RHODES UNIVERSITY

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Investigation into the biological removal of sulphate from ethanol distillery wastewater using sulphate-reducing prokaryotes

DEPARTMENT OF BIOCHEMISTRY, MICROBIOLOGY AND BIOTECHNOLOGY

MASTER OF SCIENCE THESIS

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Investigation into the biological removal of sulphate from ethanol distillery wastewater using sulphate-reducing prokaryotes

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ABSTRACT

Ethanol production wastewater is known to be toxic, and is not easily biodegradable. It also consists of a variety of coloured components adding to the complex composition of this wastewater. Disposal of this wastewater into water courses is not recommended and yet is performed all over the world.

Investigation of this wastewater found that there was a high concentration of sulphate which, in the presence of sulphate-reducing prokaryotes can cause sulphide corrosion of cement. The concentration of sulphate in the wastewater was approximately 2770 mg/L. It was also found that the wastewater pH was very low and discharge of the wastewater into the wastewater treatment works caused a negative impact on the overall quality of the final wastewater discharged to sea. It was found using FISH techniques that there were no sulphate-reducing prokaryotes present in the wastewaters but that a sulphate-reducing population existed on the sewer wall. An anaerobic contact process was designed to treat this wastewater targeting sulphate reduction to sulphide, to be converted into elemental sulphur and to increase the wastewater pH.

The process did not achieve this aim and only approximately 20-30 % reduction in sulphate from the wastewater was achieved with little to no change in the pH. A 95 % reduction in sulphate concentration was needed in order to reach acceptable discharge limits. Sulphate reduction could not be carried out, even under ideal laboratory conditions. It was found that the barrier causing the digester failure was the high concentration of phenols present in the wastewater (3.3 g/L) together with the production of high concentrations of volatile fatty acids (on average 13 g acetic/L). These two components are known to cause digester failure, especially phenols, and phenols are usually only degraded by fungal species. It was concluded that the wastewater itself was not amenable to this method of biological treatment.

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LIST OF ABBREVIATIONS

AAS	Atomic absorption spectrophotometry
ACP	Anaerobic contact process
AMD	Acid mine drainage
APHA	American Public Health Association
BOD	Biological oxygen demand
COD	Chemical oxygen demand
CSIR	Council for Scientific and Industrial Research
CSTR	Continuously stirred tank reactor
DWAF	Department of Water Affairs and Forestry
EPS	Extracelluar polymeric substance
FAU	Formazine attenuation units
FISH	Fluoresent *in situ* hybridisation
HRT	Hydraulic retention time
MLSS	Mixed liquor suspended solids
MPB	Methane producing bacteria
rRNA	Ribosomal RNA
SCWO	Supercritical water oxidation
SOB	Sulphide oxidizing bacteria
SRP	Sulphate-reducing prokaryotes
SS	Suspended solids
TDS	Total dissolved solids
TOC	Total organic carbon
TSS	Total suspended solids
UASB	Upflow anaerobic sludge blanket
VFA	Volatile fatty acid
WISA	Water Institute of Southern Africa
WRC	Water Research Commission of South Africa
WWTW	Wastewater treatment works

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Chapter 1
Literature Review

1.1 Ethanol Production

The manufacture of ethanol is a well established fermentation process of global economic importance. For example, 200 000 m^3 of ethanol spirits were produced by thirteen companies in Korea during a single year (1992). The conventional production process consists of two stages: fermentation and distillation, both of which generate wastewater. The distillation process usually produces stillage (as a waste product) at the bottom of the distillation tower (Kim *et al*., 1997). Distillation and fermentation are closely linked, where fermentation is a biochemical process that uses microbes to produce products such as baker's yeast, monosodium glutamate and ethanol, amongst others (Yeoh *et al*., 2000). The cane molasses-based distillery produces rectified spirits for both industrial purposes and human consumption. This industry is said to be one of the most polluting industries (Nandy *et al*., 2001).

Some large-scale distilleries are integrated with sugar mills. There are three kinds of waste products generated by the sugar mills. They are bagasse (the solid residue from crushing the sugarcane), pressmud (a solid generated from juice clarification) and molasses (the final residue after sugar crystallization). Bagasse can be used as fuel for boilers or in the paper manufacturing industry and molasses is used as a raw material for distillation in the alcohol production industry (Nandy *et al*., 2001). Countries like Korea, India and Malaysia produce large amounts of alcohol using molasses. For example, in India there are 285 distilleries producing 270 000 m^3 of alcohol per annum (Joshi, 1999). This production of alcohol in turn yields a vast amount of wastewater. For every 270 000 m^3 of alcohol produced by distilleries in India 400 000 m^3 of wastewater is also generated (Joshi, 1999). Hence a distillery which produces 100 m^3 of ethanol per day discharges approximately 150 m^3 of wastewater.

The distillery at which this study was carried out produces alcohol from fermented molasses generated from sugar mills. Figure 1.1 is a flow diagram representing the production process.

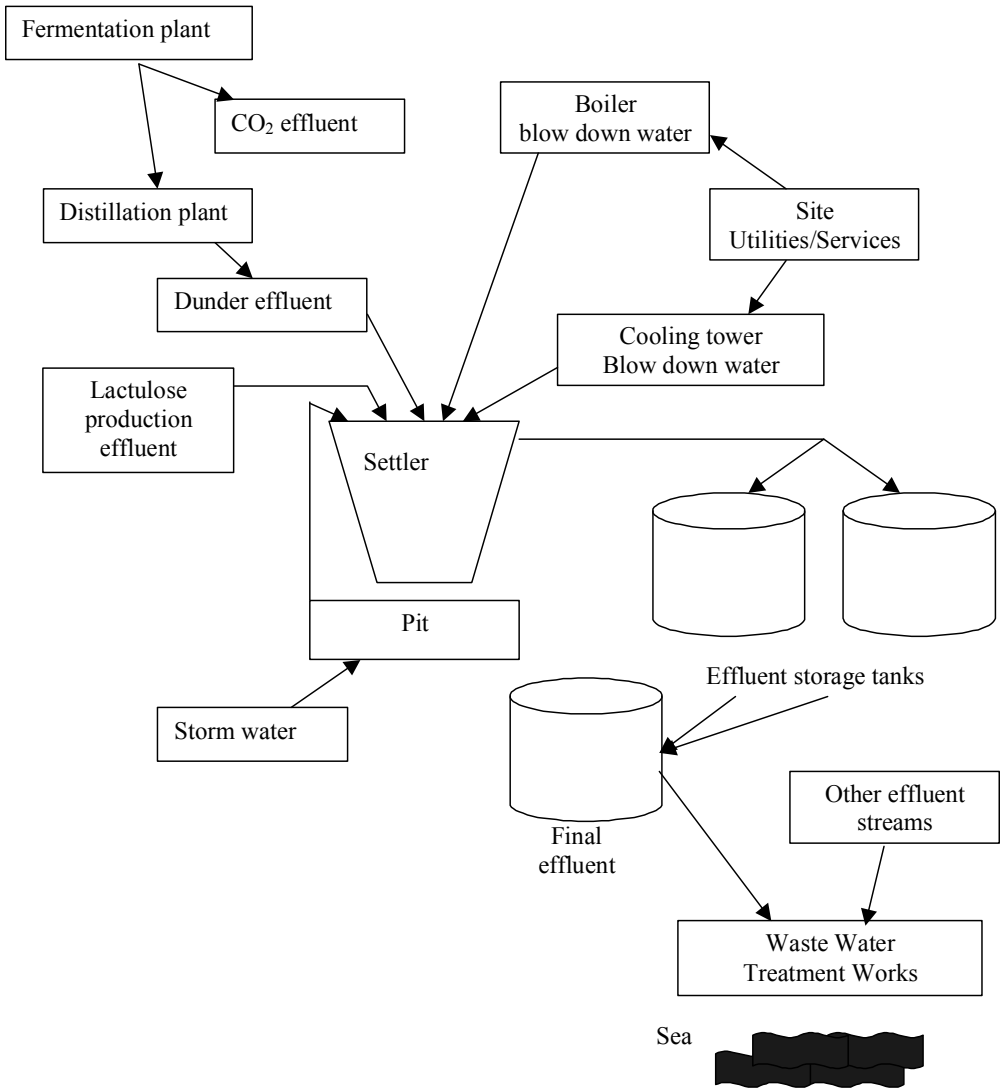

Figure 1.1 Flow diagram of wastewater flows at the industrial site.

There is a production plant that manufactures lactulose situated on the industrial site. The wastewater generated from that plant has a high sulphate content with trace amounts of sugars. Dunder wastewater is generated from the combination of molasses and water with dilute sulphuric acid and yeast cream. These are all mixed in a holding tank and then

pumped into a vat with a controlled temperature of 34 °C. Defoamer is added at this stage and CO_2 is removed. This liquid is pumped first into a tank and then through strippers in which ethanol and water vapour are removed; the remainder is the dunder. The dunder is then held in a settler along with the other wastewaters produced by the plant, as shown in Figure 1.1. A subcontracted company desludges the settler. The clarified effluent is pumped into storage tanks and then sent to the municipal wastewater treatment works (WWTW). This effluent is combined with other industrial effluents from the surrounding area and partially treated domestic wastewater and finally discharged to sea. The effluent from this particular WWTW is unique as the wastewater that is sent to the sea consists not only of the water and waste generated from the distillery process but also waste from a pharmaceutical production plant, which has its own set of characteristics.

If wastewater generated from molasses-based distilleries is disposed of untreated it can cause increased stress on watercourses, which leads to damaging effects on the aquatic environment. Distillery wastewater is characterised by its deep brown colour, low pH, high temperature, high concentrations of dissolved organic and inorganic matter and high suspended solids (Nandy *et al*., 2001). Due to the high biochemical oxygen demand (BOD), anaerobic treatment systems have been most effective in treating this wastewater (Nandy *et al*., 2001). Table 1.1 shows the range of values for characteristics of molasses distillery wastewater from the literature (Harada *et al*., 1996; Kim *et al*., 1997; Pikaev *et al*., 2001; Nandy *et al*., 2002; Jiménez *et al*., 2003; Sirianuntapiboon *et al*., 2004).

