et al*., 1995), which make it unsuitable for regions where minimal funding for waste treatment exists.

Pirbazari, Ravindran, Badriyha and Kim (1996) found that a hybrid technology known as the ultrafiltration-biologically active carbon (UF-BAC) process, a combination of adsorption, biological treatment and membrane separation removed total organic carbon (TOC) and some specific organic pollutants from two different high-strength landfill leachates. The leachates were pre-treated by either coagulation/flocculation and sedimentation or oxidation with precipitation before entering the system to enhance biodegradability. These processes reduced TOC, total suspended solids (TSS), and volatile suspended solids (VSS); as well as eliminating almost 90 % of any oil and grease that was present, thereby minimising the potential for membrane fouling. The UF-BAC process compared favourably with biological systems such as sequencing batch reactors, and also with the powdered activated carbon (PAC) method (Pirbazari *et al*., 1996).

1.7 Nitrogen removal

The high concentration of ammonia characteristic of leachates from mature landfill sites is caused by the hydrolysis and fermentation of biodegradable substrates with nitrogenous components (Onay and Pohland, 1998). The removal of this ammonia is one of the goals in the design and development of many leachate treatment technologies.

Ammonium in leachate is usually treated using biological processes such as autotrophic nitrification and heterotrophic denitrification, although the presence of high concentrations of organics and other inhibitory compounds can affect the nitrification rate significantly (Kim, Lee and Keller, 2006). Nitrifying bacteria are largely autotrophic and use ammonia nitrogen as an energy source, inorganic carbon as a carbon source and oxygen as the terminal electron acceptor

(Madigan, Martinko and Parker, 1997), but some may be heterotrophic, using an organic substrate for carbon and energy (Zumft, 1997). Most nitrate oxidisers can also grow chemoorganotrophically on glucose and some other organic substrates (Madigan *et al*., 1997). Heterotrophic bacteria also present in wastewater use organic carbon as both an energy and carbon source with oxygen as the terminal electron acceptor, thus creating competition for the terminal electron acceptor between those microorganisms that remove COD and those that remove ammonia, both of which are important contributors to the bioremediation process. Although both groups require oxygen, nitrifiers need approximately three to four times more than heterotrophs. In addition, the growth rate of nitrifying bacteria is an order of magnitude lower than the typical heterotrophic growth rate, and these factors may limit nitrification in a biological treatment system (Klees and Silverstein, 1992).

Studies on nitrification in rotating biological contactors have shown that nitrifying bacteria only compete successfully when the organic carbon is less than 15 mg.l^{-1} BOD_5. Klees and Silverstein (1992) used recirculation to dilute influent organic carbon which effectively improved nitrification in a rotating biological contactor and also increased the biodegradability of the influent stream. According to Li and Zhao (2001), a high effluent recirculation ratio of 300 – 400 % is also required in order to maintain low ammonium concentrations in conventional bioreactors. They also showed that COD removal efficiency, dehydrogenase activity and specific oxygen uptake rate (SOUR) in bioreactors used to treat landfill leachate all decreased as ammonium concentrations increased. Chemical precipitation of ammonium as magnesium ammonium phosphate was subsequently performed as a pre-treatment, but this increased the salinity significantly (Li and Zhao, 2001). Watanabe, Bang, Itoh and Matsui (1994) reported that nitrification limited nitrogen removal at high organic loading rates, while denitrification was the limiting factor when organic loading was lower. When an easily biodegradable substrate such as acetate was used as the carbon source, the optimum C:N ratio was larger than when a more recalcitrant

compound such as ethylene-glycol was used. This is likely to affect ammonia reduction in wastewaters such as leachate because mature landfills tend to produce effluents with a relatively low BOD and may also contain xenobiotic substances, which means that there may be insufficient carbon available for nitrogen removal. Hanaki, Wantawin and Ohgaki (1990) observed that assimilation of influent ammonia by heterotrophs occurred in preference to nitrification in suspended-growth laboratory scale bioreactors. The ammonia available to the nitrifiers was therefore reduced and this effect was exaggerated when the COD concentration was increased, even though an easily biodegradable carbon source was used. Organic matter thus inhibits ammonia oxidation, particularly the conversion of ammonia to nitrite, possibly due to decreased affinity between the ammonia and the ammonia oxidising bacteria. This may be partially caused by clumps of heterotrophic cells that form when carbon is readily available impeding the transport of ammonia from the bulk solution to the ammonia oxidisers (Hanaki *et al*., 1990). Attached-growth systems may therefore be preferable where ammonia removal is required.

Nitrite-oxidising bacteria (NOBs) are typically situated deep within a biofilm, beneath the ammonium-oxidisers, so the substrate generated by the latter is readily available to them. However, in bioreactors where nitrite accumulation has occurred, the NOB may be distributed on the upper surface of the biofilm because nitrite is directly available in the bulk liquid (Kim *et al*., 2006). It has been proposed that the presence of both *Nitrobacter* and *Nitrospira* (NOBs) is beneficial for nitrogen removal, enhancing nitrification rate and reducing nitrite accumulation compared to a situation where only one of these genera is present (Kim *et al*., 2006).

Denitrification is a bioenergetic process that occurs in both bacteria belonging to the Proteobacteria group and to the halophilic or hyperthermophilic archaea. This diversity of denitrifiers is an important part of microbial nitrogen-based interrelationships. Nitrate, nitrite and the gaseous nitrogen oxides (NO and N_2O)

replace dioxygen as the terminal electron acceptor and are thus reduced to dinitrogen gas in four enzymatic steps, which allows microorganisms to respire anaerobically. As the electron donors may be either organic or inorganic, the term autotrophic denitrification is used to indicate an inorganic substrate. This denitrifying part of the nitrogen cycle is crucial because it facilitates the removal of polluting intermediates; for example, nitrate can become a major problem when it contaminates water sources and nitrous oxide is a powerful greenhouse gas. As an energy generating dissimilatory process, it can be contrasted with the assimilatory route where the same nitrogenous compounds are reduced to ammonia for use in biosynthesis (Zumft, 1997).

Biological ammonia oxidation is a zero-order reaction. Nitrite accumulation is not significant when the pH is between seven and eight, and the reaction rate constant for nitrification increases as temperature increases. However, this effect is much less marked for attached nitrifying bacteria compared to their suspended counterparts. A fixed-film bioreactor is therefore more effective than a suspended-growth bioreactor at low temperatures (Watanabe, Ishiguro and Nishidome, 1980). This was pertinent to the present investigation because the low-cost objective meant that temperature control was not considered.

Attached biofilm systems are generally preferred for nitrification of landfill leachate because of the high SRT and increased robustness to environmental conditions, as opposed to systems with a suspended biomass (Kim *et al*., 2006). The high SRTs allow for increased sludge concentration and applied organic load which enables slow-growing microorganisms to develop and contribute to pollutant removal (Xue, Yang, Liu and Fu, 2009). This was another deciding factor in the design of the bioreactor used in the current study.

During complete nitrification, a syntrophic interaction between the ammonium oxidising bacteria (AOB) and NOB occurs, despite the phylogenetic distance between the two groups. However, incomplete nitrification with nitrite

accumulation has often been observed in biological wastewater treatment systems. Although this was initially considered undesirable, several strategies have been developed to take advantage of this situation (Vadivelu, Keller and Yuan, 2007). For example, new processes, such as shortcut biological nitrogen removal, have been developed to treat wastewaters containing high concentrations of nitrogenous compounds. The shortcut strategy is based on the fact that nitrate and nitrite are intermediary compounds in both the nitrification and denitrification pathways, so that partial nitrification to nitrite and denitrification from the accumulated nitrite (instead of nitrate) may be a preferable approach for use in effluent treatment. The oxygen and carbon requirements are significantly reduced and denitrification rates are greater with nitrite than with nitrate (Aslan, Miller and Dahab, 2009).

Nitrate formation is bypassed by the autotrophic anaerobic ammonium oxidation (ANAMMOX) process, in which nitrite as the electron acceptor is converted into dinitrogen gas under anaerobic conditions (Kindaichi, Tsushima, Ogasawara, Shimokawa, Ozaki, Satoh and Okabe, 2007). The advantages of this approach include lower energy expenditure, reduced biomass production and lower carbon requirements. Disadvantageous is the long start-up period (perhaps several months) that may be necessary for the anaerobic ammonium oxidising bacteria to establish themselves. However, once this has been achieved, the system is relatively inexpensive and easy to maintain (Noophan, Sripiboon, Damrongsri and Munakata-Marr, 2009). Nitrogen removal in a fixed-bed column bioreactor seeded with anammox bacteria reached 95 % efficiency after 74 days of operation, after which biofilm analyses were performed. These bacteria co-existed with nitrifiers including both aerobic ammonium oxidisers and nitrate oxidising bacteria (Kindaichi *et al*., 2007).

Ammonium oxidisers have a higher affinity for oxygen than nitrite oxidisers and nitrite oxidation can therefore be limited by maintaining the dissolved oxygen concentration at a low level within the bioreactor (Aslan *et al*., 2009). ANAMMOX

bacteria may also be inhibited by high COD concentrations (Chen, Liu, Yang, Xue and Wang, 2009). The completely autotrophic nitrogen removal over nitrite (CANON) process can be achieved by exploiting the interaction between aerobic and anaerobic ammonium-oxidisers in a single reactor, thereby reducing energy and cost requirements substantially (Chen *et al*., 2009).

Daniel, Pozzi, Foresti and Chinalia (2009) developed a sequential batch bioreactor packed with polyurethane foam and operated with intermittent aeration that was able to remove ammonium from synthetic wastewater via the nitrification-denitrification nitrite-shortcut pathway. The system remained stable over a wide range of influent ammonium concentrations and ammonium removal rates indicated that degradation rates were a product of microbial growth rates. Very little nitrate was detected in the bioreactor during the experimental period. Microbial analysis explained this result by showing that *Nitrosomonas* spp. (responsible for the conversion of ammonia into nitrite) were predominant, while *Nitrobacter* spp., which convert nitrite into nitrate, were undetected. The composition of the microbial community was assumed to be due to the immobilisation of the biomass on the polyurethane matrix. The authors believe that the polyurethane foam allowed for the development of micro-niches capable of promoting metabolic diversity within a single bioreactor. This would be advantageous in the system developed in the present project. Thus both ammonium-oxidising and denitrifying bacteria were able to co-exist and contribute to nitrogen removal (Daniel *et al*., 2009).

The shortcut strategy was also used by Aslan *et al*. (2009) with sequencing batch reactors treating a synthetic wastewater. In this case, the temperature was kept at 19^0C, whereas it was maintained at 30^0C in the research carried out by Daniel *et al*. (2009). The results showed that it is indeed possible to remove ammonium, and promote nitrite accumulation, when dissolved oxygen is limiting and the temperature is below optimum. They also indicate that different members of the microbial community are subject to different limitations so that the activity of

nitrite oxidisers can be inhibited without affecting those microorganisms responsible for oxidising ammonia. Ammonium removal and nitrite accumulation were greatest at the highest SRT of 40 days; however, the SRT had very little effect on nitrate accumulation. Although the free ammonia levels did affect nitrite accumulation by inhibiting the nitrite oxidisers, it was shown that this could not be the only factor affecting this phenomenon. The authors concluded that dissolved oxygen concentrations can be used to create sustainable conditions for nitrite build-up, and that this effect is probably favoured by free ammonia (FA) inhibition (Aslan *et al*., 2009).

A population of ammonium oxidising bacteria was developed by enrichment in a sequencing batch bioreactor inoculated with mixed nitrifying bacteria from a local wastewater treatment plant in order to demonstrate the effect of the ratio of nitrite and ammonium concentrations on the microbial community during the ANAMMOX process. Prior to enrichment, Gammaproteobacteria were most common, but Betaproteobacteria became more prevalent in the enriched culture. Most of the bacteria in the bioreactor fed with lower ammonium concentrations (S1) belonged to the *Nitrosomonas* genus, and some members of *Nitrospira* and *Candidatus* were also isolated; however, *Nitrobacter* were not detected at all (this scenario is similar to that reported by Daniel *et al*. (2009) and discussed above). At higher ammonium concentrations (S2), the bacterial population was more diverse (Alphaproteobacteria, Betaproteobacteria and Gammaproteobacteria were all present) and was dominated by *Candidatus* spp. (ANAMMOX bacteria), but *Nitrosomonas*, *Nitrobacter* and *Nitrospira* spp. were not found. Although more than 90 % of the ammonium and nitrite was removed in the S1 system, an enrichment period of approximately 100 days was required before this removal efficiency was attainable. An even higher percentage reduction of ammonia was achieved in S2, which also showed shock tolerance (Noophan *et al*., 2009).

Chen *et al.* (2009) modified the CANON process and combined it with denitrification to create a partial nitrification, ANAMMOX and denitrification

(SNAD) technology in order to achieve simultaneous nitrogen and COD removal. A novel rotating biological contactor maintained at a temperature of 35^0C and a pH between 8 and 8.2 was used to treat a synthetic wastewater in order to test the efficacy of this technique, which was shown to be a suitable option for the treatment of high nitrogen, low-COD wastewater (Chen *et al*., 2009). However, it must be pointed out that the energy input required by this system is very high.

An alternative strategy that can be used instead of heterotrophic denitrification involves the use of *Thiobacillus denitrificans*; this autotroph oxidises elemental sulphur and simultaneously reduces nitrate to dinitrogen gas without the need for additional organic substrates. This has been effectively demonstrated in packed-bed columns for the treatment of pre-nitrified landfill leachate (Koenig and Liu, 1996).

1.8 Project aims

Baker and Herson (1994a) emphasise the need to remediate contaminated sites with the development of new biological technologies focusing on the detoxification and destruction of contaminants rather than using a conventional disposal approach. The cost of landfill remediation is a major obstacle to the achievement of this goal. For example, several years ago, it was estimated that the remediation of the 4000 old landfill sites in the Netherlands would cost approximately ten billion Euro (Röling *et al.*, 2000), and this estimate is likely to have increased by many times since then. The aim of this research was to design and optimise a cost-efficient biological treatment system for the treatment of landfill leachate in countries where resources, both financial and technical, are limited.

CHAPTER 2

MATERIALS AND METHODS

2.1 BIOREACTOR DESIGN AND CONFIGURATION

2.1.1 Reactor type

An upflow packed-bed aerobic system of unique design was used for this research. An aerobic approach to treatment of the leachate was adopted for the reasons given in the **Literature Review**. Unlike most aerobic systems, both the waste stream and air supply were pushed through the system from the bottom of the bioreactor in an upflow direction (typical for anaerobic packed-bed

processes). Although the bioreactors were aerated, anoxic and anaerobic zones would still have occurred.

2.1.2 Tank bioreactor

A laboratory-scale upflow packed-bed bioreactor was constructed using a glass tank. Glass partitions divided the tank into six equal-sized chambers and the dimensions of the bioreactor are illustrated in **Figure 2.1**. The tank was fitted with an opaque fibreglass lid to reduce evaporation and prevent algal growth (**Figure 2.2 A**). Each of the six chambers had a capacity of approximately 22 litres, giving a total bioreactor capacity of 132 litres. Each chamber contained a removable fibreglass box filled with a solid support matrix for cell attachment and biofilm growth. Various matrices were used over the course of the experiments. A fibreglass lid on each box prevented the matrix from floating to the surface and causing blockages in the outflow pipe. Sampling ports were cut into the lids to allow for removal of matrix for analysis. Both the bottom of the box and the lid were perforated allowing leachate to flow through the box. Each compartment had a separate sparging system comprising a PVC pipe (15 mm diameter) with three 1 mm holes evenly spaced along its length. The spargers were installed below the boxes to supply air to each chamber. A Resun AC 9906 air pump with six outlets was connected to these pipes using thin-walled plastic tubing (inner diameter: 3 mm). The air pump was used at its maximum setting providing 1.1 – 2.2 L air.min^{-1} to give an aeration rate of 0.05 – 0.1 L air.L leachate^{-1}.min^{-1}. The upward air flow was chosen because it minimises air stripping of any volatile substances that are present in the influent waste stream, or that are produced due to biodegradation (Jou and Huang, 2003). Leachate was pumped through the bioreactor using a Watson-Marlow 504U peristaltic pump fitted with six pump heads. Marprene II tubing (inner diameter: 8 mm) was used in the pump heads, and clear silicon tubing (inner diameter: 8 mm) connected this tubing to the bioreactor compartments and/or reservoirs. Depending on the mode of operation

(see below), the recycle rate or hydraulic retention time (HRT) was controlled by this pump, as used in some other bioreactors; for example, the RBC described by Chen *et al*. (2009).

The six-chambered bioreactor could be operated either in parallel or as a cascade system. Initial experiments were performed using the parallel mode so that each chamber was kept independent of the others, allowing different operating conditions to be applied and compared simultaneously. Leachate was cycled between each chamber and its 22 litre capacity reservoir so that approximately 44 litres of leachate were treated in each section of the bioreactor. Like the reactor tank, each reservoir was fitted with an opaque lid to minimise evaporation and prevent algal growth (**Figure 2.2 B**). Samples were taken from the outflow or return pipe connecting each chamber to its corresponding reservoir **(Figure 2.3)**. All samples were stored at 4^0C until analysed.

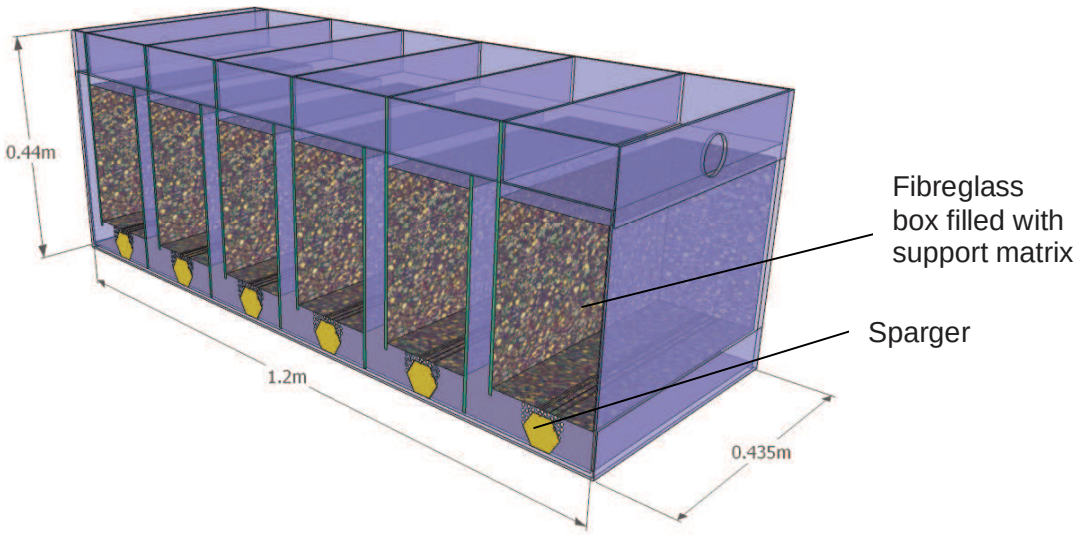


Figure 2.1: Schematic diagram of the six-chambered laboratory-scale upflow packed-bed bioreactor showing the dimensions of the tank (image created using Google SketchUp 7).

Later experiments were performed using the cascade (continuous) mode. To this purpose the bioreactor was modified so that leachate flowed from one chamber into the next, moving through all six chambers before leaving the reactor **(Figure 2.4)**. The system was therefore a plug-flow system. Untreated leachate was pumped from a 220 litre storage drum into a small five litre reservoir that acted as a sediment trap; a Watson-Marlow 504U peristaltic pump with a single pump head controlled the flow rate of the leachate from the storage drum into the sedimentation reservoir. An adjustable ball valve controlled leachate flow from the sedimentation reservoir into the first chamber of the bioreactor. Samples were taken from ports connected to each chamber, as well as from the influent leachate reservoir. All samples were stored at 4^0C until analysed. When functioning in the cascade mode, the system could be classified as pilot-scale as the 132 litre operating capacity is much larger than that of most laboratory-scale bioreactors described in other studies. For example, Aghamohammadi *et al*. (2007), treated landfill leachate in laboratory-scale continuous flow activated sludge reactors, each with a capacity of only 16 litres.

Figure 2.2: The glass laboratory-scale upflow packed bed bioreactor
A: The six component chambers; B: The bioreactor and reservoirs as configured when the six chambers were operated independently of one another

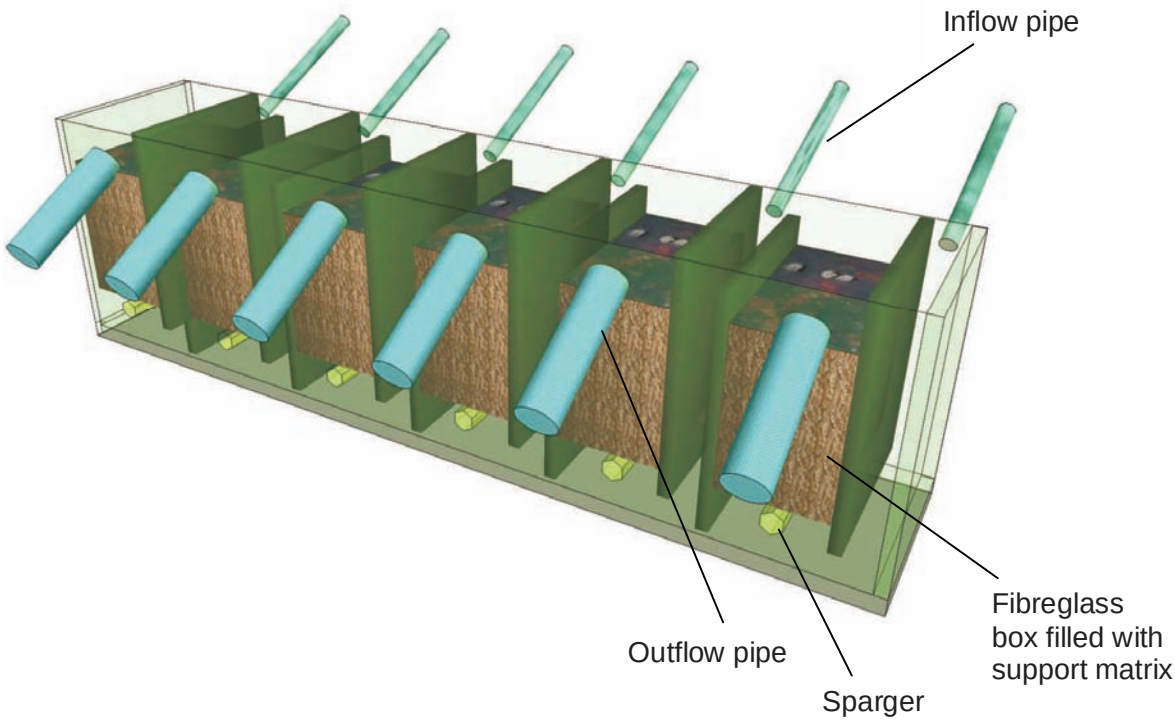


Figure 2.3: Schematic diagram of the six-chambered laboratory-scale upflow packed-bed bioreactor showing the inflow and outflow pipes when operated in batch mode. Samples were taken from the outflow pipes (image created using Google SketchUp 7).

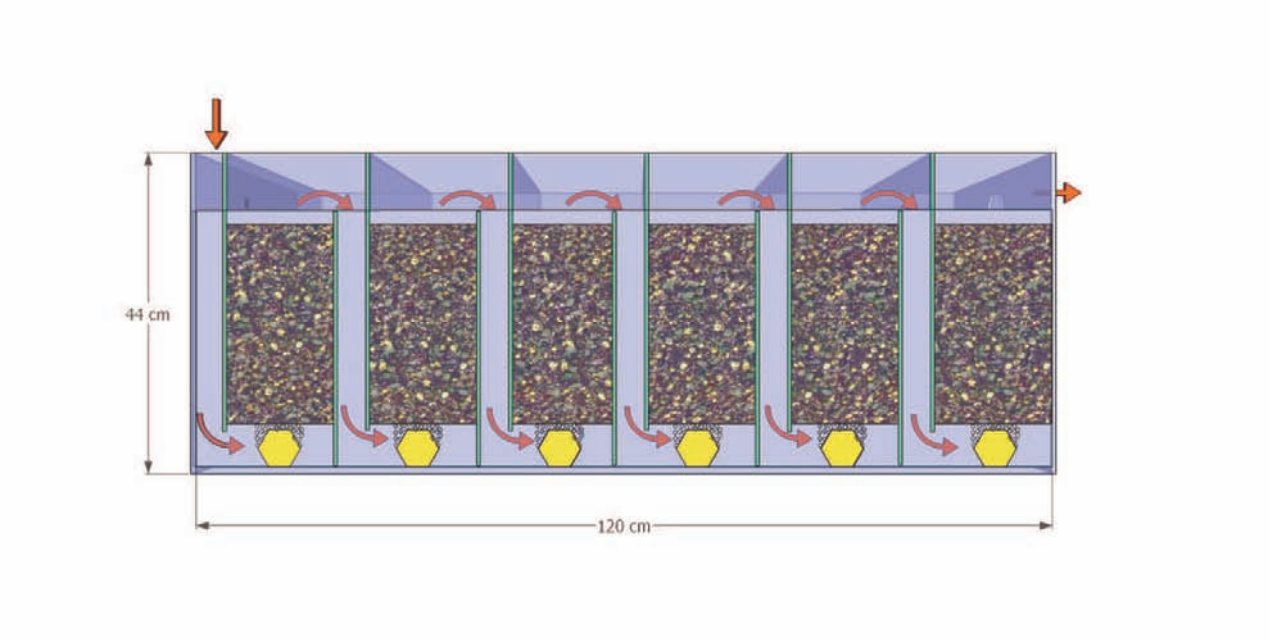


Figure 2.4: Schematic diagram showing the flow pattern of the waste stream through the laboratory-scale upflow packed-bed bioreactor when operated in the cascade mode (diagram created using Google SketchUp 7).

2.1.3 Bucket bioreactors

Plastic containers were used to construct six separate bioreactors. Each of these reactors was equivalent to a single chamber in the tank bioreactor, and had a volume of approximately 22 litres. However, no reservoirs were used. The

amount of leachate treated in each of these bioreactors was therefore half of that treated in one chamber of the tank bioreactor when cycling was employed. A PVC pipe (15 mm diameter) with three 1 mm holes evenly spaced along its length, was attached to the bottom of each bucket (**Figure 2.5 A**). Plastic tubing (inner diameter: 3 mm) connected this pipe to a Jumbo Jet Aquarium Air Pump (Super 7800) set at the maximum air flow providing 1.1 – 2.2 L air.min^{-1} to give an aeration rate of 0.05 – 0.1 L air.L leachate^{-1}.min^{-1}. A perforated, removable plastic disc covered the sparger and prevented blockage of the air holes (**Figure 2.5 B**). Each disc had 34 perforations (diameter: 3 mm) arranged in three concentric circles. The bioreactor was then filled with a solid support matrix (which varied depending on the experiment in progress) for microbial colonisation and biofilm growth (**Figure 2.5 C**). A solid, removable plastic disc wedged above the support material prevented it from floating to the surface (**Figure 2.5 D**). Each bioreactor was fitted with a submersible pump (Resun SP 980 Internal Pump) for leachate circulation. This pump was situated just below the upper disc. Liquid was pumped from the top of the bioreactor, through a short loop (inner diameter: 8 mm) on the outside of the bucket, and re-entered below the perforated disc at the bottom of the bioreactor. This recreated the upflow characteristic of the tank bioreactor. Samples were taken directly from each bucket at regular intervals (these are specified in the relevant chapter sections) and stored at 4^{0}C until analysed. These bioreactors were used to perform batch-type experiments only, and could not be connected to form a cascade system. They were used to examine various process variables while the tank bioreactor was in use.

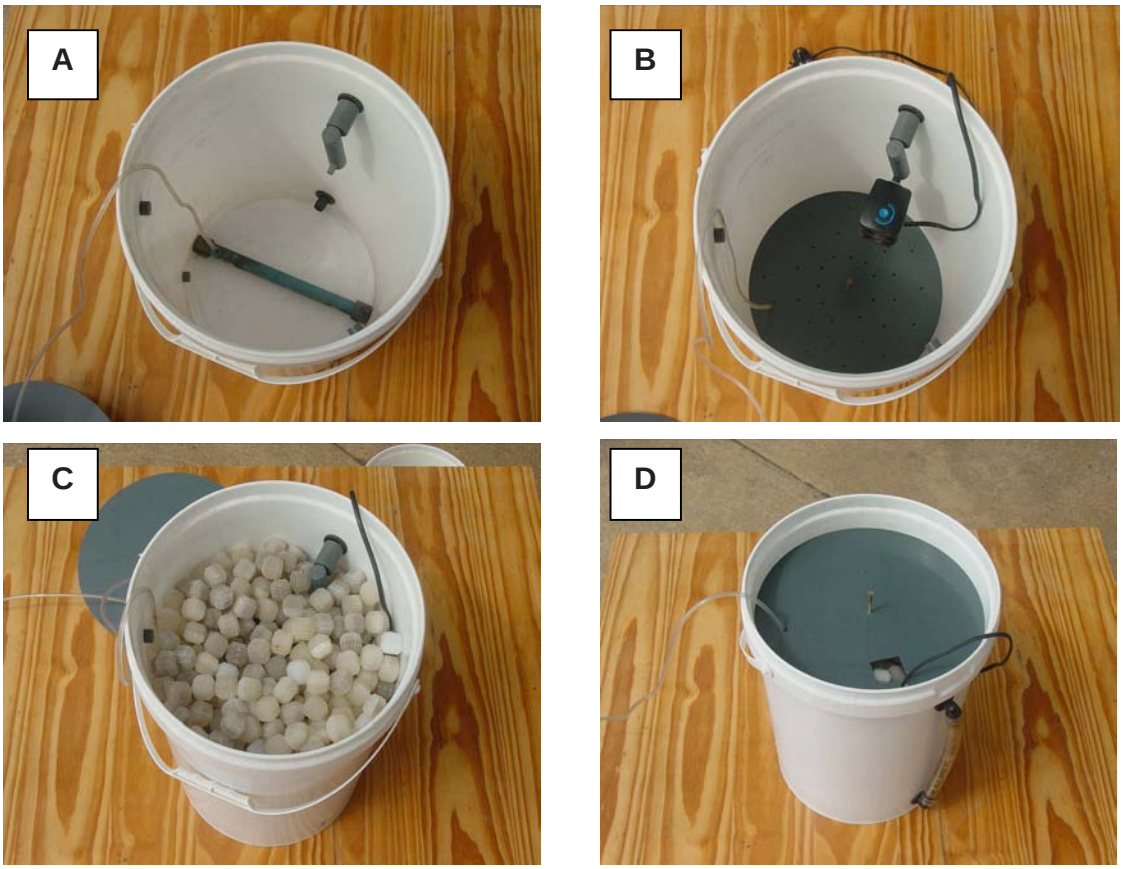


Figure 2.5: Design and components of a single bucket bioreactor
A: The PVC air sparging system installed at the bottom of the bioreactor; B: The lower perforated plastic disc and the submersible pump; C: The bioreactor filled with solid support matrix (plastic bioballs, in this case); D: The upper solid plastic disc used to contain the support material within the bioreactor and the re-cycle loop on the outside of the bucket.

The laboratory in which both the tank and bucket bioreactors were housed and operated was an outdoor structure made of rigid double-walled plastic, with a automatic fan and wet-wall system that was activated when the temperature rose above 25 ^{0}C. However, this system did not guarantee that temperatures were

maintained at or below this point. There was no heating system in operation; this was desirable since the bioreactors were exposed to temperature fluctuations similar to those that would occur in an outdoor treatment system on a landfill site.

2.2 LEACHATE SELECTION AND COLLECTION

Each batch of leachate collected had slightly different characteristics, depending on the amount of precipitation that had fallen on the landfill site, refuse composition and moisture content, ambient temperature, and operating practices (Bagchi, 1994). Although using synthetic leachate may provide a compositionally more consistent waste-stream, a treatment method must be able to effectively bioremediate leachates of various qualities if it is to be used at industrial-scale. For this reason, real leachate was used for the duration of this project.

Several different leachates from various landfill sites in the Durban-Pietermaritzburg area of KwaZulu-Natal were used over the course of this study. Each site was a formally recognized landfill serving an urban or industrial zone. All sites were lined and had leachate collection systems in place. The landfills were: Umlazi (South Durban), Shongweni (Outer Durban), Marianhill (Pinetown), Bul-Bul Drive (Chatsworth, Durban), Bisarsar Road (Springfield, Durban) and New England Road (Pietermaritzburg). Umlazi, Shongweni, Bul-Bul Drive and Bisarsar Road are semi-hazardous landfill sites, while the Marianhill and New England Road sites are permitted to accept only general waste. Each landfill site and its leachate will be discussed in detail in subsequent chapters.

Leachate was collected in 220 litre plastic drums and stored at room temperature. The volumes collected were too large to permit storage at 4^0C. However, the leachates used had already been exposed to fluctuations in ambient temperature for significant lengths of time at each landfill site, as large volumes are stored in dams or reservoirs before discharge or disposal. This uncooled storage also ensured that the environmental conditions were as similar

to those at the landfill site as possible in order to promote the survival and growth of the indigenous microbial population, which was considered more important than the small amount of degradation that occurred (Dilek Sanin *et al*., 2000).

As already mentioned in **Chapter 1**, biodegradation rates in leachate contaminated water show considerable spatial variation (Adrian *et al*., 1994), and the specific sampling sites could therefore have significantly influenced the results obtained in this study. Both the microbial population that developed in the bioreactor, and the nature of the effluent used would have been a product of the specific site from which the raw leachate was drawn. It is important to be aware of the possibility that results could have differed greatly if leachate from different locations of the dams or storage reservoirs at each particular landfill were used. It was outside the scope of this work to investigate this potential, but it may be relevant for any future research carried out using the described system.

2.3 INOCULUM

The indigenous microbial populations in all the leachates used served as a natural source of inoculum throughout this study. According to Dilek Sanin *et al*. (2000), microorganisms within the waste mass at a landfill site are exposed to trace contaminants for long periods of time due to the distinctive nature of this habitat. Thus it was expected that these acclimatised organisms would contribute significantly to the degradation of the organic contaminants in their native leachates.

In experiments where an additional inoculum was introduced, activated sludge from the Hammarsdale Sewage Works was used. As this plant serves a manufacturing area and treats effluents from a diverse range of industries, it was thought to be a likely source of microorganisms able to withstand and degrade a variety of organic and inorganic contaminants. In support of this contention, a comparative study on the biodegradation of [^{14}C] phenol by microorganisms

indigenous to a secondary treated domestic wastewater and a landfill leachate showed that the community in the domestic sewage exhibited immediate biodegradation of this substrate, while there was a definite lag phase before the leachate microorganisms started to break it down and they never achieved the removal efficiencies reached when domestic sewage was used (Deeley, Skierkowski and Robertson, 1985). As mentioned in **Chapter 1**, such compounds are often components of landfill leachate and this illustrates that the addition of activated sludge could enhance the bioremediation potential of treatment systems for this type of wastewater. Fong and Tan (2000) have also shown that the microorganisms in activated sludge can be successfully used to treat other, more specific types of waste; in their case, waste from the food industry. They isolated a consortium of nine species from an activated sludge sample, which removed BOD from food waste with almost the same efficiency as the activated sludge, illustrating that the microbial community was acclimatised to the influent components of the wastewater. Activated sludge thus contains a diverse group of microorganisms that can be used to treat a variety of waste streams (Fong and Tan, 2000).

2.4 ANALYTICAL TECHNIQUES USED TO MONITOR LEACHATE BIOREMEDIATION AND THE MICROBIAL POPULATION IN THE BIOREACTORS

2.4.1 pH

pH was measured using a Crison Micro pH 2000 meter.

2.4.2 Chemical Oxygen Demand (COD)

Chemical oxygen demand (COD) is a critical measurement in wastewater treatment. It represents the amount of oxygen consumed in the complete oxidation of the carbonaceous matter in an effluent sample (Porteous, 1992).

Karrer *et al*. (1997) state that the organic content of wastewaters is usually measured as COD. This practice, in the form of the closed reflux, colorimetric method (Standard Method 5220D), was thus used throughout this research. Merck method 1.14555 with a range of 500 – 10 000 mg.l^{-1} was chosen because of the high COD in all the untreated leachate samples examined. Pre-prepared commercial reagents (2.2 m*l* Merck COD Solution A and 1.8 m*l* Merck COD solution B) were mixed with 1 m*l* undiluted and unfiltered leachate sample in glass tubes. The tubes were tightly sealed and shaken to ensure that the contents were properly mixed. A Hach COD reactor was used to digest the samples for two hours at 148^{0}C. A Merck Photometer SQ 2000, later replaced by a Merck Spectroquant Nova 60, was used to analyse the digested samples. All samples were analysed in triplicate.

2.4.3 Biological Oxygen Demand (BOD)

Biological Oxygen Demand (BOD) measures the amount of oxygen required to biologically degrade organic matter present in a sample (Standard Method 5210). BOD_5 was measured using the WTW OxiTop Control system. This is an automatic manometric respirometric method. A direct measurement is made of the oxygen consumed by microorganisms in a closed vessel, under conditions of constant temperature and agitation. Any CO_2 generated is removed using an absorber, and the resulting decrease in pressure can therefore be correlated with BOD. The microbial oxidation of ammonia and organic nitrogen can exert nitrogenous demand, especially in samples of secondary effluent or polluted waters which may contain significant numbers of nitrifying bacteria. If an inhibitory chemical is not used, the BOD measured must be considered the sum of carbonaceous and nitrogenous demand. Such a measurement is unsatisfactory for assessing the oxygen demand of organic material in a sample. A nitrification inhibitor was therefore used in all BOD determinations in order to obtain carbonaceous BOD_5 ($CBOD_5$). Typically, the respirometric method of BOD

determination is used for comparative analyses (Standard Methods), as was the case in this investigation. Roppola, Kuokkanen, Nurmesniemi, Rämö, Pöykiö and Prokkola (2006) used this system to determine the BOD_7 of wastewater from a pulp and paper mill. Although results differed slightly from those obtained using conventional chemical methods, they were consistent. The method was found to offer many advantages, including reduced sample preparation time, use of non-diluted samples and easy reading of the measuring data. These features favoured the use of this method for leachate analysis in the current investigation.

The measurement range 0-4000 $mg.l^{-1}$ was selected for BOD analysis in this investigation. Undiluted sample (22.7 m*l*) and one drop of nitrification inhibitor were placed in a dark bottle, which was sealed with a rubber sleeve containing two NaOH pellets for CO_2 absorption. The measuring head was then attached and activated, before incubation at $20^0C \pm 0.5^0C$ for five days. Samples were agitated, using a stirrer plate, for the duration of the incubation period. The OxiTop controller was then used to obtain the required data from the measuring heads. Due to the time-consuming nature of this measurement and the limited number of measuring heads available, the BOD of each sample was only analysed once.

2.4.4 Total Carbon

Total carbon (TC) was measured using a Shimadzu Total Organic Carbon Analyser (Model TOC-5000A). This instrument performs high temperature catalytic oxidation (HTOC), converting the oxidisable material in a sample into gaseous form by injecting it on a platinum catalyst at 680^0C in an oxygen-rich environment to produce carbon dioxide. The concentration of carbon dioxide is then measured with a non-dispersive infrared detector, after going through a moisture trap and halide scrubber to remove any components that could interfere with the measurement. HTOC is useful for the analysis of complex effluents such

as landfill leachate because it is able to oxidise high molecular weight organics that may be undetected using other methods and it was therefore suitable for use in this study. Samples were filtered using a nylon, supported, plain 0.45 µm membrane filter (Osmonics Inc.) in order to remove large particulates and microbial cells, then diluted 50-fold before analysis. This method was used only for the first experiment with leachate from the Umlazi landfill site as the instrument was subsequently irreparably damaged. A modified Walkley-Black method was tested as a result of this, but the results were highly variable and were not used. COD is more widely used than TC as a measure of pollutant removal in studies relating to landfill leachate treatment and was therefore used as the primary indicator of bioremediation efficiency in subsequent experiments.

2.4.5 Nitrate

Nitrate (NO_3^-) levels were monitored using a Bran + Luebbe Continuous Flow Analyser (Model TRAACS 2000). Prior to analysis, samples were filtered through a nylon, supported, plain 0.45 µm membrane filter (Osmonics Inc.), but were not diluted as nitrate concentrations were expected to be low. Hydrazine in alkaline solution, together with a copper catalyst, was used to reduce any nitrate in the sample to nitrite. Zinc was added to the reducing agent to suppress the formation of complexes between the copper and organic material. The sample was then reacted with sulphanilamide and N-1-napthylethylenediamine di-HCl (NEDD), forming a pink compound which was measured spectrophotometrically at 520 nm. Phosphoric acid was added to reduce the pH and avoid the precipitation of calcium and magnesium hydroxide (Bran + Luebbe, TRAACS Method no. GB-353-87). Nitrate standards of 10, 5 and 1 $mg.l^{-1}$ were used to calibrate the instrument at the beginning of each run. The reagents were made up as follows:

Working hydrazine sulphate solution: 10 m/ stock $CuSO_4$, 10 m/ stock $ZnSO_4$ and 200 m/ stock $N_2H_4.H_2SO_4$ were added to 600 m/ distilled water, mixed thoroughly and diluted to 1000 m/.

Stock copper sulphate solution: 1 g $CuSO_4.5H_2O$ was dissolved in 600 m/ distilled water, and diluted to 1000 m/.

Stock zinc sulphate solution: 10 g $ZnSO_4.7H_2O$ was dissolved in 600 m/ distilled water, and diluted to 1000 m/.

Stock hydrazine sulphate solution: 10 g $N_2H_4.H_2SO_4$ was dissolved in 600 m/ distilled water, and diluted to 1000 m/.

Sodium hydroxide solution: 10 g NaOH was dissolved in 600 m/ distilled water, after which 3 m/ conc. H_3PO_4 was carefully added; the solution was then diluted to 1000 ml with the addition of 1 m/ Brij-35 (30% solution).

Colour reagent: 10 g sulphanilamide was dissolved in 600 m/ distilled water. To this, 0.5 g NEDD and 100 m/ conc. H_3PO_4 were added, and the solution diluted to 1000 m/. This reagent was stored in a dark bottle to prevent deterioration.

Stock nitrate standard solution(1000 mg.l^{-1} as N): 7.218 g KNO_3 was dissolved in 600 m/ distilled water and diluted to 1000 m/. Working standards were made up as required by appropriate dilution.

All samples were analysed in triplicate using the autosampler attached to the continuous flow analyser. However, the instrument showed only a mean of the three measurements and it was therefore impossible to calculate standard deviation. The print-out did indicate whether the range of measurements for each sample in a particular run was acceptable or not.

2.4.6 Ammonia

Ammonia (NH_3) was also measured using the Bran + Luebbe Continuous Flow Analyser (Model TRAACS 2000). Prior to analysis, samples were filtered through a nylon, supported, plain 0.45 µm membrane filter (Osmonics Inc.). The high concentrations of ammonia present, typical of landfill leachates, necessitated

diluting the samples 100 fold. Salicylate and dichloro isocyanuric acid are combined with the sample in the instrument to generate a blue compound that is detected at 660 nm. Nitroprusside served as catalyst for this reaction (Bran + Luebbe, TRAACS Method no. GB-352-87). Ammonia standards of 10, 5 and 1 $mg./^{-1}$ were used to calibrate the instrument at the beginning of each run. Reagents were made up as follows:

Sodium salicylate solution: 40 g sodium salicylate was dissolved in 600 m/ distilled water, 1 g sodium nitroprusside was added and the solution diluted to 1000 m/.

Tri-sodium citrate solution: 40 g tri-sodium citrate was dissolved in 600 m/ distilled water and diluted to 1000 m/ with the addition of 2 m/ Brij-35 (30% solution).

Dichloro isocyanuric acid solution: 20 g NaOH and 3 g dichloro isocyanuric acid were dissolved in 600 m/ of distilled water before diluting the mixture to 1000 m/.