Table 1.1 Ranges of some important wastewater characteristics.

Parameter	**Range**
pH	3.5-5.7
BOD (g/L)	7-95
COD (g/L)	15-176
Temperature °C	71-105
Suspended Solids (g/L)	1-13
Colour (A_{475}-AU)	84.4

1.2 Wastewater from ethanol distilleries and problems associated with treatment

Waste and wastewater treatment is an important and urgent subject with regards to environmental protection all over the world. The development of an inexpensive reliable treatment technology for industrial or municipal wastes is highly desired (Goto *et al*., 1998). Industrial effluents contain high concentrations of toxic substances, for example sulphide, heavy metals and nitrate, and this is reason for great concern. The discharge of such wastewater into surface waters leads to mineralisation and corrosion potential of receiving waters (du Preez and Maree, 1994).

Ethanol distilleries face major problems with regards to environmental issues, due to the composition of the wastewater. Molasses is a very important product as it is not only of commercial value because of the carbon used in fermentation, but it is also traditionally used as a biofertiliser and feed for domestic animals (Underfolker and Hickely, 1954; Chuang and Lai, 1978). The use of molasses as a raw material is problematic as it gives rise to the high amount of coloured substances in the wastewater. Melanoidin, which is the main coloured component, is difficult to degrade by usual biological treatment processes. Melanoidins are high molecular weight polymers formed through a set of reactions that takes place between carbohydrates and amino compounds during a Maliard reaction (FitzGibbon *et al*., 1998; Peña *et al*., 2003). Melanoidin accounts for the high chemical oxygen demand (COD) in the wastewater, which is a major problem when treating wastewater (Sirianuntapiboon *et al*., 2004). Most common wastewaters containing high organic loads can be treated using conventional biological methods, for example activated sludge. However, with this particular wastewater the nitrogenous brown molasses colour remains (Nakajima-Kambe *et al*., 1999) and may even increase due to the repolymerization of the coloured compounds during biological treatment (Peña *et al*., 2003). Other contributors to the colour of the wastewater are phenolics from feedstocks, caramels from overheated sugar and furfurals from acid hydrolysis. These, including melanoidins are known to inhibit fermentation and rumen microbes and melanoidins have been found to be mutagenic (Wilkie *et al*., 2000).

Stillage characteristics and production are highly variable and dependent on the feedstocks. Wash water used around the production plant, cooling tower water and boiler blow down water may all be added to the stillage, which adds to the variability (Wilkie *et al*., 2000). Of all the different feedstocks used in ethanol production cane molasses has the highest values for biological oxygen demand (BOD), COD and COD:BOD ratio as well as sulphate, potassium and phosphorous concentrations. The crystallisation and evaporation of cane juice results in the high concentration of sugars in the molasses which increases the non-fermentable organics content. This remains in the stillage, adding to the COD and increasing the COD:BOD ratio. There are also many organic components in the stillage. The principal compounds in cane molasses distillery stillage are, lactic acid, glycerol, ethanol and acetic acid. The presence of a fulvic acid component, which is similar to fulvic acid in soils and sludge, was also noticed in cane molasses stillage but the C:N ratio was higher (Wilkie *et al*., 2000). There are also trace amounts of amino acids found in the stillage. In most ethanol distilleries, heavy metals are found in the stillage at concentrations above the specified limits, most commonly chromium, copper, nickel and zinc. These heavy metals often originate from the feedstock or chemicals used during ethanol production. However, corrosion of pipes, tanks and heat exchangers are expected to add to the heavy metals found in the stillage. Often the processing equipment is made of corrosion-resistant alloys, especially for acid hydrolysis, in order to resist high temperatures and acidic conditions. These alloys may leach into the feedstock, resulting in an increase in heavy metals concentration (Wilkie *et al*., 2000).

Despite efforts to improve quality standards or treat the wastewater it still often finds a way into watercourses. Due to the high BOD levels in this wastewater this leads to depletion of dissolved oxygen, and lower pH of receiving streams, increased organic loads and malodours. Also the colour prevents penetration of sunlight, which in turn affects photosynthetic activity of aquatic plants. The high COD values result in eutrophication of contaminated waters (Bernado *et al*., 1997; FitzGibbon *et al*.; 1998; Peña *et al*., 2003). Land addition of the wastewater can act as a soil pollutant and has the

ability to prevent seed germination, reduce the alkalinity of the soil, and decrease concentration of important minerals and damage crops (FitzGibbon *et al*., 1998).

The methods used by researchers in the past for treatment of the wastewater generated from ethanol distilleries have been both chemical and biological. Some of these methods include aerobic or anaerobic treatment, trickling filters, lagoons, evaporation-condensation and direct dispersion onto soil (Maiorella *et al*., 1983). These methods however are costly, and although can effectively treat the wastewater; tend to generate other hazardous by-products or pollutants at the same time (Jiménez *et al*., 2003). Aerobic treatment of molasses-based waste can cause operational difficulties such as sludge bulking and the inability to economically treat high BOD and COD loads. There is also a high biomass production and high energy cost involved. Anaerobic digestion is more favourable because there are less energy demands, and the bacteria are able to transform most organics into biogas, with minimal sludge formation and low nutrient requirements. There are some drawbacks; the wastewater often contains inhibitory substances, which can cause slower kinetics, reduced rates of methane production and yield coefficients. Also, due to the high salinity of cane molasses waste, osmotic pressure problems in the treatment process can cause bacteria to occur. These factors result in higher hydraulic retention times (HRT). Further, besides not removing coloured compounds, a significant fraction of the initial COD may not be removed, even when using low organic loading rates (Jiménez *et al*., 2003).

A common, problematic constituent of both natural waters and wastewaters is sulphate. Industrial wastewaters are mainly responsible for anthropogenic sulphate emissions. There are many industries that can contain thousands of mg/L of sulphate in comparison to typical domestic wastewater, which generally contains less than 500 mg/L (Silva *et al.,* 2002). Sulphate emissions do not cause direct damage as sulphate is non-toxic, but the presence of high sulphate upsets the sulphur cycle. The environmental sulphur cycle involves many physical, chemical and biological components. Sulphur in mineral form may be present as sulphides and/or sulphates and moves through the cycle due to oxidation of sulphides to sulphate and or the dissolution of sulphates (Brüser *et al*., 2001).

Figure 1.2 illustrates the biochemical oxidations and reductions of the sulphur components.

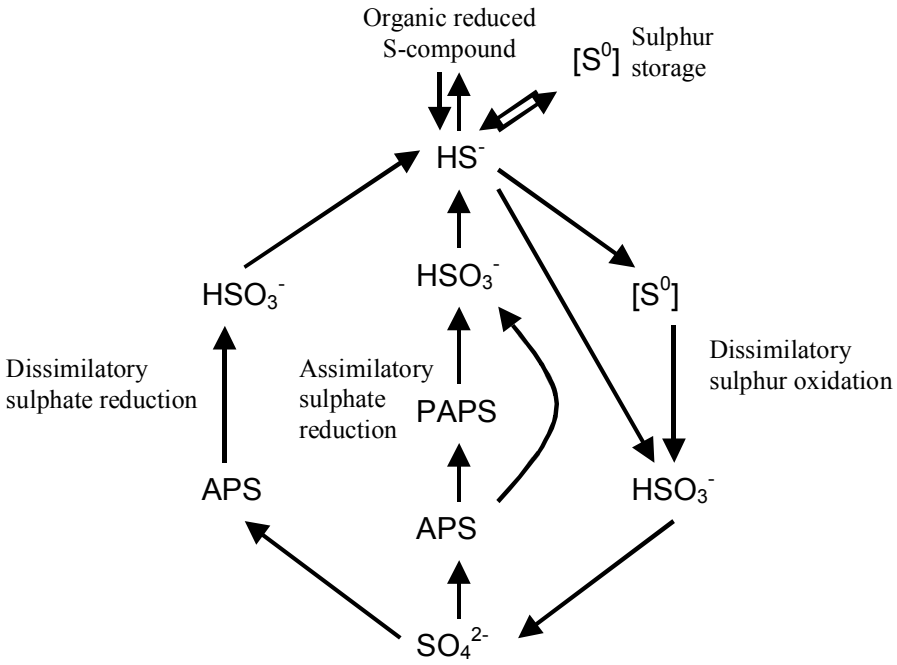

Figure 1.2 Flow diagram of the biological sulphur cycle. (Source: Brüser *et al*., 2001). APS – adenosine-5'-phosphosulphate. PAPS – 3' phosphoadenosine 5'-phosphosulphate

Industrial processes that use sulphuric acid, such as food and fermentation processes, often generate sulphate-rich wastewaters. Treatment of these wastewaters via anaerobic processes is a cause for concern because dissimilatory sulphate-reducing prokaryotes (SRP) are able to reduce sulphate and other sulphur oxides in order to support respiratory metabolism using sulphate as the terminal electron acceptor instead of molecular oxygen for degradation of organic compounds which results in the formation of hydrogen sulphide. Hydrogen sulphide is toxic, odourous and corrosive, hence high concentrations are problematic (Lens *et al.,* 2001). Dissimilatory sulphate reduction is a completely anaerobic process which usually occurs in largely stratified, anoxic water basins and sediments of wetlands, lakes and costal marine ecosystems. This process is important in marine environments because sulphate is easily available due to its high concentration in seawater (Brüser *et al*., 2001) but its unintended occurrence during anaerobic waste treatment processes can cause severe operational problems.