Stock ammonia standard solution (1000 $mg./^{-1}$ as N): 4.717 g $(NH_4)_2SO_4$ was dissolved in 600 m/ distilled water and diluted to 1000 m/. Working standards were made up as required by appropriate dilution.

As with the nitrate analyses all samples were analysed in triplicate, but the instrument only provided a mean.

The methods described above were used to calculate ratios such as C:N.

2.4.7 Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES)

An ICP Optical Emission Spectrometer (Varian 720-ES) was used to qualitatively assess the inorganic constituents of some leachate samples. An acid digestion was performed on samples of the leachate from the Umlazi landfill (**Chapter 3**) prior to ICP analysis. Two m/ of concentrated nitric acid and five ml of

concentrated hydrochloric acid were added to 100 m/ of sample. This mixture was covered and heated on a hot plate at 90-95 ^{0}C until the volume had been reduced to approximately 20 m/. After cooling, the sample was filtered through a 0.45 µm filter to prevent the nebuliser from clogging and the volume was adjusted to 100 m/ with deionised water. This aqueous sample was drawn into the instrument where it passed through a nebuliser for atomisation before being introduced into the stable, high temperature plasma flame generated using argon gas. This caused the sample to break up into its component charged ions, which were transformed into the gaseous atomic state, giving off characteristic elemental wavelengths that could then be detected.

2.4.8 Gas Chromatography – Mass Spectrometry (GC-MS)

This technique was used to identify some of the organic contaminants in leachate from the Shongweni landfill site (**Chapter 4**). One litre of leachate was extracted with 150 m/ methylene chloride and concentrated under vacuum at 35^{0}C to obtain a final volume of one m/. An HP 6890 gas chromatograph and an HP 5793 mass selective detector with HP Chemstation software (version b.02.05, 1989-1997) was used to analyse one µ/ of the prepared sample. A DB-5MS capillary column (30 m x 250 µm x 0.25 µm) with a stationary phase that consisted of 5 % diphenyl and 95 % dimethylpolysiloxane was used for chromatographic separation. The initial oven temperature was 50^{0}C, which was increased to the final temperature of 300^{0}C at a ramp rate of 10^{0}C per minute. The injection mode was splitless and the injector temperature was 250^{0}C. Compounds were identified using the Wiley275 spectral library; only those compounds with a quality match over 80 % were named. This analysis was performed by Umgeni Water (Pietermaritzburg).

2.4.9 Environmental Scanning Electron Microscopy (ESEM)

Conventional Scanning Electron Microscopy (CSEM) requires that a specimen be completely dehydrated and coated with a conductive, electron dense medium. This procedure can affect biological material significantly and cast doubt on the validity of any results obtained. For this reason Environmental Scanning Electron Microscopy (ESEM) was used since it allows hydrated specimens to be viewed in their natural state under stable conditions.

A Philips XL 30 Environmental Scanning Microscope was used to examine biofilm-covered samples of the support matrices from various experiments. Small pieces of sample were cut using a scalpel and attached to stubs using double-sided carbon tape for viewing.

2.4.10 Scanning Electron Microscopy (SEM)

The Environmental Scanning Electron Microscope described in **2.4.9** can also be operated as a conventional, high vacuum SEM to view dehydrated and coated specimens. This mode of operation was necessary when the morphological structure of the cells present in the biofilm was obscured by the thick layer of extracellular material produced by the microbial population.

All samples were fixed in buffered glutaraldehyde (3 %) for at least eight hours before dehydration. This involved successive immersion of samples in ethanol of increasing strength for ten minute periods. The series used consisted of 30, 50, 70, 80, 90 and 100 % ethanol respectively. Immersion in 100 % ethanol was repeated for a further ten minutes with fresh ethanol. Critical point drying (CPD) was then carried out under alcohol. This technique avoids the damage that can occur when a specimen is subjected to the surface tension forces created in a gradually evaporating liquid. A transitional fluid, liquid carbon dioxide in this case, is driven beyond the critical point at which it is converted into a gas without a change in density or latent heat of vapourisation leaving a critical point dried specimen (Bruton, 2000).

Dried specimens were mounted on stubs (10-15 mm diameter) using double-sided carbon tape, and coated with gold/palladium (60:40) using the sputter coating method (Bruton, 2000).

CHAPTER 3

TREATMENT OF LEACHATE FROM THE UMLAZI LANDFILL SITE

3.1 SITE HISTORY AND CLASSIFICATION

The Umlazi landfill site **(Figure 3.1)** is situated just south of Durban, Kwa-Zulu Natal. The site is managed by EnviroServ (Pty) Ltd. Site closure was completed in May 2007 and waste is no longer accepted. This landfill was classified as a H:h site and was therefore permitted to accept both liquid and solid waste in the non-hazardous, domestic and low hazardous waste categories. There is no leachate treatment plant on the site, and the small volumes of leachate that are still produced are currently disposed to sewer[1]. During the experiments described below, the site was still operational and all leachate generated flowed into an open dam.

3.2 LEACHATE CHARACTERISTICS

The Umlazi leachate was analysed using ICP. Very few inorganic constituents were identified in high concentrations. Metals such as Cu, Zn, Cd and Ni were not detected, but a peak corresponding with arsenic was observed. The raw leachate was alkaline and a pH over 8 was consistently measured during the experimental period. Initial COD values were variable (3000 to 5500 $mg.l^{-1}$) and appeared to reflect recent rainfall at the time of collection. Little nitrate (approximately 0.3 $mg.l^{-1}$) was present, but significant concentrations of ammonia were recorded (between 300 and 2000 $mg.l^{-1}$), and these values were also affected by precipitation.

[1] Govender, K. 2007. Landfill site manager, EnviroServ (Pty) Ltd. Personal communication.

Figure 3.1 Aerial photograph of the Umlazi landfill site situated approximately 30 km south of Durban, a large port city in Kwa-Zulu Natal, South Africa.

3.3 PERFORMANCE OF PINE BARK AS THE SOLID BIOFILM SUPPORT MATRIX IN AN UPFLOW PACKED-BED BIOREACTOR

3.3.1 Introduction

The main objective of this project was to develop a low-cost technology for the treatment of landfill leachate, particularly in the Kwa-Zulu Natal region. The ideal packing material for the purpose-designed bioreactor under investigation would

therefore conform to this approach. Pine bark was selected as a potential solid support matrix because it is inexpensive, and easily obtained in South Africa (du Plessis, Strauss, Sebapalo and Riedel, 2003). Although it is uncommon for an organic material to be used as a support matrix in a bioreactor, it does have some advantages. Pine bark has a coarse, irregular surface, which facilitates the attachment of microbial cells and prevents them from being easily dislodged. Bark is also biodegradable, which would reduce the amount of waste produced by the system.

Some of the contaminants present in landfill leachate may be adsorbed by pine bark. The support matrix would thereby contribute to the remediation process. Pine bark has been used as a biosorbent to remove both inorganic and organic pollutants from aqueous solutions. For example, Lens, Vochten, Speleers and Verstraete (1994) reported that pine bark was a suitable material for use in a laboratory-scale percolator column for the treatment of primary domestic wastewater. Total COD was reduced by 63%, while 64% of the ammonia and 35% of the total nitrogen present was eliminated. The effluent had an acceptable pH of 7.5 (Lens *et al*., 1994). Al-Asheh and Duvnjak (1997) effectively sorbed cadmium in this way. Pine bark pre-treated with formaldehyde was found to have a high adsorption capacity for cadmium and mercury and was as effective as other biosorbents in sorbing these metals (Vásquez, González-Álvarez, Freire, López-Lorenzo and Antorrena, 2002). Haussard, Gaballah, Kanari, de Donato, Barrès and Villieras (2003) showed that pine bark is an effective sorbent for hydrocarbons and lipids. When used as a natural, and cheaper, alternative to activated carbon for the removal of lindane and heptachlor, which are organochlorine pesticides classified as persistent organic pollutants, respective removal efficiencies of 80.6 and 93.6% were obtained (Ratola, Botelho and Alves, 2003).Pine bark waste material has also been used to remove Fe (II) ions from an aqueous solution (Acemioğlu, 2004). Brás, Lemos, Alves and Pereira (2005) also studied pine bark as a low-cost, natural and easily available sorbent for hydrophobic organic compounds, using it to successfully remove

pentachlorophenol. Metals have also been removed successfully from a low-strength landfill leachate by this material (Nehrenheim, Waara and Westholm, 2008).

A bioreactor that simulated the landfill environment was developed by Onay and Pohland (1998) in order to explore nitrogen transformations in leachate, and an organic matrix was used as biofilm support. This matrix was composed primarily of compost, but pine bark chips were used as a bulking agent to encourage uniform gas exchange and distribution, especially of air moving from the bottom to the top of the bioreactor. This compaction resisting property was a potential advantage for the experiment described here as air was introduced at the bottom of the reactor and bubbled up through the matrix.

However, the diverse native microbial population supported by this natural material is the primary reason for using it in a biological waste treatment technology. In addition, when pine bark is composted for use as a growing medium for plants, environmental conditions are maintained at ideal levels for microbial growth. This facilitates the development of those bacteria and fungi that are capable of oxidising the wide variety of organic compounds in pine bark (Davis, Hinch, Donkin and Germishuizen, 1992), which could provide additional resources for the biodegradation of pollutants in landfill leachate. This is illustrated by a methane oxidation biofiltration system designed for use in worked underground coal mining chambers in South Africa (du Plessis *et al*., 2003). Composted pine bark was used as the support medium, and no methanotrophic bacteria were introduced because this would be impractical in large-scale operations. Instead the microbial population naturally present in pine bark was relied upon to degrade the methane (du Plessis *et al*., 2003). Clarke, Kirby and Rose (2004) examined the community structure of the microbial population that developed in a lignocellulose packed-bed treatment system designed for the bioremediation of acid mine drainage. Pine chips and grass were used as the support medium and the sole carbon source for the microorganisms in the

bioreactor. This study was also performed in South Africa and justified the use of these materials because of their availability, and the resulting economic viability of the project. A diverse microbial population was identified using various molecular methods and a clear hierarchy was observed within the community. Simple organisms dominated at the top of the downflow bioreactor, while more specialised phenol degraders, and eventually cellulose degrading organisms, including those found in ruminants, dominated the lower levels. This experiment revealed the formation of distinct nutritional niches in a reactor packed with organic support materials and designed for the bioremediation of a liquid effluent (Clarke *et al*., 2004).

The objective of this study was to determine whether pine bark could be used as a low-cost, effective, solid biofilm support matrix for the biological treatment of landfill leachate in a specially designed six-chambered upflow packed-bed bioreactor **(Section 2.1.2)**.

3.3.2 Experimental design

The following experiment conducted in the six chambered tank bioreactor **(Section 2.1.2)** was designed to determine whether pine bark is suitable for use as a solid biofilm support matrix in the above bioreactor. In addition, it investigated whether the type and grade of pine bark used have any effect on bioremediation of landfill leachate, especially with regard to performance differences between composted and uncomposted bark. Composted grades are reportedly durable over long time periods as they are composed mainly of non-labile and recalcitrant large molecular weight carbon compounds. Labile components are both oxidised and polymerised during the composting process, creating a product rich in lignin-type molecules that are not readily degraded (du Plessis *et al*., 2003).

Six different bark grades including four grades of uncomposted pine bark, viz. 10-15 mm chips, 16-24 mm chips, 50-70 mm chips, and Mandini bark, and two grades of composted pine bark were investigated. Mandini bark is a heterogeneous mixture of waste bark pieces of irregular size, while the two grades of composted pine bark selected were mulch and coarse potting soil. All bark was sourced from Gromed (Pty) Ltd, South Africa (now Earth2Earth Organics (Pty) Ltd). Each chamber of the bioreactor contained one of the six bark grades **(Table 3.1)**. The fibreglass box inside each chamber was packed to two centimetres from the top. All six chambers and their respective reservoirs were filled with undiluted leachate obtained from the Umlazi landfill site. The system was run in parallel mode, cycling leachate between each chamber and its dedicated reservoir for the duration of the experiment; the recycle rate was kept as consistent as possible throughout. After 24 hours, each reservoir was topped-up to the original level to compensate for the leachate absorbed by the pine bark.

TABLE 3.1: Arrangement of pine bark grades in the six chambered tank bioreactor used to treat leachate from the Umlazi landfill in a 43 day experiment to assess their suitability as biofilm support materials

Chamber	Bark grade
1	10 – 15 mm bark chips
2	16 – 24 mm bark chips
3	50 – 70 mm bark chips
4	Mandini bark
5	Mulch
6	Coarse potting soil

No additional nutrient sources were added to the leachate and no extraneous inoculum was introduced. The experiment lasted for 43 days. Samples (25 m*l*) for analysis were taken daily from the outflow pipe of each chamber.

3.3.3 Results

Bioremediation occurred in all six chambers. COD removal ranged between 30 and 40 percent, while reduction in TC was between 66 and 75 percent. Ammonia levels decreased significantly **(Table 3.2)**.

TABLE 3.2 Percentage removal of COD, TC and ammonia from Umlazi leachate after 43 days in a six-chambered upflow packed-bed bioreactor with different pine bark types and grades as biofilm support matrices

Bark grade or type	COD removal (% ± SD)	TC removal (% ± SD)	Ammonia removal (%)
10-15 mm bark chips	34 (± 5.70)	75 (± 0.43)	92
16-24 mm bark chips	35 (± 4.61)	71 (± 0.57)	89
50-70 mm bark chips	40 (± 4.27)	67 (± 0.59)	86
Mandini bark	36 (± 4.62)	69 (± 0.61)	88
Mulch	30 (± 4.70)	68 (± 0.53)	87
Coarse potting soil	35 (± 4.61)	66 (± 0.52)	89

SD = standard deviation

COD reduction occurred largely in the first seven to ten days of remediation. Thereafter, the concentration remained relatively unchanged with little further removal occurring. Some fluctuations were observed, especially in the chambers containing mulch and 50-70 mm pine bark chips, where significant but erratic increases in the COD concentration occurred on days 21 to 25. Otherwise, all chambers exhibited similar trends for the duration of the trial **(Figure 3.2)**.

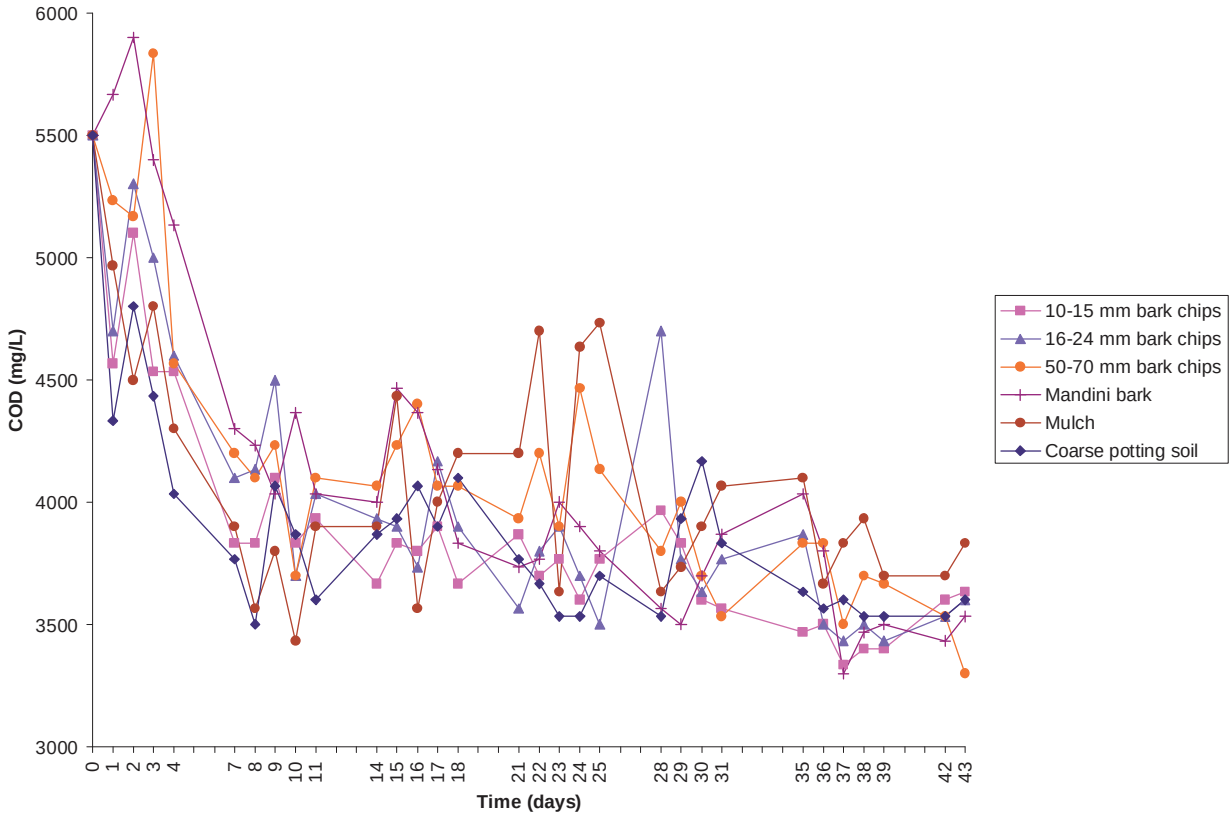


Figure 3.2 Changes in COD concentration during bioremediation of Umlazi landfill leachate in an upflow bioreactor packed with different grades/types of pine bark as biofilm support matrix. Although SD was calculated, the error bars were not easily distinguishable on the graph and were therefore omitted.

TC levels continued to decline significantly even after COD reduction had ceased. Maximum removal was achieved only after 28 to 29 days. An increase in the TC concentration was recorded between days 36 and 38, but thereafter it decreased until termination of the experiment. This was most obvious in the chamber containing the 50-70 mm pine bark chips. The leachate treated in this chamber consistently exhibited higher TC concentrations than that in the other chambers (**Figure 3.3)**.

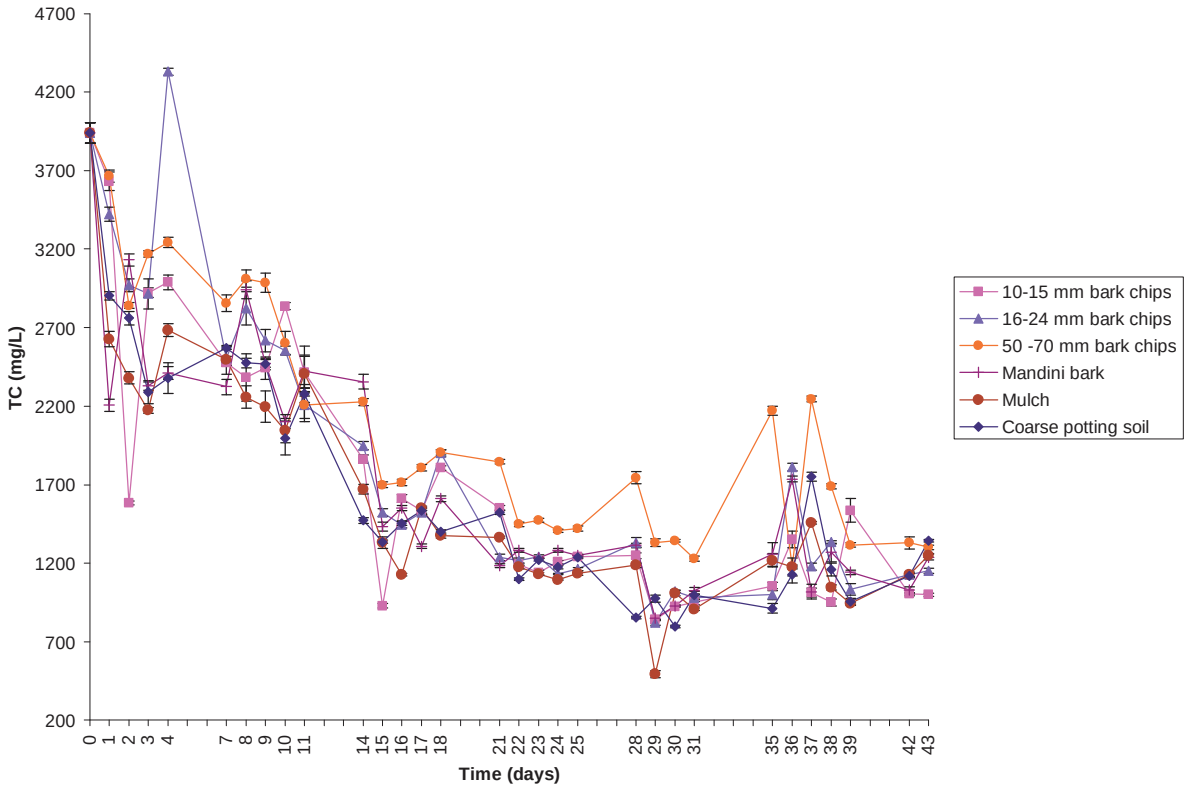


Figure 3.3 Changes in TC concentration during bioremediation of Umlazi landfill leachate in an upflow bioreactor packed with different grades/types of pine bark as biofilm support matrix. Standard deviation is indicated by error bars.

Nitrate levels remained low (< 1 mg.l^{-1}) for the first 28 days thereafter showing a fluctuating increase up to day 40 before decreasing again in the last few days. This effect was particularly evident in the chambers containing mulch and coarse potting soil (both composted pine barks), although the nitrate concentration never exceeded five mg.l^{-1}. However, after 24 hours an anomalous spike was observed in these two chambers where the nitrate concentration increased to over seven mg.l^{-1} **(Figure 3.4)**.

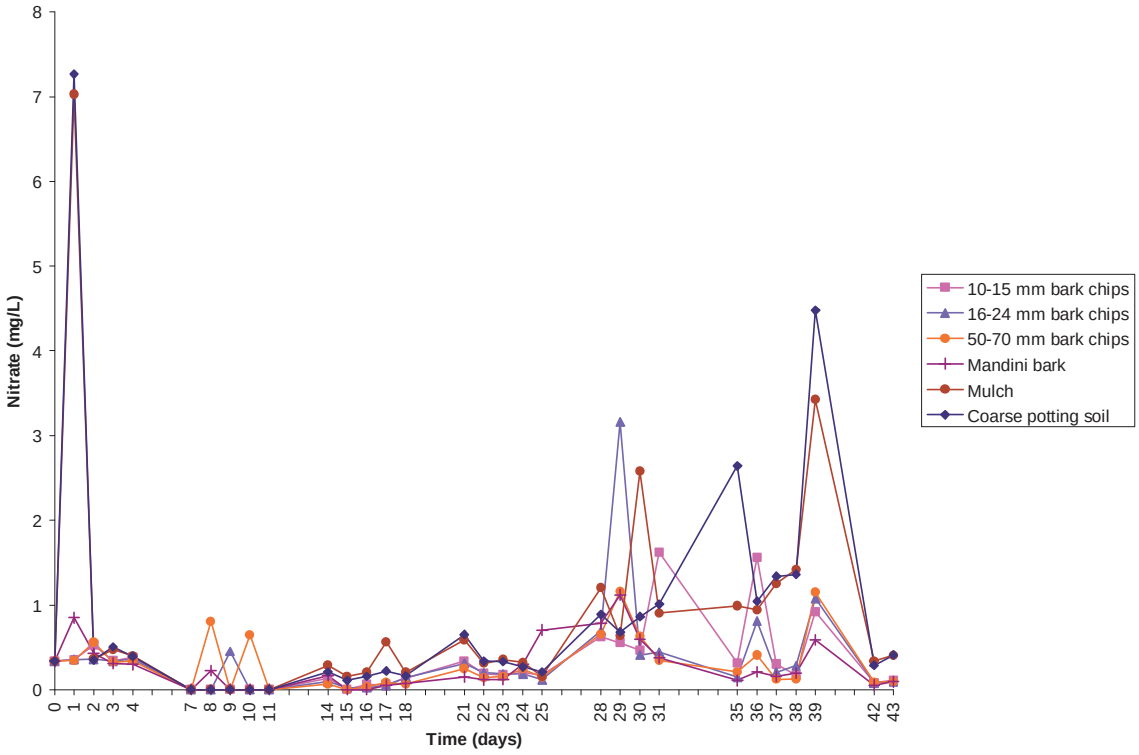


Figure 3.4 Changes in nitrate concentration during bioremediation of Umlazi landfill leachate in an upflow bioreactor packed with different grades/types of pine bark as biofilm support matrix.

Ammonia concentrations fluctuated greatly over the course of the experiment **(Figure 3.5)**. Levels decreased over the first three days, after which an increase to considerably above the initial concentration was observed in all chambers except those filled with the 10-15 and 16-24 mm pine bark chips. A significant decrease occurred from days five to ten after which a slight increase was seen over the next seven or eight days. Maximum removal of ammonia was recorded after approximately 21 days and the concentration remained at this level for about five days. All chambers exhibited a dramatic increase between days 26 and 30. Thereafter, the ammonia concentrations decreased with some fluctuation until termination of the experiment after 43 days. A spike was observed in most chambers on days 38 and 39. The ammonia concentration on day 43 was not as low as it had been over certain periods during the middle stages of the

experiment. All chambers were fairly consistent with one another, but the highest spikes were observed in the chambers with the 50-70 mm pine bark chips, mulch and coarse potting soil. Least fluctuation occurred in the chambers containing the 10-15 and 16-24 mm pine bark chips. In general, ammonia removal was more efficient in the chambers filled with uncomposted, rather than composted, pine bark.

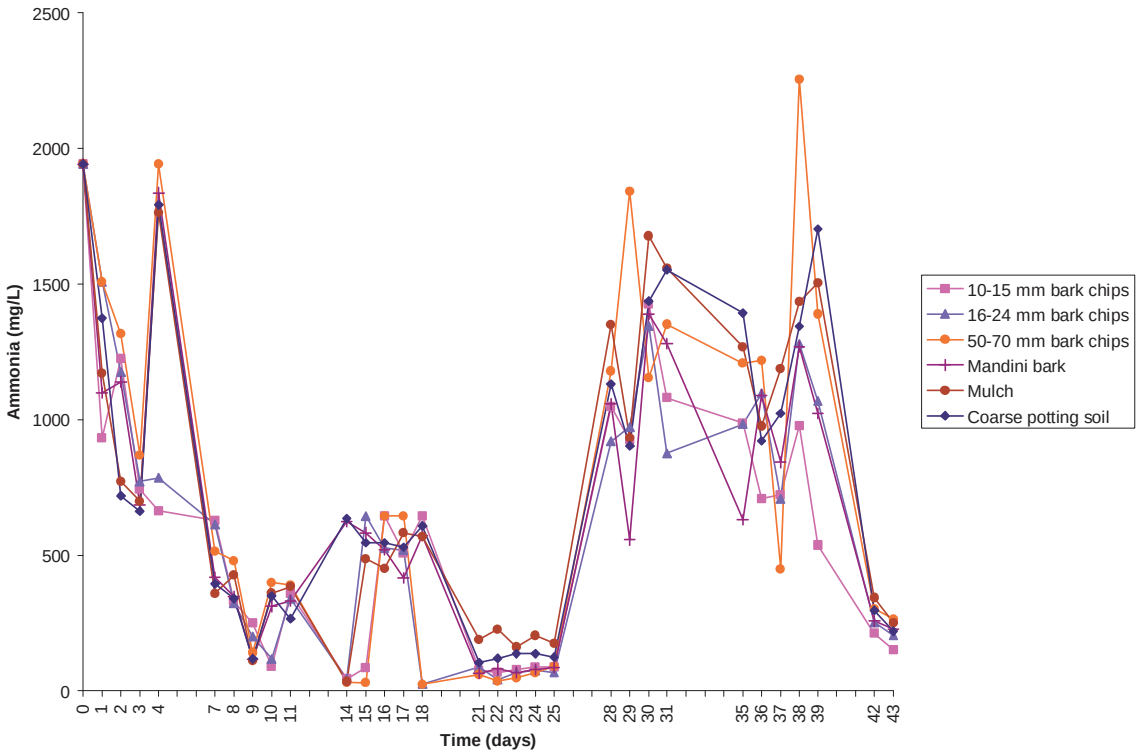


Figure 3.5 Changes in ammonia concentration during bioremediation of Umlazi landfill leachate in an upflow bioreactor packed with different grades/types of pine bark as biofilm support matrix.

SEM showed that a substantial and diverse biofilm formed on the surface of all the grades and types of pine bark used. An example of the biofilm structure and cell morphology from one chamber is shown in **Figure 3.6**, which illustrates a heterogeneous community containing rods, cocci and filamentous

microorganisms. The microbial community shown in this micrograph is similar to that of biofilms in the other chambers, which are not included here.

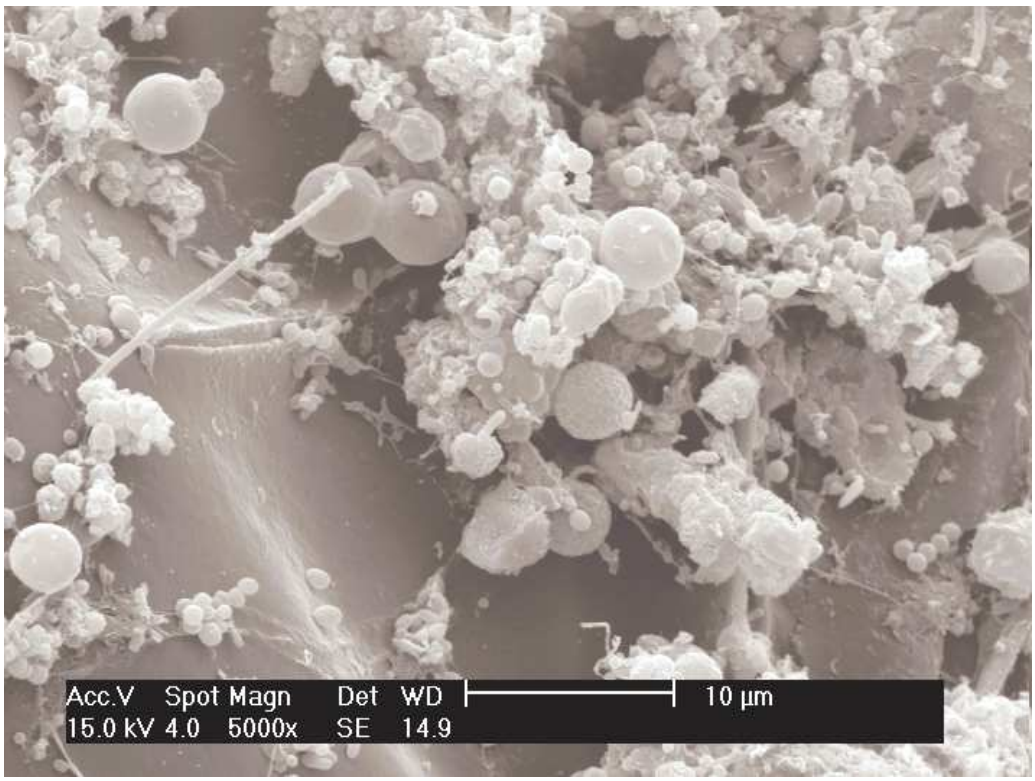


Figure 3.6 Scanning electron micrograph of the biofilm formed on a 16 – 24 mm pine bark chip after 43 days in an upflow packed-bed bioreactor.

Fine particles from the coarse potting soil and mulch tended to clog the bioreactor by escaping from the retaining boxes and blocking the tubing **(Figure 3.7)**. Chambers containing the composted pine barks therefore required more maintenance and were more difficult to manage than those filled with uncomposted pine bark chips.

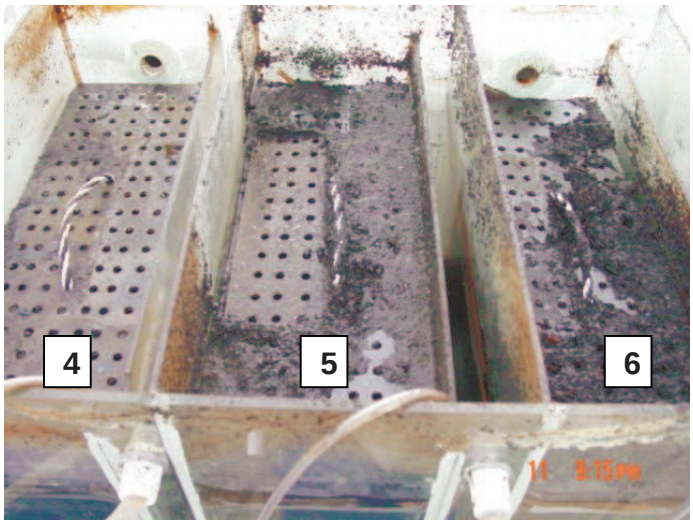


Figure 3.7 Clogging of chambers five and six (mulch and coarse potting soil, respectively) at the completion of an experiment to test the effect of pine bark type and grade on bioremediation of Umlazi landfill leachate. Clogging was caused by fine particles emanating from both composted pine barks. Note the absence of clogging in chamber 4 which contained Mandini bark.

3.3.4 Discussion

The results showed that pine bark was suitable as a biofilm support matrix in the upflow packed-bed aerobic bioreactor used in this investigation. Satisfactory COD removal was achieved, especially considering that the leachate was undiluted and had a high initial concentration relative to values reported in the literature. The reported COD concentrations of a number of leachates from landfills of a variety of ages in many locations ranged from 100 to 70 900 mg.l^{-1}, but the average value for leachates from medium age or old sites was 3850 mg.l^{-1}, which is considerably lower than the COD concentration of the Umlazi leachate (5500 mg.l^{-1}) (Renou *et al*., 2008). The reduction in TC was good as this parameter includes both organic and inorganic carbonaceous matter. The leachate did not need to be retained in the system for 43 days; maximum COD

removal was reached after only ten days, while all the degradable carbon had been eliminated after approximately 30 days.

Aerobic heterotrophic bacteria were probably the major contributors to COD removal, but denitrifying bacteria may also have consumed some COD, using it as an electron donor during the conversion of nitrate to dinitrogen gas (Chen *et al*., 2009).

Although the concentration of ammonia was erratic during the course of the experiment, the overall reduction was substantial and compared well with other treatment technologies. The fluctuations in the ammonia concentration merit some further discussion as there are a number of factors that could have contributed to this effect.

It is not immediately obvious that nitrification did, in fact, occur since decreases in the ammonia concentration were not reflected by corresponding increases in the nitrate concentration. However, it was assumed that any nitrate that was produced was due to biological reactions, as no external nitrate was added in this or any subsequent experiments. The same assumption was made by Aghamohammadi *et al*. (2007) in their work on bioremediation of semi-aerobic landfill leachates using continuous flow activated sludge bioreactors. This phenomenon occurred also in an experiment using a very similar packed-bed bioreactor to treat dairy dirty water; nitrate levels remained low throughout the experimental period, but the ammonia levels were reduced (Cannon *et al*., 2000). An RBC designed to remove both nitrogen and COD from a synthetic wastewater using the SNAD process (see **Literature Review**) also generated similar results, with influent ammonium being removed but little nitrate produced. It was suggested that conditions of oxygen limitation led to initial partial nitrification by ammonium-oxidising bacteria in the aerobic zone of the biofilm, consuming oxygen and creating an anaerobic microhabitat in the inner section of the biofilm

where the nitrite generated was converted into dinitrogen gas by ANAMMOX bacteria. Nitrate production was therefore by-passed (Chen *et al*., 2009).

Onay and Pohland (1998) also observed that nitrate was temporarily converted to ammonia, with no nitrogen gas being produced before denitrification was initiated during the initial stages of operation in their simulated landfill bioreactor incorporating aerobic, anoxic and anaerobic zones. However, once the denitrification process had started, the nitrate was reduced and an increase in the concentration of nitrogen gas was noted. It is possible that a similar phenomenon was responsible for the patterns observed in the present investigation.

Another study in which partial nitrification was initiated successfully in a membrane bioreactor used to treat synthetic wastewater identified temperature and dissolved oxygen (DO) concentration as key factors. Temperatures were maintained above 30 ^{0}C in order to increase the specific growth rate of ammonium-oxidising bacteria, thereby allowing them to dominate the microbial population at the expense of nitrite-oxidising bacteria (Xue *et al*., 2009). Temperature was not regulated in the present experiment, and despite the cooling system (described in **Section 2.1.3)**, the air temperature regularly rose above 25 ^{0}C during the day, even when the fans were in operation. However, the temperatures would have been much lower at other times and without more specific data it would be difficult to speculate on the effects this fluctuation would have had on the microorganisms responsible for nitrogen removal. Xue *et al*. (2009) found that ammonium-oxidising bacteria tended to out-compete nitrite-oxidisers at DO concentrations below 1 mg.l^{-1} and that a DO concentration between 0.3 and 0.5 mg.l^{-1} was suitable for only partial nitrification. Although the DO concentration was not measured directly in the present study, the volume of oxygen used to aerate the bioreactor was moderately low, and the presence of contaminants would have reduced the oxygen mass transfer coefficient compared to that when pure water is used. It is therefore likely that the DO

concentration was low enough to promote the growth of ammonium-oxidisers over nitrite-oxidisers.

Noophan *et al*. (2009) successfully removed nitrogen from a synthetic medium using a sequencing batch reactor in which a microbial population rich in ANAMMOX bacteria had been developed. However, an extended start-up period (approximately 100 days) occurred, as ammonium and nitrite oxidisers are autotrophic with low biomass yield, while anammox bacteria grow relatively slowly (Noophan *et al*., 2009). There was no enrichment phase in the current experiment and the bioreactor was operated for a total of only 43 days. This may mean that ANAMMOX bacteria were not the major contributors to nitrogen transformation due to insufficient start-up and running time. However, it is also possible that the raw leachate used already contained a population of ammonium oxidisers which may have proliferated in the conditions provided by the bioreactor. Other biological processes could also have impacted the bacterial community, and contributed to the observed reduction of ammonium concentrations. Some possibilities are discussed in the following paragraphs.

It is possible that simultaneous nitrification and denitrification occurred in the bioreactor; a phenomenon recorded by a number of researchers. For example, Watanabe *et al*. (1994) used a completely mixed bioreactor with partially and fully submerged rotating biological contactors to remove nitrogen from a synthetic wastewater by simultaneous nitrification and denitrification. Denitrification occurred in the submerged biofilm even though the dissolved oxygen concentration was relatively high. Although the system used in this research was aerated, the amount of air introduced was moderately low. Anoxic zones would certainly have developed within each chamber, thus providing microenvironments where denitrification could have taken place. The nitrogen in the leachate would have been converted to gaseous forms and released into the atmosphere via denitrification. As mentioned previously, this process may also have been responsible for some of the observed COD and TC reduction.

It is typically assumed that denitrification is carried out by heterotrophic microorganisms, but, especially in heavily loaded wastewaters, nitrogen elimination which seems inexplicable according to these conventional ideas may occur. For example, there are reports of more nitrogen removed than is theoretically possible according to the BOD:N of the influent stream, as in the leachate treatment plant in Mechernich, Germany (Hippen, Rosenwinkel, Baumgarten and Seyfried, 1997). Although BOD was not measured in the present experiment, the leachate is unlikely to have been highly biodegradable as the Umlazi landfill was a mature site, nearing closure and would thus be expected to produce a leachate containing a high proportion of refractory compounds **(See Chapter 1)**.

This raises the possibility of aerobic deammonification, which can explain both the direct transformation of ammonia into N_2 in conditions where oxygen concentrations are limited and the postulated existence of a denitrifying enzyme system that is active in aerobic environments. This is considered a promising avenue by some researchers as it could considerably reduce costs associated with supplying additional carbon sources (Hippen *et al*., 1997). It is conceivable that aerobic deammonification did occur in this experiment, because no supplementary nutritional sources were added to the bioreactor; the C:N ratios were initially quite low (see discussion below), and the carbon present may have been in a largely refractory form. *Nitrosomonas* and *Thiosphaera* are examples of bacteria that can consume ammonia with the production of nitrogen containing gases (NO, N_2O and N_2), even in aerobic systems, if the partial pressure of oxygen is low (Anderson and Levine, 1986). This effect was proposed by Baumgarten and Seyfried (1996) in a full-scale landfill leachate treatment plant in Germany, as an average of 60 % of the influent nitrogen was eliminated in the nitrification stage of the biological treatment phase, before entering the activated sludge section intended for denitrification.

Nitrite levels were not measured during this experiment, but nitrite accumulation may also explain the lack of correlation between the ammonia and nitrate concentrations in the bioreactor. Nitrite accumulation can occur due to incomplete nitrification or denitrification as both processes proceed via this intermediate. Allemann (1985) noted that low temperatures, high pH or the presence of free ammonia adversely affect nitrification. Temperature and pH were not controlled in this system. The Umlazi leachate, like many other landfill leachates, was very alkaline. The reactor was also subject to daily, climatic temperature changes. Temperature has a more pronounced effect on nitrification than on carbonaceous oxidation (Kim *et al*., 2006), which may explain why COD and TC removal were much more consistent than nitrogen removal. Kim *et al*. (2006) noted that in a municipal leachate treatment plant the nitrification efficiency was very much lower than the organic oxidation rate obtained during the colder season. Watanabe *et al*. (1994) achieved a maximum ammonia removal of higher than 80 % when the temperature of the influent waste stream was maintained at 25^0C. However, when no temperature control was applied and the average water temperature fell to 17^0C, overall nitrogen removal efficiency dropped to 60 %. This illustrates the dramatic effect that lower temperatures can have on this process. Temperature was also considered to be an influential factor that could have limited conversion of nitrite into nitrate during the treatment of dairy dirty water in a submerged aerated filter bioreactor by Cannon *et al*. (2000). Kim *et al*. (2006) investigated the seasonal failure of a municipal landfill leachate treatment plant using an attached biofilm technology and found that nitrite accumulation was severely affected by temperature variation, as well as by high pH and free ammonia concentration. Nitrification efficiency increased with increasing temperature within the limits 12 to 33^0C and complete conversion of ammonia was recorded only at 20^0C. The activity of the nitrite-oxidisers was reduced as the free ammonia concentration was increased. The leachate used also contained compounds with a very strong inhibitory effect on nitrification; however, these authors also suggested that there may be some organic compounds that promote nitrifying activity, or that the use of a biofilm created a

pH gradient that mitigated the effect of the ammonia on the microbial population (Kim *et al*., 2006). In contrast, Klees and Silverstein (1992) found that temperature did not have a significant effect on nitrification within the range 13 – 21^0C in a rotating biological contactor treating effluent from a wastewater treatment plant. However, the reported pH in their study was between 7.2 and 7.9, which is acceptable for nitrification, and the wastewater also had a much lower ammonia concentration (8.4 – 34.4 mg.l^{-1}) than that treated in the present project.

Aktas and Çeçen (2001) showed that many landfill leachate constituents contribute to the inhibition of nitrification in activated sludge bioreactors. This occurred even when the leachate was mixed with domestic wastewater. The presence of free ammonia caused nitrite build-up, even at low concentrations, and led to complete inhibition of nitrification at higher concentrations. The basic pH and high concentrations of ammonia nitrogen in the leachate resulted in high concentrations of free ammonia. The same effect was observed by Bae *et al*. (1997), also in activated sludge plants where nitrite accumulation appeared to be caused by high concentrations of free ammonia. In a packed-bed bioreactor designed for the treatment of dairy dirty water, the presence of free ammonia also seemed to account for the inhibition of complete nitrification (Cannon *et al*., 2000). This is likely to have occurred in the Umlazi leachate, which may also have contained compounds inhibitory to nitrifying bacteria. *Nitrobacter* is much more sensitive to free ammonia and free nitrous acid than *Nitrosomonas*, and this leads to the characteristic accumulation of nitrite (Vadivelu, Keller and Yuan, 2006).

The C:N ratio of the waste stream also plays an important role in nitrogen removal. Both low and high C:N ratios can be responsible for unstable conditions. Martienssen and Schöps (1997) reported that nitrite accumulation was caused by carbon deficiency when landfill leachate was treated in an activated sludge system. This was primarily due to incomplete denitrification.

Nitrite accumulation and reduction were correlated with changes in the microbial community. Nitrite sensitive bacteria became more prevalent in the system when the TOC:N ratio was higher than 2.5 and the denitrification ability of the bacterial population was reduced. A shift towards nitrite tolerant strains occurred when the TOC:N ratio was lowered to 1.5 (Martienssen and Schöps, 1997). The COD:N ratio of the Umlazi leachate was 2.8 and the TC:N ratio was 2, values much lower than those conventionally required for efficient microbial metabolism. Jun, Park, Park and Lee (2004) used total COD to measure C:N ratios in a sample of domestic sewage, and reported a value of about 3, which they considered very low.