1.3 Current treatment of wastewater and distillery wastewater

Molasses distillery wastewater has proven to be one of the more difficult industrial wastewaters to treat (Pollution Research Group, 1987). It can be characterised as having a high biodegradable dissolved solids content, ash content, temperature and low pH. The waste stream also has high concentrations of potassium, calcium, chloride and sulphate ions and as mentioned previously high BOD with values up to 35 g/L. The characteristics of different distillery wastewaters differ considerably according to the fermentation feed stock (and location) and hence the variability in reported treatment processes and their success or otherwise (Pollution Research Group, 1987).

Due to the high BOD values in raw distillery wastewater the application of anaerobic treatment technologies has been reported to be quite effective (Nandy *et al*., 2001). Biogas generated from these processes can be utilized to meet energy requirements of the industry for steam generation in boilers. Other partial treatments have also been found to be successful, such as biomethanation with the use of biofilters, upflow and downflow fixed film reactors, upflow anaerobic sludge blanket and fluidized bed reactors (Nandy *et al*., 2001). Treatment of wastewater via anaerobic processes has a number of benefits. These include low generation of excess sludge, energy consumption and enclosure of odours and aerosol. Utilizing high rate anaerobic digesters, which retain biomass, allows high treatment capacity and therefore a small site area is required (Pérez *et al*., 1997).

The process configurations developed for high-rate digesters have been reviewed by Hickey *et al*. (1991). A number of factors influence the performance of the reactor, such as wastewater (substrate) availability, concentration and biodegradability, biomass retention capacity and specific sludge activity. It has also been found that the media used for biofilm attachment in fixed-film processes is very important and has a significant effect on the process performance. Many non-porous supports have been used at laboratory and pilot scale, (for example, glass beads and red clay) and porous supports such as polyurethane foam. Pérez *et al*. (1997) used corrugated plastic tubes and open pore sintered glass pearls (SIRAN). They found that anaerobic fluidized bed technology

was more efficient than an anaerobic filter, with removal efficiency of organics of 81 % and the maximum organic removal of 97 %. It was concluded that the anaerobic filter was suitable for treatment of easily degradable wastewater or where high COD removal is not required and that fluidized bed technology was more suitable for treatment of hazardous wastes with recalcitrant components.

Wolmarans and deVilliers (2002) discussed the start-up of an upflow anaerobic sludge blanket (UASB) treatment plant for wastewater from a distillery in Wellington, South Africa. This distillery used grape wine feedstock that was fermented and distilled to separate alcohol from the fermented liquid. The wastewater was characteristic of the wastewater described in Table 1.1, in particular with high COD values of 20 to 30 g/L and pH values between 3 and 4. Effluent from wine-based distilleries comprises mostly organic acids. The winery installed an UASB process in 1994 in order to reduce the COD levels, which were unacceptable for discharge into the municipal sewer, as anaerobic processes have been capable of COD removal efficiencies of 90 % at loading rates of up to 16 kg COD/m^3/d or more. Rajeshwari *et al*. (2000) reported that loading rates of 16 kg COD/m^3/d can be used for sugar cane distillery effluent and the maximum UASB loading rate for juice distillery effluent recommended by Driessen *et al*. (1994) was 22 kg COD/m^3/d. Wolmarans and deVilliers (2002) reviewed the monitoring programme that was performed during the last three seasons of operation of the effluent treatment plant. The set up of the treatment plant is shown in Figure 1.3.

From 1998 to 2000 the distillery operated 24 hours a day during the season of feedstock availability. While the effluent was generated the treatment plant was run on a continuous basis. Samples were collected from two points, at the inlet to the conditioning tank and before discharge into the municipal sewer. It was found that this plant, once stabilized, achieved up to 90 % COD removal. The conclusion reached was that volumetric loading rates of 18 kg COD/m^3/d could be applied to the system without a decrease in the system's performance and that UASB technology is appropriate for the pre-treatment of high strength distillery wastewater. However this can only occur once the process has had a successful start up and reached steady state. It was recommended that in order to

achieve a good start up the initial loading rate should be as low as 4-8 kg COD/m^3/d with careful monitoring of the COD removal efficiency. Once the correct removal efficiency has been achieved and maintained for some time then the loading rate can be gradually increased. Temperature was also an important factor, as high loading rates must not be applied unless the temperature range within the reactor is between 34 and 36 °C (Wolmarans and deVilliers, 2002).

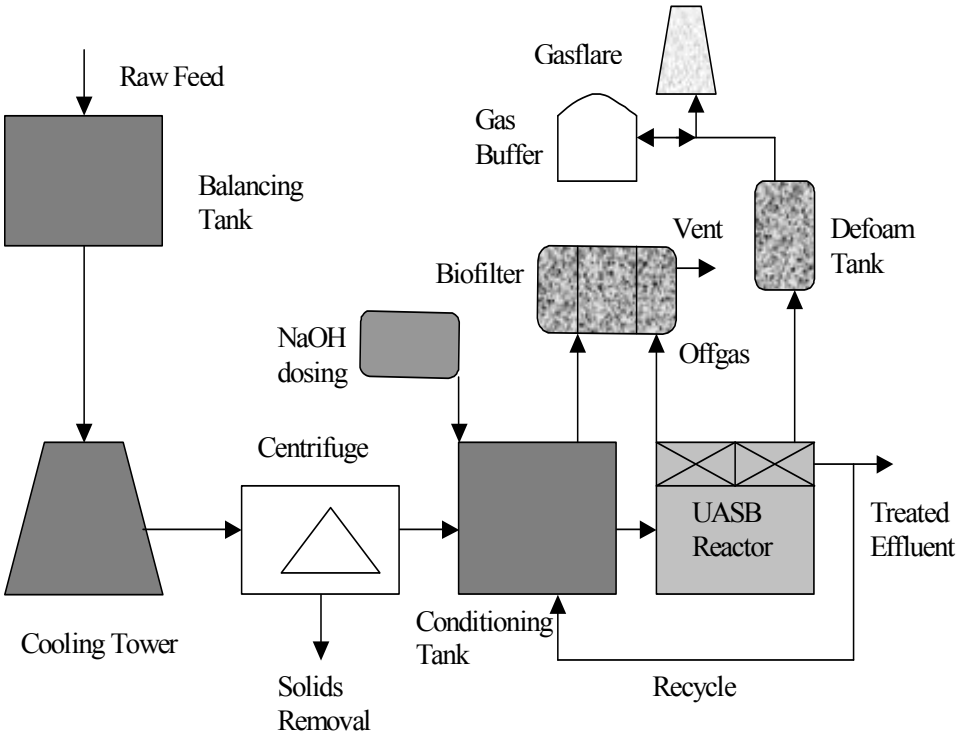

Figure 1.3 Flow diagram of Wellington distillery wastewater treatment plant (Source:Wolmarans and deVilliers, 2002).

Sulphate as mentioned is a problematic compound in wastewater, with the use of anaerobic treatments also commonly being applied for the removal of sulphates (Vossoughi *et al*., 2003). However, sulphate reduction can cause many problems in anaerobic treatment. Some of these have been studied, such as sulphate reduction to hydrogen sulphide, which inhibits methanogenesis, gives off malodours, has a high oxygen demand in effluent, can cause corrosion downstream due to the biogas released and causes SRP to compete for substrate with the bacteria associated with methane

production. Molasses slop is an example of a wastewater that is particularly high in sulphate and causes some significant problems when using sulphate reduction in an anaerobic treatment process. It has been found that during the final step of sulphate reduction and methanogenesis there is competition between SRP and methane-producing bacteria (MPB). There have been some contradictory results on the effect of sulphate reduction during anaerobic processes. Some workers have reported on competition between the two groups of bacteria (Choi and Rim, 1991; Li *et al*., 1996), while others have said that there is a syntrophic relationship (Vossoughi *et al*., 2003). Sulphate reduction in the acidogenic phase has been reported in a two-phase anaerobic digestion process for the treatment of distillery molasses slop effluent (Vossoughi *et al*., 2003). Biofilters and UASBs have been used successfully for the treatment of wastewater, especially in the removal of sulphate. One reason for their success is that the conditions created provide operation at high cell retention times. The bases of these reactors were used by Bachman *et al*. (1985) to develop a new configuration of anaerobic reactor that had high biomass retention capacity. The reactor was designed with a series of baffles and was said to be advantageous as it was simple in design and required no gas separation system. The over/under flow of the liquid decreased the amount of bacterial washout, so active biological solids were retained without the need for fixed media. Also, due to the different structure there was partial separation of acidogenesis and methanogenesis, preventing a substantial amount of the biomass from being exposed to low pH during shock loads. Due to the fact that most industrial wastewaters, especially in alcohol production, have some sulphate present which often causes a problem with the treatment system, Voussoughi *et al*. (2003) reported the performance of the baffled reactor with the use of synthetic wastewater containing a fixed COD value of 3000 mg/L and different ratios of COD:SO_4 which ranged from 16.7 to 6 (Vossoughi *et al.*, 2003).

Vossoughi *et al*. (2003) showed that when the COD:SO_4 ratio was decreased from 16.7 to 6.0 with an increase in sulphate concentration, the COD conversion did not decrease but there was slight increase in COD removal. This led them to conclude that both MPB and SRP existed within the system as a syntrophism. There was no inhibition observed when COD and sulphate concentrations were at 3000 mg/L and 500 mg/L respectively. On

increasing the sulphate concentration more COD removal occurred. The maximum COD and sulphate removal efficiencies were 86 and 97 % respectively (Vossoughi *et al.*, 2003).

Silva *et al*. (2002) also evaluated sulphate reduction efficiency and COD removal efficiency by the function of the COD:SO_4 ratio. They attempted to remove sulphate using a packed-bed anaerobic reactor at pilot scale on-site at a chemical industry. The study included both continuous and batch operation. The general conclusions from the study showed that the maximum sulphate removal efficiency was 97 % for both regimes, which in turn proves the feasibility for the reactors' purpose. The system was also low cost and can be used as a single unit or in combination with other chemical or physiochemical techniques. Silva *et al*. (2002) also found that the initial COD:SO_4 ratio did have an influence on the syntrophic and competitive relationships between the SRP and MPB and noted that addition of ethanol enhanced the sulphate reduction rate due to stimulation of the SRP. High pH maintained in the reactor resulted in the prevalence of HS^- ions, with the dissolved H_2S varying between 0.2 and 12.1 %. Even with high sulphide concentrations there seemed to be no toxicity effects on SRP; however MPB were inhibited in high HS^- concentrations (Silva *et al*., 2002).

So far only anaerobic reactors have been mentioned for the treatment of distillery wastewater, but there are some other treatment methods. One example is liquid membrane extraction, described by Kumaresan *et al*. (2003). This technique is highly sophisticated yet energy saving. This technology removes solutes and concentrates them in one step and hence is a preferred technology for high strength effluents (Kumaresan *et al*., 2003); however, the capital expenditure required precludes its use for pretreatment.

A concentration-incineration process for molasses based distillery wastewater was another option to provide a solution to the pollution problem with its main drawback being the high cost (Navarro *et al*., 2000). Concentration of the vinasse has a high energy demand so a different approach was used, bioconcentration. Some of the vinasse was continuously recycled to replace water in fermentation. The bioconcentration process

modified the energy balance of the evaporation-incineration process. This set-up also decreased the nutrient and sulphuric acid consumption. Due to the recycling of the vinasse, fermentable sugars and nutrients that are still available from incomplete fermentation can be used in other fermentations, which in turn improves the yield coefficient. From this concentration, potential by-products are also concentrated and may be more appealing for industrial exploitation. This process is also economically viable as it decreases the water consumption for fermentation from approximately 77 % to 46.2 % of the amount of water required.

Another approach for the treatment of molasses distillery wastewater is supercritical water oxidation (SCWO) (Goto *et al*., 1998). This is an environmentally acceptable technology whereby organic materials present in wastewater are oxidized to carbon dioxide, water and nitrogen (g). Supercritical water oxidation is an oxidation reaction in water above its critical point (i.e. 647 K and 22.1 Mpa). It has been applied with hydrogen peroxide as an oxidant to alcohol distillery wastewater. In a batch reactor the liquid phase product was colourless when 100 % oxidant was used, when excess oxidant was used the liquid phase was coloured, probably due to corrosion of the reactor wall, because this wastewater is high in sulphate and chloride. It was found that the total organic carbon decreased with increasing amount of oxidant and temperature. Organic acids were monitored and at 100 % oxidant there were no organic acids found above supercritical point. Ammonium ions were removed at 150 % oxidant (Goto *et al*., 1998). This seems to be acceptable for the production of cleaner wastewater.

Colour removal from wastewater is important, as releasing highly coloured wastewater into the environment has adverse effects on aquatic life. In the past chemical treatments have been to remove coloured components from molasses based wastewater (Nakajima-Kambe *et al*., 1999). Other chemical treatments are chemical precipitation, chemical adsorption and carbon adsorption. These methods are usually used on pre-treated effluent. However these treatments have disadvantages, mainly cost. The operational costs are high due to high chemical consumption. Additionally the colour removal efficiency fluctuates frequently with the generation of a large solid waste volume (Sirianuntapiboon

et al., 2004). Biological treatments using various fungi and certain bacteria have also been used to remove coloured components from the wastewater (Nakajima-Kambe *et al*., 1999). One example is the use of white-rot fungi. These organisms have a complex enzymatic system and unlike other microorganisms are capable of degrading melanoidins under nutrient-limited conditions. These enzymes are non-specific and extracellular (Gonzalez Benito *et al*., 1996). In one study *Trametes versicolor* was used for the decolouration of beet molasses wastewater from an ethanol fermentation plant (Gonzalez Benito *et al*., 1996). They investigated the influence on COD values and ammonia concentration. The tests were carried out by varying the carbon source, initial pH, nutrients and mycelia to determine their influence on the colour, COD and ammonia in the wastewater. It was found that at low sucrose concentration with only KH_2PO_4 added as a nutrient 82 % of the colour, 77 % of the COD and 36 % of the ammonia was removed from the wastewater. *Coriolus versicolor* is another white-rot fungus found to reduce colour in cane molasses based wastewater. Kumar *et al*. (1998) found that 71.5 % colour removal was obtained with a 90 % COD reduction when treating anaerobically digested cane molasses wastewater amended with glucose using *C. veriscolor*.

One example of a bacterium used for colour removal from wastewater is *Lactobacillus casei.* An immobilized isolate of this bacterium was able to reduce the colour of digested cane molasses wastewater which had been supplemented with nutrients and glucose by 52 %. There was also a reduction in COD of 57 % with the production of 11.3 mg/L of lactic acid (Shibu *et al*., 1999).

1.4 Wastewater Treatment Works

In simplified terms sewer networks are used to collect and treat wastewater from industries and households in a safe and efficient manner and discharge it into a water body. This indirectly takes into account that a sewer is also a reactor for both chemical and biological reactions. Redox conditions in the sewer network are important from a biological point of view in terms of sewer function. Aerobic conditions assist in the minimization of odour, health and corrosion problems (Hvitved-Jacobsen and Vollertsen,

2001). These conditions do however have disadvantages in that they can create problems for subsequent advanced treatment processes because readily biodegradable substrate, which is important for denitrification and biological removal of phosphorous, is degraded in the sewer network before reaching the wastewater treatment plant. Anaerobic conditions, on the other hand, also create problems within the sewer network, such as malodors, health problems and corrosion, which are all associated with the formation of hydrogen sulphide. The sewer system is complex and reactions take place in one or more of four phases; suspended water, biofilm, sewer sediments and sewer atmosphere, as well as by exchange of substances between these phases. Processes that occur in this system in turn have an effect on the urban system such as urban atmosphere and wastewater treatment plants (Hvitved-Jacobsen and Vollertsen, 2001). Figure 1.4 depicts these relationships.

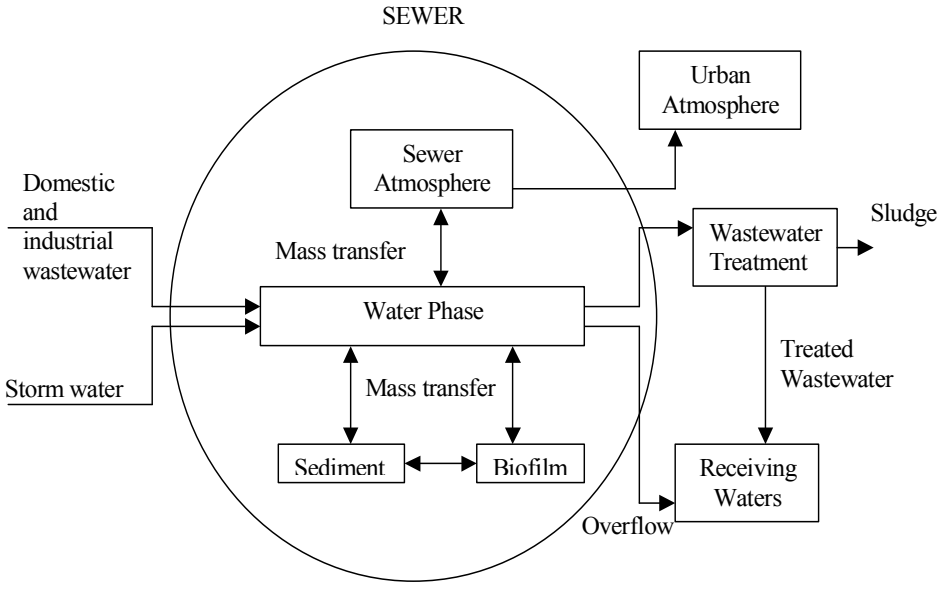

Figure 1.4 Schematic diagram of a wastewater sewer system and related surroundings. (Source: Hvitved-Jacobsen and Vollertsen, 2001)

Ongoing problems occur in areas where there is an industrial zone next to a residential area, which is the situation in which the distillery used in the present study is now placed. Pollution from industrial emissions needs to be decreased and maintained at a low level. The wastewater generated from these surrounding industries is transported to the WWTW, also situated near the residential area.

Most of the effluent and sludge from the works receiving the wastewater from the study site is discharged via a marine outfall and is currently considered an environmentally safe and acceptable means of disposal of wastewater. The ocean outfall is 4.2 km from the shore and wastewater is discharged in a water depth of 64 m through a 422 m line of diffusers. Wastewater not sent to the outfall is treated using conventional activated sludge treatment to a level prescribed by the Department of Water Affairs and Forestry (DWAF), regulated by the WWTW operator. The resulting water is then either sold to interested industries or discharged to a terrestrial water body. The wastewater entering the treatment works consists of mixed domestic and industrial wastewater streams. The wastewater from the residential area enters through a gravity sewer and the remaining wastewater is pumped to the works via rising mains. Some wastewater is pumped in from sewers owned by industry. This wastewater however bypasses the normal treatment works process and is directly discharged into the outfall. There are however some regulations and standards required to allow discharge. Tests on the wastewater are carried out by the Council for Scientific and Industrial Research (CSIR) on a regular basis to ensure the dilutions with other wastewaters are correct, which makes the mixed stream safe for discharge into the marine environment. Wastewater other than that which is discharged directly is treated by conventional screening and degritting. It is then sent to a primary settling tank, the sludge of which is removed and degritted in hydrocyclones and macerated before being added back to the primary effluent and discharged to the sea outfall. Scum is removed from the settling tanks and disposed of to land. The beaches and outfall are monitored by CSIR as an external body. The municipal wastewater management department also conducts surveys on a regular basis.