However, a carbon deficiency is unlikely to have caused the early fluctuations in ammonia concentration that characterised the present investigation as TC reduction was still occurring. This shows that degradable carbon was still available for the microbial population in the bioreactor. The C:N ratio increased several fold over the course of the experiment (to between 5 and 7 using TC values, and between 12 and 24 when COD values were used). The increase in ammonia levels at the end of the experiment may therefore have been caused by this increase in the C:N ratio and a corresponding shift towards nitrite sensitive bacteria. This would have caused the denitrification ability of the system to fall, causing accumulation of nitrite. As carbon was no longer being eliminated from the waste stream, accumulated nitrite would then have been converted into ammonia due to the lack of easily biodegradable carbon for use by the heterotrophic denitrifiers. Eventual decrease in ammonia levels must have occurred via an alternative pathway such as the aerobic deammonification process explained previously.

Organic loading can also affect nitrification by causing competition for dissolved oxygen between the heterotrophic aerobes (responsible for carbon removal) and nitrifying members of the bacterial community (Zhang, Fu and Bishop, 1994).

In a recent study Volcke, Sanchez, Steyer, Dabert and Bernet (2008) pointed out that although nitrification is performed by a great variety of bacterial species, this diversity is not usually tracked during bioreactor operation, and is largely ignored in mathematical models. However, experimental data indicates that different process conditions favour the selection of different microbial communities, which could potentially affect effluent quality in terms of the presence and concentration of nitrogenous compounds. The major parameters that cause this effect appear to be dissolved oxygen concentration (nitrite oxidisers are more sensitive to oxygen limitation than ammonium oxidisers), resulting from changes in loading rate, in conjunction with interspecies competition between different ammonium oxidisers.

Ammonia was produced at several points during the present experiment and could have been due to dissimilative nitrate reduction, which can proceed in two ways. One pathway leads to the production of ammonia, while the other generates nitrogen gas. A fairly large number of bacteria are able to reduce nitrate to ammonia, while some cannot reduce nitrate, but are able to convert nitrite to ammonia (Madigan *et al*., 1997). The factors discussed above were possibly instrumental in causing nitrite accumulation, and the subsequent conversion of this nitrite to ammonia might explain the observed increases of the latter. Akunna (1995) found that when non-fermentable organic compounds are present, nitrate reduction is likely to occur primarily via denitrification. However, if fermentable organic compounds are present, ammonification is the preferred route. High COD:NO_3-N ratios promote ammonification, while low ratios favour denitrification. The nature of the carbon source also influences the optimum ratio for denitrification (Akunna, 1995). Borzacconi *et al*. (1999) used COD:NO_3-N ratios of 4, 6 and 12 in order to promote denitrification and prevent ammonification in an upflow sludge blanket reactor used for leachate treatment. They achieved maximum denitrification efficiencies of approximately 90 % at the lowest ratio with no significant ammonification. The initial COD:NO_3-N ratio of the leachate from the Umlazi landfill was 16 418:1, which is many orders of

magnitude higher than the optimum ratio for denitrification. This may have facilitated ammonification, causing the periodic increases in ammonia concentration observed during this experiment. As the COD level was reduced, the environment may have become more conducive to denitrification, which would allow nitrogen to be removed completely from the system. Ammonia is also produced when organic nitrogen compounds are microbiologically degraded (Madigan *et al*., 1997), and, although most of the nitrogen in the influent leachate is likely to have been inorganic, some organic nitrogenous matter may also have been present. The initial high levels of ammonia (for the first eight to nine days of treatment) may be explained by inhibition of ANAMMOX bacteria due to the high concentration of TC in the influent leachate. This group would have then become more active in the removal of ammonium as the TC and COD concentrations decreased (Chen *et al*., 2009).

Henderson and Atwater (1995) used a pre-denitrifying anaerobic filter and a rotating biological contactor to remove nitrogen from a high ammonia landfill leachate and achieved ammonia removal efficiencies between 80 and 95 %, which is very similar to the results obtained in the experiment described here. Total nitrogen removal was considerably lower at 66 %, due to inhibition of the transformation of nitrite into nitrate probably caused by the high concentrations of both ammonia and nitrite in the system.

Electron microscopy revealed a diversity of morphotypes which probably contributed to the COD and ammonia reduction that occurred. Oliveira *et al*. (2004) observed a number of different morphotypes in a bioreactor used to remove formaldehyde from a synthetic waste stream. They suggested that the presence of a variety of microorganisms accounted for the high removal efficiencies achieved by the treatment, enabling the primary contaminant and its degradation products to be effectively assimilated.

Pine bark type and grade did not have a significant effect on bioremediation efficacy. Composted pine barks were more difficult to work with and are also more expensive than the bark chips. The 16-24 mm chips were therefore used in the bioreactor in all subsequent experiments that utilised pine bark as a solid biofilm support matrix. This grade was the cheapest of the uncomposted barks, the most readily available and easy to use in a packed-bed bioreactor.

A low-cost technology that does not require any specialised inoculum is clearly a viable option for the treatment of landfill leachate, especially in technologically unsophisticated countries. The autochthonous microbial population in the Umlazi leachate and any microorganisms on/in the pine bark that survived exposure to the waste stream proved to be adequate for the bioremediation of the Umlazi leachate that occurred in this experiment.

3.4 EFFECT OF AERATION ON BIOREMEDIATION OF UMLAZI LANDFILL LEACHATE IN A FORCED-UPFLOW BIOREACTOR

3.4.1 Introduction

Aeration is one of the major factors in the costs associated with leachate treatment (Armenante, 1993). It is thus important to reduce these expenses as much as possible without adversely affecting the biodegradation process to a significant degree. This is particularly pertinent in this project because of its focus on developing a low-cost solution to leachate treatment in South Africa, where resources may be limited. The advantages and disadvantages of aerobic versus anaerobic biological treatment systems were discussed in the Literature Review **(Chapter 1)**. As previously mentioned, an aerobic approach was selected for this research. However, in most aerobic biological treatment systems that use attached biomass, oxygen does not fully penetrate the biofilm. The biofilm can be divided into four major layers: bulk liquid, diffusion layer, aerobic/active biofilm

and anaerobic/inactive biofilm (Watanabe *et al*., 1980). The bioreactor used in this research would feature both aerobic and anoxic zones.

The objective of this experiment was to determine the effect of aeration on the bioremediation of the Umlazi landfill leachate in an upflow packed-bed bioreactor with uncomposted pine bark as the packing material.

3.4.2 Experimental design

All chambers of the tank bioreactor **(Section 2.1.2)** were packed with 16-24 mm pine bark chips for this experiment. The container in each chamber was packed to two centimetres from the top. The bioreactor and reservoirs were filled with leachate from the Umlazi landfill site. This batch of Umlazi leachate was collected after heavy rains, and was therefore less concentrated than the leachate used in the previous experiment. The system was operated in parallel; and, as in the first experiment, the reservoirs were filled to the original level with fresh leachate after 24 hours to replace any liquid absorbed by the pine bark. Three different oxic levels were tested in duplicate. Two chambers were left unaerated, two chambers were aerated with the aquarium pump described earlier (0.05-0.1 L air.L leachate^{-1}.min^{-1}) and two chambers were aerated with a blower (0.6-0.7 L air.L leachate^{-1}.min^{-1}). No additional nutrient sources or microbial inocula were added to the system so that the indigenous microbial population was responsible for any bioremediation that occurred. The experiment was run for 70 days with recycle. Samples (25 m*l*) were taken daily from the outflow pipes connected to each chamber for COD, nitrate and ammonia analyses.

3.4.3 Results

The percentage COD removed in this experiment was considerably higher than that removed in the first experiment in all but one of the six chambers. Those chambers that were aerated with the aquarium pump performed best, exhibiting 58 and 61% COD removal respectively. The blower-aerated and non-aerated chambers produced quite similar results with COD removals ranging from 37-46% and 43 – 45% respectively. Chamber 1 (blower-aerated) showed a sharp increase in COD concentration on the last day of the experiment because it had leaked overnight, almost emptying the reservoir so leaving only the sediment from the bottom of the reservoir for sampling. Therefore this value was omitted when the percentage COD removal in this chamber was calculated. Most of the ammonia in the leachate was eliminated (97 – 98% removal; **Table 3.2**) with the percentage reduction being higher than that obtained in the previous experiment (see **Table 3.1**). TC analysis was not possible as the only TOC analyser available at the University had irreparably broken down.

TABLE 3.3 Effect of different aeration levels on percentage COD and ammonia removal from Umlazi leachate after 70 days in an upflow packed-bed bioreactor with 16-24 mm pine bark chips as biofilm support matrix

Aeration intensity	COD removal (% ± SD)			Ammonia removal (%)		
	1	2	Average	1	2	Average
0.6 -0.7 litres air/litre leachate/min (blower)	37 (± 2.44)	46 (± 0.89)	41.5	98	98	98
0.05 – 0.1 litres air/litre leachate/min (aquarium pump)	58 (± 1.60)	61 (± 1.06)	59.5	98	98	98
None	45 (± 1.23)	43 (± 1.25)	44	98	97	97.5

SD = standard deviation

Initially COD levels decreased sharply (average 23 % reduction in three days) in all the aerated chambers. After approximately 14 days, the COD curves for the two chambers aerated with the blower flattened out, and little further reduction occurred. The COD levels in the chambers aerated with the aquarium pump continued to decline until the end of the experiment, although the rate of removal was much less after the first two weeks (on average, only 29 % of the total reduction occurred over the final 40 days). The COD in the unaerated chambers decreased initially (average 21 % removal in three days), followed by an increase from approximately 2460 mg.l^{-1} to a maximum of 2934 mg.l^{-1} before a decline over the last 60 days during which 30 – 43 % of the total COD reduction occurred **(Figure 3.8)**.

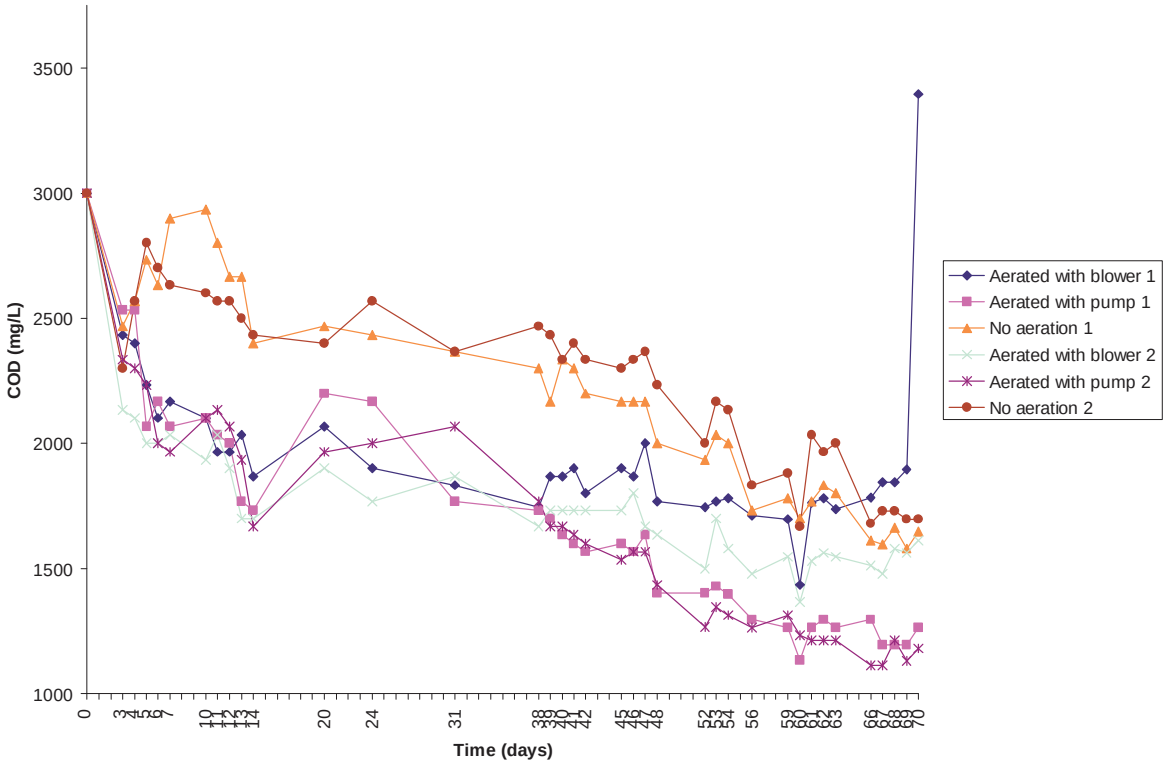


Figure 3.8 Effect of different aeration levels on COD concentrations over a 70 day bioremediation period of Umlazi landfill leachate in an upflow packed-bed bioreactor with 16-24 mm pine bark chips as support matrix. Error bars are not included on this graph as they were too small to be easily distinguished.

In the two unaerated chambers, nitrate values remained low (below 3 mg.l^{-1}) for most of the experimental period. However, in all the aerated chambers a large increase in nitrate concentrations occurred between day 14 and day 40 before a sharp decrease. The increase was much less marked in the chambers aerated with the blower than in those aerated with the aquarium pump **(Figure 3.9)**.

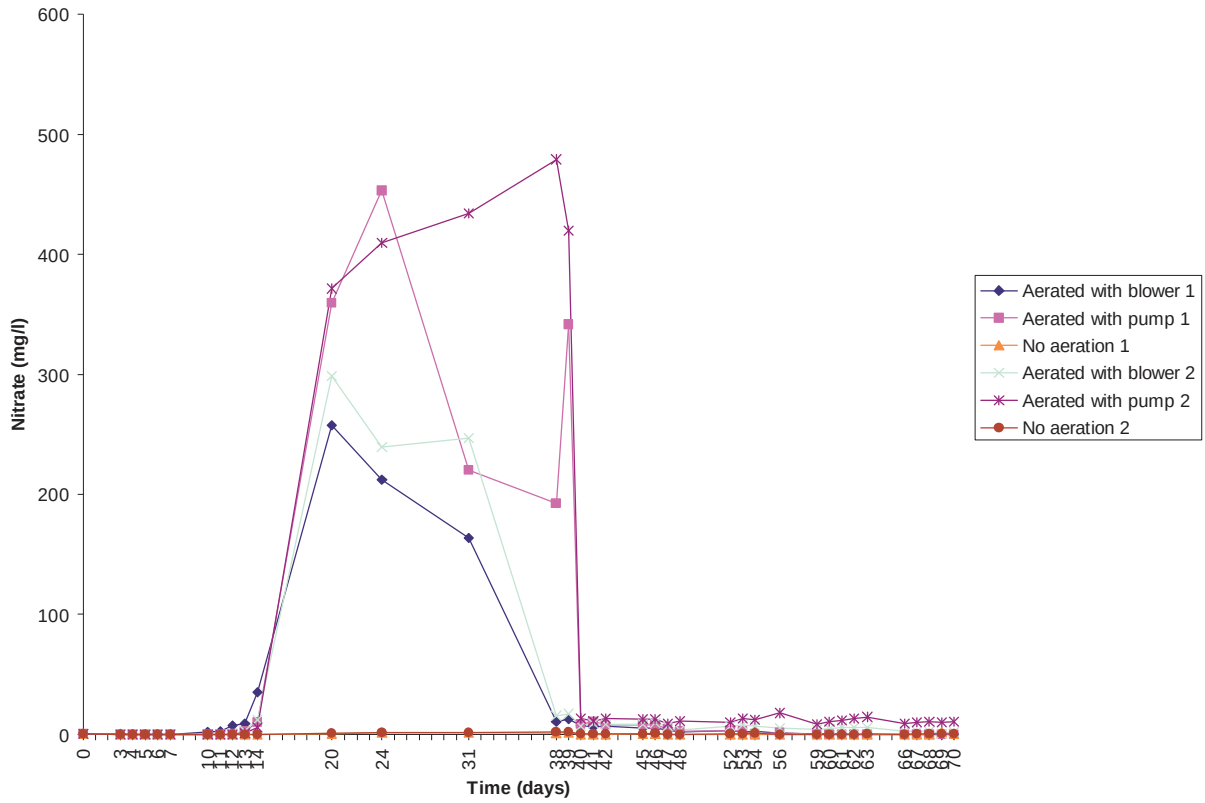


Figure 3.9 Effect of aeration levels on nitrate concentrations over a 70 day bioremediation period of Umlazi landfill leachate in an upflow packed-bed bioreactor with 16-24 mm pine bark chips as support matrix.

All the ammonia curves followed fairly similar patterns for approximately 31 days. During this time, the ammonia concentration fluctuated considerably. After this initial phase, the aerated and unaerated chambers exhibited different patterns. Ammonia concentrations in the aerated chambers decreased to below 5 mg.l^{-1}, and did not change for the remainder of the experiment. However, in the unaerated chambers they continued to vary quite substantially before gradually decreasing between days 53 and 70 to levels below 10 mg.l^{-1}. The duplicate nonaerated chambers followed virtually identical patterns **(Figure 3.10)**.

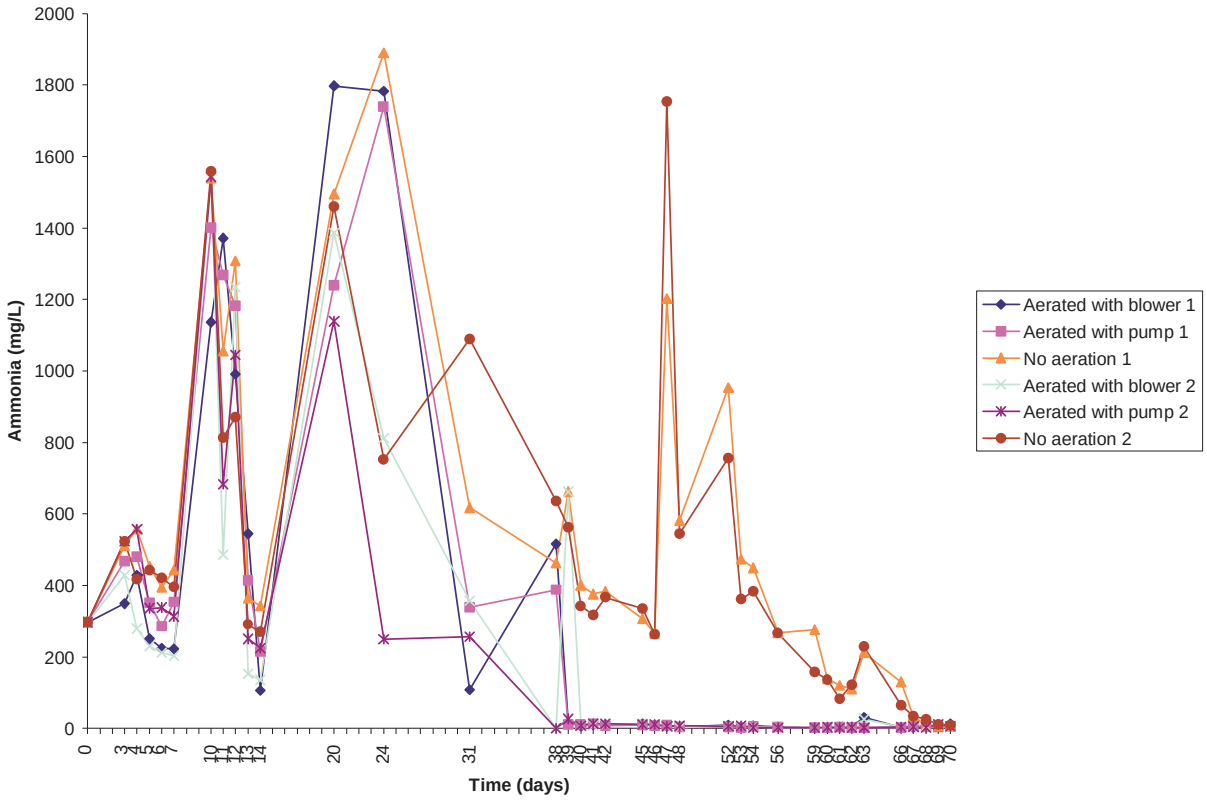


Figure 3.10 Effect of aeration levels on ammonia concentrations over a 70 day bioremediation period of Umlazi landfill leachate in an upflow packed-bed bioreactor with 16-24 mm pine bark chips as support matrix.

Although a biofilm formed on the bark in all the chambers, it grew more profusely on the bark chips in the aerated chambers. The reservoirs connected to the unaerated chambers became infested with maggots and developed an unpleasant odour. Aeration therefore created more manageable and aesthetically acceptable conditions.

3.4.4 Discussion

The results show that the bioreactor design with pine bark as solid support used in this experiment can effectively treat landfill leachate. Both COD and ammonia were removed from the waste stream.

Due to dilution by heavy rains, the initial COD of the influent leachate used here was much lower than that of the previous batch of leachate from the same site. Likewise, the concentrations of any growth inhibiting substances would have been less than in the first experiment. This may have allowed a wider range of natural microflora on the pine bark to survive exposure to the leachate and contribute to the biodegradation process, thus removing a higher percentage of the COD from the wastewater. As previously noted, biodegradation potential is greatly influenced by both temporal and spatial variation at a single landfill site (Adrian *et al*., 1994). Such variation could also have contributed to the improved efficacy of the system noted here as a different, more active microbial population may have been present. The leachate may also have contained different more easily degradable specific organic pollutants and intermediates of biotransformation.

Aeration levels did affect biodegradative processes in the packed-bed reactor with COD reduction being the most strongly influenced by the different modes of aeration used. Somewhat unexpectedly, the aquarium pump, which provided a maximum of 0.1 L air.L leachate^{-1}.min^{-1}, proved to be more effective than the blower which provided a maximum of 0.7 L air.L leachate^{-1}.min^{-1}. By contrast, Petruccioli, Duarte and Federici (2000) found that increasing the aeration rate from 0.5 – 1.0 vol.vol^{-1}.min^{-1} in an air-bubble column reactor treating winery wastewater increased the dissolved oxygen concentration, microbial biomass and COD removal rate and efficiency. However, a further increase to 1.5 vol.vol^{-1}.min^{-1} did not significantly affect COD reduction. Henderson and Atwater (1995) also observed that most organic matter was removed in the aerobic stages, and

not in the anaerobic filter phase of their leachate treatment system. This correlates with the higher COD removal efficiencies recorded in the aerated chambers of this bioreactor, compared to those obtained in the unaerated sections **(Table 3.2)**.

The patterns of ammonia removal were similar to those observed in the first experiment. Similar explanations can be used to interpret these findings. The removal of ammonia was similar in all the aerated chambers regardless of the amount of air provided. The nitrification reaction rate constant is a function of the concentration of nitrifying bacteria and aeration level (Watanabe *et al*., 1980). At high aeration intensities, the dissolved oxygen concentration increases and the average floc size decreases if the biomass concentration is fixed. When biomass concentration increases, the average floc size will also increase. This means that as aeration intensity decreases, more of the biofilm population becomes inactive, causing the reaction rate constant to drop (Watanabe *et al*., 1980). It was thus expected that the higher aeration intensity would promote increased biological removal of both carbonaceous and nitrogenous compounds in the Umlazi leachate. However, this was not observed. It may be that the lower aeration intensity allowed oxygen depleted and even anoxic regions to develop in the chambers thus encouraging the growth of a more diverse microbial population, consisting of aerobic, microaerophilic, facultatively anaerobic and anaerobic microorganisms, thereby facilitating the biodegradation of a greater variety of the pollutants in the leachate.

Although the unaerated chambers took much longer to eliminate ammonia from the Umlazi leachate, substantial removal nevertheless eventually occurred. The lack of sufficient oxygen for rapid nitrification explains the slower rate of ammonia removal observed. It was expected that overall removal in these chambers would be much less than that in the aerated chambers. This was realised in the chambers aerated with the aquarium pump, but the average removal in the chambers aerated with the blower was 2.5 % less than in the unaerated

chambers. However, the recycling of the leachate through the latter chambers and their corresponding open-to-the-atmosphere reservoirs may have introduced sufficient air into the system to allow for nitrogen removal. It is also possible for ammonia to be biologically oxidised under anoxic conditions by means of the nitrate dependent anammox reaction (Madigan *et al*., 1997).

It has been noted that, unless full nitrification is required, the inclusion of an anoxic stage is unnecessary for leachate treatment, and ammonia and nitrogen removal can thus be satisfactorily achieved using an aerobic treatment system (Henderson and Atwater, 1995). This observation was confirmed by the experiment performed here, and as the primary goal was to reduce ammonia concentrations rather than to achieve the complete removal of nitrogen, the aerobic chambers in fact produced better results in this regard than those that were left unaerated.

Energy expenditure can be significantly reduced by aerating a system with relatively low volumes of air. The aquarium pump was therefore a more economical option than the blower for the type and capacity of bioreactor used. This could also be a critical consideration should this system be scaled-up in the future. The aquarium pump was therefore used in all subsequent experiments since it met the objective of low operational cost that motivated this research.

3.5 CONCLUSIONS

The upflow packed-bed bioreactor used here is a viable option for the low-cost treatment of leachate from landfill sites similar to the one at Umlazi. An inexpensive, natural material such as pine bark can be used as a solid support matrix for biofilm attachment, and a low aeration intensity can also make the system more economical. Notwithstanding pine bark being satisfactory at the scale used in these experiments, it may not be suitable for larger scale operations because of its low mechanical strength. In addition, the inhibitory

compounds present in pine bark, including phenolics, could adversely affect the bioremediation efficacy in a full-scale bioreactor. These considerations led to other matrices being tested at a later stage in this research programme (see **Chapter 4**).

CHAPTER 4

TREATMENT OF LEACHATE FROM THE SHONGWENI LANDFILL SITE

4.1 SITE HISTORY AND CLASSIFICATION

The Shongweni landfill site **(Figure 4.1)** is, like the Umlazi site, managed by EnviroServ (Pty) Ltd. It is situated between Durban (about 30 km from the city) and Pietermaritzburg in Kwa-Zulu Natal. However, unlike the Umlazi landfill, this site is still in operation. It accepts both liquid and solid waste in the domestic, non hazardous and low hazardous categories. It is thus an H:h site[2]. Most of the waste received is industrial. Approximately 2300m^3 of leachate is generated every month. This leachate is collected at the bottom of the landfill, and pumped via a pipeline to specially constructed holding tanks at the top of the site (Mannie and Thompson, 2005). Large volumes of leachate are stored in these tanks before being transported by road to Southern Works Waste Water Treatment (SWWWT), which is about 50 km away, for disposal[3]. However, this process will not be allowed to continue indefinitely as SWWWT has strict criteria for acceptance of waste, and the high COD concentrations in the Shongweni leachate make it difficult to conform to these standards. Leachate cannot be discharged to sewer as there is no reticulation system in the area. In any case, this option is becoming less popular because of its negative impact on domestic waste treatment systems (Mannie and Thompson, 2005). Although some pilot-scale studies on leachate treatment have been conducted, there is currently no leachate treatment plant on site[4].

[2] Govender, K. 2008. Landfill site manager, EnviroServ (Pty) Ltd. Personal communication.
[3] Govender, K. 2008. Landfill site manager, EnviroServ (Pty) Ltd. Personal communication.
[4] Govender, K. 2008. Landfill site manager, EnviroServ (Pty) Ltd. Personal communication.

Leachate was collected from the large storage tanks at the top of the site for use in this research (visible in the foreground of **Figure 4.1**).

Figure 4.1 Aerial photograph of the Shongweni landfill site, located between Durban and Pietermaritzburg (an inland city) in Kwa-Zulu Natal.

4.2 LEACHATE CHARACTERISTICS

Several batches of the Shongweni leachate were collected and analysed as it was used in a number of bioremediation experiments. An initial sample of the leachate was analysed by the Darvill Wastewater Treatment Purification Works in Pietermaritzburg. Parameters relevant to the monitoring and treatment of leachate, including COD, TOC and NH_3-N, were measured **(Table 4.1)**. Gas chromatography – mass spectroscopy (GC-MS) screening was also performed. This analysis was purely qualitative, and gave an indication of some of the most

abundant organic contaminants in the leachate **(Table 4.2)**. The concentrations of these compounds are unknown. Identification was accomplished using the Wiley275 spectral library. A thick, visible layer of oil was present on the surface of the leachate in the reservoirs at the landfill site. While samples were collected mainly from below this layer, the inclusion of some oily waste was unavoidable and this may have contained either organic substances, such as hydrocarbons and lipids, or mineral oils, for example, spent diesel motor oil.

The COD of this batch of leachate (5019 mg.l^{-1}) listed in **Table 4.1** is much higher than the initial COD of the batches used in the experiments described later in this chapter. For example, the CODs of the raw, experimental leachates used in the tank and bucket bioreactors were 3823 mg.l^{-1} **(Section 4.3)**, and 2180 mg.l^{-1} **(Section 4.4)** respectively, while the leachate used for the flask experiments had an average COD of 2945 mg.l^{-1} **(Section 4.5)**. The chemical composition of the waste stream was therefore highly variable, as is typical of most landfill leachates.

A sample of leachate was independently tested to assess the biodegradability of the Shongweni landfill leachate because of the poor results obtained in both the tank and bucket bioreactors. The COD in this case was 2236 mg.l^{-1}, while the BOD was 116 mg.l^{-1}. The maximum recorded BOD:COD ratio of this sample was therefore 0.05, which is extremely low. Although the COD concentration of the raw leachate varied appreciably, BOD levels were fairly constant. This indicates that the leachate contains either highly recalcitrant or perhaps bactericidal/bacteriostatic components. Such a waste stream would typically be unsuitable for biological treatment. However, a high-strength landfill leachate with a maximum BOD:COD ratio of 0.0003 has been biologically treated with some success (Percival, Senior and Southway, 1997). These authors suggested that the high COD concentration (30 000 – 53 000 mg.l^{-1}), combined with an abundance of labile volatile fatty acids, justified the consideration of a biological approach. Although the Shongweni leachate did not have such a high COD

concentration, its BOD:COD ratio was considerably higher than that reported by Percival *et al*. (1997). It was therefore considered worthwhile trying to enhance its biodegradability in a series of flask experiments **(Section 4.5)**.

TABLE 4.1 Characteristics of Shongweni landfill leachate as determined by Darvill Wastewater Treatment Purification Works

Parameter	**Units**		**Parameter**	**Units**	
TOC	$mgC.l^{-1}$	1760	Zn	$\mu g.l^{-1}$	0.14
COD	$mgO_2.l^{-1}$	5019	Pb	$\mu g.l^{-1}$	125
Alkalinity	$mgCaCO_3.l^{-1}$	10878	Cd	$\mu g.l^{-1}$	<1.0
pH		8-9	Cr	$\mu g.l^{-1}$	1016
NO_3^-	$mgN.l^{-1}$	0.5	Hg	$\mu g.l^{-1}$	4.5
NH_3	$mgN.l^{-1}$	1102	As	$\mu g.l^{-1}$	74
Total phosphate	$\mu g.l^{-1}$	12000	Se	$\mu g.l^{-1}$	<1.0
Ca	$mg.l^{-1}$	88.2	Ni	$\mu g.l^{-1}$	218
Mg	$mg.l^{-1}$	126	B	$\mu g.l^{-1}$	5835
K	$mg.l^{-1}$	1050	Co	$\mu g.l^{-1}$	83
F	$\mu g.l^{-1}$	34800	Mo	$\mu g.l^{-1}$	24

TABLE 4.2 Some of the most abundant organic contaminants identified in the Shongweni landfill leachate using GC-MS (Only those compounds with a library match greater than 80% are shown)

Compound	Compound
1,3-Oxathiolane	7-Benzofuranol, 2,3-dihydro-2,2-di
Pyridine, 2-methyl	Dodecane, 2,6,10-trimethyl
p-Xylene	1,3,5-Triazine-2,4,6 (1H,3H,5H)-tri
2,5-Dimethylcyclopentanone	N-Tetradecane
Cyclopentanone, 2,2,2-trimethyl	Tetradecane
Methyl-5(4)-methylimidazol-4(5)-Y	Pentadecane
Benzene, 1-ethyl-2-methyl	15-Methyltricyclo[6.5.2.(13,14),0(7)
2,5-Furandione, 3,4-dimethyl	Urea, N-[5-1,1-dimethylethyl)-1,3
2-Acetyl-5-methylfuran	Benzamide, N,N-diethyl-3-methyl
2,3-Dimethylcyclopent-2-en-1-one	Hexadecane
Cyclohexanone, 3,3,5-trimethyl	2(3H)-Benzothiazolone
2-Cyclopenten-1-one, 3,4,4-trimethyl	Heptadecane
Benzenemethanol, .alpha.,.alpha.-d	Phenol, nonyl
Phenol, 2,6-dimethyl	Benzenesulfonamide, N-butyl
Phosphoric acid, triethyl ester	Anthracene
Acenaphthalene	Pyrazine, tetramethyl
Morpholine, 4-acetyl	Ametryn
Phenol, 2,4,6-trimethyl	2.8-Dimethyldibenzothiophene
Phenol, 3-(1,1-dimethylethyl)	1,6-Dimethyldibenzothiophene
10,10-Dimethyl-9-oxa-10-sila-9,10	Heptadecane, 2,6,10,15-tetramethyl
Sulphur, mol(S8)	Heptadecane, 9-octyl
Flourathene	Tetracosane
Heneicosane	Octadecane
Pyrene	Tricosane
Phenol, 4,4'-(1-methylethylidene) b	

4.3 EFFECT OF RECYCLE RATE ON THE PERFORMANCE OF THE UPFLOW PACKED-BED BIOREACTOR

4.3.1 Introduction

It is necessary to establish the optimum operating conditions for a bioreactor in order to achieve efficient bioremediation. This is a particularly important concern for a system, such as the one used in this study, which has been specifically designed as an inexpensive low-maintenance treatment option.

The six-chambers comprising the upflow packed-bed bioreactor can be run in parallel as a batch system in order to compare different operating conditions. Although a waste stream does not flow through the composite system in this mode, the leachate still moves upwards through the solid support matrix and comes into contact with the biofilm, thereby potentially mediating biodegradation. This is achieved using a recycle loop between each chamber and a corresponding reservoir **(Section 2.1.2)**. This recycle loop has other significant functions, such as mixing of the leachate which keeps particulate materials suspended. Onay and Pohland (1998) used laboratory-scale simulated landfill bioreactors to remove nitrogen from leachate. In this example, a recycle loop was used in a downward-flow batch system to transport stabilisation products from one zone of the bioreactor to the next. Each bioreactor consisted of an anoxic layer at the top, overlying an anaerobic region below which was an aerobic level. Residual carbon and nitrogen from the anaerobic zone could thus be supplied to the aerobic, and subsequently, the anoxic zone at the top of the bioreactor, allowing both nitrification and denitrification to occur. Nitrogen removal was therefore accomplished in tandem with the removal of other contaminants without the need for external treatment. A 95 % nitrogen conversion was obtained with leachate recycle, but much lower efficiencies were observed when a single-pass method without a recycle loop was used (Onay and Pohland, 1998). Although the

bioreactor used in the present study did not contain such well-defined zones, the recycle loop probably still played a similar role by transporting leachate components between the aerobic and anoxic areas that developed within each chamber, thus exposing them to different microbial populations to achieve possibly higher removal efficiencies, especially with regard to nitrogen.

Other biological treatment systems also make use of recycling to enhance process efficacy. For example, sequenced batch processes have a reactive phase during which the waste stream is re-circulated through the reactor. The duration of this phase is determined by the level of substrate removal required, and can be manipulated in response to changes in substrate loading (Kennedy and Lentz, 2000).

Recycle rate was an important parameter when the present system was operated as a batch process because it is synonymous with the leachate mixing rate. Thus leachate cycled at a faster rate through a chamber of this bioreactor was more thoroughly mixed than leachate cycled more slowly. Particulate matter in the leachate would be more likely to remain in suspension, instead of settling at the bottom of the chamber and its corresponding reservoir. At a slower recycle rate potential nutrient sources and also inhibitory substances in the leachate might settle, thus preventing their active degradation since they would become part of the largely non-bioavailable sludge at the bottom of the chambers. This would, in turn, affect the composition and size of the microbial population as well as the bioremediation rate and efficacy. Although each chamber was aerated, the additional turbulence caused by a faster recycle rate would increase the amount of dissolved oxygen available, thereby influencing the aerobes present.

Biofilm morphology, composition and stability may also be affected by recycle rate. Cao and Alaerts (1994) showed that plug flow reactors favour the formation of floc-forming, denser bacterial growth, while well-mixed systems favour filamentous growth. They also showed that shear stress influences biofilm

structure, rather than composition. Biofilms with larger surface area and smaller volumetric density tend to develop where shear stress is comparatively low. Lazarova, Pierzo, Fontvielle and Manem (1994) found that the ratio of total protein to exopolysaccharides was higher in fixed and fluidised bed reactors when compared to turbulent bed and air-lift reactors. This can be correlated with the different hydrodynamic conditions; high turbulence increases exopolysaccharide synthesis because of greater physico-chemical stress. This may increase the strength of attachment of the cells in the biofilm. Biofilms that developed in the fixed-bed and fluidised bed reactors were typically homogeneous, smooth and fragile, but biofilm characteristics also varied with location within the fixed bed bioreactor due to different shear stresses (Lazarova *et al*., 1994). As both mixing and shear stress are affected by recycle rate, these are relevant considerations in the present investigation.

Determining a suitable recycle rate could also provide a guideline for setting the flow rate or hydraulic retention time when the bioreactor is converted for use as a cascade system for continuous leachate treatment.

The objectives of this experiment were to assess the applicability of biological treatment for the Shongweni landfill leachate and to determine the effect of recycle rate on bioremediation with the upflow packed-bed bioreactor operating as a batch system.

4.3.2 Experimental design

This experiment was performed in the tank bioreactor **(Section 2.1.2)**, operating in parallel mode. Six different recycle rates were tested: 6, 10, 18, 28, 46 and 60 $l.h^{-1}$. These recycle rates are equivalent to approximately 7, 11, 20, 30, 50 and 65 reactor volumes per day (0.27, 0.45, 0.82, 1.27, 2.09 and 2.73 $vol.vol.h^{-1}$ respectively). The fibreglass box in each chamber was packed to two centimetres

from the top with pine bark chips (16-24 mm) serving as the solid biofilm support matrix. All chambers and corresponding reservoirs were filled with undiluted leachate collected from the Shongweni landfill site.

No additional nutrients or microbial inocula were added to the system. The experiment lasted for 49 days with samples (100m*l*) taken at start-up and then twice weekly from the outflow pipe of each chamber for COD, ammonia and nitrate analysis **(Section 2.4.1)**.

4.3.3 Results

Due to the poor results obtained, analysis was limited to COD. Although COD concentrations decreased initially in some of the chambers, little reduction occurred overall and the COD curves were erratic with no discernable trend **(Figure 4.2)**. All chambers except the chamber with a recycle rate of 28 $l.h^{-1}$ exhibited an increase in COD concentration over the experimental period. After a small initial increase, the chamber with a recycle rate of 46 $l.h^{-1}$ did show a promising decrease in COD up to day 28, however, a steady increase occurred thereafter until the end of the experiment. The COD of the leachate in chamber four (18 $l.h^{-1}$) declined for the first 14 days, but then became erratic and on day 49 was slightly higher than it had been initially.

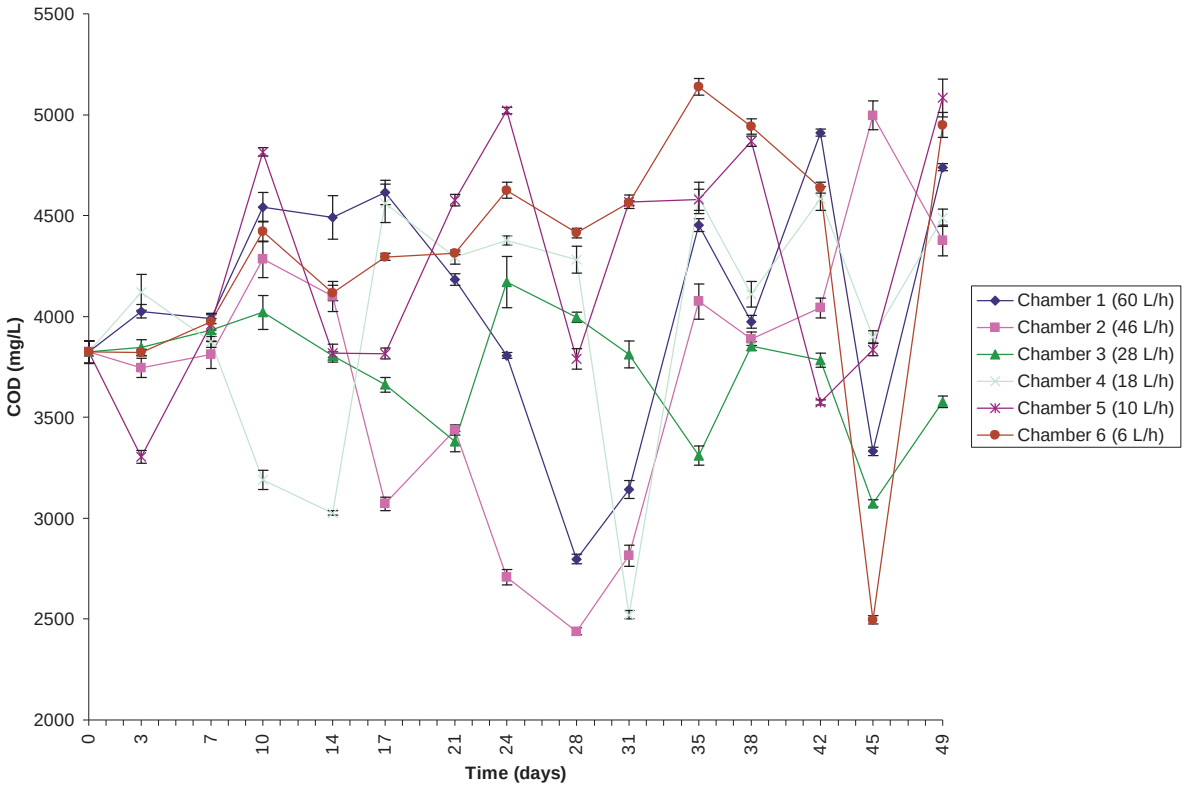


Figure 4.2 Effect of recycle rate on COD concentrations in Shongweni landfill leachate over 49 days in an upflow packed-bed bioreactor operated as a batch system.

4.3.4 Discussion

It was impossible to determine the effect of recycle rate on bioremediation of the Shongweni landfill leachate from the results obtained in this experiment. It was difficult to identify any meaningful trends in COD reduction, as the COD in each chamber fluctuated considerably with little or no overall removal. The unresponsiveness of this waste stream to biological treatment could be due to a number of factors, the most obvious being the low BOD:COD ratio mentioned in **Section 4.2** that probably indicates either the presence of recalcitrant compounds, or substances inhibitory to microbial growth. However, a visible biofilm on the surface of the pine bark chips indicated that some cell growth had

occurred. The microbial community may have been largely dormant, however, with increased production of protective exopolysaccharides creating a thicker biofilm than would otherwise have been expected as discussed in more detail below. **Table 4.1** shows that the ratio of organic carbon (TOC) to total oxidisable carbon (COD) is relatively low at 0.35. The aerobic or facultatively anaerobic heterotrophs likely to predominate in the microbial community would thus be unable to metabolise a large proportion of the contaminants present. As described in **Section 4.2**, the leachate from the Shongweni landfill had a visibly oily quality; lipids are hydrophobic and therefore form a layer on the surface of wastewaters which reduces the efficiency of the aerobic microflora in the treatment system. The reduction of COD, BOD and TOC can thus be significantly limited by the lack of oxygen in the waste stream (Haussard *et al*., 2003). This may also have contributed to the results obtained in this experiment.