As water in a useable form in this country is scarce and the country is moving towards a more environmentally responsible approach with many practices, it is essential that wastewater discharged into the marine environment is well controlled. Water Research Commission (WRC) reports have in the past stated that the treatment, disposal and proper management of industrial wastes is imperative for effective pollution control in order to conserve water. It is also important from the sewage system perspective as acceptance of industrial wastewaters into the sewer system or treatment works can cause problems. This

could prevent a well-designed process from complying with legal standards for effluents and possible re-use of water (Pollution Research Group, 1987).

For the WWTW concerned the following conditions are required for discharge into the sea outfall. No trade effluent should be accepted containing concentrations in excess of the substance stated in Table 1.2.

Table 1.2 Acceptance values from WWTW for discharge into the sea outfall sewer.

Parameter	Value
Temperature	44 ºC
pH	5.5 - 9.5
Settleable solids	2 mg/L
Oils, grease and waxes	50 mg/L
Arsenic	5 mg/L
Cadmium	1.5 mg/L
Total chromium	3 mg/L
Copper	3 mg/L
Lead	5 mg/L
Mercury	0.05 mg/L
Cyanides	10 mg/L
Nickel	10 mg/L
Zinc	20 mg/L
Sulphide	1 mg/L
Sulphate in solution	250 mg/L

1.5 Wastewater regulations

The National Water Act No. 54 of 1956 is an important part of water and wastewater treatment. This act was written for controlling the use of water for industrial purposes and the control of wastewater generated from industrial processes. Part of the act required that a person starting a factory using water, including seawater, informed the Department of Environmental Affairs of their effluent treatment processes to be in place and the potential results to be achieved. Also, if they were to use more than 300 m^3 of public water per day a permit needed to be obtained (Pollution Research Group, 1987). Purification and disposal of effluents fell under Section 21 of the Water Act 1956. Some important points were as follows:

1. Purification of any wastewater or effluent or waste produced by or resulting from an industrial purpose needs to form an integral part of the process.
2. Use of water for industrial purposes must be purified to a predetermined standard, which the minister shall lay down after consultation with the bureau of standard.
3. Water including seawater used for industry and purified to the correct standard must be returned to the stream of origin or the sea (Pollution Research Group, 1987).

This act has since been reviewed, now The National Water Act (Act 36 of 1998), with similar requirements. Section 21 of the more recent Act describes water use, which includes the discharge of waste or water containing waste into a water resource through a pipe, canal, sewer or sea outfall. It also includes disposing of water in a manner which may detrimentally impact on a water resource and disposing in any manner of water which contains waste from, or which has been heated in, any industrial or power generating process. Section 22 states that in the case of discharge or disposal of waste or water containing waste must comply with the applicable standards or management practices prescribed in section 26 of the Act. These are prescribing waste standards which specify the quantity, quality and temperature of the waste which is to be discharged, deposited or allowed into a water course and prescribing the outcome or effect which must be achieved through management practices for the treatment of waste for any class before discharge into a water course. Section 22 also states that any run-off, seepage or water containing waste which emanates from that use, to the water resource from which the water was taken, unless told otherwise by the relevant authorities. Other important points under section 26 of the new Act are that any water use needs to be registered by the responsible authority and the use of water from a water resource must be monitored, measured and recorded. Waste discharged or deposited into a water resource needs to be monitored and analysed, and methods need to be prescribed for such monitoring and analysis (The National Water Act 36 of 1998).

Water supplies in South Africa are low due to the relatively arid climate, with average rainfall of approximately 497 mm per annum in comparison to a world average of approximately 860 mm per annum. The rainfall is unevenly distributed with under 300

mm of rainfall to one third of the country per annum (Pollution Research Group, 1990). The disposal of environmentally unsafe water into the aquatic systems needs to be eliminated. There have been some ideas to move towards cleaner production instead of treatment solutions.

The idea of cleaner technology for ethanol production is that there will be the elimination of conventional biological or chemical treatment systems post production and a zero-discharge system for this industry. Although conventional treatments such as anaerobic digestion have been successful, there are disadvantages such as high energy consumption and variation in treatment efficiency with the change in raw material used in ethanol fermentation. Biological wastewater treatment uses 30 % of the total energy used for the ethanol production process. These processes also tend to take up a large area and require a high labour cost (Kim *et al*., 1997). The new production process consists of membrane filtration and reuse of stillage as brewing water for the next fermentation. Trials are still underway (Yeoh *et al*., 2000). While cleaner production is doubtless the better option for new distilleries is it is difficult to retrofit old sites, so wastewater treatment remains to be of great importance.

1.6 Problem statement and hypothesis

The distillery at which this study was carried out generated a wastewater which regularly did not meet its discharge consent limits for pH and sulphate and was suspected to cause corrosion of the concrete sewer wall during transport to the receiving WWTW. It was hypothesised that the hydrogen sulphide content of the wastewater combined with the presence of SRP biofilms in the sewer were leading to biologically mediated corrosion of the sewer wall, and that this problem could be rectified by the removal of the wastewater sulphate content by biological wastewater treatment.

1.7 Aim

To determine the chemical and microbial composition of the wastewater and find a viable solution to remove sulphate from the wastewater for final discharge into the environment at acceptable levels and thereafter test the solution at laboratory and pilot-plant scale.

1.8 Objectives

1) Chemical and microbial characterisation of the wastewater to identify the source of the hydrogen sulphide.

2) Microbial characterisation of the sewer wall biofilm to determine the potential for biologically mediated corrosion.

3) Determination of the extent of the problem.

4) Identify technically viable solution.

5) Test potential solution at laboratory and pilot scale.

Chapter 2
Wastewater Characterisation

2.1 Introduction

Industrial ethanol production releases large amounts of high-strength wastes. The wastewater is characterised by its low pH (4-5), high COD and BOD concentrations and strong colour. Conventional treatment for this wastewater is usually anaerobic digestion, however before a suitable treatment strategy can be devised, it is necessary to fully characterise the wastewater to see which components are problematic. In this case it was necessary to determine which components of the wastewater were not meeting the requirements set by the sewerage undertaker for discharge into the sewer network. This chapter describes the chemical characterisation of the wastewater produced and determines the extent of the mismatch between wastewater target and actual quality.

Microbial characterisation of wastewater is just as important as the characterisation of chemical components. Microbes use the components present as food and energy sources. In this case since the principal problem component was sulphate the threat of high concentrations of sulphide was serious only if SRP were present (due to their conversion of sulphate to produce an end product of sulphuric acid). Therefore experiments were undertaken to determine whether these microorganisms were present in the wastewater produced on-site or in the biofilm on the sewer wall, and if so in what quantities.

The method used to identify these SRP was fluorescent *in situ* hybridization (FISH), which enables rapid, specific identification of individual microbial cells. The method involves targeting rRNA molecules since they exist in all living organisms, are stable and exist in high copy numbers and they have both variable and highly conserved sequence domains (Amann *et al*., 2001)

In terms of application of this technique to wastewater treatment, it is possible to identify dominating species responsible for wastewater treatment, detect cell specific activity of

certain microorganisms, track changes in bacterial community composition and identify new species which are possibly important contributors to treatment, by using specific FISH probes (Amann *et al*., 2001). Using this technique will enable identification of the SRP and the results generated were expected to show whether there was a threat it terms of biological sulphuric acid production.

In order to fully characterise the wastewater the tests shown in Table 2.1 were performed on ten sets of samples taken from six different sampling points over a four-week period. Three sample points were on the ethanol and lactulose manufacturing site. Since there are two main streams of wastewater going into the final discharge from the site, sampling from both was used to determine where the offending components originate. The three on-site sampling points were dunder (the wastewater generated from ethanol fermentation and distillation), lactulose production wastewater and final effluent (the mixed wastewater discharged to the sewer network). To determine the effect final effluent had on the sewer network and the changes undergone by the wastewater in the distribution network, three sample points were used at the municipal WWTW. These were a few metres upstream of the final effluent outlet pipe, a few metres downstream and the effluent outfall itself. The combined wastewaters from the area are mixed in the sewer and eventually discharged from a marine outfall. Unfortunately information regarding the production processes on the site was not disclosed therefore effluent loads could not be put into context in terms of volumes and flow rates.

2.2 Materials and methods

Table 2.1 lists the tests performed on the samples. The tests included those that were set by the municipality required to meet specific standards in order to discharge wastewater into the WWTW.

Table 2.1 List of tests performed on samples collected.

Ammonium	Colour	Nitrate	Suspended solids
BOD	Copper	pH	Total dissolved solids
Cadmium	Cyanide	Settleable solids	Total solids
Chromium	Lead	Sulphate	Turbidity
COD	Nickel	Sulphide	Zinc

The pH, settleable solids, suspended solids, total dissolved solids and total solids were performed immediately after sampling on the site according to the company method of analysis which was adapted from standard methods (APHA *et al*., 1998). The tests were carried out in duplicate and the mean of the results is reported. The rest of each sample was stored appropriately according to APHA *et al*. (1998) and the remaining tests were performed at Rhodes University, Grahamstown, South Africa.

Chemical oxygen demand, cyanide, sulphide, nitrate and ammonium were performed using photometric test kits using the Merck spectroquant® NOVA 6.0. The kit numbers were 1.14541, 1.14561, 1.14779, 1.14773 and 1.14752 respectively, and were used to perform tests analogous to standard method numbers 5220D, 4500-CN^-D, 4500-S F, 4500-NO^3 F and 4500-NH^3 G (APHA *et al*., 1998). Colour and turbidity tests were determined photometrically using the Merck spectroquant® NOVA 6.0. Sulphate was performed using the Hach Sulfaver kit, analogous to standard method number 4110B (APHA *et al*., 1998). Five day BOD was determined according to standard method 5210B (APHA *et al*., 1998).

Metal concentrations were determined using atomic absorption spectrophotometry (AAS) according to standard method 3111B (APHA *et al*., 1998) using an instrument with a direct air-acetylene flame (GBC 909AA). All metal standard solutions were prepared from 1000 mg/L AAS standard solutions (Wirsam Scientific, South Africa) diluted with deionised distilled water until appropriate concentrations (see Appendix B for the standards concentration for each of the metals tested). All samples were filtered using cellulose-acetate membrane filters (25 mm diameter, 0.22 μM pore size, Merck Chemicals SA Pty Ltd) before measurement on the AAS. The measurements were recorded as mg/L.

Fluourescent *in situ* hybridization probing for microbial 16s rRNA was performed on the six wastewater samples described in earlier (dunder, lactulose, final site effluent, final site effluent at the WWTW outfall and the mixed wastewater at the WWTW upstream and downstream of the outfall). Tests were also performed on biofilm samples from the sewer wall downstream of the final effluent outfall at the WWTW. This technique uses rRNA molecules as probes. This is because they are found in all living organisms, are stable and occur in high copy numbers, also they have variable and highly conserved sequence domains. This analysis involves four steps: fixation and permeabilisation of the sample followed by hybridisation and then washing steps to remove unbound probe, lastly detection of the cells by microscopy or flow cytometry. The molecular probes used are usually 15-30 nucleotides long and are covalently linked ant the 5' end to a fluorescent dye molecule, which is used to detect the organisms (Amann *et al*., 2001). The protocol is described fully in Appendix C.

2.3 Results and discussion

The results of some tests are summarised in Table 2.2. The following figures show the trends in the tests performed at the manufacturing site. Some of these tests are performed on a regular basis at the factory as part of quality control. The target specifications for these tests are as follows; total dissolved solids <1000 mg/L, settleable solids 2 mL/L, suspended solids 2000 mg/L and pH 5.5 - 9.5.

Table 2.2 Summary of results for solids and turbidity tests.

		Dunder	Lactulose WW	Final effluent from site	Final effluent at outfall	WWTW upstream	WWTW downstream
Settleable solids (mL/L)	Max	100.0	0.0	125.0	30.0	24.0	38.0
	Mean	18.5	0.0	22.7	11.5	11.2	14.2
	Min	0.0	0.0	0.2	1.3	4.0	5.0
Total dissolved solids (g/L)	Max	27.80	21.60	19.30	18.90	2.85	2.07
	Mean	23.95	13.73	18.35	18.08	1.21	1.64
	Min	13.54	3.90	16.70	16.90	0.09	1.25
Suspended solids (mg/L)	Max	15322	226	6158	4482	416	500
	Mean	10018	127	3786	3139	156	242
	Min	5966	14	2712	648	0	0
Total solids (%)	Max	14.05	2.45	9.17	7.23	0.54	0.33
	Mean	13.26	1.47	6.35	6.39	0.21	0.26
	Min	11.08	0.44	5.62	4.56	0.11	0.19
Turbidity (FAU)	Max	9400	0	3800	6600	140	300
	Mean	5900	0	2580	3200	58	160
	Min	4400	0	1600	1200	20	20

The solids content of wastewater can affect the quality of the receiving water. High solids concentrations can prevent light penetration and thereby inhibit photosynthetic activity, resulting in a lack of dissolved oxygen, which can lead to animal and plant death in aquatic systems (DWAF, 1996). Therefore solids content is an important parameter to monitor for control of biological and physical treatment of water (APHA *et al*., 1998).

The error bars in Figure 2.1 show that the settleable solids concentrations in each of the wastewaters were highly variable, with the exception of lactulose production effluent, which contained no detectable settleable solids. The dunder and final effluent streams showed the highest degree of variability. The reason for this variability could be partially due to the times at which samples were taken. The final effluent is held in a settler which allows the solids to settle out before the effluent is discharged; samples could have been taken when some newly arrived final effluent had not yet had time to settle or when the settled sludge level was high because it had not yet been removed by the sub-contracted company. Figure 2.1 also shows that the settleable solids did not meet the specification for discharge to the WWTW (2 mL/L), as the mean value of the final effluent discharged was 22.7 mL/L. This parameter is probably a difficult one to monitor and control, as the quality of the molasses used can vary seasonally. There is also the possibility of microbial

growth in the settler, as the settler contents contain a good carbon source for microbial growth, which can add to the settleable solids present. Even though the final effluent from the site is above the specified limit at the outfall, the upstream concentration of settleable solids was also above specification, averaging 11.2 mL/L. The addition of the final effluent increased the settleable solids concentration by 3.6 mL/L. If the final effluent discharged from the site had been within the limits, mixed wastewaters in the sewage network would still have broken the limit of 2 mL/L.

Total dissolved salts concentration is a measurement of the amount of compounds dissolved in water. Due to the fact that most dissolved substances in water carry a charge, the total salts concentration is an indication of the total dissolved solids (TDS) content (DWAF, 1996). Table 2.2 shows that the wastewater generated at the site was well above the upper discharge limit of 1000 mg/L. The TDS of the final effluent discharged into the sewer was just greater than 20 000 mg/L. Total dissolved solids can greatly affect the aquatic ecosystem if the rate or duration of change in TDS is variable. This affects aquatic ecosystems more than the absolute changes of TDS. Organisms have the ability to adapt and maintain their osmotic balance but require time in which to do so (DWAF, 1996). The TDS concentrations in the dunder and final effluent stream were high (~15 000 - 25 000 mg/L) and the outfall increased the TDS of the wastewater in the sewer by 400 mg/L, making the final concentration 1640 mg/L. However, TDS was not on the list of requirements for discharge stipulated by the municipality and hence was not a priority parameter for treatment.

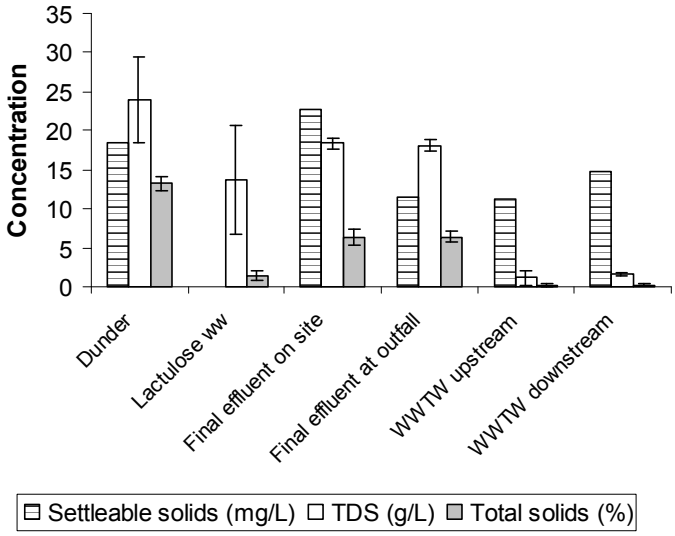

Figure 2.1 Values of solids tests performed at the industrial site.

The total suspended solids (TSS) in a water body is the amount of particulate material that is suspended in that water. Turbidity is related to the concentration of total suspended solids in water, because turbidity is the optical property that causes light to scatter and be absorbed as opposed to transmitted in a straight line in water. Suspended matter results in the scattering of light. Inorganic matter, microscopic organisms and plankton cause light to be absorbed (DWAF, 1996). Figure 2.2 illustrates the values for TSS and turbidity. High concentrations of TSS released into water bodies can result in a temperature decrease, which affects sensitive organisms in the region of the outfall. Since suspended inorganic material has an electrical charge, dissolved substances such as nutrients, and trace metal ions can adsorb to the surface of the particles. This can cause these substances to become unavailable, which is unfavourable in terms of essential nutrients (but favorable for toxic metal content). A change in TSS concentration that is not caused from natural variation such as seasonal patterns can have adverse effects on organisms. Turbidity results in less light penetration through water and therefore will affect photosynthetic activity. The associated decrease in primary production leads to a decrease in food availability higher up the food chain. Presence of suspended solids may also affect feeding mechanisms for filter-feeding species and interfere with fish gill functioning and body growth (DWAF, 1996). Although suspended solids is not on the list of criteria for allowing discharge of effluent into the sewer it is important as it can affect

receiving waters if the TSS concentration is high. In this case the final effluent outfall had a high concentration (Figure 2.2), but it did not affect the wastewater in the sewer network which had an average TSS of 242 mg/L.

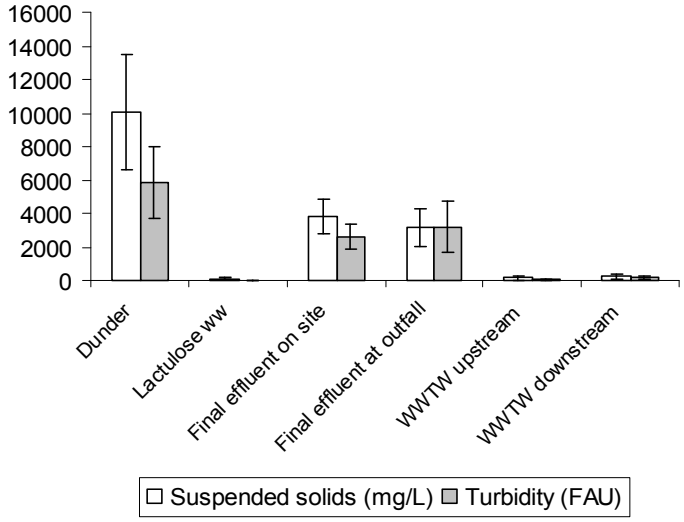

Figure 2.2 Values of suspended solids and turbidity in wastewater samples.