The overall increase in COD concentration observed in most of the chambers is difficult to explain. Lazarova *et al*. (1994) used COD to measure the oxidisable matter of biofilms as part of a determination of total amount of biofilm. However, they noted that this measurement cannot be reliably used to indicate either the activity or physiological state of the microbial community. The results presented in **Section 4.3.3** may reflect the presence of a largely dormant or inactive biomass with large quantities of extracellular material produced in response to unfavourable conditions in the bioreactor. Alternatively, cells may have died and undergone lysis, thus releasing intracellular substances responsible for the observed increase in COD concentration. This could have been a cyclical process: cells may initially have grown to form a rudimentary biofilm using the small amount of biologically oxidisable matter present, but this growth would not have been sustainable and dying cells would cause COD to increase. Some of the released components could then have been metabolised by surviving cells, causing a subsequent decrease in COD. The repetition of such a cycle would explain the fluctuations in the COD concentration. Bilgili, Demir, Akkaya and Ozkaya (2008) noted that leachate treatment processes can produce effluents

containing high levels of soluble, inert COD comprising both non-biodegradable compounds from the raw leachate and compounds produced due to microbial activity in the treatment system. The leachate from the Shongweni landfill had a low BOD:COD ratio indicating the presence of many inert compounds. **Table 4.2** also indicates the complexity of the leachate components – many of these may not have been completely broken down by the microbial population. Microbial degradation of the small amount of bioavailable matter may thus have added to the COD concentration by the production of soluble, inert carbonaceous substances during metabolism, causing the observed increases.

It is also possible that some COD was leached from the pine bark used as a support matrix, due to the characteristics of this particular leachate, which had a higher pH than the Umlazi leachate used in **Chapter 3**. Haussard *et al*. (2003) described an increase in colour in treated solutions when raw pine bark was used to remove lipids from wastewater, showing that some organic substances were released. This did not occur if the pine bark was treated, either biologically (biodegradation for one month followed by drying and grinding) or chemically (grinding, followed by a 12 hour treatment with HCl at 60 ^{0}C) before use. However, the pre-treatments described would be impractical and uneconomical for a technology such as the one developed in this study and this option was therefore not considered. Eluate tests performed in a recent study showed an increase in COD due to leaching of compounds from pine bark (Trois, Pisano and Oxarango, 2010). Trois, Coulon, de Combret, Martins and Oxarango (2010) have also suggested that the release of phenolic compounds and hydroxylated benzene rings when pine bark was used as the substrate in a system treating synthetic leachate affected microbial activity by increasing acclimatisation time and inhibiting denitrification. Similar processes could explain the increase in COD observed in the present experiment.

The results of this experiment seem to indicate that biological remediation is not an appropriate treatment option for the Shongweni landfill leachate. However,

some phases where COD reduction occurred were observed. It was therefore decided that some further attempt should be made to treat this leachate biologically and this is described in **Sections 4.4 and 4.5** below.

4.4 PERFORMANCE OF DIFFERENT BIOFILM SUPPORT MATRICES IN UPFLOW PACKED-BED BUCKET BIOREACTORS

4.4.1 Introduction

A variety of different substrates have been used as support matrices for biofilm growth in biological treatment systems. When developing a technology intended for use in developing countries, such as this bioreactor, it is crucial that all aspects of the system are considered with respect to making it both cost-effective and low-maintenance. However, it is important that treatment efficacy should not be compromised. The support matrix used in the bioreactor could significantly affect these issues, and therefore needed to be thoroughly investigated.

The possible advantages associated with the use of pine bark as an organic solid support matrix in biological water treatment systems have been discussed extensively in **Section 3.3.1**. This substrate was shown to be a viable, inexpensive and easily obtainable option for use in the upflow packed-bed bioreactor (**Sections 3.3.3 and 3.3.4**). However, its performance needed to be compared with other more conventional biofilm support matrices to assess whether the bioremediation efficacy of the bioreactor could be improved. Haussard *et al*. (2003) observed that raw and treated bark were equally effective at sorbing hydrocarbons and lipids from wastewater; however, they found that the treated effluent from the experiments in which raw bark was used were coloured, indicating that partial dissolution of soluble organic matter, such as tannins, had occurred. Obviously, this imparting of colour is undesirable, and could contribute to an increase in COD, BOD or TOC. This would be a concern

especially if the system designed for the present investigation was upscaled because the levels of inhibitory compounds, including phenols, would increase and could adversely affect the bioremediation levels achieved at laboratory-scale. This was another reason for testing other, inorganic matrices, which would not release any substances into the leachate being treated. Although Haussard *et al*. (2003) recommended that bark be biologically or chemically pre-treated before use, this may not be practical for large-scale operations. This release of colour is not always observed, and another study reported that no significant change in leachate pH or electrical conductivity was caused by contact with pine bark, and no coloured compounds or COD were released. However, the material used in this experiment was a commercial product composed of dried and granulated pine bark with some wood fibre (Nehrenheim *et al*., 2008), while Haussard *et al*. (2003) used raw coniferous bark, which is more similar to the material used in the present investigation and therefore more relevant.

A further consideration could be the difficulty of increasing the scale of the bioreactor using a support matrix such as bark that does not have the mechanical strength associated with man-made substances developed specially for this purpose. Although the pine bark chips remained stable and did not disintegrate in any of the previously described experiments, these were all carried out on a laboratory-scale for relatively short periods of time whereas a system on a landfill site may need a matrix that can be used for several years before replacement is required.

Other plant substrates such as almond and walnut shells have been used as both an organic carbon source and a support medium for denitrifying bacteria in batch reactors designed for denitrification of secondary effluents. One study compared walnut shells, almond shells and pine bark, using gravel as a control. The best results were obtained with walnut shells, while the control reactor, filled with gravel (a commonly used biofilm support matrix), did not remove nitrate at all. It was suggested that walnut shells produce a large quantity of readily

biodegradable organic matter, making them a more effective matrix than either pine bark or almond shells. However, the release of COD produced during the breakdown of these organic media was of concern, although the effluent COD was always below regulatory limits (Diaz, Garcia, Mujeriego and Lucas, 2003).

Because of this concern and the fact that inorganic solid support matrices are more typically used in this type of biological treatment system, it was decided to compare the 16–24 mm pine bark chips with more conventional materials such as moulded plastics and ceramics that have recently become popular in this field.

For example, food processing wastewater has been biologically treated in a packed-bed reactor filled with a ceramic carrier. A bacterium isolated from the wastewater to be treated was immobilised onto this material by a vacuum method, and high levels of colonisation (2.9×10^9 cfu.g^{-1} dry ceramic carrier) and COD removal (82 %) were achieved (Kariminiaae-Hamedaani *et al*., 2003).

Min, Evans, Chu and Logan (2004) point out that specific surface area and porosity of the biofilm support matrix are important factors in the performance of a fixed-bed bioreactor system. They used such bioreactors packed with either sand or plastic as immobilisation materials to treat perchlorate contaminated groundwater at pilot-scale. Although higher removal rates were obtained in the sand bioreactor, the plastic medium was preferred because of its greater consistency in removal and the lower backpressures produced. There was also no evidence of channelling or short-circuiting in the bioreactor packed with the plastic medium, whereas this was a problem in the sand-filled reactor, probably due to the low porosity of the sand and the clumping caused by growth of the biofilm (Min *et al*., 2004). Thus, although sand is a commonly used support matrix, it was not tested in the present investigation because it was considered unsuitable as a packing material for the bioreactor design used. It was felt that similar problems to those encountered when mulch and coarse potting soil were

used in previous experiments (as described in **Section 3.3.3**) would have been exacerbated if sand was used.

Two types of packing material were used by Cannon *et al*. (2000) in a submerged and aerated filter bioreactor (which was very similar to the system used in the current study). Pall rings (50 mm diameter) and Flocor RS media, consisting of corrugated tubes (20 mm diameter and length) and with a specific surface area more than double that of the pall rings, were compared. It was suggested that the greater specific surface area of the Flocor RS medium accounted for the more efficient BOD reduction observed when this packing material was used. However, the nitrifying performance of the bioreactor containing this matrix was disappointing, perhaps due to the fact that it was less intensely mixed than the one containing pall rings (Cannon *et al*., 2000). Bertin *et al*. (2001) compared silica beads and polyurethane foam cubes in two identical packed-bed aerobic bioreactors used to treat olive mill wastewaters; both support materials performed similarly when low molecular weight compounds were degraded, but the silica beads were more effective when high molecular weight phenolic compounds were removed from an anaerobic effluent. This was attributed to the higher total amount of immobilised biomass in this bioreactor, although both matrices gave rise to a microbiologically stable and biologically active biofilm (Bertin *et al*., 2001).

In a pilot-scale study for the biological treatment of oil refinery wastewater using a fixed-film bioreactor, a packed-bed constructed of mixed media was used. The support frame of the bioreactor was made of cylindrical plastic (polypropylene) pall rings and highly porous polyurethane foam slabs were used to ‘incubate’ the microbial population. This system allowed for a very large surface area to volume ratio for the attachment and growth of biofilm, while maintaining high bio-catalytic activity. It also reduced the effects of typical problems such as channelling and plugging and minimised mass transfer limitations. COD removal rates were consistently higher than those obtained when more traditional technology was

used; sludge production was also greatly reduced and the process was much more stable (Jou and Huang, 2003).

Choi *et al*. (2001) used a variety of packing materials including expanded clay, polystyrene, polyurethane and an acrylic substance in sequencing batch biofilm reactors designed to treat domestic sewage. The focus of this research was on the correlation of EPS production with nutrient removal: it was found that the type of support medium had no effect on the EPS content of the biofilms formed (Choi *et al*., 2001). However, no organic support media were tested in this study, in contrast to the work presented in this thesis.

Rostron, Stuckey and Young (2001) compared the performance of cells attached to adsorption particles made of polyurethane foam with that of PVA-encapsulated microorganisms in order to determine which had most potential as an immobilisation medium for nitrification of high strength ammonia wastewaters. This is highly relevant to the present investigation as landfill leachates usually contain high concentrations of ammonia. Although adsorption has generally been preferred as an immobilisation technique, the PVA encapsulated cells exhibited the highest volumetric nitrification rates and were also preferable to the adsorbed population in terms of the logistics of system optimisation. The encapsulated nitrifying bacteria may have been more protected from substrate inhibition effects, and also from competition with faster-growing heterotrophs due to the closed structure of the PVA particles. They are also more resistant to biofilm loss caused by increased turbulence because the biodegradative activity of these cells does not depend on adsorbed biomass (Rostron *et al*., 2001). However, encapsulation is impractical for the system used in the current research as it is not consistent with the aim of developing a low-cost, low-maintenance technology.

Although activated carbon has often been used in fluidised bed reactors, and has been shown to contribute significantly to removal of xenobiotic compounds by

adsorption (Imai *et al*., 1993), it would be difficult to use in a packed-bed system and was therefore not considered for use in these experiments. In addition, Loukidou and Zouboulis (2001) found that granulated activated carbon (GAC) did not perform as well as porous polyurethane in an attached-biomass reactor for the treatment of landfill leachate. This was due to the large amount of residual suspended solids which would need to be separated from the treated wastewater; and the increased operational costs associated with carbon addition (Loukidou and Zouboulis, 2001).

Based on the findings of others and on local availability of materials, the inorganic matrices chosen for comparison with the organic pine bark chips were 'plastic bioballs' and 'ceramic noodles'. These materials are discussed in detail in section **4.4.2**.

The objective of this experiment was to determine whether an inorganic solid biofilm support matrix would perform significantly better than pine bark chips when used in the upflow packed-bed bucket bioremediation system.

4.4.2 Experimental Design

The bucket bioreactors **(Section 2.1.3)** were used for this experiment. Initially, three support matrices were tested. These were the 16 – 24 mm pine bark chips used in all previous experiments **(Figure 4.3 A)**, plastic bioballs **(Figure 4.3 B)** and ceramic noodles **(Figure 4.3 C)**. The dimensions of each of these matrices are shown in **Figure 4.3**.

Biofilm attachment and growth on the pine bark chips has been illustrated in previous sections; the rough surface provides niches in which microbial cells can grow and generate the extracellular polysaccharides that make up a biofilm. The plastic bioballs comprise evenly spaced fins that substantially increase the

surface area for biofilm attachment and growth. The spaces between these fins also provide a protected environment for the development of a biofilm because turbulence and shear stress will be reduced in these areas relative to the outer surfaces. The ceramic noodles are hollow, and the inside of each cylindrical noodle will also create an environment conducive to biofilm growth. The source of the pine bark chips (Gromed Pty Ltd) has already been mentioned in **Section 3.3.2**, and the other two matrices were obtained from a local pet shop that supplies these materials as aquarium biofilters.

Further characteristics of each of the matrices are presented in **Table 4.3**. The surface area of the plastic bioballs and ceramic noodles were calculated geometrically, but there are slight irregularities between individual units, so the values given are necessarily approximations. Specific surface areas were determined using the approximate number of individual units (calculated according to mass) in each bucket bioreactor, and then relating this value to the volume occupied by the matrix.

Pore volume was measured by submerging ten individual units of each matrix separately in distilled water for 72 hours to allow maximum absorption to occur. The saturated samples were weighed, dried in an oven at 70^0C for 72 hours and re-weighed. The procedure was performed in triplicate and the average difference in mass was regarded as the pore volume. This value was used to calculate the pore volume per bucket bioreactor, using the average number of individual units required to fill each reactor to the predetermined level used in the experiment.

Void volume was calculated by filling a vessel with each matrix up to a known mark (1.8 *l*); distilled water was then added until this mark was reached. The amount of water required to reach the selected mark was considered to be the void volume. The experiment was performed five times for each material, and the average volume was used to extrapolate to the volume used in each bucket

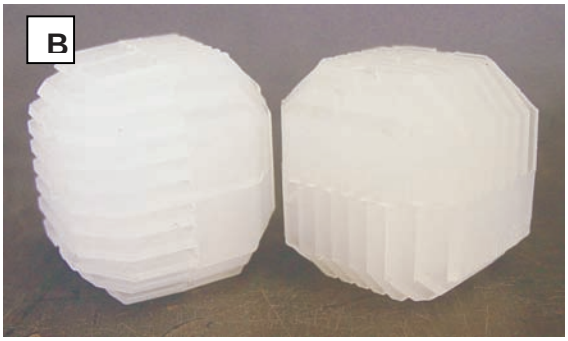
bioreactor (19.8 *l*). The plastic bioballs had the greatest void volume, while both the pine bark chips and the ceramic noodles had very similar void volumes **(Table 4.3)**.

Cost per bioreactor was calculated using either the mass or the volume of material required to fill each bioreactor, depending on the way in which prices were quoted by the suppliers; the cost of transport has not been included in this analysis. The pine bark chips are the cheapest matrix by a considerable margin, with the ceramic noodles being the most expensive (costing 87 times more than the pine bark chips).

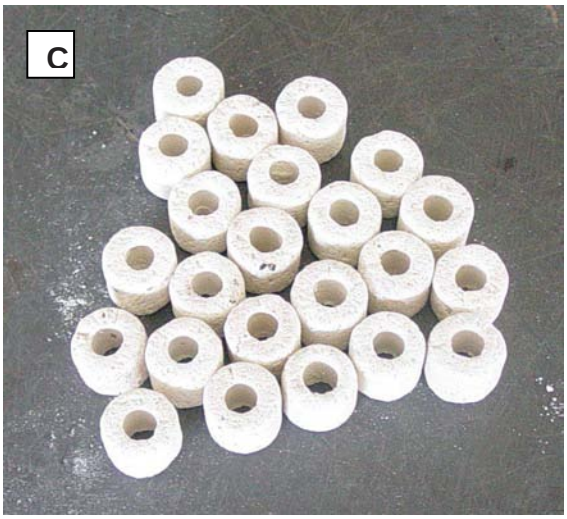
The bioremediation experiment was performed in duplicate so each packing material was used in two bioreactors. Each bucket bioreactor was set up as discussed in **Section 2.1.3**, and packed to five centimetres from the top with the appropriate material before being filled with undiluted leachate from the Shongweni landfill site. Leachate levels were corrected after 24 hours in order to compensate for any liquid absorbed by the pine bark, and to a much lesser extent the other two solid support materials.

The leachate was not supplemented with additional nutrients, but activated sludge from the Hammarsdale Sewage Works **(Section 2.3)** was used as inoculum at a ratio of 10 % (v/v). The experiment lasted for 28 days and samples were taken from the liquid at the top of each bucket at the beginning of the experiment and after 3, 7, 10, 14, 17, 21, 24 and 28 days for COD analysis **(Section 2.4.1)**.

Variable dimensions
Average diameter =
16 – 24 mm



L = 25 mm
W = 25 mm
Spacing = 1 mm



OD = 15 mm
ID = 7 mm
H = 15 mm

Figure 4.3 The three solid support matrices used for biofilm attachment and growth. Dimensions are given beside each photograph. A: 16 – 24 mm pine bark chips; B: plastic bioballs; C: ceramic noodles.

TABLE 4.3 Characteristics of the three support matrices tested in the upflow packed-bed bucket bioreactors

Matrix	**Approximate surface area per matrix unit (m^2 x 10^{-3})**	**Specific surface area per bioreactor ($m^2.m^{-3}$)**	**Pore volume per bioreactor (*l*)**	**Void volume per bioreactor (*l*)**	**Cost per bioreactor (R)**
Pine bark chips	ND	ND	2.785	10.65	14
Plastic bioballs	7.077	358	0.377	13.54	835
Ceramic noodles	1.484	317	3.506	10.46	1224

ND = not determined

4.4.3 Results

The initial COD concentrations were the lowest recorded for the Shongweni leachate (2180 mg.l^{-1} [see **Section 4.2**]). However, as noted in **Chapter 1,** temporal fluctuations (usually dependent on weather conditions and the types of waste being dumped) are not uncommon in leachates.

In both bucket bioreactors where pine bark chips were used as the solid biofilm support matrix, the COD concentration increased steadily for most of the experiment (24 days), then finally decreased between days 24 and 28 **(Figure 4.4)**. Despite this decrease the COD values at the end of the experiment were higher than the starting values. A negative COD removal efficiency was thus recorded in both cases; the average total COD reduction being -18% **(Table 4.4)**. The COD concentrations were also inconsistent with each other at all sampling times (with the exception of the final sample taken on day 28); one bucket always had a higher COD concentration than the other despite the identical set-up of the two reactors.

Although the plastic bioballs and the ceramic noodles exhibited the same average total COD removal efficiency (17 %; **Table 4.4**), the overall results from the two bioreactors filled with the plastic bioballs were much more consistent with one another than those observed in the two reactors packed with the ceramic material **(Figure 4.4; Table 4.4)**. However, anomalous COD increases occurred in all six of the bioreactors, especially between days 17 and 24, after which a period of rapid reduction occurred. These factors meant that the cumulative reduction values were extremely erratic, and quite often negative **(Table 4.4)**.

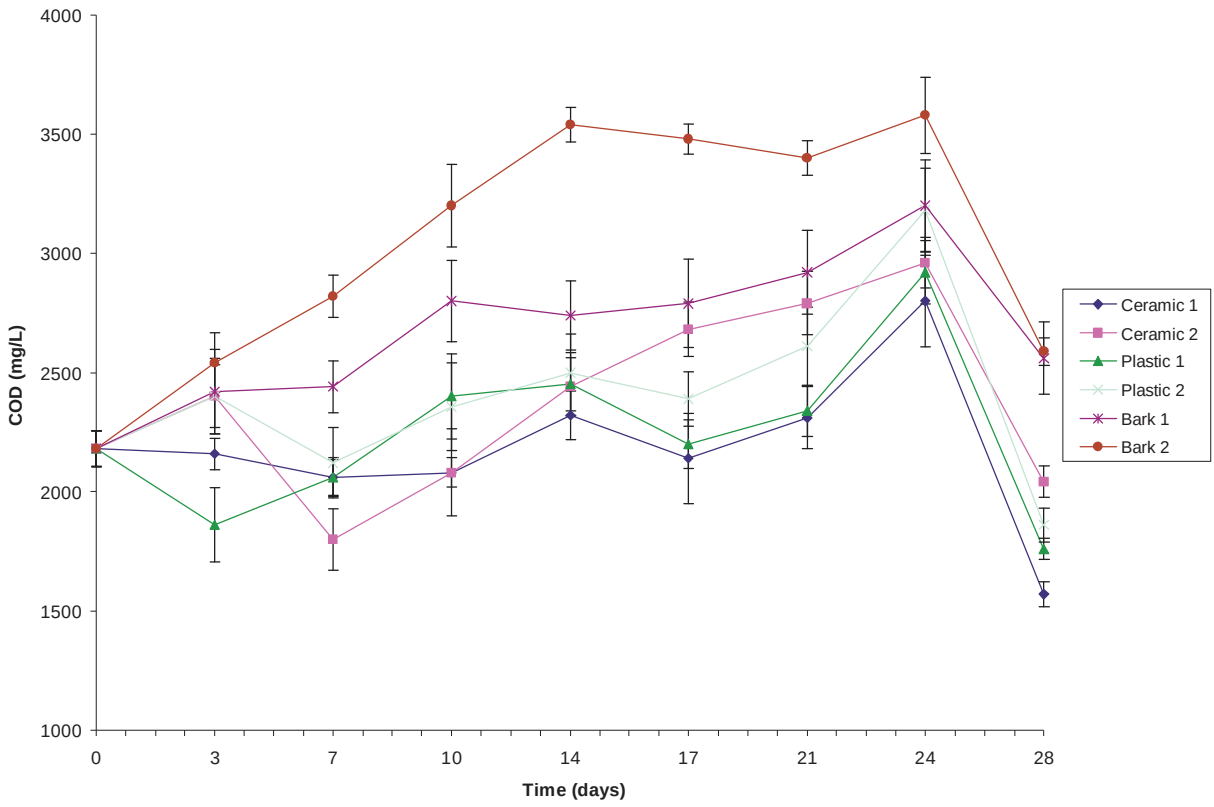


Figure 4.4 Comparison of COD removal efficiencies over 28 days in upflow packed-bed bucker bioreactors with pine bark, plastic bioballs and ceramic noodles serving as biofilm support matrices. Error bars indicate standard deviation (n=3).

TABLE 4.4 Total COD removal efficiencies in a 28 day experiment performed in upflow packed-bed bucket bioreactors filled with different support matrices

Time (days)	COD removal efficiency expressed as a percentage with standard deviation																	
	Pine bark						**Plastic bioballs**						**Ceramic noodles**					
	Total			**Cumulative**			**Total**			**Cumulative**			**Total**			**Cumulative**		
	1	**2**	**Avg.**	**1**	**2**	**Avg.**	**1**	**2**	**Avg.**	**1**	**2**	**Avg.**	**1**	**2**	**Avg.**	**1**	**2**	**Avg.**
3	-11 (±7.85)	-17 (±6.09)	**-14**	n/a	n/a	**n/a**	15 (±6.76)	-10 (±7.16)	**3**	n/a	n/a	**n/a**	1 (±3.98)	-10 (±6.20)	**-5**	n/a	n/a	**n/a**
7	-12 (±5.47)	-29 (±5.27)	**-15**	-1	-11	**-6**	6 (±4.33)	3 (±6.56)	**5**	-11	12	**1**	6 (±4.15)	17 (±5.66)	**12**	5	25	**15**
10	-28 (±7.83)	-47 (±8.20)	**-38**	-15	-13	**-14**	-10 (±7.81)	-8 (±8.03)	**-9**	-17	-11	**-14**	5 (±7.79)	5 (±3.74)	**5**	-1	-16	**-9**
14	-26 (±6.88)	-62 (±5.69)	**-44**	2	-11	**-5**	-12 (±6.23)	-15 (±7.25)	**-14**	-2	-6	**-4**	-6 (±5.16)	-12 (±5.94)	**-9**	-12	-17	**-15**
17	-28 (±8.32)	-60 (±5.43)	**-44**	-2	2	**0**	-1 (±5.06)	-10 (±5.66)	**-6**	10	4	**7**	2 (±8.12)	-23 (±5.85)	**-11**	8	-10	**-1**
21	-34 (±8.08)	-56 (±5.52)	**-45**	-5	2	**-2**	-7 (±5.32)	-20 (±7.50)	**-14**	-6	-9	**-8**	-6 (±6.08)	-28 (±6.53)	**-17**	-8	-4	**-6**
24	-47 (±8.86)	-64 (±8.07)	**-56**	-10	-5	**-8**	-34 (±6.71)	-46 (±8.27)	**-40**	-25	-22	**-24**	-28 (±8.52)	-36 (±5.84)	**-32**	-21	-6	**-14**
28	-17	-19	**-18**	20	28	**24**	19	15	**17**	40	42	**41**	28	6	**17**	44	31	**38**

Scanning electron micrographs showed microbial growth and biofilm formation on all three support matrices (**Figures 4.5, 4.6** and **4.7**). However, it was difficult to obtain a good image of the cells on the plastic bioballs due to rapid charging, which created bright areas on the screen when this matrix was examined. The greatest diversity in microbial morphology was associated with the pine bark, while few individual cells were observed on the ceramic noodles and plastic bioballs. This may have been due to the presence of a large amount of extracellular polysaccharide covering the matrix surfaces in the bioreactors containing these materials. Such polysaccharides would not have been removed during preparation as ESEM was used to view the samples.

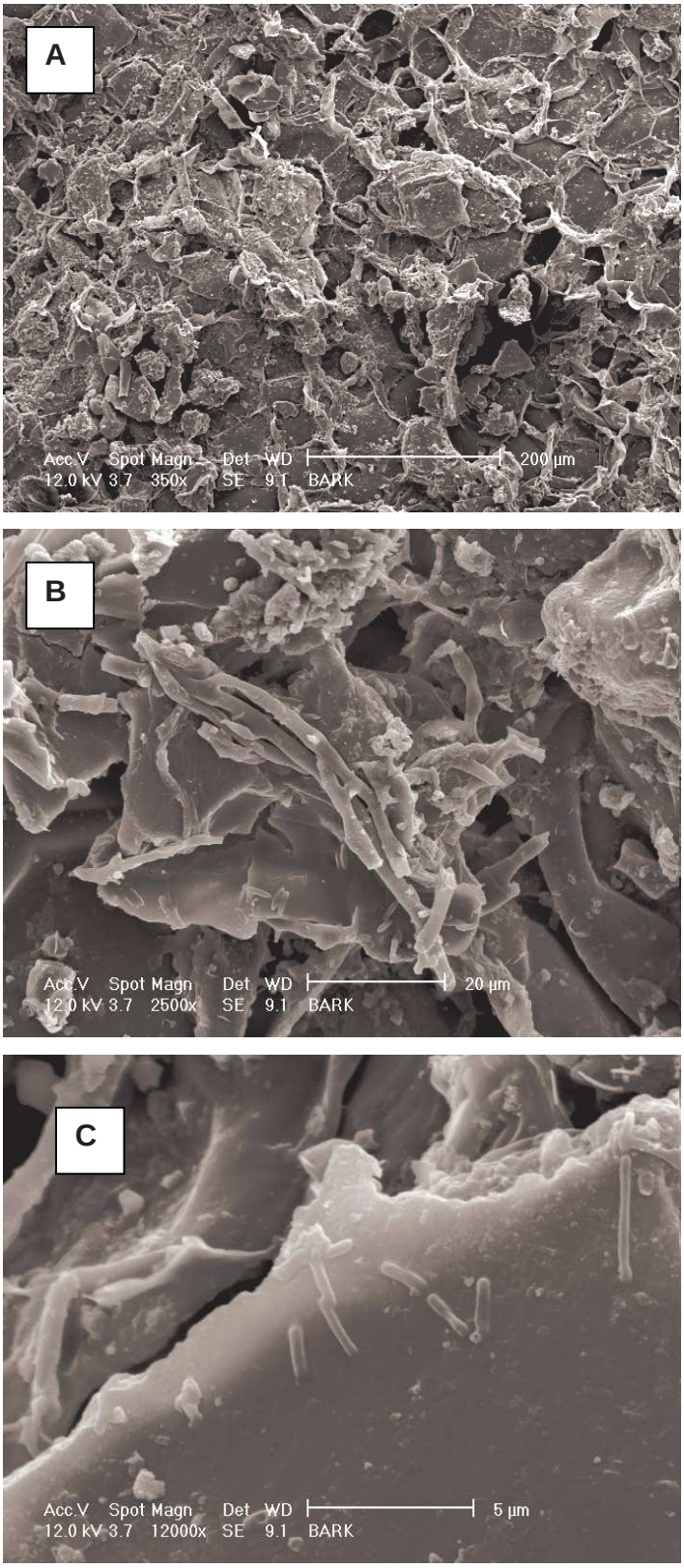


Figure 4.5: Scanning electron micrographs of pine bark serving as biofilm support matrix during bioremediation of Shongweni landfill leachate in an upflow packed-bed bioreactor. A: 350 x magnification; B: 2500 x magnification; C: 12000 x magnification.

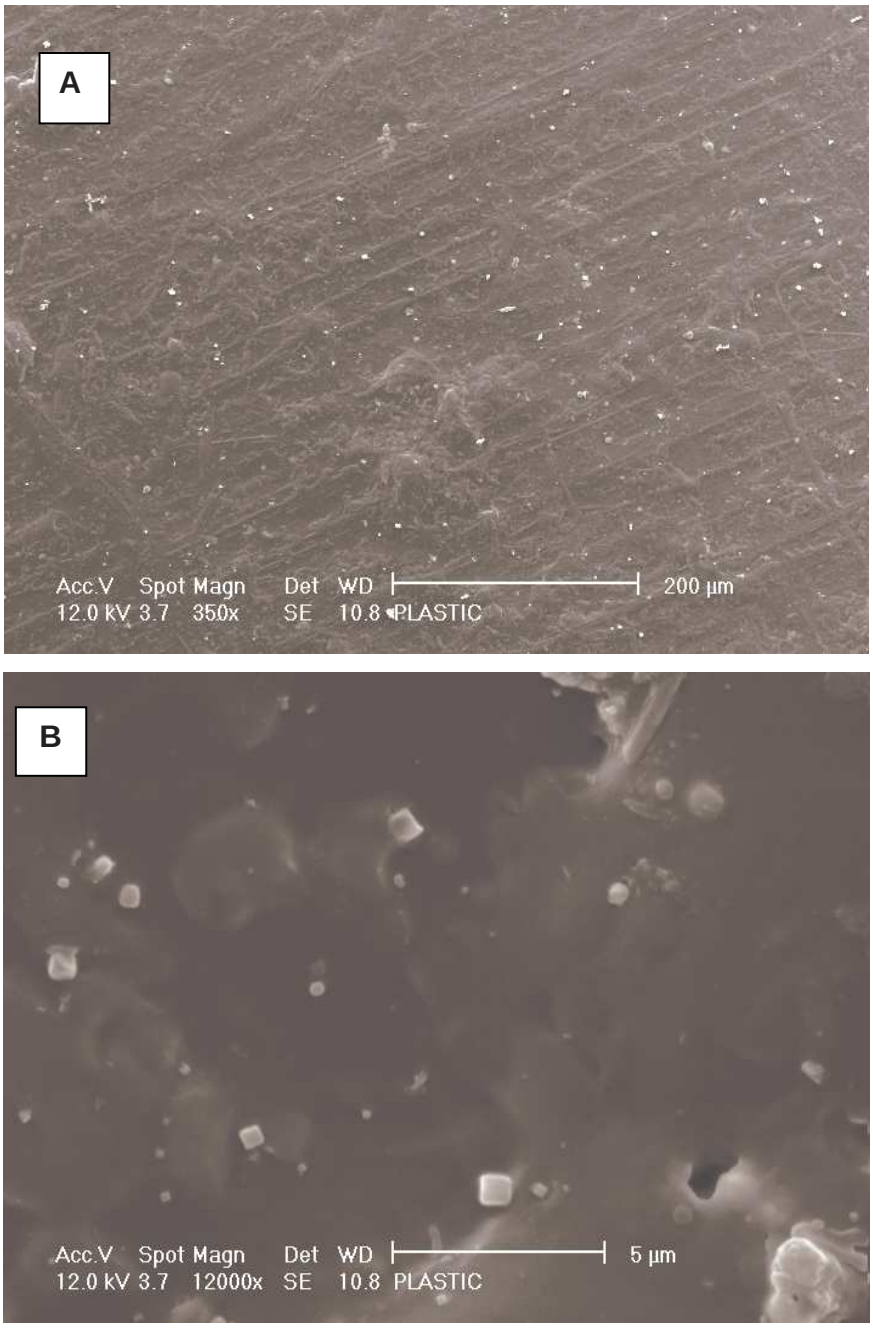


Figure 4.6: Scanning electron micrographs of a plastic bioball serving as biofilm support matrix during bioremediation of Shongweni landfill leachate in an upflow packed-bed bioreactor. A: 350 x magnification; B: 12000 x magnification. Note the almost total absence of attached biota.

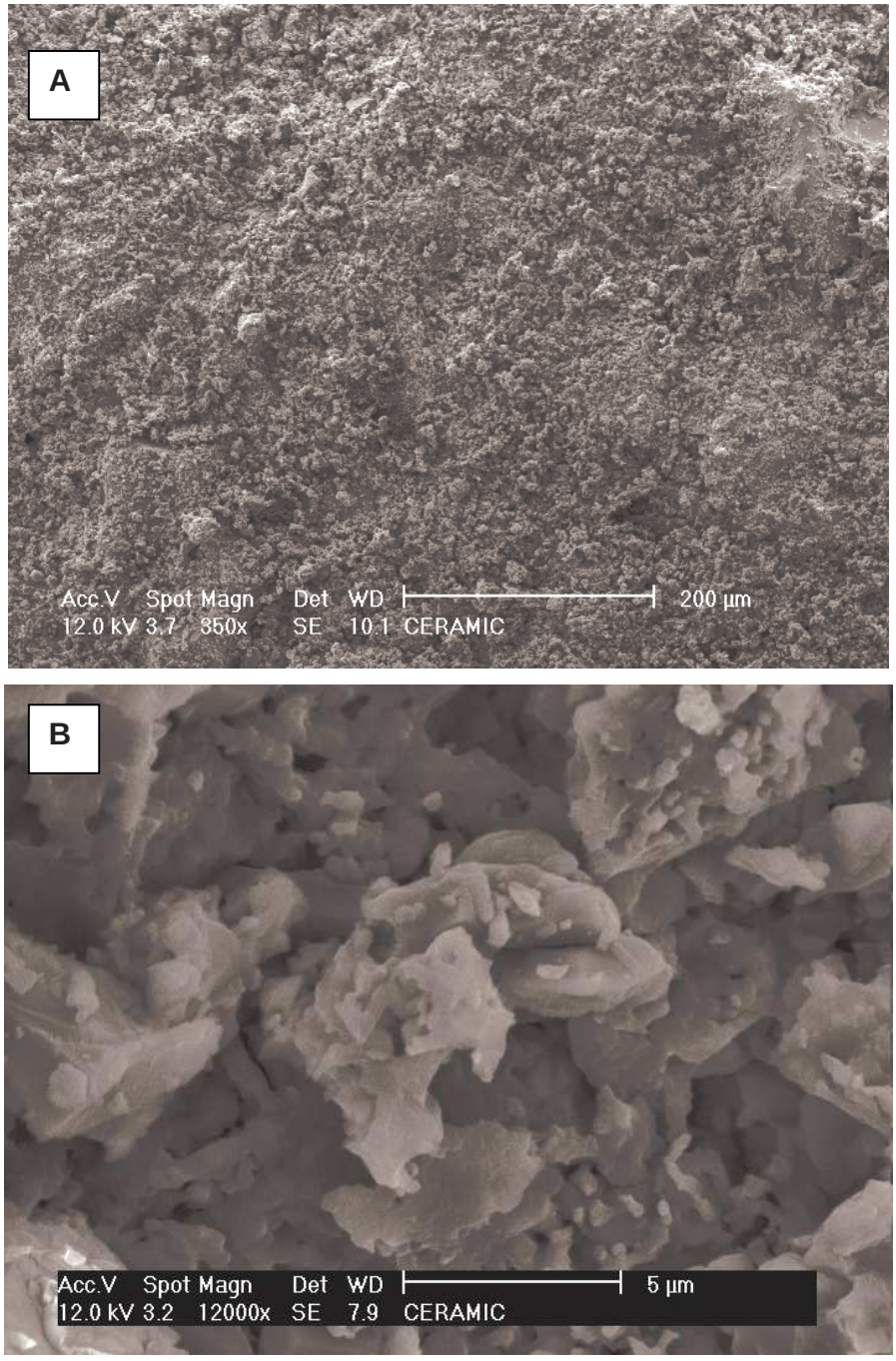


Figure 4.7: Scanning electron micrographs of a ceramic noodle serving as biofilm support matrix during bioremediation of Shongweni landfill leachate in an upflow packed-bed bioreactor. A: 350 x magnification; B: 12000 x magnification. Note the paucity of attached microbial cells.

In terms of manageability, the ceramic matrix proved problematic because the noodles near the bottom of the packed-bed were crushed by the weight of those above them. This produced a fine, white powder which clogged the circulation pumps and aeration systems in addition to creating cloudiness in an already highly coloured effluent.

4.4.4 Discussion

Although the COD removal efficiencies observed in this experiment were low compared to those of the other leachates investigated in this project and with those reported by other researchers, a recognisable trend was apparent; in contrast to the experiment described in **Section 4.3**.

It is clear that the inorganic matrices consisting of plastic or ceramic materials performed much better than the organic pine bark matrix. It has been shown that direct aqueous extraction of pine bark in alkaline solutions yields catechin-type tannins, as well as significant quantities of sugars and lignin, even at a relatively low temperature of 20^0C (Fradinho, Neto, Evtuguin, Jorge, Irle, Gil and de Jesus, 2002). All of these substances would contribute to COD concentrations measured in solution. The leachate from the Shongweni landfill site is typically alkaline (pH 8-9), and the relatively long exposure of the pine bark chips to this effluent could have released such compounds into the liquid being treated. In a batch system such as the one used here, the toxins would not have been removed and the COD concentration would increase over time as more of these bark constituents were leached from the matrix. Subsequent decreases in COD could then be due to microbial degradation or adsorption of these substances by the adapting biofilm. For example, microbial degradation of some of these compounds may require inducible enzymes, which would need to be produced before these substances could be attacked.

Similar processes would not occur in a bed packed with an inorganic matrix consisting of inert plastic or ceramic components as they would not contribute any carbonaceous compounds to the solution in the bioreactor, and thus would have no effect on the COD levels of the liquid. However, some anomalous COD increases were also observed in these bioreactors. It is possible that some of the intermediates produced during bioremediation increase the soluble, and therefore, measurable COD before being metabolised by other inhabitants of either the biofilm or the free-living microbial population. This effect would be particularly marked in batch systems such as the bucket bioreactors used in this experiment, that were closed systems from which samples were simply drawn from the surface of the liquid. In the case of the ceramic noodles, the powder resulting from the crushing effect described in **Section 4.4.3** may have interfered with the COD analyses; an effect which may also explain the large differences between the two identically prepared bucket bioreactors containing this support medium.

The morphological diversity of the microorganisms in the bioreactors containing pine bark probably reflects the presence of both the indigenous population from the Shongweni leachate and that on the pine bark chips. Although one of the suggested advantages of using an organic support matrix such as pine bark was that the additional source of potential biodegradative pathways could enhance pollutant removal, this did not seem to be the case in this experiment. Despite the paucity of attached cells observed on the plastic bioballs and ceramic noodles, the bioreactors containing these matrices performed much better than those with the pine bark. This may suggest that a significant proportion of the COD reduction was due to the planktonic microbial population. It is possible that the free-living microorganisms in the bioreactors packed with pine bark were inhibited by the release of toxic compounds from this material and that largely inactive cells were able to survive in the biofilm due to the production of protective extracellular substances.

Thus, although there are many potential advantages to the use of pine bark as a support for biofilm attachment in a wastewater treatment system (discussed in detail in **Section 3.3.1**), this experiment showed that inert matrices that do not contribute to the carbonaceous load in the bioreactor result in a dramatic improvement in bioremediation capability. While the average COD removal efficiency in the bucket bioreactors containing pine bark was -18 %, the bioreactors containing plastic bioballs and ceramic noodles both achieved 17 % removal.

The results of this experiment also prompted further efforts to promote biological remediation of this recalcitrant leachate.

4.5 NUTRITIONAL SUITABILITY OF SHONGWENI LEACHATE FOR MICROBIAL GROWTH

4.5.1 Introduction

The results of the previous experiments **(4.3 and 4.4)** indicate that the Shongweni landfill leachate has a low biodegradability. Biodegradability is affected by three main factors. Firstly, the nature of the chemical contaminants involved. It is likely that this leachate contains many recalcitrant compounds that are resistant to microbial attack. This is supported by the results of GC-MS shown in **Table 4.2**. Phenols, substituted phenolics, benzene derivatives and various aromatics, which may have been inhibitory as well as difficult to degrade, were among the most abundant organic compounds identified in the leachate. Secondly, a viable microbial population capable of metabolising the contaminants concerned must be present in order for bioremediation to occur. Finally, environmental conditions should be suitable for such microbial activity (Philp, Bamforth, Singleton and Atlas, 2005). It was felt that perhaps the Shongweni landfill leachate could be more effectively biodegraded if some of these factors

were optimised; there is little that can be done about the chemical contaminants in the leachate, but microbiological inocula can be added and environmental conditions adjusted. Biostimulation and bioaugmentation were therefore legitimate approaches to overcoming the poor COD removal observed in **Sections 4.3 and 4.4**.

Biostimulation involves enhancing the activities of those autochthonous microorganisms capable of degrading the pollutants of concern (Atlas and Philp, 2005). The environmental conditions can be modified to eliminate restraints on biodegradation. Nutrient concentrations, the availability of molecular oxygen and redox potential are some of the factors that can be controlled in order to achieve this (Atlas and Bartha, 1993). Leachate from the Shongweni landfill has a C:N:P:K ratio of 1.6**:**1**:**0.01**:**0.95 and a pH between eight and nine **(Table 4.1)**. Such conditions are not conducive to microbial growth, especially in conjunction with the consistently low BOD:COD ratio of the leachate, and could potentially be responsible for the observed lack of biodegradation. Optimisation of nutrient concentrations and pH levels was therefore attempted.

Bioaugmentation is the introduction of either specific strains or consortia of microorganisms to enhance the ability of a system to bioremediate organic contaminants. This approach is particularly valuable where the indigenous microbial population is not capable of degrading a persistent recalcitrant compound. Addition of allochthonous microorganisms increases genetic diversity and hence catabolic potential thus improving removal rate and efficiency of biodegradation in many cases (Philp and Atlas, 2005). Although the Shongweni landfill leachate does contain indigenous microorganisms, they may function sporadically and thus perform unpredictably in a bioreactor. This is characteristic of microbial communities that colonise industrial waste, and has been observed in fluidised bioreactors in which such microbial populations proved to be dynamic and unstable (Fernandez, Huang, Seston, Xing, Hickey, Criddle and Tiedje,

1999). Bioaugmentation may make the system more reliable, as well as improve degradative ability (van der Gast, Knowles, Starkey and Thompson, 2002).

As previously referred to in **Section 2.3**, Deeley, Skierkowski and Robertson (1985) found that better removal of [^{14}C] phenol from spiked samples of domestic wastewater and landfill leachate was obtained with microorganisms from domestic sewage as opposed to the microbial population indigenous to the leachate. They suggested that the absence of a lag period in the case of the consortium from the domestic sewage may have been due to the presence of microorganisms already acclimatised to phenol, or a similar substance; this was likely as such populations are generally highly diverse and large. In contrast, the samples of landfill leachate used may have lacked any microorganisms able to metabolise phenol, or they may have been present in insufficient numbers especially as the sample was taken down-gradient from an active landfill site, and not from an actual leachate collection facility (Deeley *et al*., 1985). Jun, Park, Park and Lee (2004) introduced Archaea into an intermittently aerated system that was already seeded with activated sludge and noted that a symbiosis seemed to develop between the anaerobic Archaea and aerobic bacteria, possibly due to the excretion of stimulatory compounds by the Archaea, or because of their scavenging activities and the resultant removal of non-biodegradable compounds, metabolic by-products and/or cell debris. Total COD reduction was higher in this bioreactor than in those without Archaea, and stable nitrification was also achieved, while denitrification appeared to be enhanced. Addition of Archaea also reduced sludge production probably by mineralising solid pollutants and decaying biomass (Jun *et al*., 2004). Thus the addition of a suitable inoculum such as that from a domestic wastewater plant could conceivably improve the COD removal efficiencies from the Shongweni leachate.

The objective of the following experiments was thus to determine whether Shongweni landfill leachate could be more effectively and efficiently biodegraded using either biostimulation or bioaugmentation techniques.

4.5.2 Experimental design

A series of flask experiments was performed to assess the suitability of Shongweni landfill leachate for microbial growth. The undiluted leachate used previously may have contained high concentrations of toxic compounds inhibitory to microbial growth. The first experiment **(Section 4.5.2.1)** was therefore designed to determine whether dilution of the leachate would promote biodegradation by the native population. As noted in the introduction to this section, the C:N ratio of this leachate is much lower than that required for vigorous microbial growth. Thus a second experiment **(Section 4.5.2.2)** was set up to investigate the effect of increasing the C:N ratio to a physiologically suitable level. **Section 4.5.2.3** describes an experiment designed to assess the effect of agitation and, consequently, the availability of oxygen, on the bioremediation of the leachate. Finally, an experiment **(Section 4.5.2.4)** was performed to determine whether addition of an allochthonous inoculum would improve COD removal efficiency. This experiment also investigated the effect of adjusting the pH of the Shongweni leachate from 8-9 to 6.9, especially to accommodate the organisms present in the introduced inoculum.

4.5.2.1 Effect of dilution on the bioremediation of Shongweni landfill leachate

This experiment was set up in two litre Erlenmeyer flasks. Raw leachate was diluted with distilled water to 25, 50 and 75 %. An undiluted sample served as a control. The high concentrations of ammonia in the Shongweni leachate may have inhibited biodegradation so air stripping (Jumbo Jet Super 7800 set at the maximum air flow rate for two hours) was employed as a pre-treatment in one sample to reduce the amount of ammoniacal nitrogen present (Britz, 1995). The contents of each flask were as follows:

Flask 1: 150 m/ leachate; 450 m/ dH_2O

Flask 2: 300 m/ leachate; 300 m/ dH_2O

Flask 3: 450 m/ leachate; 150 m/ dH_2O

Flask 4: 600 m/ leachate

Flask 5: 600 m/ leachate (pre-aerated)

The experiment was conducted at room temperature in a GFL 1083 reciprocal shaker waterbath with the speed control set at 30%. COD **(Section 2.4.1)** was measured at the beginning of the experiment, and after five, seven and ten days. Aliquots of 100 m/ were taken at each sampling time.

This experiment acted as a control for **4.5.2.2**, which was carried out simultaneously.

4.5.2.2 Effect of increased carbon:nitrogen ratio on the bioremediation of Shongweni landfill leachate

Raw leachate was diluted with distilled water to 25, 50 and 75 % and amended with sufficient glucose (D+ glucose, Saarchem AR grade, anhydrous) to adjust the C:N ratio to 15:1. Pre-aerated and unaerated undiluted leachate samples were similarly supplemented with glucose. As indicated above, experiment **4.5.2.1** was carried out at the same time and was used as a control. Two litre Erlenmeyer flasks were used. The contents were as follows:

Flask 1: 150 m/ leachate; 450 m/ dH_2O; 11.00 g glucose

Flask 2: 300 m/ leachate; 300 m/ dH_2O; 22.01 g glucose

Flask 3: 450 m/ leachate; 150 m/ dH_2O; 33.01 g glucose

Flask 4: 600 m/ leachate; 44.01 g glucose

Flask 5: 600 m/ leachate (pre-aerated); 44.01 g glucose

The experiment was performed at room temperature in a reciprocal shaker waterbath (GFL 1083) with the speed dial set at 30 %. COD **(Section 2.4.1)** was measured at the start of the experiment and then after five, seven and ten days. Samples of 100 m/ were taken each time.

Although addition of glucose would not be considered in a large scale leachate treatment operation, it was used here as an easily biodegradable source of carbon to determine whether the C:N ratio has any significant impact on the efficacy of bioremediation. Waste carbon sources from other processes, such as spent yeast, cornsteep liquor, cannery effluents or chicken litter, could be investigated if carbon addition was found to encourage co-metabolism and enhance biodegradation.

4.5.2.3 Effect of agitation on the bioremediation of Shongweni landfill leachate

Three 2L Erlenmeyer flasks were each filled with 600 m/ undiluted, unsupplemented leachate. One flask was placed in a reciprocal shaker waterbath (GFL 1083) with the speed dial set at 30%. This ensured that mixing and a certain level of aeration occurred. The second flask was simply left standing on a bench top, with no agitation or aeration. The third flask was flushed with nitrogen gas at the start of the experiment in order to exclude oxygen. The flask was sealed with a rubber bung and was re-flushed with nitrogen gas each time liquid samples were removed for analysis.

The experiment was done at room temperature. Samples were taken for COD analysis **(Section 2.4.1)** at the start of the experiment, and then after four, seven and ten days.

4.5.2.4 Effect of adding an allochthonous inoculum in the form of activated sludge on the bioremediation of Shongweni landfill leachate

Microorganisms occurring naturally in activated sludge **(Section 2.3)** are acclimatised to pH levels that are close to neutral and thus may perform poorly in a leachate with a high pH, such as that from the Shongweni landfill site. The contents of some flasks were therefore acidified to pH 6.9, using conc. HCl. A sample of pure leachate was similarly acidified and served as a comparison. The seven 2L Erlenmeyer flasks used in the experiment were prepared as follows:

Flask 1: 600 m/ unacidified leachate (pH 8 – 9)
Flask 2: 600 m/ leachate; acidified to pH 6.9 using conc. HCl
Flask 3: 540 m/ unacidifed leachate (pH 8 – 9); 60 m/ activated sludge
Flask 4: 540 m/ leachate; 60 m/ activated sludge; acidified to pH 6.9 using conc. HCl
Flask 5: 450 m/ unacidified leachate (pH 8 – 9); 150 m/ activated sludge
Flask 6: 450 m/ leachate; 150 m/ activated sludge; acidified to pH 6.9 using conc. HCl
Flask 7: 600 m/ activated sludge

The experiment was conducted at room temperature in a reciprocal GFL 1083 shaker waterbath with the speed dial set at 30 %. Samples (100 m/) were taken at time zero, and then after four, seven, eleven and fourteen days. COD analyses **(Section 2.4.1)** were performed on all samples.

4.5.3 Results

4.5.3.1 Effect of dilution on the bioremediation of Shongweni landfill leachate

Dilution had very little effect on COD reduction with COD removal being low in all flasks. The lowest percentage removal (5 %) was observed in the 25 % leachate dilution. All the other flasks exhibited similar removal efficiencies of 9, 9, 11 and 9 % for the 50 % leachate, 75 % leachate, 100 % leachate and 100 % pre-aerated leachate respectively **(Figure 4.8)**.

Although dilution did not appear to influence COD removal, the microbial populations in each flask were quite different at the end of the ten days. The 25 % leachate contained filamentous bacteria, possibly actinomycetes **(Figure 4.9 A)**, which were not present in the undiluted leachate (both non-aerated and pre-aerated), which typically contained mainly bacilli and coccobacilli (**Figures 4.9 B and 4.9 C** respectively). The morphotypes observed in the latter two treatments were similar.

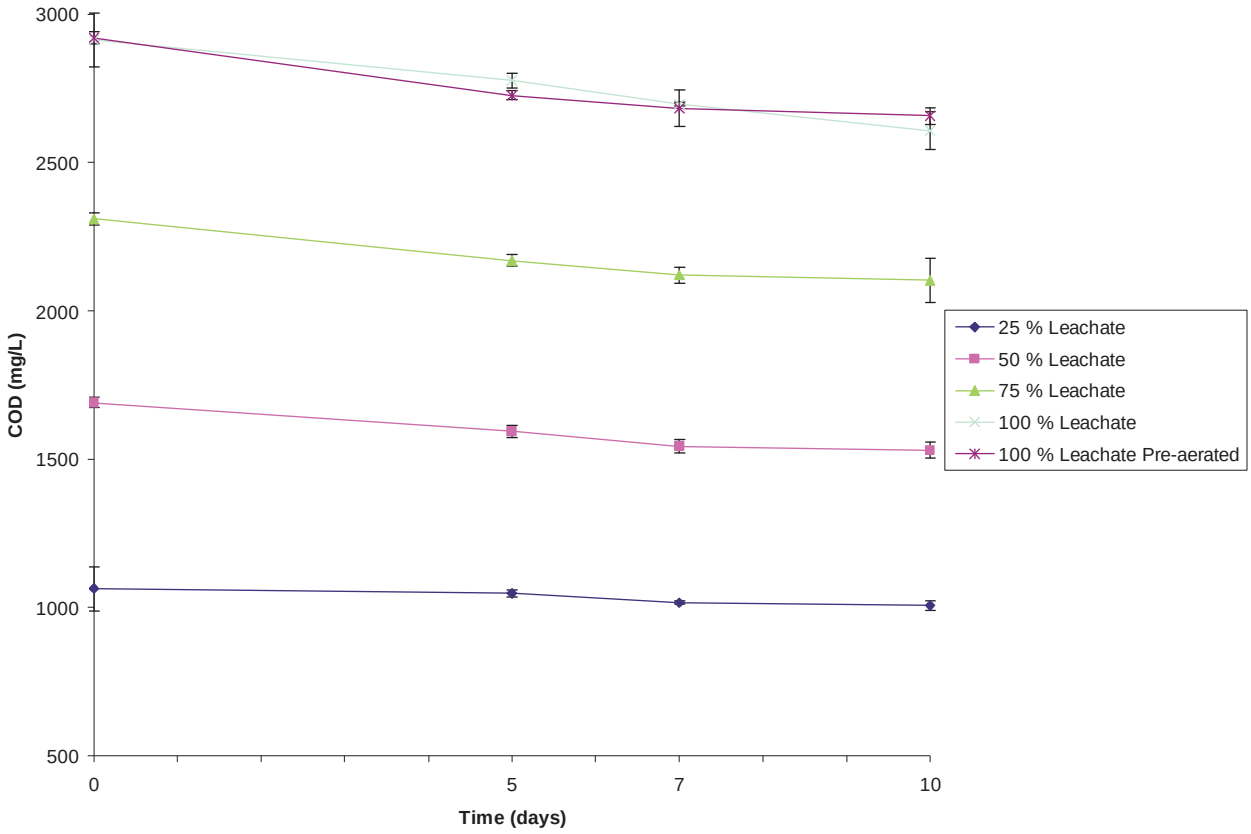


Figure 4.8 Changes in COD concentrations over ten days bioremediation of full strength and variously diluted Shongweni landfill leachate. Error bars indicate standard deviation (n=3)

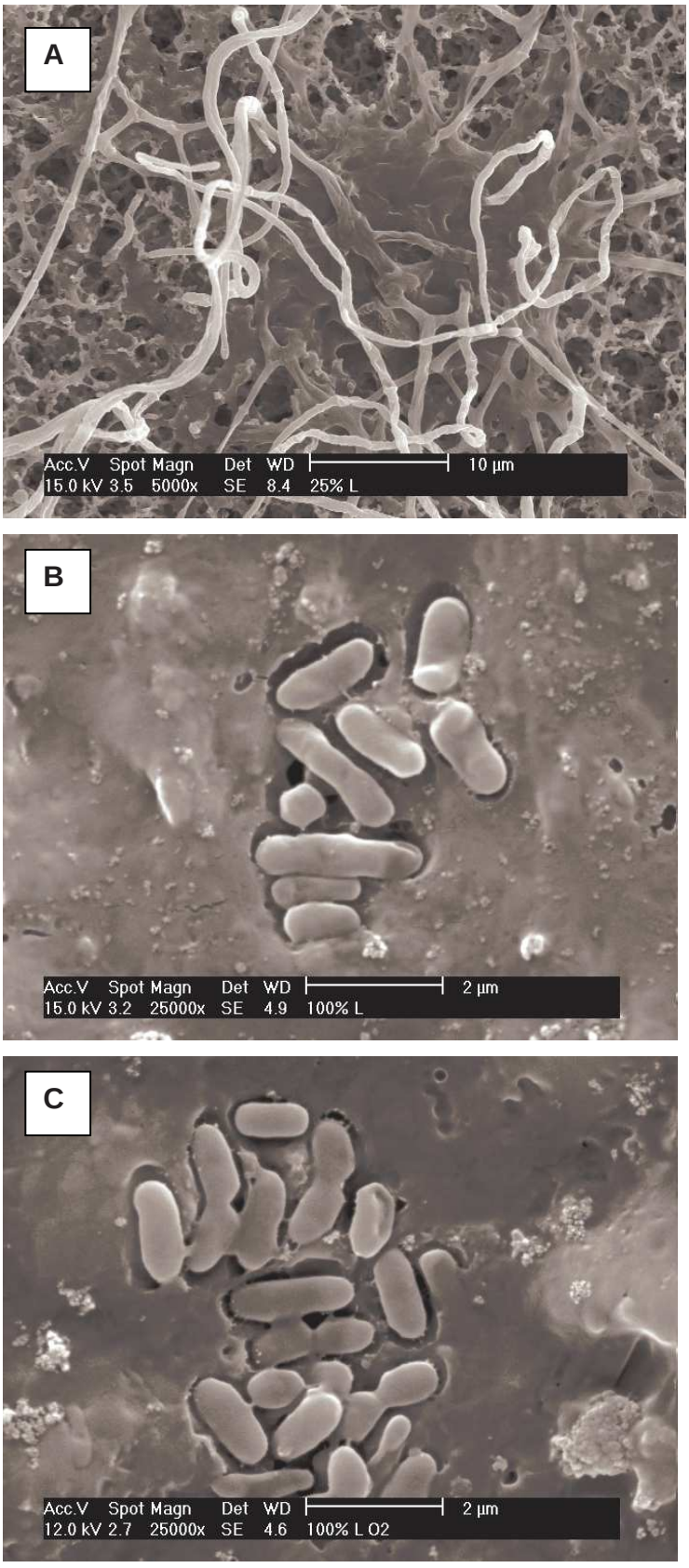


Figure 4.9: Scanning electron micrographs of microorganisms present after a ten day bioremediation period of different strength leachate from the Shongweni landfill site. Samples were filtered using cellulose acetate filters in a Büchner funnel and small pieces of each filter were mounted for viewing. **A:** 25 % leachate (x5000); **B:** 100 % leachate (x25000); **C:** 100 % pre-aerated leachate (x25000).

4.5.3.2 Effect of increased carbon:nitrogen ratio on the bioremediation of Shongweni landfill leachate

The COD of the leachate increased dramatically on addition of glucose. However, COD reduction was not significantly better than that observed in the unsupplemented leachate **(Section 4.5.3.1)**. The highest removal efficiency (13 %) was recorded in the flask containing leachate diluted to 25 %. In this experiment, COD reduction in the pre-aerated undiluted leachate (9 %) was greater than that in the undiluted leachate that was not pre-treated (3 %). The COD removal efficiencies (3 – 13 %) covered a similar range to those in the control experiment (5 – 11 %).

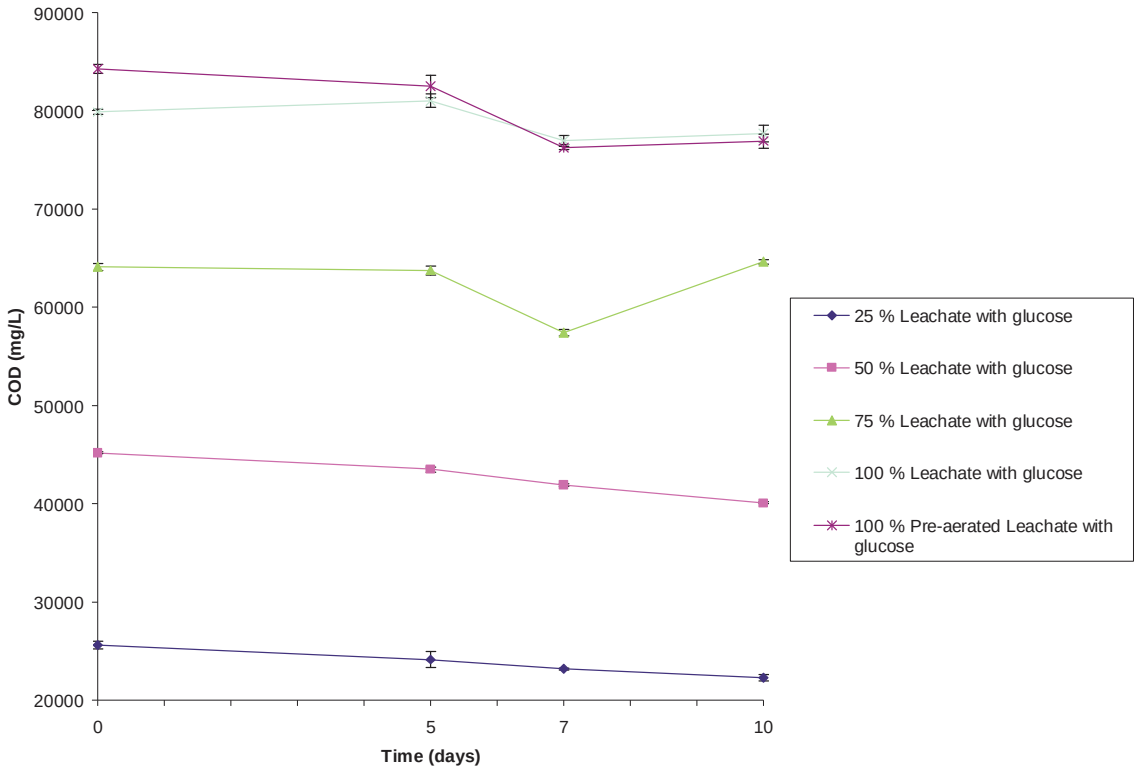


Figure 4.10 Changes in COD concentrations over ten days bioremediation of full strength and variously diluted Shongweni landfill leachate with adjusted C:N ratios. Error bars indicate standard deviation (n=3).

4.5.3.3 Effect of agitation on the bioremediation of Shongweni landfill leachate

Again, COD concentrations were not significantly reduced in this experiment **(Figure 4.11)**. The highest removal efficiency of 7 % was observed in the leachate subjected to agitation. Very low removal efficiencies were recorded in the other two flasks: 2 % for the leachate with no agitation, and 3 % for the leachate that was flushed with nitrogen gas.

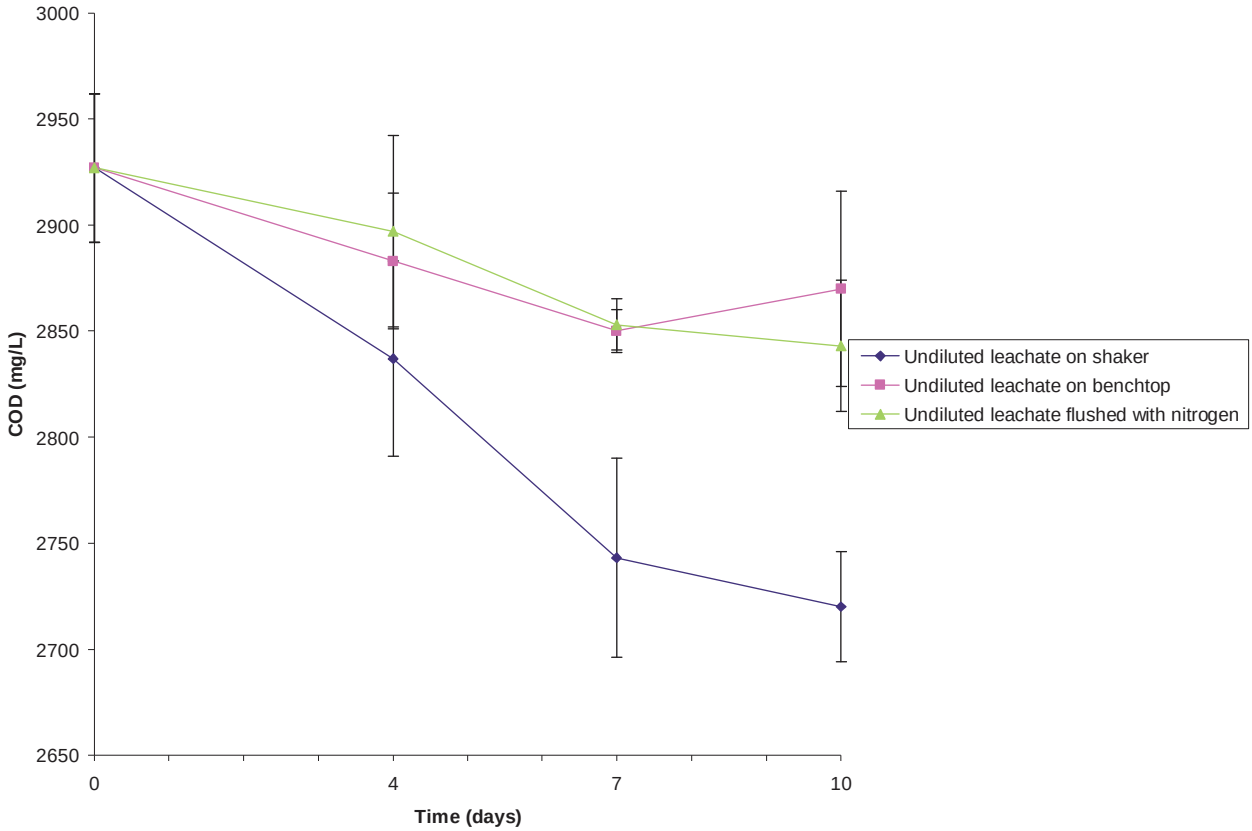


Figure 4.11 Effect of agitation on COD removal over ten days bioremediation of Shongweni landfill leachate. Error bars indicate standard deviation (n=3).

4.5.3.4 Effect of adding allochthonous inoculum in the form of activated sludge on the bioremediation of Shongweni landfill leachate

The addition of extraneous microorganisms did not improve bioremediation of this leachate. Similarly to the other experiments described above, little or no significant COD reduction was observed **(Figure 4.12)**. The COD of the activated sludge declined steadily, eventually decreasing by 33 %. A slightly better removal efficiency (15 %) was recorded in the leachate amended with 10 and 25 % inoculum when compared with the uninoculated leachate (6 %). However, the addition of activated sludge also increased the initial COD of the leachate. Adjusting the pH did not produce a positive effect and only 4 and 11 % of the total COD was removed in the flasks at pH 6.9 containing leachate with 10 and 25 % activated sludge respectively. Almost no change was recorded in the uninoculated leachate in which the pH had been adjusted to 6.9 **(Figure 4.12)**.

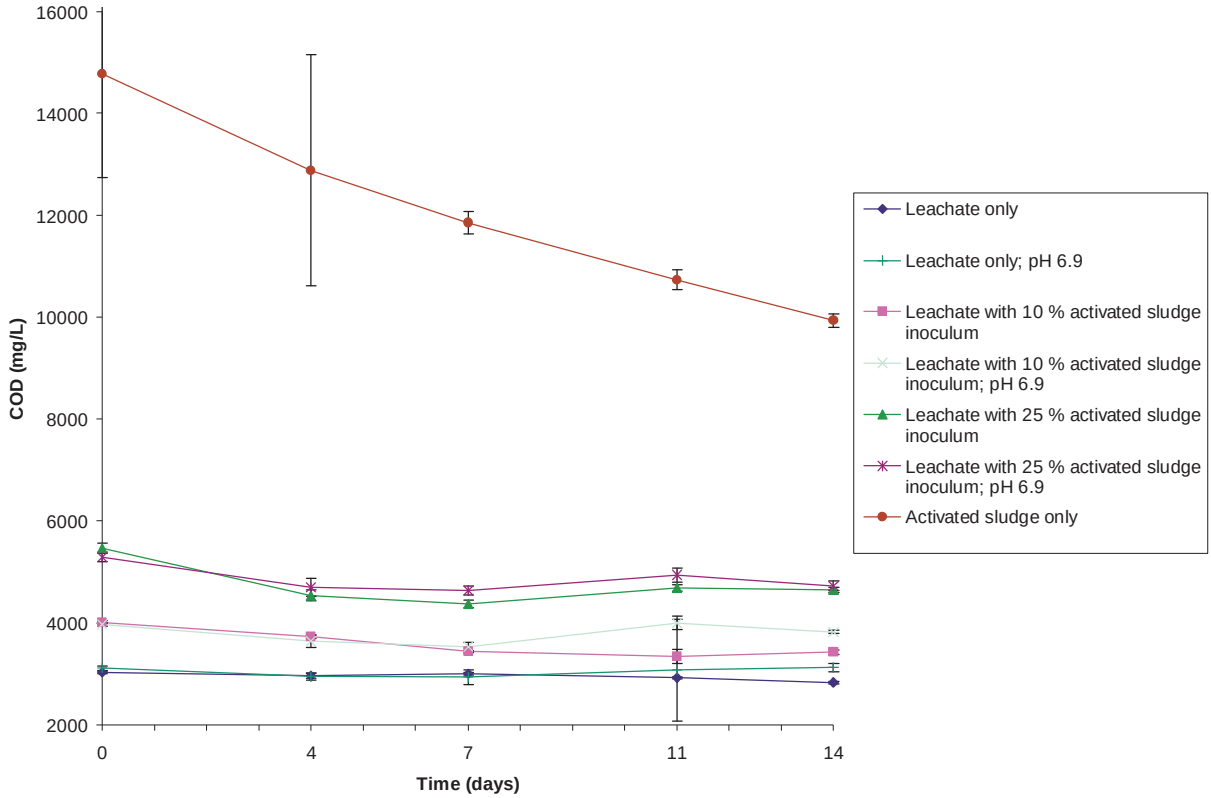


Figure 4.12 Changes in COD concentrations over 14 days bioremediation of Shongweni landfill leachate supplemented with activated sludge as an inoculum. Error bars indicate standard deviation (n=3).

4.5.4 Discussion

4.5.4.1 Effect of dilution on the bioremediation of Shongweni landfill leachate

Although Percival *et al*. (1997) reported that a high-strength landfill leachate required dilution to 25 % before it responded to aerobic biological treatment, dilution did not enhance biodegradation in the current instance. The indigenous microbial population in the Shongweni leachate was relied upon to reduce COD on the assumption that these microorganisms were probably adapted to the toxic or inhibitory compounds present. Dilution would have reduced not only the antagonising compounds, but also the amount of carbon available for metabolism. This could explain the lower removal efficiency observed in the flask containing only 25 % leachate.

Pre-aerating the leachate did not improve COD reduction at all, possibly because of ineffective air stripping of ammonia. This technique is strongly influenced by the pH of the waste stream, which must be above nine to facilitate the formation of ammonia gas (Britz, 1995). The batch of leachate used in this experiment had an initial pH of 9.19; however, the pH was not regulated at all during the air stripping procedure. Many other factors, including temperature, aeration rate and surface area, affect the efficacy of this process (Britz, 1995). To reiterate, the aim of this project was to develop a low-cost technology for treatment of landfill leachate and if pre-aeration with the prerequisite pH and temperature control was required in order to improve biodegradability of the Shongweni leachate, costs would increase considerably. This option was therefore not considered for further investigation.

4.5.4.2 Effect of increased carbon:nitrogen ratio on the bioremediation of Shongweni landfill leachate

The results showed that addition of easily metabolisable glucose to bolster carbon content did not improve the bioremediative capacity of the autochthonous microorganisms in the Shongweni leachate. Even though the BOD:COD ratio was significantly increased, the population was still unable to reduce the COD. Bae, Kim and Chang (1997) successfully used sucrose as a BOD source to facilitate COD reduction in the treatment of landfill leachate. However, this was done in the polishing stage of their process after the leachate had already been treated in activated sludge reactors and subjected to Fenton's treatment. COD was removed via coagulation and some recalcitrant compounds were also converted into more biodegradable forms during the chemical phase, thus allowing further COD removal which may otherwise have been impossible to achieve in the final biological phase. Cometabolism could also have been responsible for the additional COD reduction (Bae *et al*., 1997). In this study, no chemical phase was incorporated into the treatment process and, therefore, no transformation of recalcitrant compounds took place if they were not metabolised biologically. Compounds resistant to microbial attack would thus remain in the leachate, contributing to the COD concentration, and perhaps also inhibiting microbial growth. This may explain why increasing the BOD:COD ratio was ineffective, and why even the easily biodegradable additional carbon was not eliminated from the leachate.

It is possible that a co-substrate other than glucose was required in order to achieve transformation of the carbonaceous pollutants present in this leachate. However, it is not viable to find and provide co-substrates for all of the organics present in a heterogeneous waste stream such as landfill leachate. Therefore this was not investigated in this study. Also, it is unlikely that this would be cost-effective, even if suitable growth-supporting substrates were found.

Adjusting the C:N ratio to a biologically functional level is therefore not a viable approach to treating this particular leachate as the COD concentrations are dramatically increased without a corresponding increase in bioremediation efficiency. This could also indicate that the low BOD:COD ratio was perhaps not the only factor that limited biological treatment of this particular leachate.

4.5.4.3 Effect of agitation on the bioremediation of Shongweni landfill leachate

Although the COD removal efficiency obtained in the agitated leachate (7 %) was disappointing, it was higher than that obtained in either the unagitated (2 %) or nitrogen-flushed leachate (3 %). High-strength organic wastewaters often tend to be treated anaerobically, but Percival *et al*. (1997) found that high-strength landfill leachate with a low BOD:COD ratio was best treated aerobically. This was, therefore, the preferred option for the Shongweni leachate, which also has a low BOD:COD ratio.

Although agitation may have had some enhancing effect on the removal of carbon compounds from this waste stream, other factors were clearly limiting this process and therefore agitation, which caused mixing and a slight degree of aeration, did not significantly improve biodegradation.

4.5.4.4 Effect of adding allochthonous inoculum in the form of activated sludge on the bioremediation of Shongweni landfill leachate

Adding activated sludge as inoculum did improve COD removal efficiency somewhat, but the COD of the leachate was increased by this supplementation. This effect was not mitigated by the enhanced bioremediation so the COD concentration after 14 days was still higher than in the unsupplemented leachate. Previous experiments showed that it is unlikely that the higher percentage COD

removal observed was due to biodegradation of leachate constituents. The COD concentration of the activated sludge culture decreased consistently during the experiment, and it is probable that a similar effect caused the enhanced removal efficiencies observed in the inoculated leachate containing flasks. The introduced cells were therefore unable to metabolise the recalcitrant carbonaceous compounds in this leachate. The microorganisms in the activated sludge may also have been unable to adapt to conditions in the leachate, possibly because of the presence of a variety of inhibitory substances. Jun *et al*. (2004) cautioned that bio-augmentation may not always work because the survival and maintenance of the introduced microorganisms will only occur if they become acclimatised to the native microbial communities. The two microbial consortia present in the experiment described were possibly incompatible with one another and therefore did not produce the desired synergistic result. They may even have been antagonistic, impinging biodegradation by both populations.

Dignac *et al*. (2001) observed that carbohydrates in treated wastewater samples could originate from xenobiotic carbon compounds present in the untreated influent, as well as from refractory soluble microbial products that are produced by the microorganisms in activated sludge. This may account for the increased COD concentrations in the flasks containing this inoculum.

Adjusting the pH to 6.9 resulted in lower COD removal efficiencies than in the more basic original leachate (pH 8-9). This effect was most noticeable in the uninoculated acidified leachate in which no net reduction in COD occurred. Clearly, the natural microbial population is adapted to the high pH of the Shongweni leachate and the cells may have been stressed by the sudden change in environmental conditions. The negative effect of adjusting the pH became less as the percentage of activated sludge added increased. However, the alkaline pH of the leachate does not seem to be the cause of the minimal biodegradation observed when inoculum was added to the leachate.

Adding allochthonous microorganisms to treat Shongweni leachate was therefore not considered a worthwhile option.

4.6 CONCLUSION

The combined results of the various experiments suggest that the biological approach is not a viable option for the treatment of leachate from the Shongweni landfill site. Despite the presence of an indigenous microbial population, albeit a small one, no significant COD reduction was achieved. Techniques such as biostimulation and bioaugmentation were ineffective suggesting that some recalcitrant waste streams, including the Shongweni leachate, are probably better treated using one or a combination of the physico-chemical techniques discussed in **Chapter 1**. Although the treatment of this leachate using the upflow packed-bed bioreactor was largely unsuccessful, the experiments performed were still valuable since they illustrated that low-cost biotechnology of this type is not appropriate for treatment of all landfill leachates. They also showed that, although COD is the standard measurement used in wastewater treatment, BOD is a crucial aspect when biological processes are under consideration.

CHAPTER FIVE

A COMPARATIVE STUDY: TREATMENT OF LEACHATES FROM SELECTED URBAN LANDFILL SITES IN KWA-ZULU NATAL

5.1 SITE BACKGROUNDS AND CLASSIFICATIONS

Four urban landfill sites in the Durban-Pietermaritzburg area of KwaZulu-Natal were selected for exploratory treatment in the upflow packed-bed bioreactor. These sites were: New England Road (Pietermaritzburg), Marianhill (Pinetown, Outer Durban), Bisarsar Road (Durban) and Bul-Bul Drive (Chatsworth, South Durban). A brief description, including the classification of each site, is given in **Sections 5.1.1 – 5.1.4**.

5.1.1 New England Road Landfill

The New England Road landfill site **(Figure 5.1)** is a general, large, leachate-generating site (G:L:B+). Although the area has been used as a dump for the last 54 years, it was only permitted to operate as a municipal site in the early 1990s, and is expected to be in use for a further 8 – 10 years before closure. However, this period could be extended if waste minimisation initiatives are implemented[5].

This landfill accepts solid waste from most of the Pietermaritzburg area. It is used for all general waste collected by the municipality, as well as for all local

[5] Naidoo, C. 2008. Landfill site manager, uMgungundlovu District Municipality. Personal communication.

industries, including restaurants. However, no hazardous waste may be dumped[6].

The site is lined, and leachate is drained to a central sump. A pneumatic pressure pump is then used to transport the liquid to the nearby Darvill Waste Water Treatment Works. There is very little available space for a leachate treatment system on-site and proximity to a small stream also makes this difficult. The leachate collection system was recently re-engineered (October 2007), so data on leachate generation can only be given for the period since then and does not include many possible sources of variation; for example, seasonal rainfall changes. So far an average of 710 m^3 of leachate has been produced each month for the last five months[7].

Figure 5.1 The New England Road landfill site in Pietermaritzburg.

[6] Naidoo, C. 2008. Landfill site manager, uMgungundlovu District Municipality. Personal communication.
[7] Naidoo, C. 2008. Landfill site manager, uMgungundlovu District Municipality. Personal communication.

5.1.2 Marianhill Landfill

This landfill is also a G:L:B+ site that accepts only solid waste. Most of the waste accepted is general domestic waste, but there is also some rubble, garden refuse and commercial/industrial waste materials (polystyrene, for example). In addition, mixed loads, condemned foods, whole tyres, light waste, sanitary waste and perishable foods may occasionally be dumped. Over the period 1997 to 1999 an average of 100 764 tonnes were deposited at this site annually (Durban Metro online, 1999). This has increased to the 700 tonnes of waste per day that is currently accepted. Marianhill landfill was licensed in 1996 to start operation in 1997, and will probably be viable for another five to ten years[8].

The site is lined with a leachate collection system and a combined biological-chemical treatment is operational. However, the leachate used in this research had not been through this process and was therefore a raw leachate. Approximately 1200 m^3 of leachate are generated on a monthly basis, and the treatment plant is well within its capacity as it is able to treat 50 m^3 per day[9].

5.1.3 Bisarsar Road Landfill

Although the Bisarsar Road landfill is also classified as a G:L:B+ site, it is run on a much larger scale than the Marianhill site currently accepting about 5000 tonnes of waste every day. The composition of the waste is very similar to that dumped at the Marianhill landfill and consists mainly of municipal domestic refuse, with some garden refuse, builders' rubble and waste from businesses in the area[10]. Other components of the waste mass include condemned foods, sand, asphalt, polystyrene, sanitary wastes and perishable foods with the

[8] Govender, K. 2008. Landfill site manager, EnviroServ (Pty) Ltd. Personal communication.
[9] Govender, K. 2008. Landfill site manager, EnviroServ (Pty) Ltd. Personal communication.
[10] Moodley, L. 2008. Operations manager, Durban Solid Waste, EThekwini Municipality. Personal communication.

occasional load of delisted hazardous waste (Durban Metro online, 1999). The site has been used as a dump since the 1980's, but was officially recognised as an authentic landfill only in the early 1990's, which is when it was lined and started operating in accordance with regulations. At current waste deposition rates, the site could remain viable for another four or five years. However, if the amount of waste that is landfilled can be reduced, this time span could be increased[11].

There is no leachate treatment system at Bisarsar Road because of the site's close proximity to a wastewater treatment works. It is thus more economical to discharge the leachate to sewer and pay for the additional COD load, than to construct a specialised plant at this stage. The site generates at least 6000 m^3 of leachate monthly[12].

5.1.4 Bul-Bul Drive Landfill

This landfill is permitted to accept waste with a hazard rating of three or four; it is thus a H:h site. It accepts both solid and liquid waste including general waste, domestic and garden refuse, bark, ash, sludge, industrial wastes and hazardous liquids[13]. The site opened in 1990 and is expected to remain operational until 2012[14].

Although there is no leachate treatment plant on-site, the landfill is lined and has a leachate collection system consisting of two tanks. Raw leachate collects in the first tank, which feeds into the second tank allowing for the separation of oils and

[11] Moodley, L. 2008. Operations manager, Durban Solid Waste, EThekwini Municipality. Personal communication.
[12] Moodley, L. 2008. Operations manager, Durban Solid Waste, EThekwini Municipality. Personal communication.
[13] Chetty, N. 2008. Operations manager, Waste Services (Pty) Ltd. Personal communication.
[14] Gerber, R. 2008. Landfill site manager, Waste Services (Pty) Ltd. Personal communication.

grease. Leachate from the second tank is discharged to sewer. An average of about 4300 m^3 of leachate is produced every month[15].

5.2 LEACHATE CHARACTERISTICS

Leachate samples from each of the four landfills were analysed for both COD and BOD_5 in order to determine the BOD_5:COD ratio and therefore assess the suitability of a biological approach for treatment of these waste streams. It should be noted that these values represent 'snapshots' of the leachate from each site, and are not necessarily 'typical' if leachate variation over time, and with season, is considered.

5.2.1 New England Road Landfill

This leachate contained very low COD concentrations (212 $mg.l^{-1}$) and was therefore not considered for further treatment. Leachate produced by this landfill needs no specialised treatment and can simply be disposed to sewer.

5.2.2 Marianhill Landfill

Leachate from the Marianhill landfill site had a fairly low COD of 1951 $mg.l^{-1}$. However, the BOD was 931 $mg.l^{-1}$, which represents only about 48 % of the COD value.

This COD concentration correlates fairly well with the typical value (between 2000 and 3000 $mg.l^{-1}$) obtained when leachate monitoring is carried out at the site. The pH of the raw leachate is generally below five, but increases to neutral

[15] Chetty, N. 2008. Operations manager, Waste Services (Pty) Ltd. Personal communication.

after it has been through the on-site treatment system[16]. However, the raw untreated leachate was used in this study.

5.2.3 Bisarsar Road Landfill

The initial sample of leachate from this site had a relatively high COD of 4342 mg.l^{-1} and a BOD of 2566 mg.l^{-1}, which means that theoretically it should be possible to biologically degrade 59 % of the carbonaceous compounds present.

According to the landfill site manager[17], the COD concentrations are usually much lower than this being similar to those of the leachate produced at the Marianhill landfill (2000 – 3000 mg.l^{-1}). The leachate generated at this site is also acidic, usually below pH five.

5.2.4 Bul-Bul Drive Landfill

The COD concentration (3790 mg.l^{-1}) of this leachate was slightly lower than that of the Bisarsar Road leachate. Approximately 74 % of this appeared to consist of biodegradable compounds as the sample had a BOD of 2790 mg.l^{-1}.

Routine leachate monitoring data show that the COD concentrations of the Bul-Bul leachate vary widely from approximately 3000 mg.l^{-1} to over 6000 mg.l^{-1}, but the value recorded above correlates well with the concentrations measured on site during the time when these samples were taken. The BOD is also variable; however it always seems to constitute a fairly large proportion of the

[16] Moodley, L. 2008. Operations manager, Durban Solid Waste, EThekwini Municipality. Personal communication.
[17] Moodley, L. 2008. Operations manager, Durban Solid Waste, EThekwini Municipality. Personal communication.

corresponding COD concentration. The pH of the leachate generated at this site is slightly alkaline, typically between seven and eight[18].

5.3 TREATMENT OF THREE DIFFERENT LEACHATES IN THE UPFLOW PACKED-BED (PINE BARK) BIOREACTOR OPERATED AS A BATCH SYSTEM

5.3.1 Introduction

Although the initial focus of this study was specifically the treatment of the Umlazi landfill leachate, this later became impossible as the site was undergoing closure and the volumes of leachate available became insufficient to operate the bioreactor properly. Leachate generated at the site was no longer pumped into an open dam, but stored in underground sumps from which it was impracticable to extract large volumes. It thus became necessary to use leachates from other landfills to continue the research project. The leachate from the Shongweni landfill site was chosen as the replacement because it accepts the same types of wastes as the Umlazi site had done, although it has not been in operation for as long. The latter is mentioned because landfill age is known to influence leachate quality (see Literature Review; **Chapter 1**). Also it produces huge quantities of leachate, which is stored in large reservoirs on site and is relatively easy to collect **(Section 4.1)**. However, biological treatment of this leachate in the upflow packed-bed bioreactor was unsuccessful, as discussed in **Chapter 4**, indicating that not all leachates are suited to biological treatment. For this reason, a number of other leachates with different characteristics were treated in the upflow packed-bed bioreactor to determine the range of its application and its technological sustainability.

[18] Chetty, N. 2008. Operations manager, Waste Services (Pty) Ltd

The diversity of factors that contribute to leachate quality and, therefore, to its suitability for biological treatment (see **Chapter 1**), mean that different leachates could respond very differently to the technology under investigation. The objective of this experiment was to determine whether the Marianhill, Bisarsar and Bul-Bul landfill leachates were biologically treatable using the upflow packed-bed bioreactor under evaluation.

5.3.2 Experimental design

The box in each chamber of the tank bioreactor was filled to two centimetres from the top with 16-24 mm pine bark chips for this experiment. Three different leachates were treated in the system, each in duplicate. Two chambers and their corresponding reservoirs were filled with leachate from the Marianhill landfill site, two with leachate from the Bisarsar Road landfill site and two with leachate from the Bul-Bul landfill site. Each chamber was aerated using the fish tank pump described in **Section 2.1.2**. The reactor was operated in batch mode.

No extraneous carbon sources were used, but activated sludge from the Hammarsdale Sewage Works **(Section 2.3)** was added at a ratio of 1:10 (v/v) to the leachate which had already been placed in each chamber. However, as the system was being evaluated for use in developing countries where large quantities of leachate require treatment with minimum cost, no acclimatisation was performed. The activated sludge was therefore immediately exposed to full-strength leachate. No temperature or pH control was implemented during the 27 day experimental period. Samples (100 m*l*) were collected from the outflow pipe connected to each chamber twice per week for COD, ammonia and nitrate analyses **(Sections 2.4.1, 2.4.4 and 2.4.5)**.

5.3.3 Results

All three leachates were treated successfully in the bioreactor; however, COD removal differed significantly **(Figure 5.2; Table 5.1)**.

The COD reduction achieved in the chambers containing the Marianhill leachate was relatively low **(Table 5.1)**. Both chambers followed a very similar trend with removal taking place largely over the first three days, with a marked reduction in rate thereafter. Maximum removal was recorded after 10 to 13 days (34 and 26 % in chambers 1 and 2 respectively), after which the COD concentrations increased slightly.

The highest reduction in COD concentrations was observed in the chambers containing the Bul-Bul leachate with total removal of 46 and 51 % in chambers 3 and 4 respectively (**Table 5.1)**. In this case, COD concentrations continued to decline throughout the experiment with a relatively small decrease in removal efficiency over time. Again, the two chambers exhibited consistent results except for the difference in initial concentrations, which were 5370 and 5837 $mg.l^{-1}$ respectively.