The results from the tests for heavy metals were all within the limits set by the municipality (Table 2.3). Presence of heavy metals in industrial wastewater can affect receiving waters and soils downstream. Although some of these metals are needed as essential trace nutrients for plants and animals, high concentrations can cause serious environmental hazards. Measurement of the metals content in the wastewater tested from the site (results not shown) and sewage treatment works shows presence of very low concentrations of metals with some negligible values. Although some of these values may exceed those shown in the South African water quality guidelines for discharge to the environment (DWAF, 1996), they met the requirements necessary for discharge into the WWTW.

Table 2.3 Concentrations of metals found in the wastewater at the WWTW

Metal	WWTW upstream (mg/L)	Final effluent at outfall (mg/L)	WWTW downstream (mg/L)	WWTW outfall quality limit (mg/L)
Cadmium	0.016	0.007	0.009	1.5
Chromium	0.36	0.45	0.29	3
Copper	0.015	0.181	<LOD*	3
Lead	1.08	1.65	0.67	5
Nickel	0	0	0	10
Zinc	0.61	0.99	0.60	20

*LOD = limit of detection

Dissolved oxygen is important for the survival of aerobic organisms. It is essential for respiration; therefore maintenance of sufficient dissolved oxygen concentrations is critical in aquatic systems. Chemical oxygen demand and BOD are important measurements for determination of water quality requirements for effluents that are discharged into aquatic systems more than for aquatic systems themselves (DWAF, 1996). Knowledge of these concentrations aids in limiting the impact of these effluents on the aquatic ecosystems if the effluents are then treated to reduce the BOD and COD concentrations.

Reduction in dissolved oxygen concentration can be caused from the presence of oxidisable organic matter from wastewater discharges. High amounts of suspended material in water affect the saturation concentration of the dissolved oxygen. This happens either chemically, due to the oxygen-scavenging attributes of suspended particles, or physically, by reducing the volume availability of water for solution (DWAF, 1996).

Chemical oxygen demand by definition is the amount of oxygen needed for chemical oxidation of compounds in water. Biological oxygen demand by definition is the amount of oxygen needed for biodegradation of compounds via microorganisms that are present in aerobic conditions (Milner, 1996). The COD test is advantageous over the BOD test as it takes three hours to obtain a result in comparison to five days. However, COD concentrations are not a substitute for BOD and need to be considered as an independent

measurement. In most cases the values for COD are higher than those of BOD in the same sample, but this may not always be so depending on the various wastes (Dugan, 1999). All the samples taken for analysis of COD and BOD in this study follow the trend of BOD < COD (Figure 2.3). These two parameters are also assessed as a ratio of COD: BOD. A ratio > 100 suggests that the compound is relatively non-biodegradable and < 10 is that a compound is highly degradable (Milner, 1996). In this case all the values of COD: BOD ratios were below 10 for the various wastewaters (results not shown), therefore biodegradability should not be an obstacle. Comparing the values in Table 1.1 (Chapter 1) with those in Figure 2.3 it can be seen that both BOD and COD values of the wastewater tested on site fall within the ranges given in that table for typical molasses based distillery wastewater. These high values result in decreased oxygen supply and may result in eutrophication of contaminated watercourses (FitzGibbon *et al*., 1998). Methods need to be undertaken to decrease these values to more acceptable levels.

Another major factor affecting the downstream effect of discharging molasses based distillery effluent into an aquatic environment is colour. Highly coloured wastewater can decrease sunlight penetration into water preventing oxygenation of water by photosynthesis, which in turn affects the aquatic organisms living in that water (FitzGibbon *et al*., 1998). This dark pigmentation of wastewater also causes an increase in COD (Nakajima-Kambe *et al*., 1999). Figure 2.4 shows the colour of all the wastewater tested.

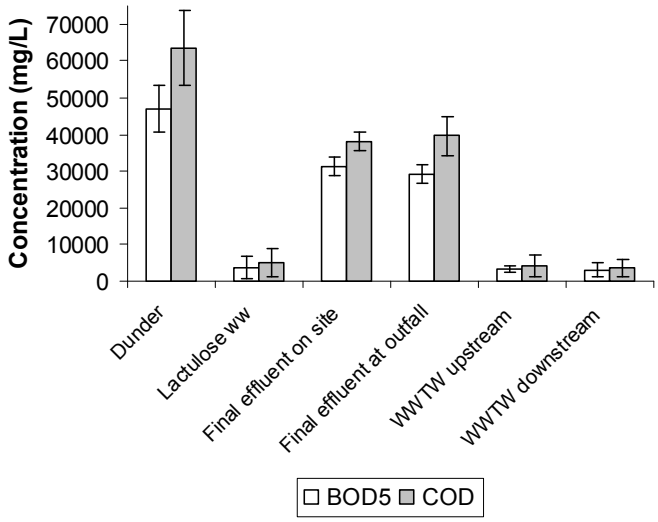

Figure 2.3 Chemical and biological oxygen demand concentrations for wastewater streams.

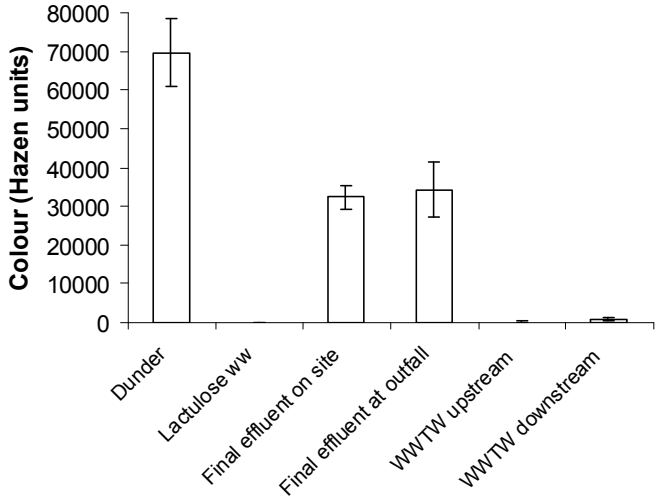

Figure 2.4 Colour measurements of the wastewater streams.

These results showed that only dunder had high colour, causing the colour in the final effluent. The lactulose production wastewater was almost colourless, at an average of 21 Hazen. The WWTW upstream colour reading was also low, 87.56 Hazen, with an increase in intensity in the downstream sample to 386.58 Hazen owing to the addition of the industrial site final effluent outfall (7084.80 Hazen). Colour was not one of the criteria for discharging wastewater in to the WWTW.

The pH of water plays a very important role in the downstream effects of discharge into aquatic systems. Industrial activities usually cause an increase in acidity levels rather than an increase in alkalinity (DWAF, 1996). The pH standards for discharge into the WWTW are 5.5 - 9.5. Figure 2.5 shows the pH values of the wastewaters and the effect of that pH on the receiving water stream at the WWTW.

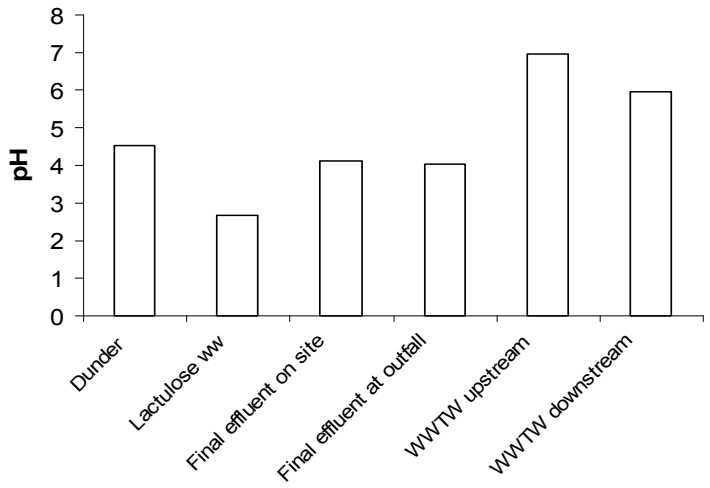

Figure 2.5 pH values of wastewater on-site and at the wastewater treatment works.

The pH value for the dunder was low. Although the lactulose production effluent tended to be highly acidic, a pH of 2.5, it did not appear to affect the pH of the final effluent sent to the WWTW. The final effluent pH (4.11) did not however meet the requirements of the municipality. The low pH of the effluent from the outfall decreased the pH of the wastewater at the WWTW from ~ pH 7 to pH 6. Although the pH dropped, it was still within the municipal specification limits, due to the dilution with other wastewater streams. It was not known whether the specifications for the on-site wastewater were the same for the WWTW to discharge into the sea. If they were, then the low pH of the wastewater did not reduce the pH level below its limits.

Sulphate is often found in natural waters and wastewaters. It is especially found in high concentrations in industrial wastewaters of the pulp and paper industry, pharmaceutical industry and food or beverage industries that use or produce molasses in their processes (O'Flaherty *et al*., 1997; Percheron *et al*., 1997; Khanal and Huang, 2003). High sulphate

concentrations in wastewaters also occur when sulphuric acid is used in an industrial process (O'Flaherty, *et al*., 1997; Maree *et al*., 2004). Sulphate itself is not toxic but high concentrations in wastewater can upset the natural biological sulphur cycle if present with SRP, resulting in the formation of hydrogen sulphide gas which is harmful (Silva *et al*., 2002). Sulphide gas has an unpleasant odour, can result in the corrosion of materials such as concrete, affects human health and can lower the quality of biogas generated during treatment of high sulphate water (Khanal and Huang, 2003). Therefore sulphide is an important parameter to monitor, especially if anaerobic treatment is to be used for decreasing the sulphate concentration in wastewater. Figures 2.6 and 2.7 show the sulphate and sulphide concentrations for the wastewaters sampled.