The initial COD values of the Bisarsar leachate in the two chambers containing this waste stream differed significantly, viz. 3800 and 4800 $mg.l^{-1}$. This led to a notable difference in total removal efficiency (27 and 42 % in chambers 5 and 6 respectively); however, the final COD concentration was very similar in both chambers **(Table 5.1)**. A gradual decrease in removal efficiency occurred over the duration of the experiment, with the highest removal occurring in the first three days after which the cumulative reduction rates slowed appreciably.

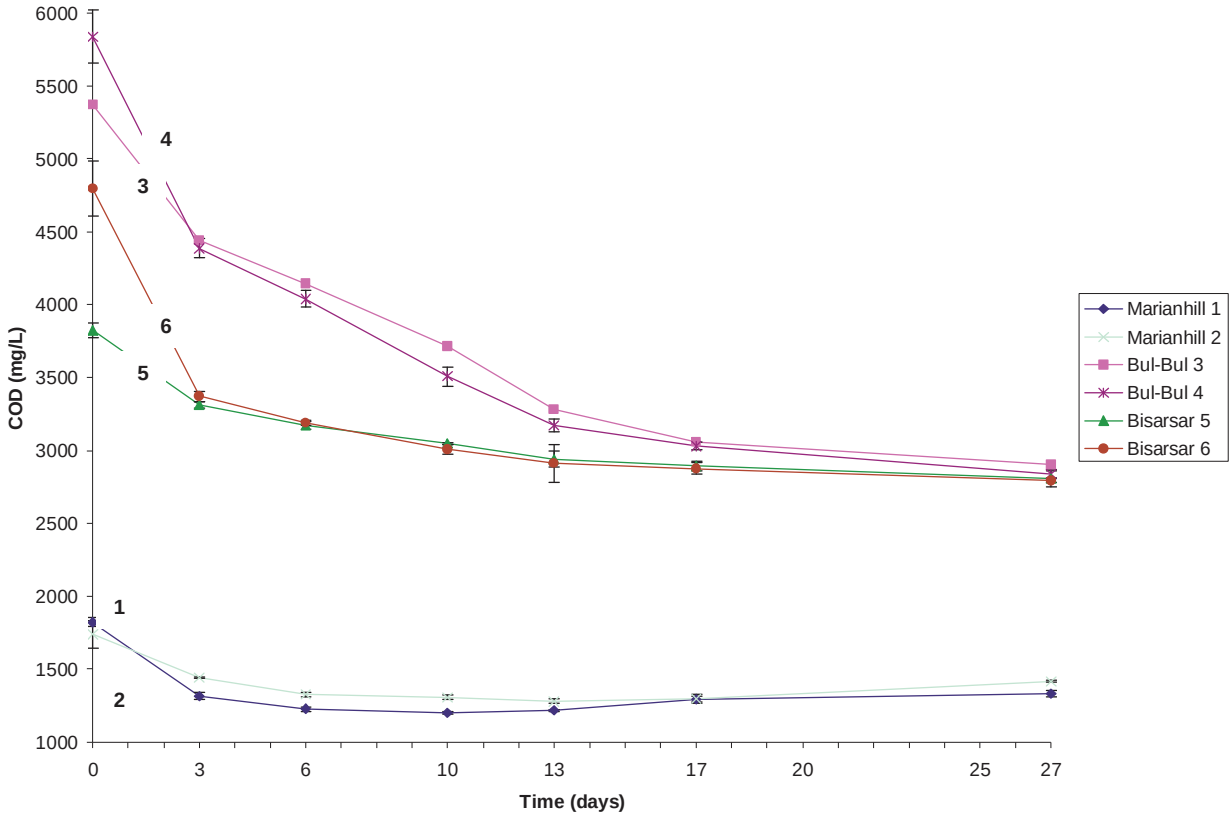


Figure 5.2 Reduction in COD concentrations of the Marianhill, Bul-Bul drive and Bisarsar Road leachates over 27 days treatment in the upflow packed-bed bioreactor operated as a batch system. Error bars indicate standard deviation (n=3).

TABLE 5.1 Total and cumulative COD reduction of the Marianhill, Bul-Bul Drive and Bisarsar Road landfill leachates over 27 days treatment in the upflow packed-bed bioreactor operating as a batch system

Time (days)	**COD reduction expressed as a percentage with standard deviation**																	
	Marianhill						**Bul-Bul**						**Bisarsar**					
	Total Chambers			**Cumulative Chambers**			**Total Chambers**			**Cumulative Chambers**			**Total Chambers**			**Cumulative Chambers**		
	1	**2**	**Avg**	**1**	**2**	**Avg**	**3**	**4**	**Avg**	**3**	**4**	**Avg**	**5**	**6**	**Avg**	**5**	**6**	**Avg**
3	28 (±1.52)	17 (±3.93)	**22.5**	n/a	n/a	**n/a**	17 (±1.19)	25 (±2.27)	**21**	n/a	n/a	**n/a**	13 (±1.07)	30 (±2.42)	**21.5**	n/a	n/a	**n/a**
6	33 (±1.22)	24 (±3.68)	**28.5**	7	8	**7.5**	23 (±1.03)	31 (±2.05)	**27**	4	8	**6**	17 (±1.10)	34 (±2.24)	**25.5**	4	5	**4.5**
10	34 (±1.07)	25 (±3.63)	**29.5**	2	2	**2**	31 (±0.97)	40 (±1.92)	**35.5**	10	13	**11.5**	20 (±0.89)	37 (±2.15)	**28.5**	4	6	**5**
13	33 (±1.01)	26 (±3.58)	**29.5**	-1	2	**0.5**	39 (±1.19)	46 (±1.63)	**42.5**	12	10	**11**	23 (±1.52)	39 (±3.10)	**31**	4	3	**3.5**
17	29 (±1.43)	25 (±3.87)	**27**	-6	-1	**-3.5**	43 (±0.93)	48 (±1.46)	**45.5**	7	4	**5.5**	24 (±1.09)	40 (±2.12)	**32**	2	1	**1.5**
27	27 (±1.45)	19 (±3.86)	**23**	-3	-9	**-6**	46 (±1.61)	51 (±1.39)	**48.5**	5	6	**5.5**	27 (±1.49)	42 (±1.94)	**34.5**	3	3	**3**

Ammonia concentrations were erratic over the course of the experiment **(Figure 5.3)**. It was difficult to identify any consistent trends, but the initial levels in the Bisarsar leachate were much higher than those in either of the others. Indeed, the only significant decrease in ammonia was observed in the chambers containing this particular leachate, viz. chambers 5 and 6 with 80 and 88 % reduction respectively. Ammonia concentrations increased slightly overall in chamber 1 treating the Marianhill leachate, and significantly in both chamber 2 also treating leachate from Marianhill and chamber 4, which contained the Bul-Bul leachate.

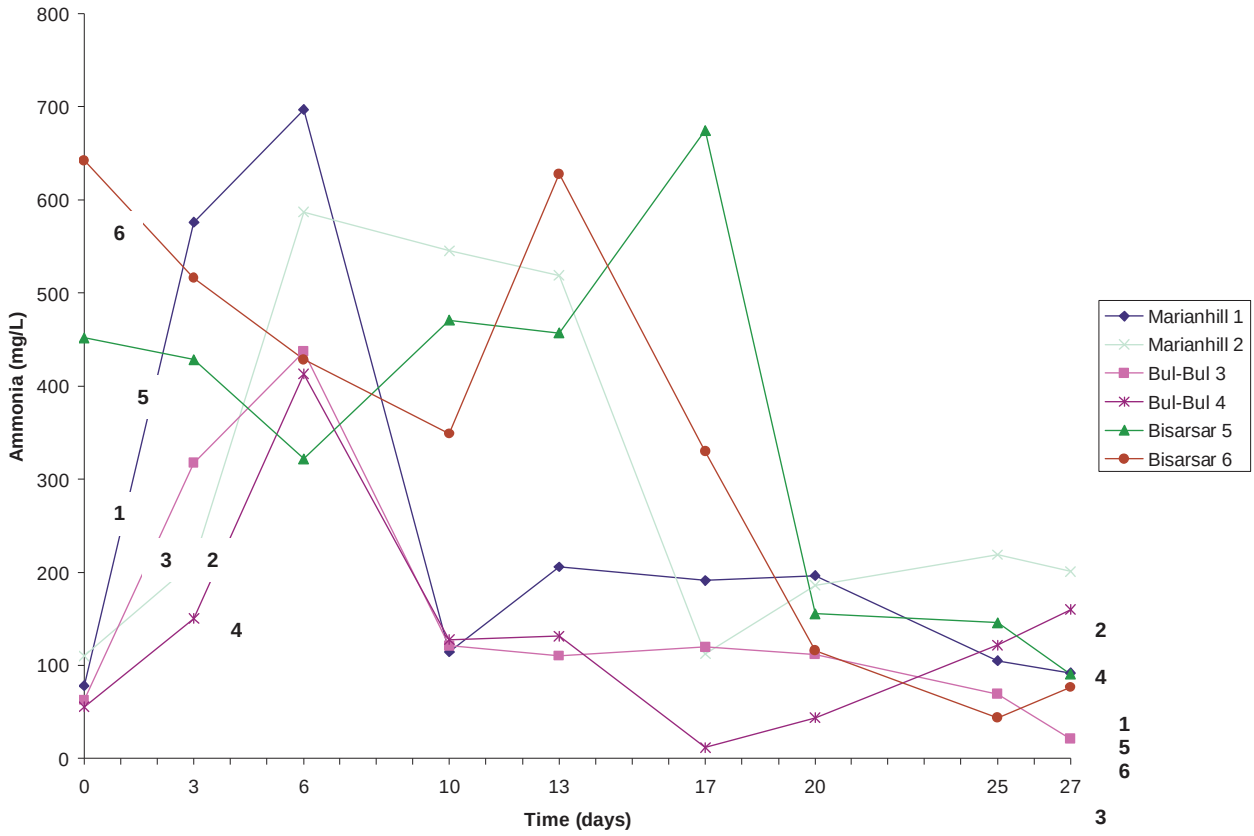


Figure 5.3 Changes in ammonia concentrations in the Marianhill, Bul-Bul Drive and Bisarsar Road landfill leachates over 27 days treatment in the upflow packed-bed bioreactor operating as a batch system.

Nitrate concentrations in the Bul-Bul leachate showed little change, remaining very low throughout the experimental period. In contrast, both the Marianhill and Bisarsar Road leachates exhibited a large increase in nitrate levels over the 27 days. The level in the Marianhill leachate began to rise gradually after approximately 10 days, ultimately reaching about 300 mg.l^{-1}. A much steeper increase in nitrate concentration was observed in the chambers containing the Bisarsar Road leachate; levels only began to rise after 20 days, but reached 650 – 850 mg.l^{-1} at final sampling **(Figure 5.4)**.

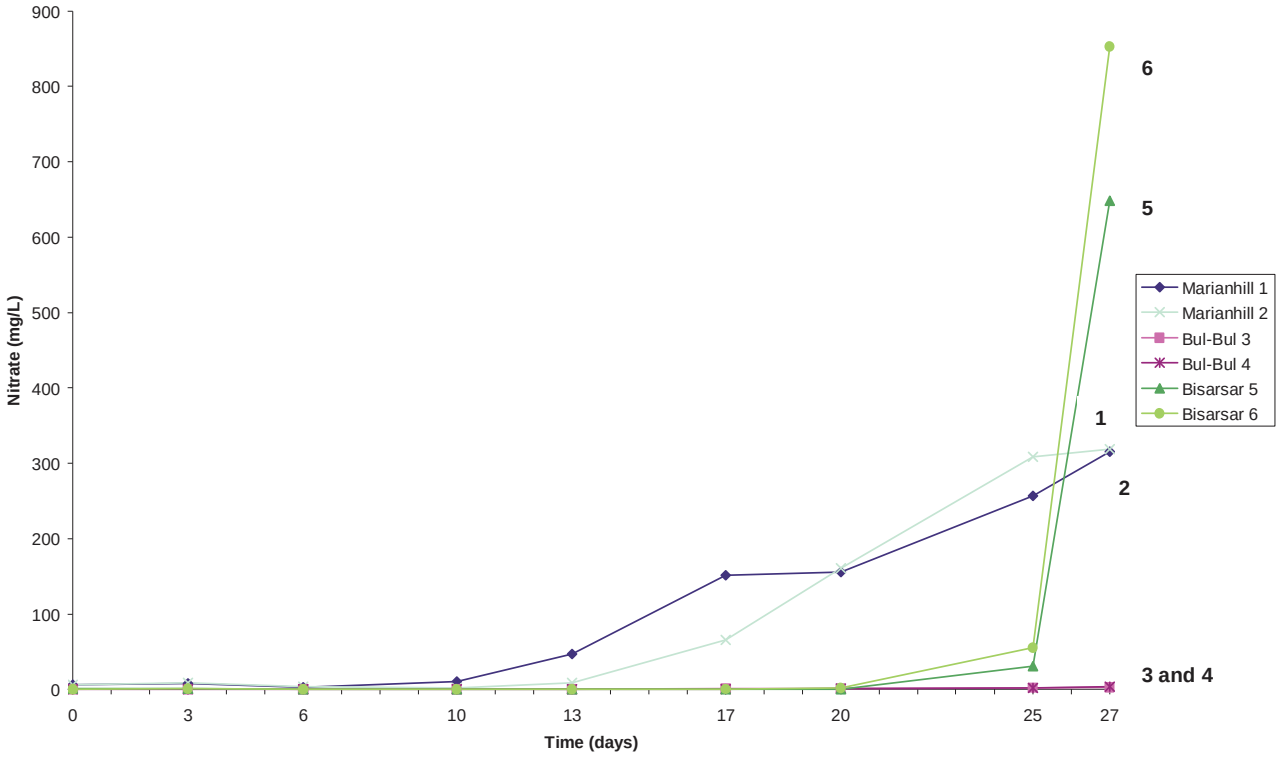


Figure 5.4 Changes in nitrate concentrations in the Marianhill, Bul-Bul Drive and Bisarsar Road landfill leachates over 27 days treatment in the upflow packed-bed bioreactor operating as a batch system.

5.3.4 Discussion

The bioremedial COD removal efficiencies achieved here were much better than those obtained with the Shongweni landfill leachate. Most of the results were similar to those obtained with the Umlazi leachate. The Marianhill leachate had a relatively low COD concentration (1951 mg.l^{-1}) as well as a relatively low proportion of biodegradable organics (0.47), and, consequently, COD removal efficiency was poor. However, the COD reduction recorded for the Bul-Bul leachate (49 %) was much greater than for any of the other leachates used in this study, which is consistent with its relatively high BOD:COD ratio. Wiszniowski, Surmacz-Górska, Robert and Weber (2007) examined the effect of landfill leachate composition on the removal of both organics (as COD) and nitrogen in a laboratory-scale activated sludge system. In their experiments, leachate from the same landfill was used throughout, but influent COD and ammonia concentrations changed markedly over time. COD removal efficiency varied widely, peaking at 85 % when the readily biodegradable organic fraction was high, reflected in a BOD_5:COD ratio of 0.6, and dropping to an average removal of 38 and 37 % when the BOD_5:COD ratios were 0.5 and 0.3 respectively. Higher average COD reduction was expected at the 0.5 level, but fluctuations in percentage removal suggested that the microorganisms in the system were not completely acclimatised to the changing composition of the influent leachate. The lowest initial BOD_5:COD ratio (0.3) corresponded with the lowest initial COD concentration (1020 mg.l^{-1}) and the smallest COD reduction (Wiszniowski *et al*., 2007). Other aspects of leachate composition, such as the presence of inhibitory or xenobiotic compounds, may also have affected percentage COD removal in the Marianhill leachate.

In none of the leachates did the percentage COD removed reach the anticipated levels based on their BOD:COD ratios **(Section 5.2)**. Biodegradation was still

occurring in the Bisarsar and Bul-Bul leachates at the end of the experimental period, although cumulative COD reduction rates had declined, so it may simply be that more time was required for all the biologically removable carbon to be metabolised.

The autochthonous microbial population would very likely have been quite different in each of the three leachates used, and would thus have responded differently to the environmental conditions in the bioreactor as well as to the introduction of the allochthonous microorganisms from the activated sludge. Roth and Lemmer (1994) sampled biofilms from sewers discharging either domestic or trade wastewater. Both biofilms contained high population densities of bacteria belonging to several metabolic groups including heterotrophs, proteolytes, amylolytes, lipolytes, ammonifiers, nitrate reducers and anaerobes. Most of these counts were in the same or in a higher order of magnitude than those found in high load activated sludges. In addition, enzyme activity in the biofilm from trade wastewater, which contained high concentrations of chromium and nickel, was higher than that in the biofilm from domestic sewage (Roth and Lemmer, 1994). Ludvigsen *et al*. (1999) noted that the viable biomass decreased and biofilm community composition shifted to less diverse populations in a leachate contaminated aquifer as distance from the source increased and concentrations of both inorganic and organic contaminants declined. Another study showed that unique microbial communities characterised each waste layer at a landfill site. The microorganisms present depended on solid waste composition, moisture content, temperature and pressure at different depths. The microbial population of the leachate produced showed great diversity and reflected the communities identified in various waste layers (Sawamura, Yamada, Endo, Soda, Ishigaki and Ike, 2010).

The microbial heterogeneity that occurs within a single landfill site is likely to be emphasised across populations from sites at different locations, especially if the waste composition differs. This would also affect the microorganisms found in

leachates from these sites. Marianhill and Bisarsar Road landfills accept only general waste, while Bul-Bul Drive is also permitted to accept hazardous waste. The indigenous microbial population in leachates from the former may have been metabolically less diverse, resulting in the poor COD reduction observed in the Marianhill leachate. The greater diversity of materials landfilled at Bul-Bul Drive could have facilitated the development of a more resilient, adaptable and metabolically active biofilm within the chambers containing this leachate.

The survival and growth of the microbial community introduced via the added activated sludge also needs to be considered, especially when the lack of an acclimatisation period is taken into account. Li and Zhao (1999) showed that high levels of ammoniacal nitrogen could significantly reduce the microbial functioning of activated sludge, indicated by a decline in COD removal efficiency, dehydrogenase activity and specific oxygen uptake rate. Thus high ammonia levels not only inhibit biological nitrification, but also biological decarbonation. These authors recommended that high ammonia concentrations in leachate be lowered to levels less than 100 mg.l^{-1} by pre-treatments such as air stripping or chemical precipitation in order to improve the biological treatment process. The Bisarsar Road leachate had an initial ammonia concentration well above 100 mg.l^{-1} and this may have affected the activated sludge community, contributing to the mediocre COD removal efficiency observed. Although the initial ammonia concentrations of the other two leachates were close to 100 mg.l^{-1}, concentrations did increase dramatically to several times this amount during the course of the experiment. This could have influenced the efficacy of the allochthonous microorganisms introduced via the activated sludge. However, it must be remembered that all activated sludge communities are different having adapted to the presence of different compounds dependant on the type of waste stream being treated. The activated sludge used in this study as an additive may have been able to tolerate higher concentrations of ammonia than the community used by Li and Zhao (1999).

Chappell and Evangelou (2002) proposed that the nitrifying activity of any attached microbial community will be directly linked to the surface formed by the EPS layer of the biofilm and its potential to adsorb ammonia from solution. Other factors, such as the presence of metal cations (especially those of higher oxidation states) and low pH levels, may suppress nitrification rates. On the other hand oxyanions, such as phosphate, enhance nitrification. Biofilm thickness also affects ammonia removal; in general, the thicker the biofilm, the greater the rate of removal up to a certain maximum point where some of the cells become inactive (Chappell and Evangelou, 2002). Since different leachates supporting different indigenous microbial populations were used in the current experiment, the biofilms generated in each chamber would have varied. This could therefore have impacted the nitrifying action of the microbial community because the surface properties of the biofilm influence substrate oxidation (Chappell and Evangelou, 2002).

The Marianhill landfill is the youngest of the three sites and should thus be producing the most biodegradable leachate; however, as noted in **Section 5.2**, this was not the case. The oldest landfill site (Bul-Bul Drive) in fact generated the leachate with the highest BOD_5:COD ratio. At this site the leachate is stored in large tanks for significant periods of time, and this accounts for the higher pH of the liquid. It could also mean that the oxidation-reduction potential was more positive in this waste stream (Tatsi and Zouboulis, 2002; **Section 5.3.1**), thus encouraging the growth of a more heterogeneous microbial community that could take better advantage of the varied aerobic-anoxic environment within the bioreactor. Therefore the age of the landfill did not seem to be a major factor influencing bioremediation potential in this case.

According to Wiszniowski *et al*. (2007), the organic matter in landfill leachate can be used as a carbon source for denitrification via nitrate, provided that the BOD_5:N ratio is at least 4:1. As this requirement was met for all three leachates used in this experiment, i.e. the BOD_5:N ratios were 10, 47 and 5 for the

Marianhill, Bul-Bul and Bisarsar Road leachates respectively, high levels of nitrogen removal should have occurred. A fairly good average percentage removal (84 %) was obtained in the chambers treating the Bisarsar Road leachate and, in these cases, the decrease in ammonia concentrations could be correlated with an increase in nitrate concentrations indicating that nitrification had taken place. Despite the satisfactory BOD_5:N ratios, ammonia removal was not consistent in the chambers containing the Marianhill and Bul-Bul leachates, although the rise in nitrate levels in the Marianhill leachate does correspond to declining ammonia concentrations towards the end of the experimental period. Despite the greater final ammonia removal efficiencies obtained in earlier experiments using the Umlazi landfill leachate, large fluctuations in ammonia levels occurred **(Section 3.3.3; Figure 3.5)**. However, both experiments conducted on the Umlazi leachate were of longer duration than the experiment currently under discussion. It is possible that more time was required to eliminate the nitrogen in the Marianhill and Bul-Bul Drive leachates. As noted in **Section 3.3.4**, Martienssen and Schöps (1997) have reported that very high C:N ratios, such as those characteristic of the Bul-Bul Drive leachate (averaging 95:1), can cause unstable conditions for both nitrification and denitrification. It is also possible that as the amount of organics in the leachate decreases over time and biodegradability of the remaining compounds is reduced, an external metabolisable carbon source would be required in order to facilitate complete nitrogen elimination (Wiszniowski *et al*., 2007). This may have occurred in the case of the Marianhill leachate, which had the lowest initial COD concentration and BOD_5:COD ratio.

Marttinen *et al*. (2003) noted the influence of storage on the composition of landfill leachates. They found lower concentrations of pollutants in leachate that had been stored in large reservoirs on the studied site, than in freshly produced leachate. This was attributed to sedimentation of hydrophobic contaminants that sorbed to particulate matter; the possibility that some biodegradation occurred in the storage tanks was also considered. However, variation in pollutant

concentrations could also be due to seasonal effects because leachate was retained in the reservoirs for approximately three months (Marttinen *et al*., 2003). Tatsi and Zouboulis (2002) recorded a similar trend when comparing 'fresh' leachate with 'old' leachate taken from a leachate pond where it had been for some considerable time and was thus considered partially stabilised. Both COD and BOD values, as well as many other contaminant concentrations (both organic and inorganic) were much higher in the 'fresh' samples. The oxidation-reduction potential was negative in the recently produced leachate and positive in the leachate collected from the pond; again reflecting a degree of stabilisation. An increase in pH was also observed, which may indicate the anaerobic consumption of volatile fatty acids. Although organic pollutant load decreased with leachate age, the ammoniacal nitrogen levels remained high, so that this compound was the major contaminant in the 'old' leachate. However, nitrate concentrations became relatively low. According to these authors, the low phosphorus content in the stored leachate could also be influential for subsequent biological treatment as this nutrient is an important limiting factor in many biotechnological systems.

These concerns are relevant to the current research, because the length of time that leachate was stored before collection differed: both the Marianhill and Bisarsar Road leachates were collected as 'freshly' produced samples, while leachate from Bul-Bul Drive had been stored in large reservoirs for a period of several weeks **(Section 5.1.4)**. Changes in oxidation-reduction potential and pollutant concentrations during storage may have made the Bul-Bul Drive leachate more amenable than the other leachates for biological treatment.

5.4 CONCLUSION

The upflow packed-bed bioreactor with pine bark as a support for biofilm development was suitable for treating different leachates from various urban landfill sites in the Durban-Pietermaritzburg area. However, the Bul-Bul leachate

was more successfully bioremediated, in terms of COD removal efficiency, than the Marianhill and Bisarsar Road leachates, and it thus became the focus for further evaluation of the system **(Chapter 6)**. The removal of nitrogen was much less convincing than that achieved previously with the Umlazi leachate, but as the emphasis of this research was on the removal of organic carbon so the Bul-Bul leachate was selected in preference to that from the Bisarsar Road and Marianhill sites. The bioreactor under investigation seemed better suited for the treatment of more alkaline leachates with a relatively high BOD_5:COD ratio.

CHAPTER 6

TREATMENT OF LEACHATE FROM THE BUL-BUL DRIVE LANDFILL SITE

6.1 SITE BACKGROUND AND CLASSIFICATION

The site background and classification have already been mentioned in **Section 5.1.4**. The Bul-Bul Drive site is an H:h landfill, and can therefore accept some low hazardous waste material.

6.2 LEACHATE CHARACTERISTICS

The characteristics of the Bul-Bul leachate were given briefly in **Section 5.2.4**. It usually has a relatively high BOD:COD ratio with variable COD concentrations depending on the season and type of waste dumped at the site. The pH of the leachate is slightly alkaline[19]. As noted in **Section 5.4**, this leachate was selected for further investigation because of the promising results obtained in the experiment described in **Chapter 5**. Some aspects of the upflow packed-bed bioreactor still required investigation and optimisation, and the performance of the system as a continuous treatment method needed to be evaluated. The Bul-Bul Drive landfill leachate was therefore used for all remaining experiments.

[19] Chetty, N. 2008. Operations manager, Waste Services (Pty) Ltd. Personal communication.

6.3 PERFORMANCE OF DIFFERENT BIOFILM SUPPORT MATRICES IN THE UPFLOW PACKED-BED BUCKET BIOREACTORS

6.3.1 Introduction

Despite the relatively poor results obtained with pine bark as support material in the experiment comparing different matrices for the bioremediation of leachate from the Shongweni landfill site, it continued to be used for comparison with plastic bioballs in the treatment of the Bul-Bul Drive leachate **(Section 6.3.2.2)** for three reasons. Firstly, the ceramic noodles had been eliminated due to their instability in the bioreactors. Secondly, large quantities of the ceramic matrix were required to fill each reactor, and since this material was the most expensive of those tested, the system would become compromised in terms of cost-effectiveness. Additionally, pine bark chips had been used in all previous experiments because of their viability as a low-cost solid biofilm support matrix. It was considered important to maintain continuity within the research programme as a whole, and to determine the extent to which biodegradation is negatively affected by the use of this cheaper, organic matrix as opposed to a more conventional, but relatively expensive inorganic matrix such as the plastic bioballs.

The objective of this experiment was to determine whether inorganic plastic bioballs would perform significantly better than pine bark chips when used as a solid biofilm support matrix in this upflow packed-bed bioremediation system.

6.3.2 Experimental Design

The bucket bioreactors **(Section 2.1.3)** were used for this experiment, and two packing materials were tested: the 16 – 24 mm pine bark chips **(Figure 4.3 A)**

and the plastic bioballs **(Figure 4.3 B)**.The characteristics of these two matrices are described in detail in **Section 4.4.2**.

The experiment was performed in triplicate so each packing material was used in three bioreactors. Each bucket bioreactor was set up as discussed in **Section 2.1.3**, and packed to five centimetres from the top with the appropriate material before being filled with undiluted leachate from the Bul-Bul Drive landfill site. Leachate levels were corrected after 24 hours to compensate for any liquid absorbed by the pine bark.

The leachate was not supplemented with any nutrients, but activated sludge from the Hammarsdale Sewage Works **(Section 2.3)** was used as inoculum at a ratio of 1:10 (v/v). The experiment continued for 26 days and samples were taken from the liquid at the top of each bucket at the beginning of the experiment and after 3, 6, 10, 18 and 26 days for COD analysis **(Section 2.4.1)**.

6.3.3 Results

The COD reductions in this experiment were superior to those obtained with the Shongweni leachate. In this case, the initial COD concentration (4262 mg.l^{-1}) was much higher than that of the previous leachate, and fell within the normal range expected for this particular effluent **(Section 5.2.4)**. The starting values shown in **Figure 6.1** may vary slightly because they reflect the addition of the 10 % (v/v) activated sludge, which is a heterogeneous inoculum, to each bioreactor. However, the initial values for two of the bioreactors, one containing bark and the other plastic bioballs, were much higher than those of the other four.

COD removal in the three bioreactors containing the plastic bioballs was significantly better than that in those containing pine bark chips. The former bioreactors also gave much more consistent results. An initial decrease in COD

concentrations was observed in all the bioreactors, but after six days COD removal efficiencies in those reactors containing pine bark were greatly reduced and cumulative COD removal percentages declined to below zero. This continued until day 18, after which COD removal improved for the last eight days **(Figure 6.1)** of the experiment resulting in an overall reduction of 29 % **(Table 6.1)**. In contrast, the cumulative COD removal percentages for the bioreactors with the plastic matrix were always positive. The most active reduction took place over the first ten days, after which the removal efficiency decreased **(Figure 6.1)**. The total average COD removal efficiency in these bioreactors was 60 %, which is very satisfactory for a leachate of this nature **(Table 6.1)**.

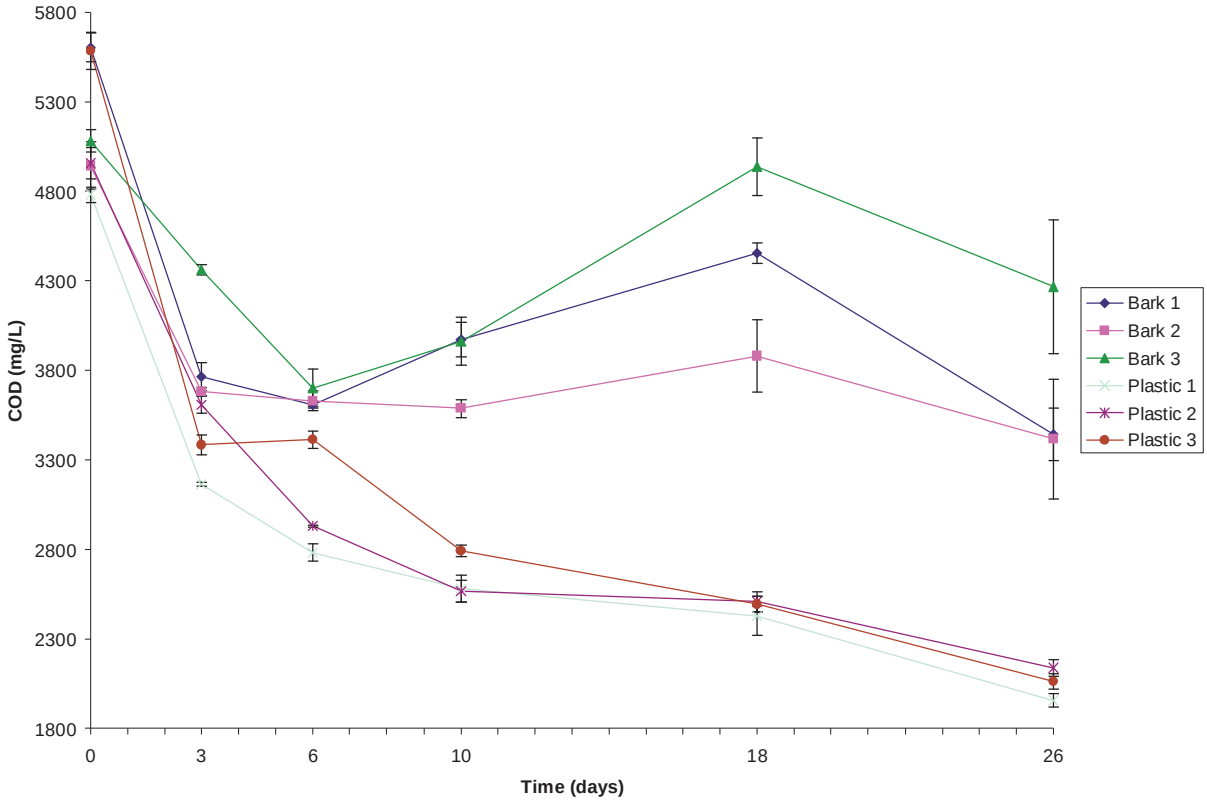


Figure 6.1 Comparative changes in COD concentrations over time in upflow packed-bed bucket bioreactors with 16 – 24 mm pine bark chips and plastic bioballs as biofilm support matrices. Error bars indicate standard deviation (n=3).

TABLE 6.1 Total and cumulative COD reduction over time in upflow packed-bed bucket bioreactors with 16 – 24 mm pine bark chips and plastic bioballs as biofilm support matrices

Time (days)	**COD removal efficiency expressed as a percentage with standard deviation**															
	Pine bark								**Plastic bioballs**							
	Total				**Cumulative**				**Total**				**Cumulative**			
	1	**2**	**3**	**Avg.**	**1**	**2**	**3**	**Avg.**	**1**	**2**	**3**	**Avg.**	**1**	**2**	**3**	**Avg.**
3	33 (±1.47)	26 (±1.76)	14 (±1.03)	**24**	n/a	n/a	n/a	**n/a**	34 (±0.54)	27 (±1.39)	39 (±1.28)	**33**	n/a	n/a	n/a	**n/a**
6	36 (±0.86)	27 (±1.96)	27 (±1.97)	**30**	4	1	15	**7**	42 (±0.99)	41 (±0.91)	39 (±1.22)	**41**	12	19	-1	**10**
10	29 (±1.72)	27 (±1.88)	22 (±2.41)	**26**	-10	1	-7	**-5**	46 (±1.45)	48 (±1.34)	50 (±0.94)	**48**	7	12	18	**12**
18	20 (±1.33)	22 (±3.95)	3 (±2.94)	**15**	-12	-8	-25	**-15**	49 (±1.99)	49 (±1.23)	55 (±1.00)	**51**	6	2	11	**6**
26	39 (±2.37)	31 (±6.07)	16 (±6.40)	**29**	23	12	14	**16**	59 (±0.75)	57 (±1.04)	63 (±0.90)	**60**	19	15	17	**17**

The results of this experiment are similar to those obtained with the Shongweni leachate **(Section 4.4)** comparing pine bark chips and plastic bioballs. However, much improved COD removal efficiencies were recorded for both matrices, probably because of the much higher initial COD concentration and BOD:COD ratio of the Bul-Bul Drive leachate. The higher BOD:COD ratio reflects the presence of more soluble and more easily biodegradable organic compounds that are metabolisable by a large proportion of the microorganisms in the system as opposed to the Shongweni leachate used in **Section 4.4**, which was probably largely comprised of xenobiotic substances as indicated by the much lower ratio. Alternatively, the autochthonous microbial population and leachate constituents could have simply been different due to the temporal and spatial variability that is often observed in leachate polluted wastewaters (Adrian *et al*., 1994).

Once again, the bioreactors containing the plastic bioballs removed COD much more effectively and efficiently than those with the organic pine bark matrix. The COD concentrations in the three bioreactors containing the plastic bioballs were consistent with one another, and showed no anomalous increases. The increase in cumulative reduction efficiencies observed with both matrices in the last eight days of the experiment **(Table 6.1)** could be attributed to changes in the leachate constituents that occur as biodegradation progresses. For example, microorganisms that are able to metabolise leachate components may release by- and/or waste products, making nutrients available to others and facilitating their growth. This possibility will be discussed in more detail in the following section **(6.4)**, where a similar, but even more marked, trend was observed. These results suggest that COD removal may be biphasic, and that it could prove worthwhile to retain leachate in the system for longer periods of time to allow this phenomenon to occur. This would improve the quality of the treated effluent as the effect was significant, especially in the bioreactors containing pine bark.

The increases in COD levels between days 10 and 18 in the bioreactors containing pine bark chips could be explained by the release of compounds such

as various types of sugars, lignin and tannins as discussed for the similarly treated Shongweni leachate **(Section 4.4)**. Perhaps the significant increase in cumulative COD reduction recorded here over the last eight days of the experiment could be explained by the biodegradation of some of the substances that may have leached into solution from the pine bark matrix. This would make them accessible to the microbial population, after a possible “lag phase” required for the biofilm population to adapt to these released substances.

However, the COD concentrations recorded at each sampling time in the three bioreactors containing pine bark differed widely from one another. This variability could not be accounted for by the initial differences in COD. Indications are that the plastic bioballs facilitate more reliable and efficient COD removal. Similarly, Min *et al*. (2004) found that a plastic medium exhibited more consistency than sand for perchlorate removal in a fixed-bed bioreactor, although higher retention times were necessary to achieve complete removal. However, in their investigation, the sand did have a higher specific surface area than the plastic matrix which accounts for the longer retention time required, whereas the specific surface area of the plastic bioballs used in the current investigation was relatively high compared to the other matrices used. In contrast to these bioballs, the plastic used for perchlorate removal was cheaper than sand, which meant that it was chosen as the preferred support medium (Min *et al*., 2004).

A matrix for attached-growth wastewater treatment processes should compromise between a high specific surface area (for maximum efficiency) and a large void volume (to circumvent possible blockages and clogging). The plastic bioballs had both the highest specific surface area and the maximum void space of the solid support materials assessed, and were thus the most desirable matrix from a theoretical point of view. The higher COD removal efficiency achieved in the bioreactors containing this matrix relative to those filled with pine bark chips were anticipated based on the characteristics of this material **(Table 4.3)**. It also had the smallest pore volume and largest void volume, which would reduce

channelling effects in the bioreactor. However, support media with higher porosities can reduce the retention time required for adequate treatment of a waste liquid; for example, perchlorate-contaminated groundwater (Min *et al.*, 2004).

The metabolic capacity of biofilms is also affected by the substratum surface, according to Chappell and Evangelou (2002); however the effect appears to be unpredictable. It is clear that any effect that the support surface has on biofilm hydrophobicity is important, because the stability of the biofilm depends on the hydrophobicity/hydrophilicity ratio and if this changes, there will be an impact on enzymatic activity (Chappell and Evangelou, 2002). Although no detailed characterisation of the biofilms formed in this study was performed, these factors may have influenced the biodegradative efficacy of the reactors filled with different matrices.

The results obtained from both experiments comparing support matrices (those on the Shongweni and Bul-Bul Drive landfill leachates) indicated that a change in the solid support matrix to be used in the larger tank bioreactor should be considered. It should be noted that, although the plastic bioballs are more expensive than the pine bark at the laboratory-scale **(Table 4.3)**, if used on a commercial scale the cost could be significantly reduced by bulk manufacturing a similar matrix from recycled plastic. For example, a similar plastic matrix can be obtained from a local supplier (Talbot & Talbot Pty Ltd) at R 910 per m^3, which is equivalent to only R 18 per bucket bioreactor. Thus the aim of developing a low-cost technology would not necessarily be adversely affected by using plastic as the support for biofilm attachment and growth since it would be far more durable than other potential materials, including pine bark which tends to disintegrate fairly rapidly when used in large volume reactors. All further experiments were therefore conducted using plastic bioballs as the solid support matrix.

6.4 EFFECT OF RECYCLE RATE ON THE PERFORMANCE OF AN UPFLOW PACKED-BED BIOREACTOR DURING BIOREMEDIATION OF THE BUL-BUL DRIVE LEACHATE

6.4.1 Introduction

This experiment was performed with the same rationale as that presented in **Section 4.3.1** when investigating the Shongweni leachate. In essence, the influence of recycle rate on mixing and its consequent effects on biofilm development and COD reduction.

However, in this case the leachate was more suitable for biological treatment in the upflow packed-bed bioreactor. This was illustrated in the trials described in **Chapter 5**, and also by the encouraging results obtained in the earlier experiments discussed in this chapter (see **Section 6.3**).

The objective of this experiment was to determine whether recycle rate has any effect on bioremediation rate or effectiveness, and if so, whether a relatively fast or relatively slow recycle rate is preferable for improved performance of the bioreactor when operating as a batch type system.

6.4.2 Experimental Design

The tank bioreactor **(Section 2.1.2)** was used for this experiment and plastic bioballs served as the solid support matrix for biofilm attachment based on the results of the experiments discussed in **Section 6.3**. The bioreactor was operated in parallel mode and six different recycle rates were tested. These were: 6, 14, 23, 27, 37 and 42 $l.h^{-1}$, which were equivalent to approximately 7, 15, 25, 29, 40 and 46 reactor volumes per day. The packing material was loaded into each of the six fibreglass boxes to approximately two centimetres from the

top, and each chamber and corresponding reservoir was then filled with undiluted raw leachate from the Bul-Bul Drive landfill site. Leachate volumes did not need to be corrected after 24 hours as in previous experiments **(Chapters 3, 4 and 5)** as the plastic bioballs absorb very little liquid **(Table 6.1)** and the volume of leachate being treated in each compartment was not significantly reduced.

The plastic bioballs used were the same ones used in the experiment described in **Section 6.3**; they were, however, washed in 10% hydrogen peroxide and rinsed several times with tap water before packing into the bioreactor chambers. A simple qualitative test based on the oxidation of the iodide ion to iodine by hydrogen peroxide with resultant colour change from clear to a yellowish brown was performed to ensure that no hydrogen peroxide, which could have affected the microbial community in the bioreactor, remained on the bioballs. A sample of the washed bioballs was suspended in a freshly prepared solution of potassium iodide solution (10 %) and shaken vigorously. The solution remained clear, even after a period of 15 to 30 minutes, indicating that no hydroperoxides were present. By contrast, a control sample, which had been washed in hydrogen peroxide but not rinsed with tap water, gave a clear yellow solution, illustrating that the chemical was still present on the bioballs. This procedure was thus deemed suitable for cleaning plastic bioballs for re-use. This is useful because bioballs are durable, and can be used for long periods of time without disintegrating, thus reducing replacement costs associated with maintaining the support medium.

No additional nutrients were added to the bioreactor, but 10 % (v/v) activated sludge from the Hammarsdale Sewage Works **(Section 2.3)** was added to each chamber as additional inoculum.

As before, samples from the outflow pipe (where the leachate flowed into a reservoir for recycle) of each chamber were taken at the start of the experiment, and then on days 3, 7, 10, 14 and 21. In this instance, however, samples were

also taken from the bottom of each chamber on days 7, 10, 14 and 21. This was done because it was thought that recycle rate may have affected both the amount of sludge generated, and the settling of particulate matter present in the waste fluid. COD analyses were performed on all samples **(Section 2.4.1)**.

6.4.3 Results

COD removal occurred at all the recycle rates used varying between 48 and 51 %. This verifies the results obtained in the comparative experiment described in the previous chapter, where 48.5 % removal was recorded for the leachate from the Bul-Bul Drive landfill site **(Table 5.1)**. The curves for all the chambers were very similar, although there were slight differences in initial COD concentration **(Figure 6.2)**. The final COD removal efficiencies were slightly lower (approximately 10 %) than those obtained in the bucket reactors packed with plastic bioballs **(Table 6.1)**. However, the previous experiment was run for five days more than this one, with volumes approximately half those used here when the tank bioreactor and reservoirs operating as a batch system are taken into account **(Section 2.1.2)**.

Interestingly, a similar trend to that observed in the previous experiment comparing support materials in the bucket bioreactors **(Figure 6.1; Figure 6.2)** can be discerned here. There is an initial, rapid decrease in COD concentration over the first three days, followed by a more gradual reduction until day ten. During the next period (days 10 – 14), very little or no COD reduction occurred with a slightly negative cumulative removal efficiency being recorded in some cases. This suggested that all the biodegradable matter had been metabolised by the biofilm and that the treated leachate could be removed from the system. However, a second phase of COD reduction occurred after this “stationary” period (viz. days 14-21) during which the cumulative reduction efficiencies

peaked. Analyses of samples taken after day 21 indicated that the rate of decline in COD concentration did not continue as rapidly thereafter (results not shown).

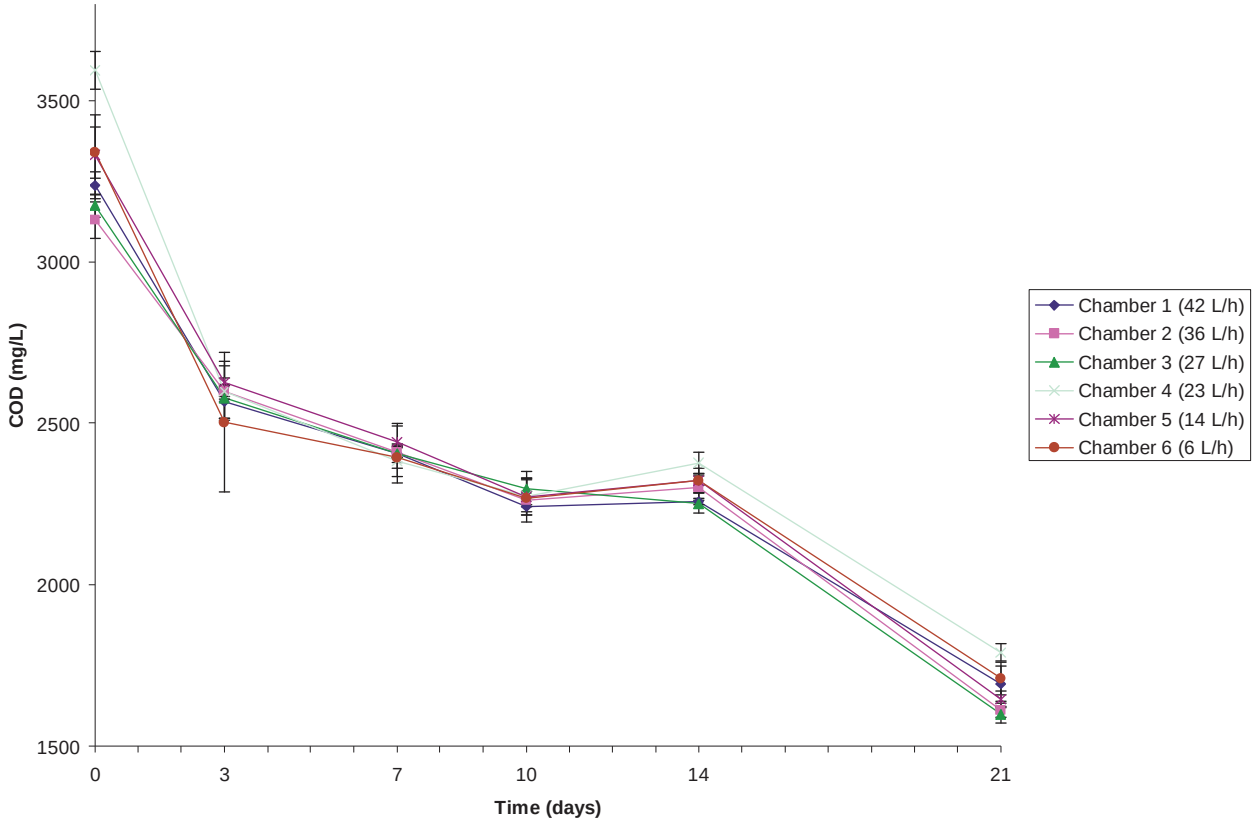


Figure 6.2 Changes in COD concentrations over 21 days bioremediation in an upflow packed-bed bioreactor operating as a batch-type system at different recycle rates. Error bars indicate standard deviation (n=3).

TABLE 6.2 Total and cumulative COD removal efficiencies over a 21 day bioremediation period in an upflow packed-bed bioreactor operating at different recycle rates. The overall percentage COD removal in each chamber is highlighted.

	COD removal efficiency expressed as a percentage with standard deviation											
Sampling time (days)	**Recycle rate (*l*.h^{-1})**											
	42		**37**		**27**		**23**		**14**		**6**	
	Tot.	**Cum.**	**Tot.**	**Cum.**	**Tot.**	**Cum.**	**Tot.**	**Cum.**	**Tot.**	**Cum.**	**Tot.**	**Cum.**
3	21 (±1.68)	n/a	17 (±1.40)	n/a	19 (±1.87)	n/a	28 (±2.42)	n/a	21 (±2.84)	n/a	25 (±5.84)	n/a
7	26 (±2.57)	6	23 (±1.31)	7	24 (±1.04)	7	34 (±1.49)	8	27 (±2.66)	7	28 (±1.71)	4
10	31 (±1.07)	7	28 (±2.20)	6	28 (±1.65)	5	37 (±1.49)	5	32 (±2.61)	7	32 (±1.49)	5
14	30 (±0.84)	-1	26 (±1.62)	-2	29 (±1.06)	2	34 (±1.22)	-5	30 (±2.42)	-2	30 (±1.48)	-2
21	**48** (±1.56)	25	**48** (±1.00)	30	**50** (±0.85)	29	**50** (±0.95)	25	**51** (±1.70)	29	**49** (±1.67)	26

Although the COD concentrations in samples taken from the outflow pipes were not significantly different from one another, those in samples from the bottoms of the chambers did vary with the recycle rate. At the end of the experiment, the COD concentration of the sludge in the chamber with the fastest recycle rate (42 $l.h^{-1}$) was 1662 $mg.l^{-1}$, whereas that in the chamber with the slowest recycle rate (6 $l.h^{-1}$) was 3472 $mg.l^{-1}$. This represents an approximate 200 % increase in the sludge COD as the recycle rate decreased seven fold. In general, the COD concentrations of the sludge rose for the first ten days and then decreased until termination of the experiment on day 21. The “sludge” in each chamber of the reactor was flocculent and spongy.

6.4.4 Discussion

Recycling, specifically across the range of rates tested, had no significant effect on either the rate or effectiveness of bioremediation of the Bul-Bul Drive landfill leachate, although it did affect the way in which carbon removal occurred. This was illustrated by the differences in COD concentrations of samples taken from the sludge at the bottom of each chamber. It is clear that more carbonaceous matter is able to settle at the bottom of the bioreactor when the recycle rate is slow, and particulates as well as biomass probably tend to accumulate here. At faster recycle rates, this does not happen as the insoluble materials in the leachate do not easily settle, and COD is therefore more likely to occur throughout the chamber and therefore in closer contact with the biofilm attached to the surface of the support matrix.

However, even though this effect was noted, it was outside the scope of this study to explore the different mechanisms involved in carbon removal during the treatment of effluents within the bioreactor. The purpose of this research was to determine whether the bioreactor under investigation was suitable for the treatment of liquid wastes such as landfill leachate. Therefore no effort was made

to elucidate the location of carbon compounds removed from the treated leachate within the bioreactor.

Although the different recycle rates applied had no significant effect on COD removal, it would probably be better to operate the system at a relatively high mixing rate to circumvent the problem of having to dispose of large quantities of sludge containing high concentrations of incompletely oxidised carbon compounds. It may be that leachate could be more successfully treated using fairly low retention times were the bioreactor to be operated as a cascade, or continuous, system.

Despite the fact that the COD removal efficiencies were indistinguishable at the different recycle rates, the overall COD reduction in all chambers of 49 % compared favourably with the results of others working on similar leachates. The similar trends observed over the range of recycle rates tested suggest that it might be worth retaining the leachate in a treatment system of this type even when cumulative COD reduction seems to be declining, as suggested in **Section 6.3.4.2**. All the chambers exhibited a second phase of rapid COD removal that occurred after the leachate had been in the bioreactor for two weeks. Initially, the leachate would contain many pollutants that are easily degradable by both members of the autochthonous population, and microorganisms introduced in the activated sludge used as inoculum. This accounts for the high COD removal efficiencies observed in the first three days of the experiment. Some of these organisms may also have been responsible for the partial degradation of certain leachate components, releasing more recalcitrant (and perhaps more toxic) substances into the system. As the more easily biodegradable compounds disappeared and growth-limiting conditions arose, the microbial population would enter an apparent stationary phase, during which the numbers of some of the faster-growing heterotrophs may have declined. This would allow slower-growing, metabolically diverse organisms possibly suppressed in the biofilm originally to increase in number and metabolise the more refractory compounds

remaining in the leachate, thereby causing the second phase of COD removal observed. This pattern of COD removal may also have been responsible for the similar trend described in **Section 6.3.3.2 (Figure 6.1)**.

The average COD removal efficiency (49 %) achieved is the same as that obtained previously for this leachate (see **Section 5.3**), where COD removal from leachates from different landfills was compared in the same tank bioreactor, but with pine bark chips as the support matrix for biofilm attachment and growth. However, initial COD concentrations were over 2000 mg.l^{-1} higher in the latter experiment than in the current experiment. The final COD concentrations also differed with the former approximately 1000 mg.l^{-1} higher than the latter. This indicates that the support medium may not affect bioremediation to a significant extent when larger volumes such as those in the tank bioreactor are treated. Although poor COD removal efficiencies were recorded when using the bucket bioreactors packed with pine bark to treat Shongweni leachate (see **Section 4.4**), this support material may be viable for the large scale bioreactors that would be required if this technology were to be installed on landfill sites. Although it has been noted that costs of plastic media could be reduced if they were used on this large scale, pine bark chips would still be cheaper initially, although the longevity and transport costs of each type of medium would have to be considered before any realistic cost comparison could be made.

6.5 PERFORMANCE OF THE UPFLOW PACKED-BED BIOREACTOR AS A CONTINUOUS FLOW CASCADE SYSTEM

6.5.1 Introduction

Although many batch-type systems are used in wastewater treatment, the particular system developed in this research was intended to operate as a continuous flow system for the treatment of landfill effluents. The batch experiments described up to this point were used as a tool to assess the potential performance of a cascade system operated under different conditions. Aeration levels, different support matrices and recycle rates were optimised using this approach. However, leachate is generated continuously while a landfill is in operation and for a considerable time after closure; this means that a continuous biological process is necessary if a technology is to be viable at large-scale. Large storage facilities would be required if a batch process was used on-site (Armenante, 1993), and this is impractical where large volumes of hazardous liquid waste are produced. Continuous processes are also more amenable to automation, thus reducing labour costs and the possible impacts of human error on the system (Williams, 2002). The cost-efficiency aspect is crucial in a low-maintenance system, such as this one, which is intended for use in developing countries where skilled labour is likely to be less readily available than in developed countries.

Continuous operation also means that more specialised microbial communities can develop in each compartment of the bioreactor, depending on the substrates and inhibitory compounds present. Oliveira *et al*. (2004) also noted that continuous flow reactors have been reported to attain higher levels of pollutant removal than batch reactors. They used a horizontal-flow anaerobic immobilised biomass (HAIB) reactor to remove formaldehyde from a synthetic waste stream, obtaining a removal efficiency as high as 99 %. This result was attributed to the

hydrodynamic properties of the reactor, which allowed the microorganisms to acclimatise to the primary substrate and the intermediate products in different sectors along the length of the system, so that much of the population was not exposed to potentially toxic compounds.

The following experiments were performed to assess the performance of the upflow packed-bed tank bioreactor as a continuous treatment system for high strength effluents such as the Bul-Bul Drive landfill leachate.

6.5.2 Experimental Design

6.5.2.1 Operation of the bioreactor as a cascade system with a recycle loop

This experiment was performed to test the operation of the upflow packed-bed bioreactor as a cascade, and to acclimatise the microorganisms in the system to the cascade mode. It is acknowledged that the recycle loop would not necessarily have facilitated the development of different microbial populations in different chambers. However, time was still allowed for the attachment and formation of a functional biofilm consisting of a heterogeneous community of microorganisms from the leachate itself, and from the activated sludge inoculum.

The tank bioreactor was converted to cascade mode (as described in **Section 2.1.2**) for this experiment. In this case, untreated leachate (150 *l*) from the Bul-Bul Drive landfill site was recycled following passage through all six chambers. A 10 % (v/v) inoculum of activated sludge from the Hammarsdale Sewage Works **(Section 2.3)** was added to the leachate, but no exogenous nutrients were provided. This mixture was then pumped through the bioreactor from a single large (220 *l*) reservoir. When the system was filled to capacity, the reservoir was

disconnected and replaced by a recycle loop between the outlet of chamber six and the inlet of the first chamber.

Plastic bioballs were used as the biofilm support material in all six chambers. They were not removed from the bioreactor and washed on completion of the previous experiment **(Section 6.4)** in the hope that the biofilm established thereon would provide a source of microorganisms already adapted to the prevailing conditions in the system. All chambers were aerated with fish-tank pumps as described in **Section 2.1.2**.

The experiment lasted for two weeks, with samples being taken for COD and BOD analyses (**Sections 2.4.1** and **2.4.2**) from each chamber at the start, and on days seven and 14.

6.5.2.2 Operation of the bioreactor as a continuous cascade system

The aim of this experiment was to determine whether the upflow packed-bed bioreactor is an efficient and effective continuous treatment method for high strength effluents such as landfill leachate, and thus whether it would be appropriate to investigate its use at a larger scale, for example, on a landfill site.

This experiment used the tank bioreactor, set up in the cascade mode, as a continuous system with an influent waste stream entering the reactor at one end, and treated liquid removed at the other, after passing through all six chambers in sequence (set-up described in **Section 2.1.2**).

Untreated and undiluted leachate from the Bul-Bul Drive landfill site was used as the influent waste stream. In this instance, no activated sludge was added as inoculum because, as already stated, the colonised bioballs used in the previous experiment **(Section 6.5.2.1)** were not removed from the bioreactor chambers, or

washed, before the start of this experiment, so it was assumed that an acclimatised microbial population already existed in the system. These cells would be augmented by the autochthonous microorganisms in the fresh leachate. The small volume of liquid remaining in the bioreactor was gradually replaced with the fresh leachate, although obviously some mixing would have occurred. All the raw leachate used was collected at the same time, but was stored in three separate drums each with a capacity of 220 *l* which were used successively as the influent liquid over the 80 days of the experiment.

The residence time of approximately 12 days meant that it took about 48 hours for the leachate to flow upwards through each chamber of the bioreactor, and all calculations are based on the assumption that flow rate remained constant. However, some minor fluctuations in the flow rate would have been inevitable during such a long-term experiment.

Samples were taken for COD and BOD analyses (**Sections 2.4.1** and **2.4.2**) every 48 hours from the influent leachate stream, as well as from the sampling ports connected to each chamber. As described in **Section 2.1.2**, these sampling ports were located at the point where the waste stream cascaded from one chamber into the next.

6.5.3 Results

6.5.3.1 Operation of the bioreactor as a cascade system with a recycle loop

As shown in **Figure 6.3**, COD concentrations decreased rapidly for the first week of the experiment, and then continued to decrease more gradually until day 14. The curves for all the chambers were almost identical. BOD removal in four out of the six chambers followed a similar pattern to that of COD reduction **(Figure**

6.4). However, in chambers four and six, the curves showing the decline in BOD concentration were virtually straight lines indicating a constant rate over the entire experiment. Note that the differences in initial concentration are more pronounced when BOD, as opposed to COD, is measured. The initial BOD concentration is clearly higher, and very similar, in the first three chambers and then declines in each of the remaining three chambers.

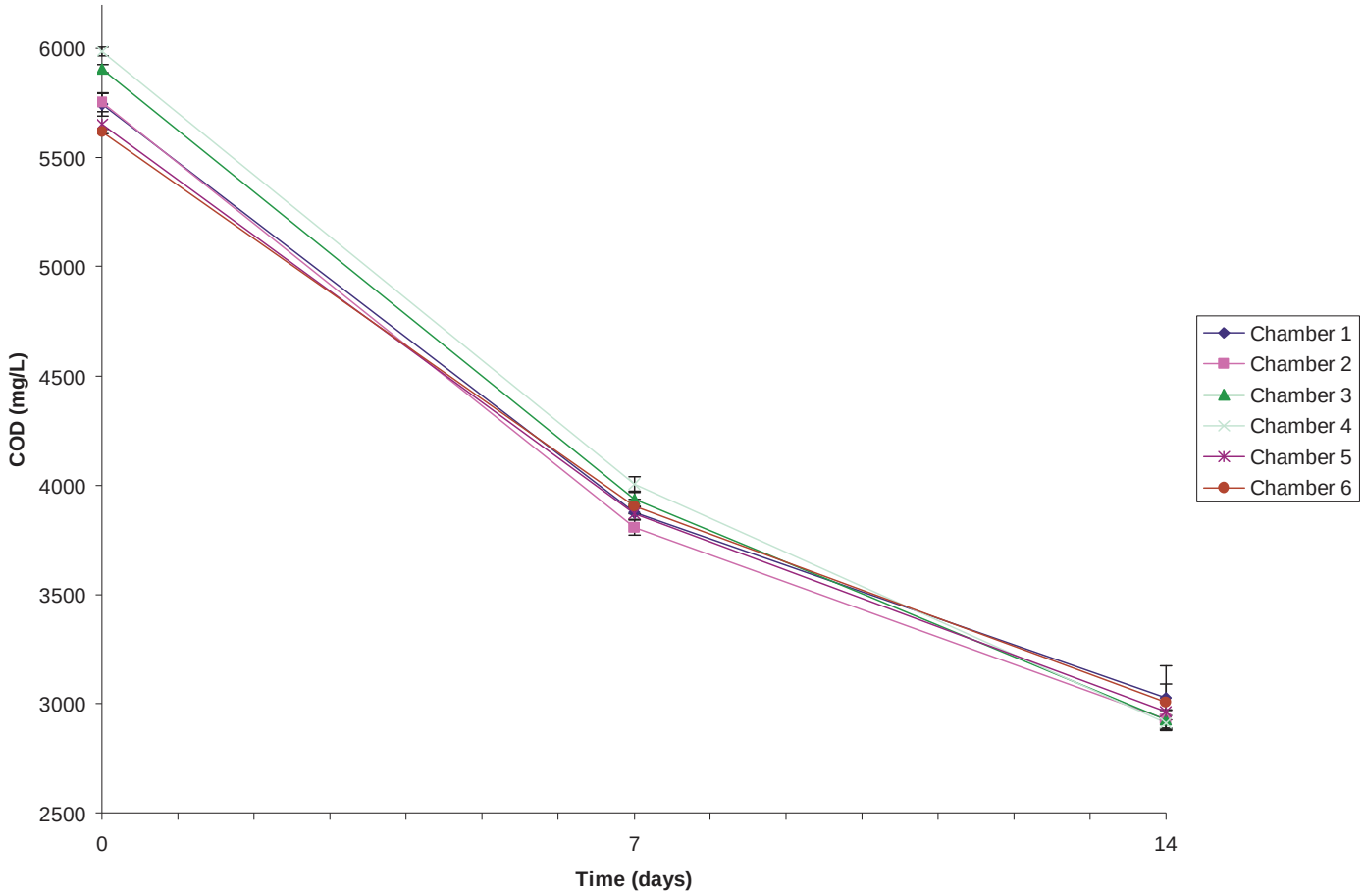


Figure 6.3 Changes in COD concentrations over 14 days with the bioreactor configured as a six-chambered batch series.

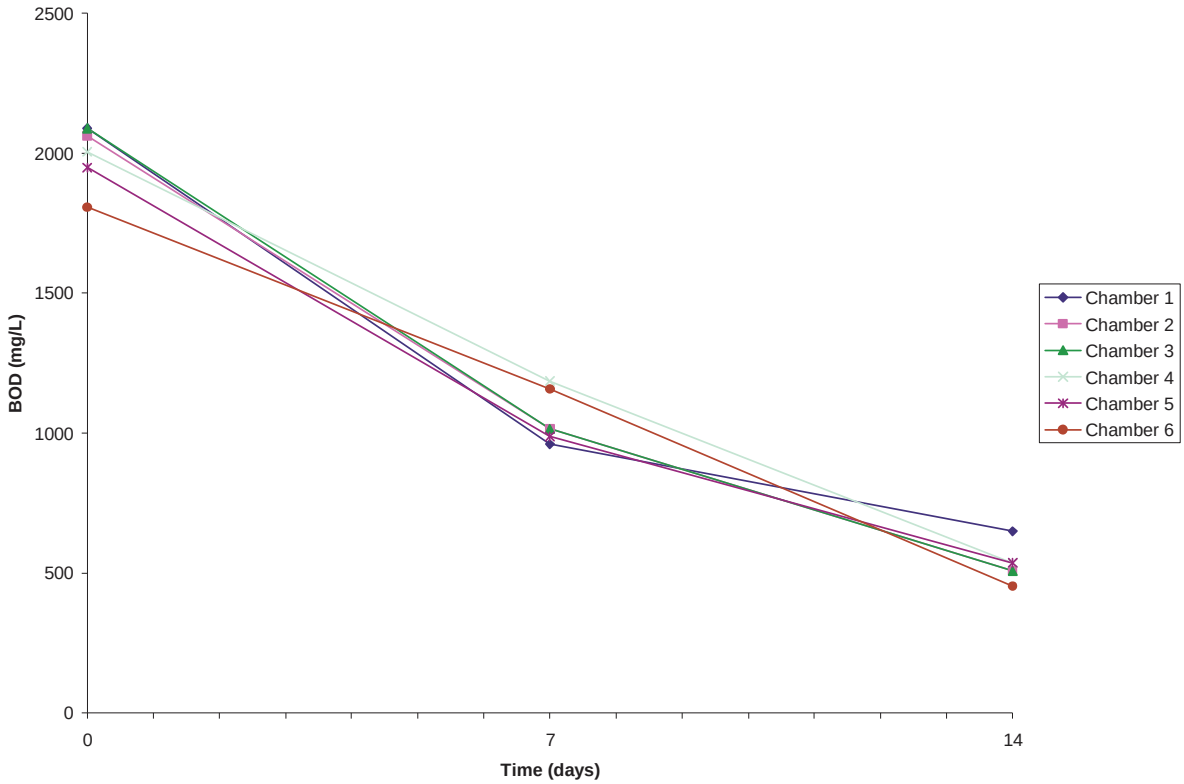


Figure 6.4 Changes in BOD concentrations over 14 days with the bioreactor configured as a batch series of six chambers

The BOD:COD ratio decreased each week, with a total decrease of 16 % from 34 to 18 % over the whole experimental period; while final COD and BOD removal efficiencies were 49 and 73 % respectively **(Table 6.3)**. Both removal efficiencies are satisfactory for a typically strong effluent such as the Bul-Bul Drive landfill leachate. **Table 6.3** also illustrates that, while most of the reduction in COD concentrations took place in the first week, this was not true of BOD removal which remained constant in all chambers, except chamber one, for the duration of the experiment.

TABLE 6.3 Total and cumulative BOD and COD removal efficiencies and BOD:COD ratios over 14 days in the upflow packed-bed bioreactor operating as a cascade system with recycle. Final COD and BOD reduction percentages, as well as the average BOD:COD ratios at each sampling time are highlighted (n/a represents not applicable)

Time (days)	Chamber	BOD:COD (%) with SD	BOD reduction (%)		COD reduction (%) with SD	
			Total	Cumulative	Total	Cumulative
0	1	36 (±0.33)	n/a	n/a	n/a	n/a
	2	36 (±0.28)	n/a	n/a	n/a	n/a
	3	35 (±0.11)	n/a	n/a	n/a	n/a
	4	33 (±0.11)	n/a	n/a	n/a	n/a
	5	34 (±0.06)	n/a	n/a	n/a	n/a
	6	32 (±0.09)	n/a	n/a	n/a	n/a
	Average	**34**				
7	1	25 (±0.18)	54	n/a	33 (±0.68)	n/a
	2	27 (±0.25)	51	n/a	34 (±0.70)	n/a
	3	26 (±0.63)	51	n/a	34 (±1.41)	n/a
	4	30 (±1.20)	41	n/a	33 (±2.40)	n/a
	5	26 (±0.13)	49	n/a	32 (±0.33)	n/a
	6	30 (±0.28)	36	n/a	31 (±0.59)	n/a
	Average	**27**	47		33	
14	1	21 (±1.01)	69	32	44 (±2.25)	22
	2	17 (±0.24)	75	50	50 (±0.66)	23
	3	17 (±0.27)	76	50	51 (±0.97)	26
	4	18 (±0.21)	73	55	51 (±0.79)	27
	5	18 (±0.50)	72	46	48 (±1.42)	24
	6	15 (±0.34)	75	61	48 (±1.05)	23
	Average	**18**	**73**	49	**49**	24

6.5.3.2 Operation of the bioreactor as a continuous cascade system

The COD of the influent leachate decreased from 6377 to 5150 mg.l^{-1} over the first eight days of the experiment before flattening over the remaining 72 days. However, despite this change, the COD concentration in the treated liquid exiting chamber six was relatively constant, averaging 2737 mg.l^{-1} **(Figure 6.5)**. This meant that the rate of COD removal declined steadily throughout the experimental period, with a maximum value of 62 % and a minimum of 33 % **(Figure 6.8)**. Points on this graph are only shown from day 12 onwards because that is the stage at which the bioreactor would theoretically have been filled with fresh untreated leachate and all liquid from the previous batch of leachate that was still present in the system initially would have been replaced (refer to **Section 6.5.2.2**).

The process settled only after approximately 60 days of operation when COD reduction in each of the six chambers showed clear trends. These tendencies were, however, discernable prior to this point despite the fluctuations in COD levels observed during the early stages. The COD concentrations in all chambers are very similar at time zero because of the treated leachate that remained in the system from the previous experiment. **Figure 6.5** shows that a significant proportion of the COD, averaging 15 %, was removed in chamber one. A smaller reduction occurred in chamber two and almost none in chamber three. However, another significant drop in COD concentration occurred in chamber four. After this very little further COD removal occurred in chambers five and six. This trend is clearly evident when the total and cumulative COD removal efficiencies from day 60 to day 80 are tabulated **(Table 6.4)**. This twenty day period was more closely examined as the system performed consistently during this time.

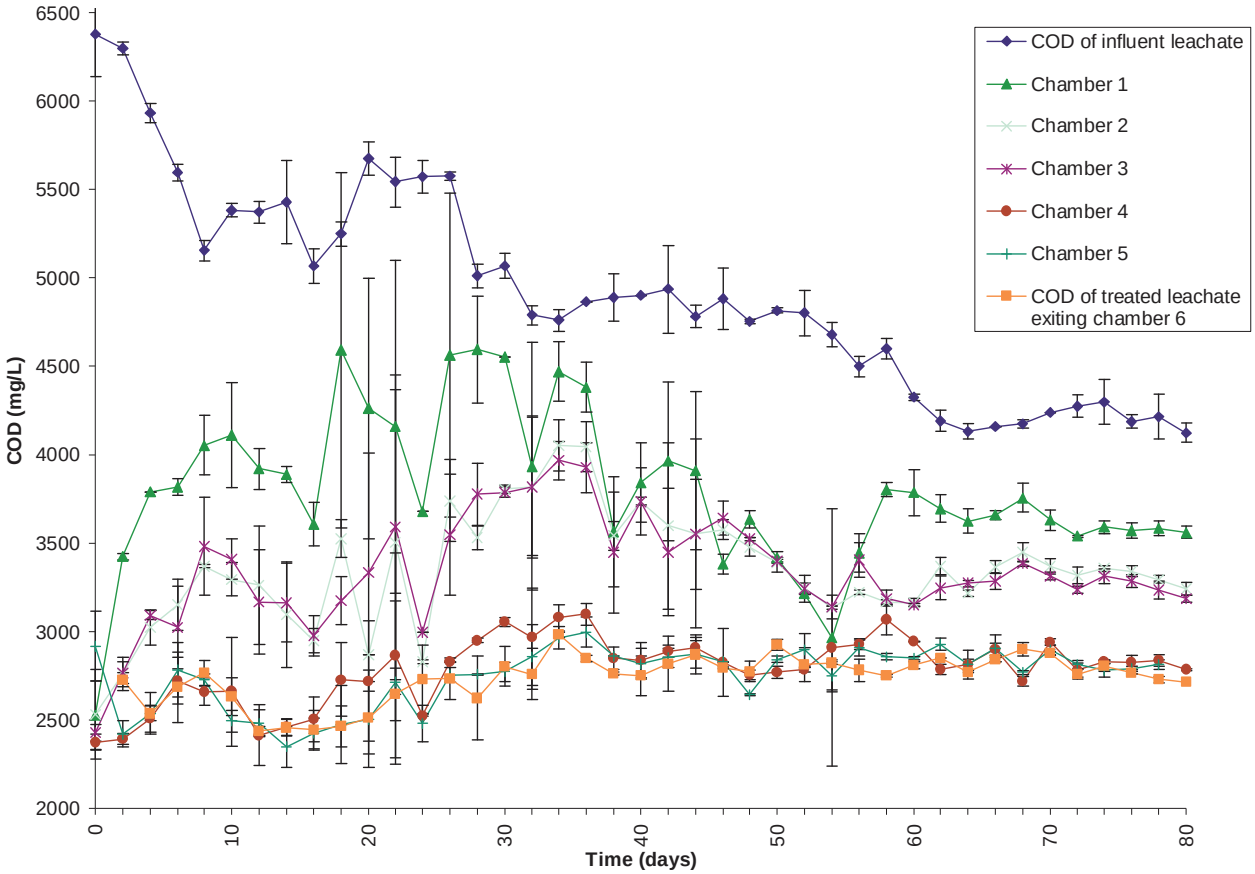


Figure 6.5 Changes in COD concentrations of both the influent leachate and after it had passed through each of the six chambers over an 80 day period with the upflow packed-bed bioreactor operating as a continuous cascade system during treatment of the Bul-Bul Drive landfill leachate.

TABLE 6.4 Total (tot.) and cumulative (cum.) COD removal efficiencies from days 60 to 80 during bioremediation of the Bul-Bul Drive landfill leachate in the upflow packed-bed bioreactor operating as a continuous cascade system (n/a represents not applicable)

Time (days)	**COD reduction expressed as a percentage**											
	Chamber											
	1		**2**		**3**		**4**		**5**		**6**	
	Tot.	**Cum.**	**Tot.**	**Cum.**	**Tot.**	**Cum.**	**Tot.**	**Cum.**	**Tot.**	**Cum.**	**Tot.**	**Cum.**
60	18	n/a	30	17	33	1	39	8	41	7	41	2
62	15	n/a	27	11	28	-3	40	12	39	0	41	0
64	14	n/a	26	13	29	3	37	13	40	-1	42	6
66	11	n/a	20	7	24	-2	37	11	35	-3	39	-1
68	10	n/a	17	6	19	-1	37	17	40	5	36	0
70	13	n/a	19	10	20	4	30	12	33	-7	37	-4
72	16	n/a	20	9	22	4	33	16	33	4	36	5
74	16	n/a	21	5	21	0	32	12	33	0	33	0
76	17	n/a	22	7	22	2	32	15	33	1	33	1
78	14	n/a	23	8	24	3	33	14	33	0	34	2
80	16	n/a	23	10	26	3	35	14	35	3	35	4
Average	**15**	**n/a**	**23**	**9**	**24**	**1**	**35**	**13**	**36**	**2**	**37**	**1**
	(±2.46)		**(±3.83)**		**(±4.27)**		**(±3.22)**		**(±3.36)**		**(±3.29)**	

Influent BOD concentrations decreased over the course of the experiment, but fluctuated throughout. The treated leachate cascading from chamber six had a relatively constant BOD concentration, at an average of 145 mg./$^{-1}$ **(Figure 6.6)**. BOD removal efficiency (averaging 89 %) was more consistent than COD removal efficiency, although it showed a tendency to decrease over the last week of the experiment when the influent BOD concentration was declining **(Figure 6.8)**.

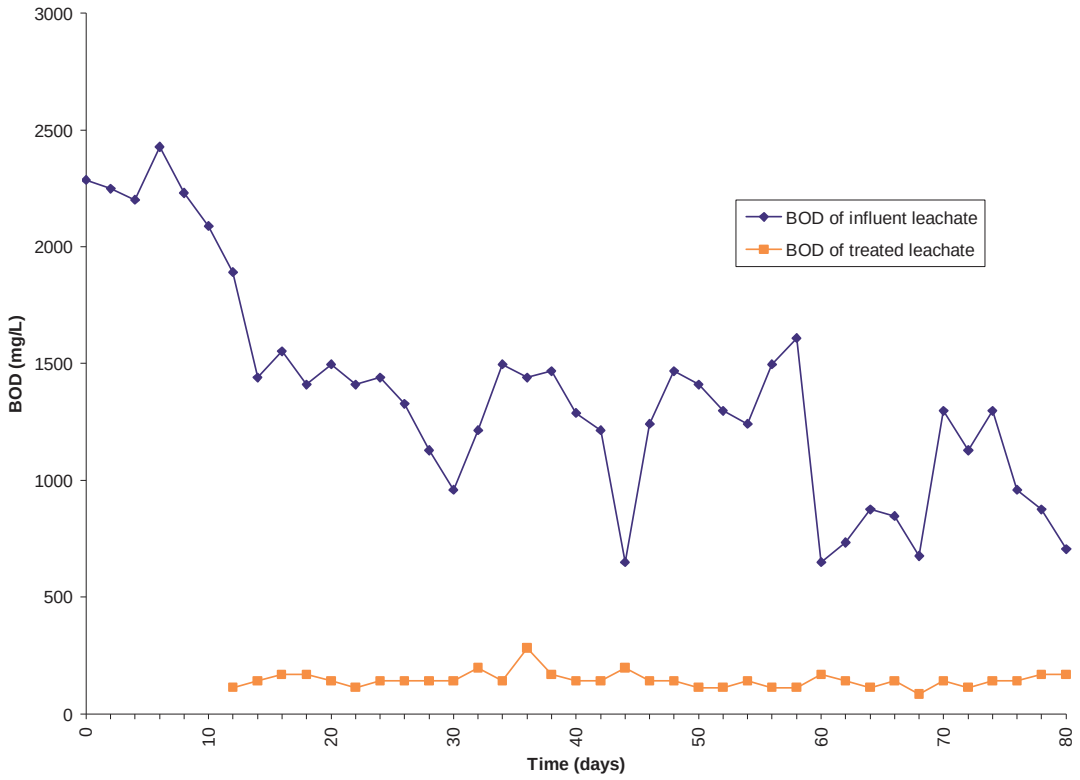


Figure 6.6 Changes in BOD concentrations of both the influent leachate and after it had passed through all six chambers over an 80 day period with the upflow packed-bed bioreactor operating as a continuous cascade system during treatment of the Bul-Bul Drive landfill leachate.

These trends are illustrated in **Figure 6.7**, which shows the influent COD and BOD concentrations and the corresponding effluent values after passage through

all six chambers. The COD concentrations of the treated leachate were always higher than the influent BOD values; this effect became especially marked as the experiment progressed. However, the total amount of COD removed was always greater than the equivalent influent BOD value.

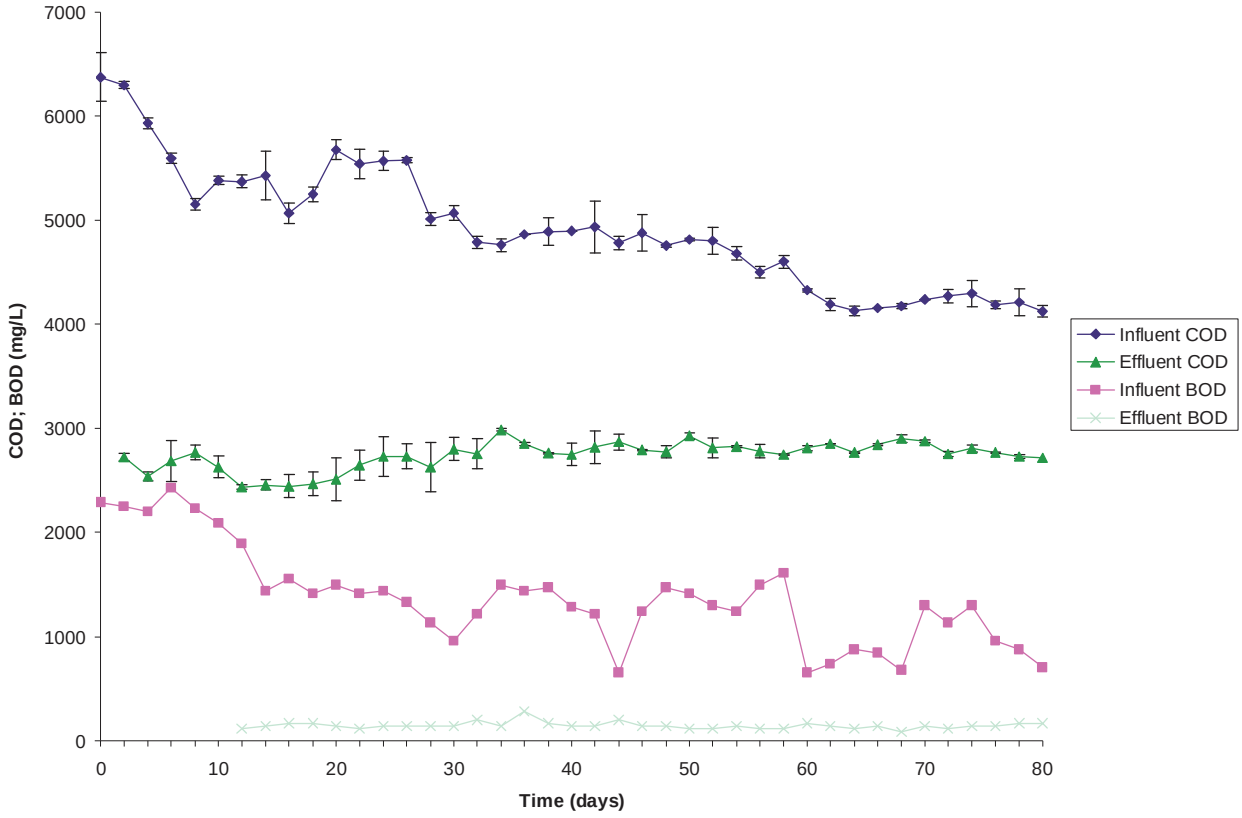


Figure 6.7 Changes in COD and BOD concentrations of the influent and the treated leachate over 80 days bioremediation in the upflow packed-bed bioreactor operating as a continuous cascade system.

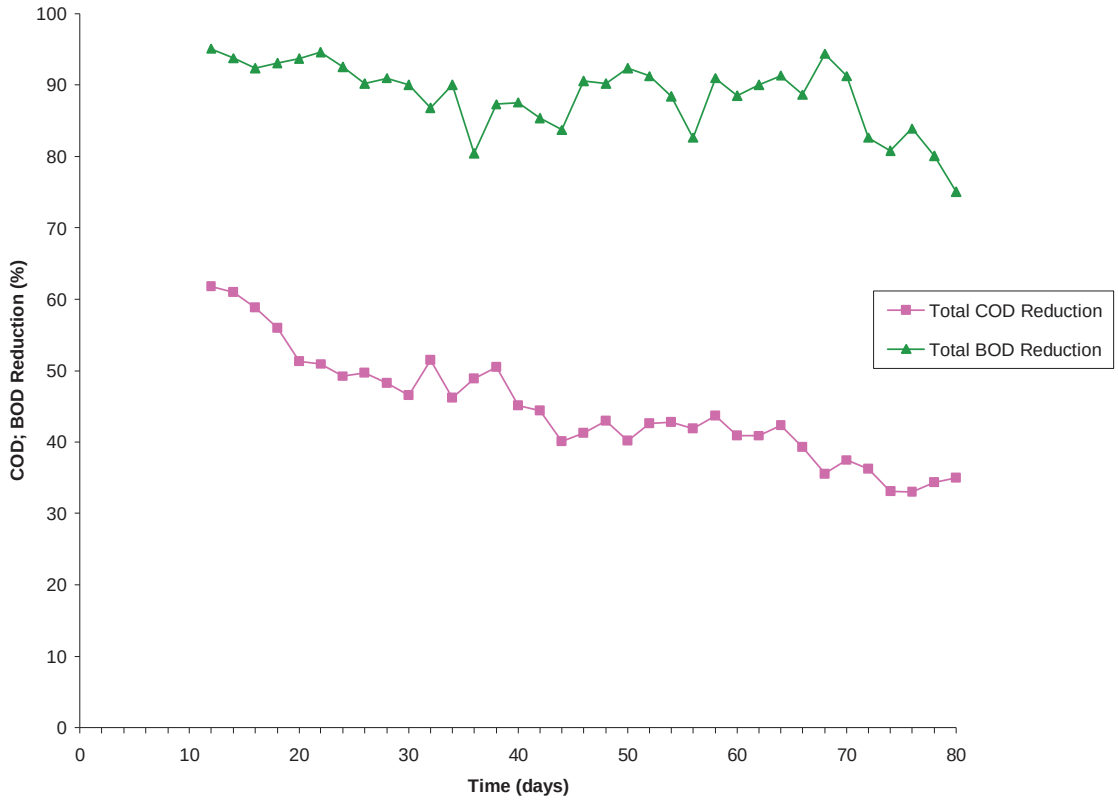


Figure 6.8 COD and BOD removal efficiencies over 80 days bioremediation in the upflow packed-bed bioreactor operating as a continuous cascade system.

The BOD:COD ratio of the influent leachate fluctuated significantly during the experimental period (the average was 26 %); however, after treatment the BOD:COD ratio was relatively consistent at an average of 5 % **(Figure 6.9)**. The highest biodegradability of the raw leachate was measured on day eight (43 %) and this decreased rapidly up to day 30 after which a cyclical fluctuation occurred between days 31 and 80. The pattern of the influent BOD:COD curve strongly resembles the changes in influent BOD concentration illustrated in **Figures 6.6** and **6.7**, despite that fact that influent COD was decreasing over the course of the experiment.

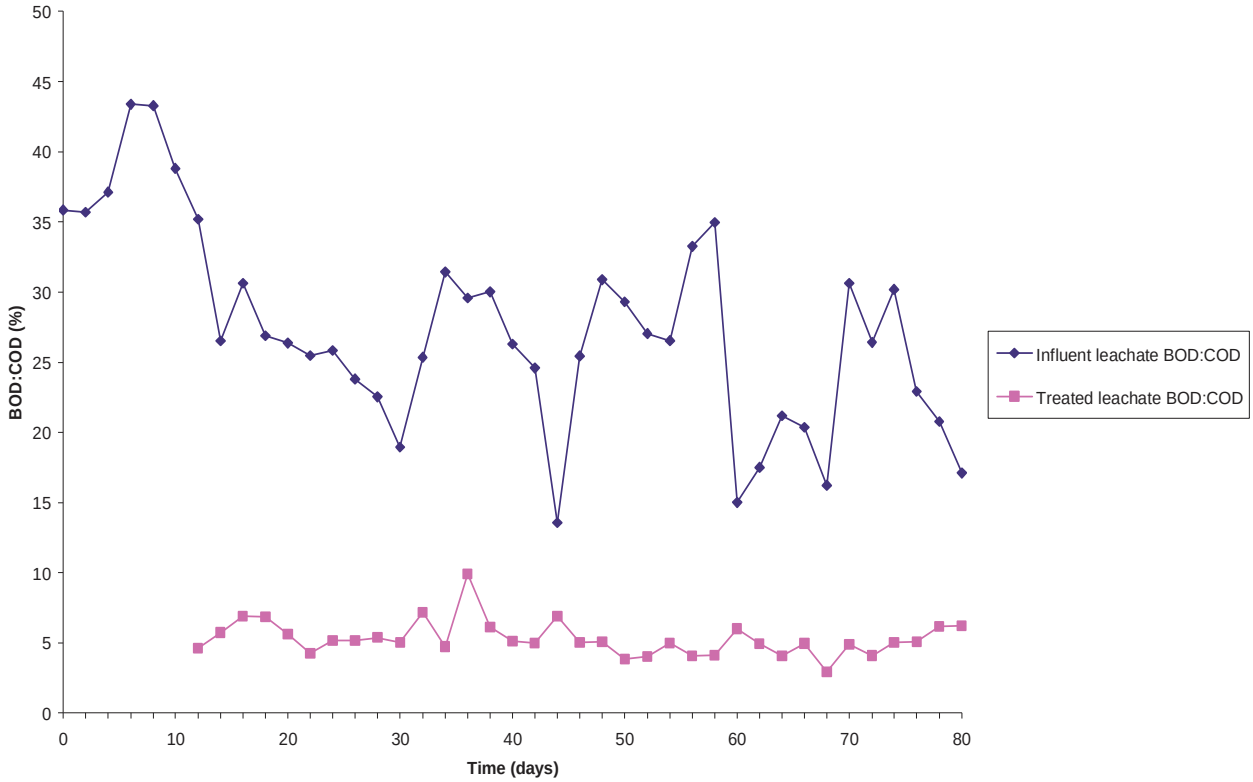


Figure 6.9 BOD:COD ratios of the influent (raw) leachate and the effluent (treated) leachate over 80 days bioremediation in the upflow packed-bed bioreactor operating as a continuous cascade system.

A biofilm developed on the bioballs within each of the six chambers **(Figures 6.10 – 6.15)**, showing changing patterns of growth across the bioreactor. The biofilm was typically patchy and formed an appreciable extracellular matrix in the first chamber **(Figure 6.10)**, while the cells in the next two chambers were part of a heterogeneous, multi-layered structure containing a diverse array of morphotypes **(Figure 6.11; Figure 6.12)** with filamentous microorganisms present. Microbial growth was sparser in the fourth chamber **(Figure 6.13)**, but the cell population became more complex again in the fifth chamber, which exhibited features similar to the biofilm in the second and third chambers **(Figure 6.14)**. The final chamber **(Figure 6.15)** contained fewer cells than the preceding chamber and was characterised by a large amount of extracellular material,

although the biofilm was more intricate than that observed in chamber one **(Figure 6.10)**.

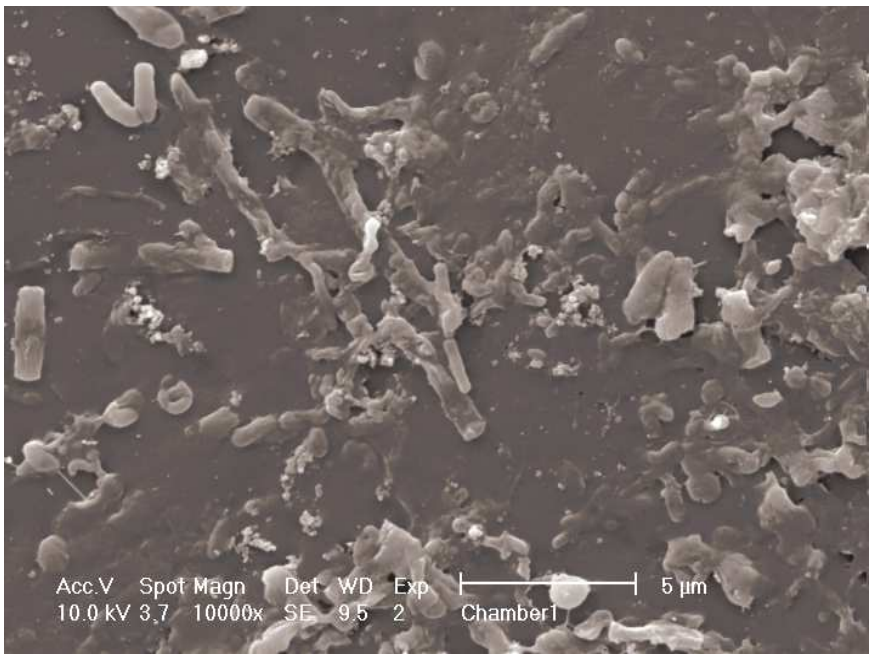


Figure 6.10 Scanning electron micrograph of the biofilm that developed in chamber one after 80 days bioremediation of the Bul-Bul Drive landfill leachate in the upflow packed-bed bioreactor operating as a continuous cascade system (x 10 000).

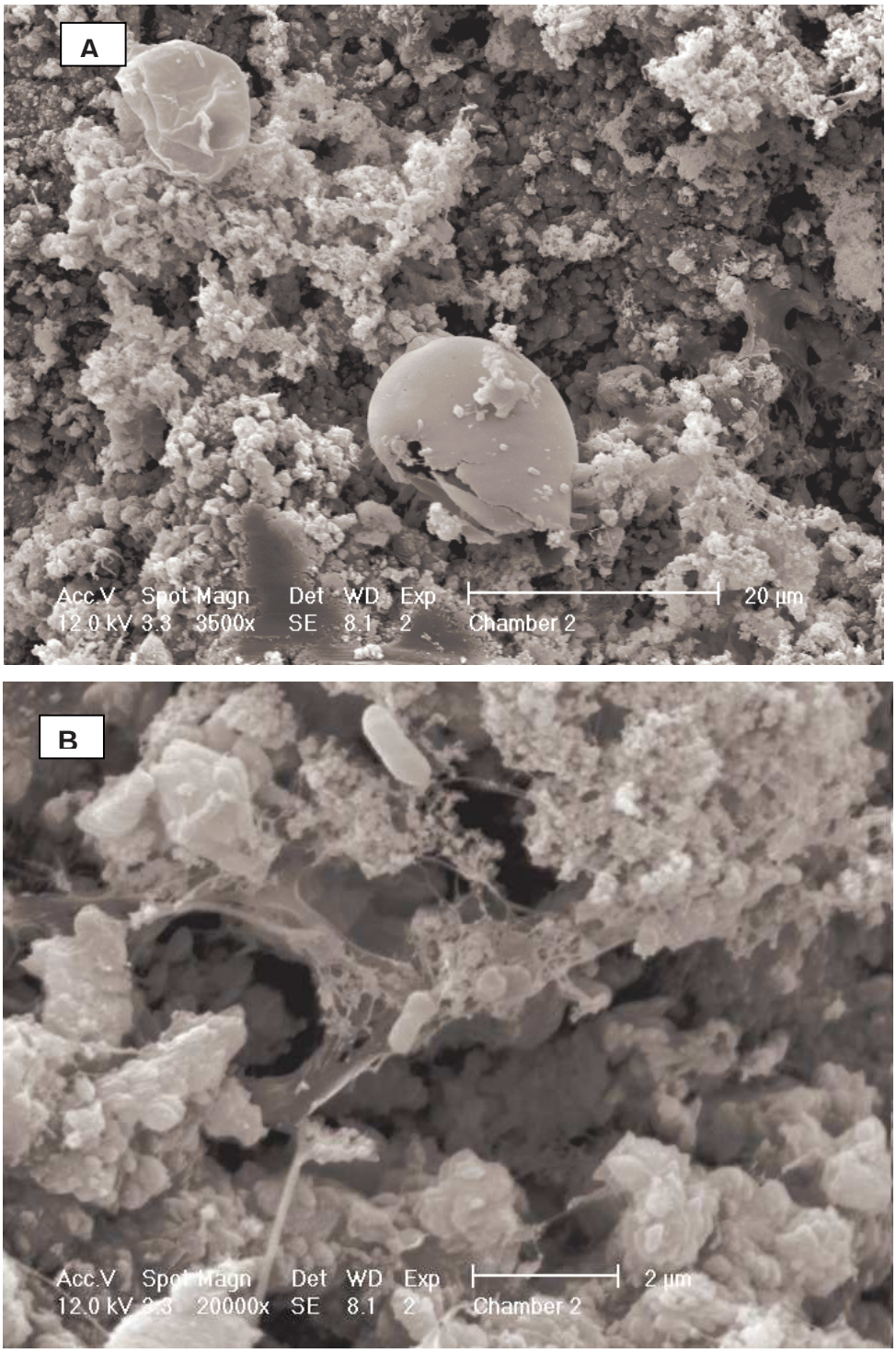


Figure 6.11 Scanning electron micrographs of the biofilm that developed in chamber two after 80 days bioremediation of the Bul-Bul Drive landfill leachate in the upflow packed-bed bioreactor operating as a continuous cascade system. **A:** x 3500; **B:** x 20 000

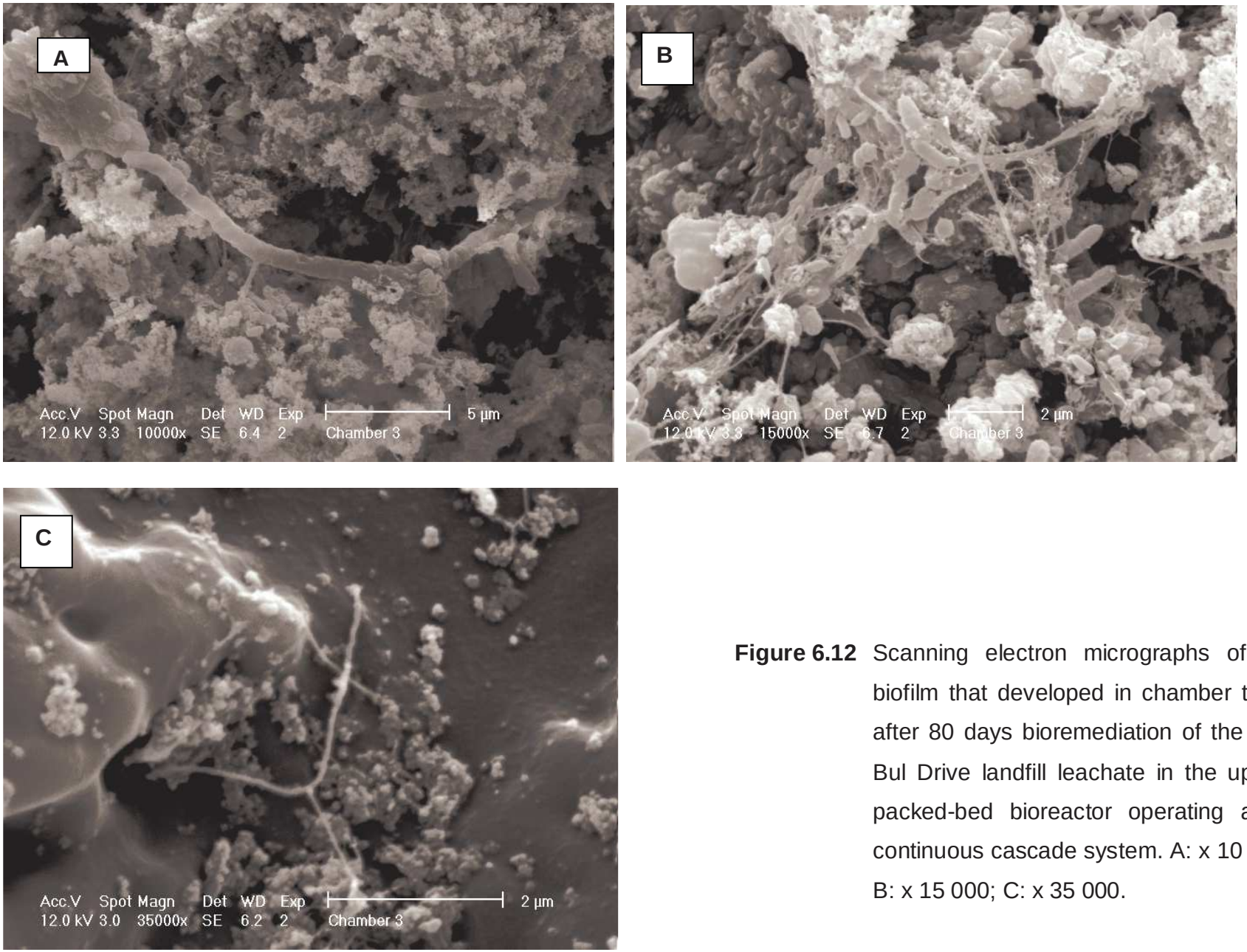


Figure 6.12 Scanning electron micrographs of the biofilm that developed in chamber three after 80 days bioremediation of the Bul-Bul Drive landfill leachate in the upflow packed-bed bioreactor operating as a continuous cascade system. A: x 10 000; B: x 15 000; C: x 35 000.

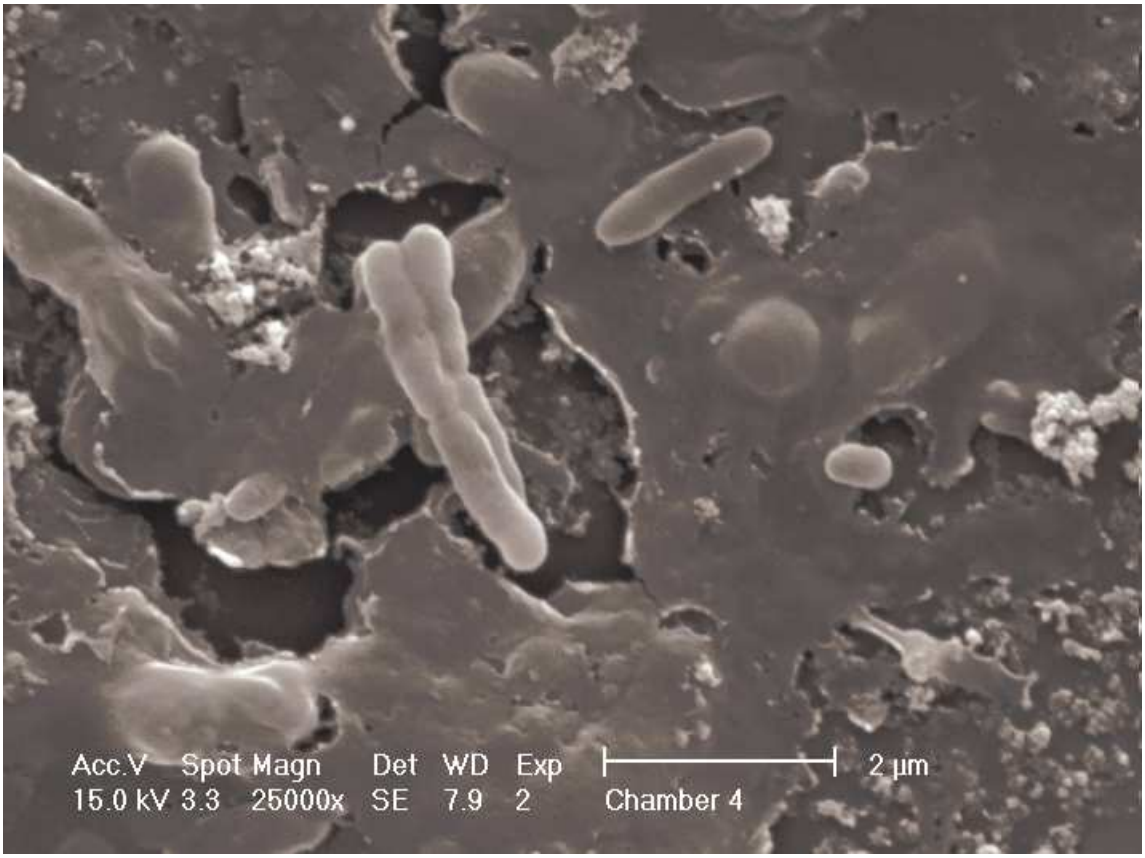


Figure 6.13 Scanning electron micrograph of the biofilm that developed in chamber four after 80 days bioremediation of the Bul-Bul Drive landfill leachate in the upflow packed-bed bioreactor operating as a continuous cascade system (x 25 000).

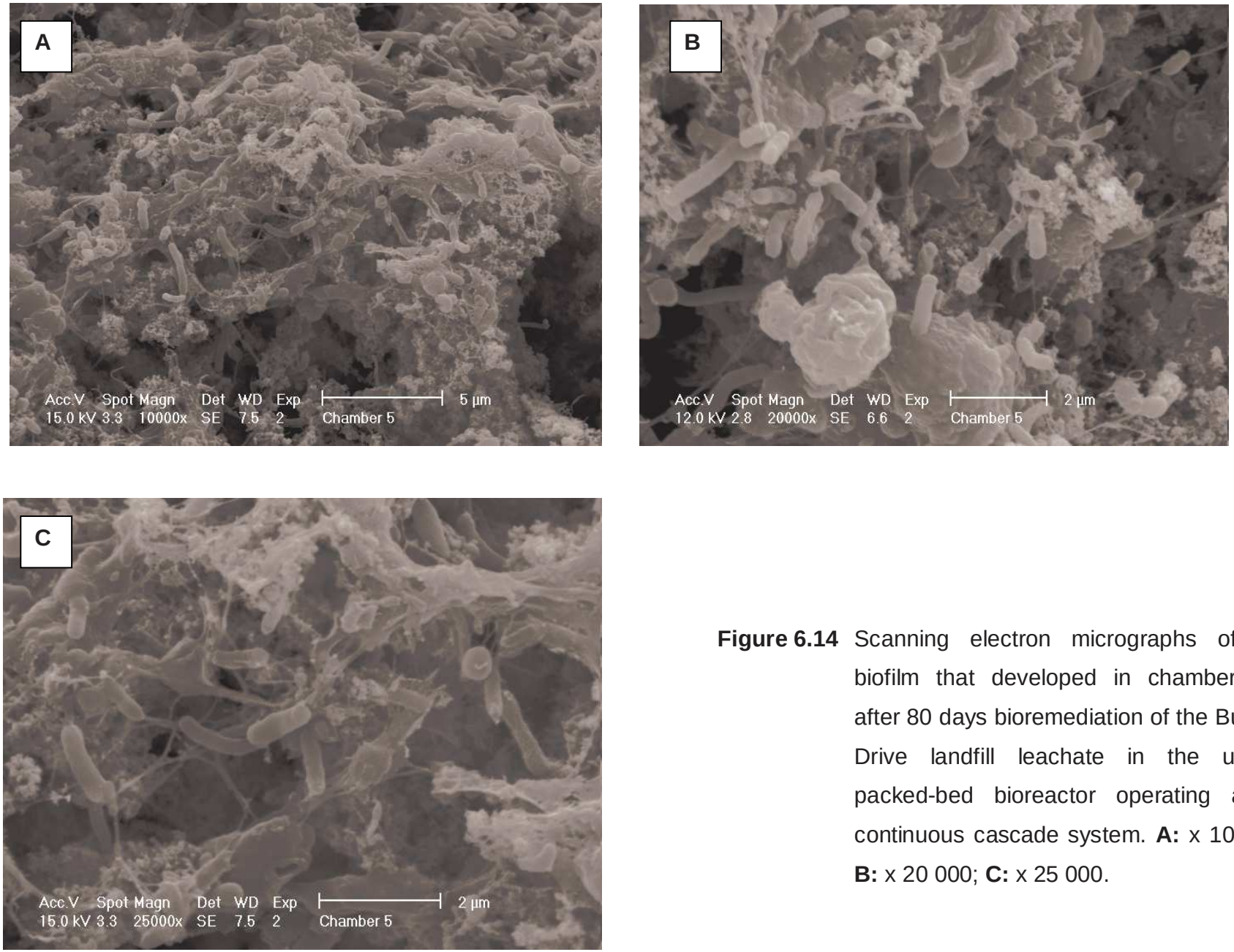


Figure 6.14 Scanning electron micrographs of the biofilm that developed in chamber five after 80 days bioremediation of the Bul-Bul Drive landfill leachate in the upflow packed-bed bioreactor operating as a continuous cascade system. **A:** x 10 000; **B:** x 20 000; **C:** x 25 000.

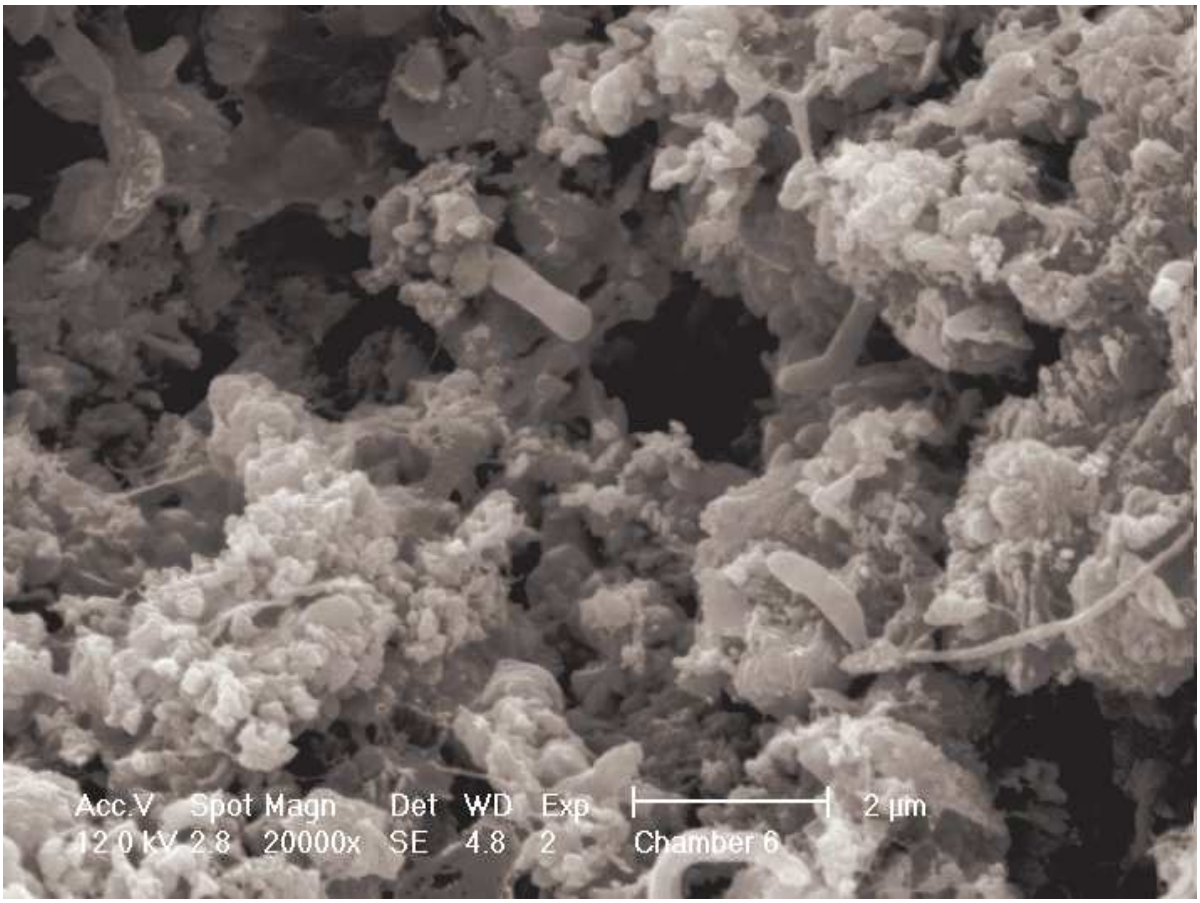


Figure 6.15 Scanning electron micrograph of the biofilm that developed in chamber six after 80 days bioremediation of the Bul-Bul Drive landfill leachate in the upflow packed-bed bioreactor operating as a continuous cascade system (x 20 000).

6.5.4 Discussion

6.5.4.1 Operation of the bioreactor as a cascade system with a recycle loop

Both BOD and COD were successfully removed when the leachate was recycled after passage through all six chambers in series. The reduction in COD concentration correlated well with the results obtained with the tank bioreactor when it was operated in parallel, using leachate from the same landfill site **(Sections 5.3.3 and 6.4.3)**. This indicated that the system is capable of producing consistent results over time, even when temporal fluctuations in leachate composition occur.

The decrease in the BOD:COD ratio over time indicated that the microorganisms in the bioreactor were actively degrading the biologically available compounds which constitute the BOD in the leachate. In contrast, the COD concentration includes substances which are biodegradable as well as those that are recalcitrant. Such xenobiotic compounds may only be removed when physico-chemical processes are used in combination with biological treatment. This explains why the BOD removal efficiency (73 %) is so much higher than the COD removal efficiency (49 %).

More COD was removed during the first week of the experiment than during the second. This is characteristic of a batch system, as most of the compounds that are relatively easy to metabolise will be quickly degraded during the initial stages of biofilm development, leaving the more recalcitrant compounds to be broken down more slowly by fewer, more specialised microorganisms. Some of the COD constituents may never become available as nutrients to any of the microorganisms present in the system and will not be biologically removed (Gourdon, Comel, Vermande and Veron, 1989). However, this trend was not

observed when removal of BOD was considered. This is difficult to explain, especially as the BOD:COD ratio, which can be used to assess biodegradability (Fan, Shu, Yang and Chen, 2006), was lower after seven days than it had been at the start of the experiment.

The noticeable differences in initial BOD concentration in the six chambers could be attributed to the way in which the bioreactor was filled. Leachate and inoculum were pre-mixed before being pumped through the system, so some of the bacterial flocs present in the activated sludge, and other suspended solids, may have been trapped on the plastic bioballs in the first chambers, thus reducing the initial BOD flowing into the subsequent chambers of the bioreactor.

6.5.4.2 Operation of the bioreactor as a continuous cascade system

As noted above, the influent COD and BOD concentrations changed significantly over the experimental period, although all the raw leachate used was collected from the landfill site at the same time. The 220 *l* drums used for storage were not mixed until they were used so some settling of particulate matter may have occurred, thus reducing the COD of the influent leachate in successive drums. The stored leachate would also have contained free-living indigenous microorganisms that may have degraded some of the more bioavailable compounds. In contrast, the influent BOD concentrations did not decline uniformly, but rather fluctuated in a cyclical manner with each 'cycle' corresponding to a switch in drum. The storage drums were sealed and not aerated and the lack of oxygen may have restricted the amount of biodegradation that occurred compared to that in the drum in use. The drum in use would have been exposed to air and undergone some mixing, especially as the leachate level therein became lower and it would thus have been a more favourable environment for microbial activity. Biodegradation occurring within the drum as

the leachate was removed would cause the BOD concentration of the influent stream to chamber one to decline.

COD removal efficiencies ranged from a high of 62 % at the beginning of the experiment to a low of 33 % towards the end of the experimental period. However, the average removal efficiency of 45 % compares favourably with the results of other investigations on the biological treatment of 'difficult-to-remediate' liquids such as landfill leachate. This is especially gratifying since it was achieved without the addition of nutrients and without either temperature or pH control, all factors that can add considerably to treatment costs.

The COD removal pattern displayed across the six chambers of the reactor suggested that it may be possible to use either a bioreactor with fewer chambers, or a faster flow rate. Little reduction occurred in chambers five and six, and their presence therefore added little value to the efficacy of the bioreactor. Klees and Silverstein (1992) studied carbon and nitrogen removal in a staged RBC and noted that in the final stages the biofilm was thinner due to the reduced carbon concentration. They found less diversity of microorganisms, and many dead cells, as well as cell debris; the population was also more prone to predation. The specific growth rate of autotrophs relative to that of heterotrophs increased which accounted for the poor carbon removal exhibited at this point in the system (Klees and Silverstein, 1992).

A similar effect may explain the poor performance of chambers five and six in the upflow packed-bed bioreactor. This section of the bioreactor was susceptible to precipitate build-up and/or clogging, and this caused channelling and short-circuiting, which reduced the exposure of the biofilm to an already recalcitrant waste stream. Similar problems were reported by Keenan *et al*. (1993), as being responsible for reduced COD removal and ultimately this caused the recycle pump to fail. They proposed that the activity of sludge microorganisms decreases when the fixed solids content reaches a critical value, probably because of mass-

transfer limitations due to formation of inorganic precipitates and the lack of contact between viable bacteria and their substrate (Keenan *et al*., 1993). The present study did not focus on heavy metal removal, but it may be that if the concentration of these contaminants is high, some form of pre-treatment may be required in order to reduce precipitate formation, which would occur especially in those zones of the bioreactor that developed anoxic or even anaerobic regions.

The pattern shown when the bioreactor was operating as a continuous cascade system (**Section 6.5.3.2)** indicates the development of specialised biofilms or microbial communities that are adapted to the changing composition of the leachate in different chambers. However, this effect does not occur in each successive chamber, but it seems that the microbial populations that became established in chambers one, two and four differed from one another. This is deduced from the low cumulative reduction recorded in chamber three and after the leachate has passed through chamber four. The populations in these groups of chambers are therefore probably quite similar, although in the latter case, as mentioned above, there may simply be very few or no available carbon sources for heterotrophic organisms remaining by the time the waste stream flows through the last two chambers. It may be that some of the constituent compounds in the leachate can be broken down only by inducible enzymes, which are produced as the microorganisms in each chamber become accustomed to the characteristic composition of its influent. Carbon limiting conditions promote enzyme induction and place the population under a selective pressure to metabolise recalcitrant substances (Enzminger, Robertson, Ahlert and Kosson, 1987). Such conditions would be more likely to occur in the latter chambers of the bioreactor. Some compounds may only be present in the latter stages of the system, being waste- or by-products of metabolic reactions which have taken place in previous chambers. The products of incomplete microbial mineralisations such as co-metabolic transformations are often degraded by other microorganisms (Enzminger *et al*., 1987). The effect discussed here may

also be related to the biphasic patterns of COD removal observed in previous experiments where the bioreactor was operated as a batch type system.

BOD reduction was relatively consistent, and the 89 % removal efficiency achieved illustrated that biological processes are important in treating landfill leachate. However, the fact that some of the BOD was not removed suggests that the performance of the system could be enhanced by the addition of allochthonous microorganisms capable of metabolising the compounds that make up this more recalcitrant component of the biologically degradable material in the leachate.

The system considered as a whole performed better than could have been expected, considering its purely biological nature. This is clear from the fact that the amount of COD removed was greater than the influent BOD concentration (often by a considerable margin), which is, theoretically, the highest reduction that could be predicted. However, this conflicts somewhat with the BOD concentration of the treated effluent, which was still above 100 mg.l^{-1} at the end of the process.

The complexity and amount of biofilm that formed in each of the six chambers **(Figures 6.10 – 6.15)** reflected the COD removal efficiencies obtained across the bioreactor. The structure of the biofilm and the presence of different morphotypes indicated that the microbial population in the first chamber was noticeably different from that in the next two chambers.

The change in COD removal from the leachate that occurred in chamber four also correlated with a change in microbial type and abundance from that in chamber three **(Figures 6.13 and 6.12)**. The complexity of the biofilm in chamber five was unexpected, however, as the cumulative COD reduction was not significant in this section. The sparse biofilm with substantial extracellular matrix in chamber one may indicate that the high concentrations of COD and ammonia

in the raw leachate affected the microbial population and encouraged the secretion of protective substances as suggested by Sponza (2003) who noted that active secretion of EPS increased when microorganisms were under stress due to the presence of toxic compounds. These microorganisms would have removed some of the pollutants, either by metabolising them in the case of biodegradable organics, or via adsorption onto the biofilm for both xenobiotic organic and inorganic materials (Flemming and Wingender, 2001). This would make the waste stream passing into the next chamber more tolerable for a wider physiological range of microorganisms. This may account for the lack of a visible extracellular matrix and the increased morphological diversity, including filamentous bacteria in chambers two and three that was observed in the present investigation. Cao and Alaerts (1995) reported data that suggests that filamentous bacteria prefer the lower substrate concentrations that exist in the later chambers of multi-chambered bioreactors.

Availability of nutrients may have been limited by the time the leachate passed through the fourth chamber. Additionally, most of the organic compounds remaining would be more recalcitrant than those degraded by the microbial communities in the earlier chambers of the bioreactor. This can be deduced from the smaller microbial population, reduced variety of morphotypes, increased secretion of exopolymeric substances, and the trends in COD degradation across the cascade system.

The results obtained with the bioreactor operating as a continuous system are comparable with those reported by other researchers using various types of bioreactor, and some selected examples are discussed below. Aghamohammadi *et al*. (2007) investigated the aerobic biodegradation of semi-aerobic leachate in continuous flow laboratory-scale activated sludge reactors, both with and without the addition of powdered activated carbon (PAC). Although the BOD:COD ratio of their samples was lower than that of the Bul-Bul leachate, the initial COD concentration was also significantly lower. The maximum COD removal efficiency

obtained without PAC addition was 29 % and with PAC addition, 46 % removal was achieved. The performance of the system used in the present investigation was thus relatively good when compared to that of the unamended system used by Aghamohammadi *et al*. (2007), especially when one considers that pH was also controlled in their reactors. They also noted that even the PAC augmented bioreactor did not produce a treated effluent that would meet the local discharge standards and could therefore be used only as part of a more comprehensive treatment system (Aghamohammadi *et al*., 2007).

The microorganism-attached activated carbon fluidised bed process used by Imai et al. (1993) removed 60 % of the refractory organics and 70 % of the total nitrogen from a mature landfill leachate, but a considerable proportion of the reduction was due to adsorption rather than biodegradation. Also their experiment was conducted on a very small scale as mentioned in **Chapter 1** (Imai *et al*., 1993).

Bae *et al*. (1999) reduced the BOD of a leachate from a coastal landfill site by 98 % using an activated sludge process. However this leachate was highly biodegradable with a BOD:COD ratio of 1.3, which is much higher than the corresponding ratio for the Bul-Bul leachate (with an average of 0.26 over 80 days).

A leachate treatment system consisting of biological nitrification and denitrification, Fenton's treatment and a final activated sludge phase achieved an excellent 97 % COD removal. However, this system was temperature controlled at 35^0C (which adds a significant energy cost), and was run at a relatively small scale with an effective volume of four litres (Bae *et al*., 1997). In contrast to the technology described in this thesis, Bae *et al*. (1997) combined both biological and chemical approaches to obtain their results.

Borzacconi *et al*. (1999) used an upflow sludge blanket denitrifying reactor to remove carbon and nitrogen from sanitary landfill leachate and achieved COD removal efficiencies as high as 82 % at bench scale (a reactor volume of 4.6 *l*). Their results were, however, highly variable and the lowest recorded removal efficiency was 10 %. The system was heated to 30^0C and potassium nitrate was added to ensure that an ideal COD:NO_3-N ratio was maintained throughout the operation (Borzacconi *et al*., 1999). It was also quite different from the technology developed here as temperature control and nutrient amendment were used, which are not cost-efficient at a larger scale.

Henderson and Atwater (1995) treated landfill leachate with a high ammonia concentration of 2140 mg.l^{-1} and a relatively low BOD:COD ratio of 0.14, using a pre-denitrifying anaerobic filter and a rotating biological contactor. They achieved an average BOD removal efficiency of 92 % and a COD removal efficiency of 49 %. These authors also noted that it is typical for the amount of COD removed to exceed the BOD of the leachate (Henderson and Atwater, 1995), as found in the current study.

A constructed wetland achieved an average COD removal efficiency of 68 % over a five year period, but the average BOD removal efficiency over the same period was much less at only 46 %. Ammonia was also monitored, and an average reduction of 81 % was measured. There was, however, a high variability in the results obtained each year (Bulc *et al*., 1997). The results achieved in the current work compare favourably with the results obtained by the authors mentioned in the above paragraphs.

A combined biological and chemical system consisting of alternating fluidised bed reactors and ozonation units achieved a very high COD removal of 98 %, but this was only attained after several treatment phases and the initial COD of the leachate was low (600 mg.l^{-1}) compared to the leachate used in the current research. Ozonation on its own removed only 20 % of the COD present, whereas

even an initial biological unit managed to attain a COD removal efficiency of 40 % without ozonation (Karrer *et al*., 1997). This emphasises the role of biodegradation in the treatment of landfill leachate, and illustrates that even when physicochemical approaches are used, it is usually preferable to include some form of biological treatment as well.

6.6 CONCLUSION

The upflow-packed bed bioreactor design was successfully used to bioremediate leachate from the semi-hazardous Bul-Bul Drive landfill site. The research performed showed that the bioreactor was not only efficacious as a batch-type system, but was also applicable to continuous treatment of an effluent that is constantly generated, usually in relatively large volumes. Its operational volume (132 *l*) was almost large enough to be considered pilot-scale, which would be an advantage if scale-up to commercial size was undertaken. In contrast, many of the bioreactors used by other researchers have very small operational volumes, making it more difficult to predict performance when larger volumes are treated. Although plastic bioballs performed best as the solid support matrix when small volumes were treated, pine bark may still be an appropriate option for a more cost-effective system designed for use in developing countries, particularly if its durability could be increased. The effect of the antimicrobial compounds that are known to be present in pine bark also needs to be investigated further before it can be used in large-scale systems.

CHAPTER 7

CONCLUSION: LEACHATE TREATMENT USING A NOVEL PACKED-BED BIOREACTOR

The bioreactor design used in this study was intended to remove organics and ammoniacal nitrogen from landfill leachate, which is a highly variable and heterogeneous wastewater. Several different leachates were treated in batch mode with the system configured to create six chambers, and COD removal efficiencies ranged from a minimum of 23 % for the Marianhill leachate to a maximum of 63 % for the Bul-Bul Drive leachate over the course of these experiments. The average COD removal efficiency of all the experiments carried out with the bioreactor operating in this mode was 42 %; however, it is important to remember that these experiments were carried out under different conditions and lengths of time. The results obtained using the Shongweni landfill leachate have not been included in this overview as it was concluded that this effluent was not suitable for biological treatment. When treating leachate from the Bul-Bul Drive landfill site, the bioreactor achieved a lower COD removal efficiency when operated as a continuous cascade system (37 %) as opposed to a cascade system with recycle (49 %).

Although it is difficult to compare treatment technologies for this type of wastewater because a variety of different factors must be taken into account to determine the potential of a specific design, data from other systems can be used to evaluate the success of the method being investigated. Reported COD removal efficiencies from leachates cover a wide range from as little as 6.7 % removal using a 45 *l* SBR to treat an alkaline leachate with an initial COD concentration of 1183 mg.l^{-1} up to 98 % removal in an activated sludge reactor treating an alkaline leachate containing 7439 mg.l^{-1} COD. In a recent review of

landfill leachate treatment, the average COD removal efficiency of all the relevant aerobic biological treatment systems surveyed was 64 % (Renou *et al*., 2008). Although this is somewhat higher than the average percentage removed by the packed-bed bioreactor used in this research, the system can nevertheless be considered successful in the removal of carbonaceous matter from landfill leachate. This becomes more apparent when the operating parameters of most of the systems discussed in the abovementioned review are considered.

One of the principal benefits of the system studied in this research is that there is no need to supplement the waste stream prior to treatment, which contrasts with many other technologies described in the literature. Temperature must often be controlled and maintained close to 30 ^{0}C, creating a need for significant energy input. Leachates may also need to be diluted, or their pH corrected to avoid destabilising the microbial population in the bioreactor (Baker and Herson, 1994a; Bollag and Bollag, 1995; Renou *et al*., 2008). Many studies do not consider the implications of treating the large volumes of leachate generated by landfill sites; the technologies described would often be prohibitively expensive or require significant technical expertise (which is rarely available) if applied at full scale. The approach taken throughout the current investigation is thus a novel one and it demonstrates that a technologically undemanding, low-cost bioremediation strategy can be successfully applied especially where, as in this case, autochthonous microorganisms from the effluent itself are used (some additional inoculum in the form of activated sludge was added to supplement biodegradative activity). This makes the system much simpler to operate with little maintenance required, which is appropriate for countries such as South Africa where there are not only financial constraints, but also a shortage of skilled labour in the waste treatment sector. The bioreactor used in this project was specifically designed in the hope of providing a solution to these problems and the results show that it successfully treated several of the leachates generated in local landfills.

Gotvajn, Tišler and Zagorc-Končan (2009) compared various treatment strategies for industrial leachate from a mature landfill where waste from a large tannery operation is deposited. They reported that they were unable to meet the regulatory requirements for release into surface waters, and no method was sufficiently effective on its own. Similarly, the technology used in the current work could be used to produce an effluent suitable for discharge into a local wastewater treatment facility rather than surface water, or it could be combined with other physicochemical technologies to obtain increased COD removal efficiencies. The abovementioned authors reported that Fenton's oxidation was the most effective treatment for removing organics and a COD removal efficiency of 86 % was reached. This pilot biological treatment plant was used only to remediate diluted leachate of up to 30 % volume and was able to remove 80 % of the COD and 90 % of the BOD (Gotvajn *et al*., 2009). In contrast, the system presented here was able to cope with full strength leachate which had not been pretreated in any way. Larger volumes of leachate could therefore be treated at one time. Costs associated with storage and pretreatment were also avoided, which would be a significant advantage when applying the technology *in situ* on a larger scale. Although the COD removal efficiency reported by Gotvajn *et al*. (2009) is higher than that achieved in the current investigation, the BOD removal efficiency is very similar (89 % reduction was recorded in the experiment treating Bul-Bul Drive leachate continuously).

Gotvajn *et al*. (2009) also investigated nitrogen removal and concluded that air stripping at a high pH (11) was the most effective strategy. Ammoniacal nitrogen was reduced by 80 % using this method. However, only 35 % of the ammonia was removed without the addition of concentrated NaOH at pH 8. In addition, nitrification was severely inhibited if the leachate was not diluted. The bioreactor used in the present study compares favourably with this example and was able to remove between 87 and 98 % of the ammonia in the slightly alkaline undiluted Umlazi leachate (also pH 8). Carbon and nitrogen removal could therefore take place simultaneously without the use of sophisticated, expensive equipment.

The current study is also particularly relevant because many sites in South Africa, including the one at Bul-Bul Drive, do not have a treatment plant and leachate must be disposed to sewer in small amounts at considerable cost depending on the concentration of COD and other contaminants. This recurrent expense could be reduced by installing a bioreactor such as the one investigated in the current study to treat leachate before disposal without incurring significant construction and maintenance costs. A potential environmental and human health hazard is created by the large volumes of raw leachate that are typically stored on landfill sites in KwaZulu-Natal. A bioreactor that treats leachate as it is generated would reduce the risk associated with this practice by decreasing leachate strength and increasing the amount of effluent that can be disposed to sewer within a given time period.

The present work was largely preliminary but could be expanded in future studies in order to further improve and optimise the functioning of the system. For example, it would be useful to investigate nitrogen removal when the bioreactor is operating as a continuous system. Such an investigation would be very valuable as it could lead to the construction of a full-scale system that could effectively remove both the carbonaceous contaminants and the typically high concentrations of ammonia occurring in landfill leachate. A full scale version of the bioreactor could then be installed *in situ* to determine whether it could deal with fluctuating leachate quantities and composition in real time. There are a number of landfill sites in the Kwazulu-Natal area where this could be done.

The bioreactor could also be used to investigate microbial ecology; Daims, Taylor and Wagner (2006) suggest that systems used for wastewater treatment are highly amenable for ecological studies because they are chemically and physically well-defined, they can be manipulated for experimental purposes and modern molecular techniques can easily be used to examine complex microbial communities in such reactors. This approach could be used to improve the

performance of the bioreactor, as well as to target specific pollutants found in particular effluents. For example, future research could focus on the ammonia removal that occurs in the bioreactor – it is important to determine the role of the various Bacteria and Archaea involved in the nitrogen cycle to clarify the fate of ammonia in the system. Cultivation independent approaches have recently been used to assess biodiversity and population dynamics of ammonia oxidisers and denitrifiers in a membrane bioreactor, revealing many gene sequences that are not closely related to those in any classified microorganisms (Wan, De Wever, Diels, Thoeye, Liang and Huang, 2011). Such techniques may provide valuable information about the microorganisms in this bioreactor. It is also possible that eukaryotic microorganisms such as yeasts play a part in biodegradation of organic compounds. This could also be explored in future ecological studies.

One of the most important and unique aspects of this work was its localised nature. All the components of the bioreactors and the solid support matrices that were used in the current study were sourced within the areas served by the landfill sites from which leachate was collected. This has a number of advantages. The use of low cost and/or waste materials that are produced in the relevant region makes construction and maintenance of leachate treatment facilities more affordable while benefiting the local economy. The environmental impact of such a plant would also be minimised by using either recycled materials or those that would otherwise need to be disposed (probably to landfill).

Although there is much potential for further work on the system, the objectives of this work **(Section 1.8)** were met. The effect of various aspects of bioreactor design such as the nature of the solid support matrix, aeration level and recycle rate on bioremediation efficiency were investigated and optimised for the treatment of local landfill leachates. The performance of the system as a continuous treatment strategy was assessed and satisfactory COD and BOD removal efficiencies were achieved.

The work described illustrates that a low-cost plant that is relatively easy to maintain can be used to achieve significant levels of pollutant removal from effluents to facilitate disposal into conventional wastewater treatment plants that may otherwise be disrupted. The leachates from several landfill sites in Kwazulu-Natal were successfully bioremediated using the technologically undemanding, but effective novel upflow packed-bed bioreactor designed and assessed in this study. The unique approach taken throughout the investigation led to the development of an inexpensive treatment technology specifically designed to overcome the problems of leachate generation and treatment in a localised area. Other landfill sites, or any leachate-generating operations, could benefit by using such a locally determined treatment strategy.

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