The limit for sulphate concentration for discharge into the WWTW was 250 mg/L. Figure 2.6 clearly shows that the sulphate levels are well above this limit. The actual value an order of magnitude higher than the limit. Both lactulose and alcohol production use sulphuric acid in their processes and hence the high amount of sulphate present. The greatest concern about this high concentration of sulphate comes from the WWTW operator. The reason is because there is concern over sulphide corrosion of the cement at the launder on the works site. Figure 2.7 shows the sulphide concentrations. Sulphide levels in the dunder were highest of all the samples but dilution with the lactulose wastewater, which contained very little sulphide, resulted in the concentration in the final effluent on site being substantially lower. This concentration was not within the specifications for discharge set by the municipality (2 mg/L), however the sulphide in the wastewater did not appear to increase levels of sulphide downstream of the final wastewater outfall.

It is interesting to note that the addition of high sulphate concentration in the final effluent outfall at the WWTW (2770.43 mg/L) resulted in an overall downstream sulphate concentration of 21.23 mg/L due to the dilution with other wastewater streams, which contained no sulphate. The overall sulphate concentration in the mixed wastewater discharged to sea was low.

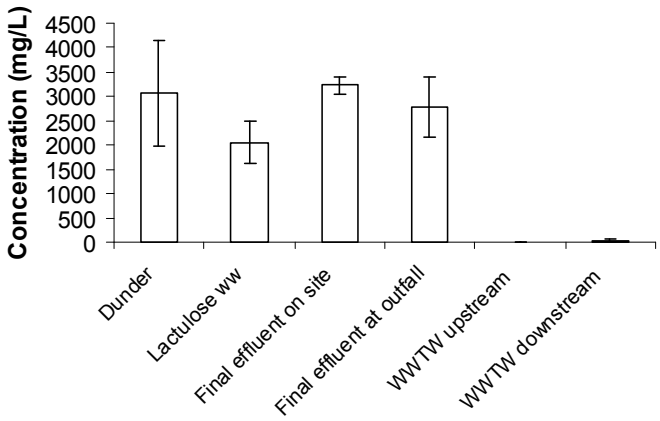

Figure 2.6 Sulphate concentrations in the wastewater samples.

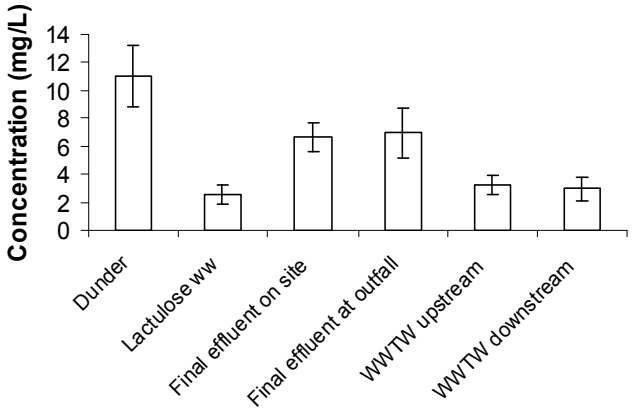

Figure 2.7 Sulphide concentrations in the wastewater samples.

Ammonia (NH_3), and ammonium (NH_4^+) are the reduced forms of inorganic nitrogen, and their proportions are controlled by pH and temperature of the water. They can be present as dissolved ions or suspended solids. Nitrite, on the other hand, is an intermediate produced during oxidation of organic nitrogen and ammonia, with nitrate as the end product of this reaction. Of the two products, nitrate is the more stable and abundant in the aquatic ecosystem (DWAF, 1996). Table 2.4 lists the concentrations of these two forms of nitrogen in the wastewater samples tested. The highlighted values were of most importance, as it was this stream of wastewater that affected the water discharged to sea.

Ammonia is present in water in two forms with the ionized form NH_4^+ having little to no effect on aquatic life, but may contribute to eutrophication. Toxicity of ammonia lies in

the un-ionized form, NH_3 (DWAF, 1996). The concentration of ammonium discharged from the final effluent outfall was low at 2.89 mg/L. The final effluent contained 3.84 mg/L ammonium, some of which was degraded during transport in the distribution network. However, the amount of ammonia degraded did not account for the increase in nitrate recorded between the release of final effluent from the site and its arrival at the WWTW.

Table 2.4 Concentration of nitrate and ammonium found in the wastewater samples.

Sample	Nitrate (mg/L)	Ammonium (mg/L)
Dunder	5700	7.46
Lactulose production WW	410	3.62
Final effluent on site	3100	3.84
Final effluent at outfall	**5100**	**2.89**
WWTW upstream	280	19.28
WWTW downstream	290	11.02

High concentrations of nitrate were observed at the point of discharge into the WWTW. Although the addition of lactulose production effluent lowered the nitrate concentration of the final effluent compared to the dunder, the levels were still high. When industrial effluent containing high nitrate concentrations are impacted on aerobic waters the concentration of background inorganic nitrogen increases. This results in the decrease of dissolved oxygen present in the water and increasing BOD, COD and pH values (DWAF, 1996). Another problem involving high concentrations of nitrate is its toxicity to mammals, as nitrates have the capability to convert to nitrites in the gastrointestinal tract. The nitrites are then able to combine with amino acids to release nitrosamines, which are believed to be carcinogenic. Nitrites can also bind to haemoglobin, which then forms methaemoglobin, which is unable to carry oxygen in the blood. The formation of methaemoglobin can cause anemia, which can become lethal. In the European community the highest levels of nitrate permitted in ground and surface water is between 80 and 100 mg/L (Chazal and Lens, 2000). The Nitrates Directive requires the EU Member States to designate all known land, that drain into surface and groundwaters where nitrate concentration is greater that 50 mg/L as nitrate-vulnerable zones. This is also the case if the there is a rising trend in nitrate concentration or if there is evidence of eutrophication. In order to comply with the European Court of Justice ruling, the sates must apply the Action Programme measures designed to reduce the nitrate concentration

losses to surface or groundwaters to the whole country or designate the rivers and lake catchments where nitrate concentration exceeds 50 mg/L, a rising trend in concentration or eutrophication evidence (Goodchild, 1998). The level of nitrate in the final effluent outfall was very high. The overall effect this stream had on the mixed WWTW wastewater being discharged to sea was an increase of approximately 10 mg/L. However, the WWTW operator has not set any limits for nitrate or ammonium levels for the wastewater being discharged.

The last test performed was cyanide. Cyanide levels of <10 mg/L were required for discharge into the WWTW. Figure 2.8 shows the concentrations of cyanide in the wastewaters, the highest concentration being less than 2 mg/L in the dunder. The concentration in the final effluent discharged to the WWTW was 0.7 mg/L, well below the limit. The upstream and downstream concentrations of cyanide differ by 0.016 mg/L.

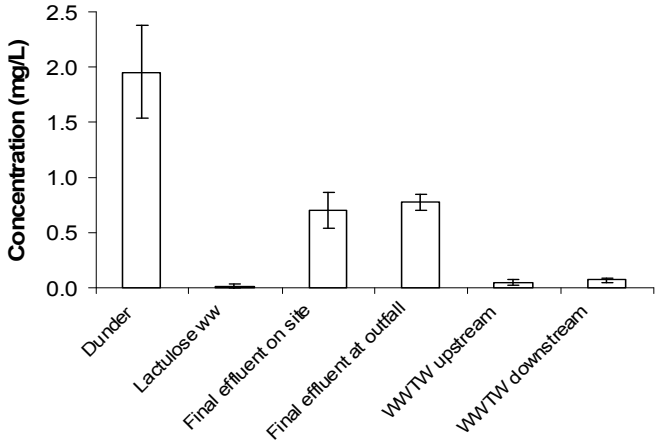

Figure 2.8 Cyanide concentrations in the wastewater samples.

Looking at all the characteristics it appears that the sewer network transporting the final effluent from the manufacturing site to the WWTW does not significantly affect the concentration of the constituents. Below is a table showing the average values from the final effluent sample before it enters the sewer network and the effluent sample that is discharged at the outfall into the WWTW.

Paired t-tests indicated that pH, sulphate, copper and nickel were the only components that showed a significant difference between the two sample points (at the 95%

confidence interval), even though the percentage change was high for some of the other components measured. This showed that there was very little change to the overall final effluent composition from the site before entering the WWTW.

Table 2.5 Mean values of constituents measured in the final effluent samples.

Component	On site	At outfall	% Change	t-value
pH	4.11	4.05	1.46	**2.80**
Settleable solids (mL/L)	23	11	49.44	1.05
Suspended solids (mg/L)	3800	3100	17.09	1.87
Total solids (%)	6.35	6.39	-0.63	1.33
Total dissolved solids (mg/L)	18.35	18.08	1.47	0.88
COD (mg/L)	38000	40000	-4.10	-1.08
BOD (mg/L)	32000	29000	7.1	0.23
Sulphate (mg/L)	3200	28000	14.10	**2.52**
Sulphide (mg/L)	6.64	7.0	-0.60	-0.95
Nitrate (mg/L)	3100	5100	-65.05	-6.04
Ammonium (mg/L)	3.84	2.9	24.74	1.53
Colour (Hazen)	32000	34000	-5.62	-1.10
Turbidity (FAU)	2580	3200	-24.03	-14.02
Cyanide (mg/L)	0.70	0.78	-11.43	-1.10
Cadmium (mg/L)	0.01	0.01	0.00	0.76
Copper (mg/L)	0.56	0.18	67.86	**4.48**
Chromium (mg/L)	0.22	0.45	-104.54	-19.00
Lead (mg/L)	1.69	1.65	2.37	0.15
Nickel (mg/L)	0.06	0.00	100	**7.95**
Zinc (mg/L)	1.02	0.99	2.94	0.65

Using the paired t-test, t-values were generated for the information on wastewater upstream and downstream of the final effluent outfall at the WWTW (Table 2.6). From these it was noted that pH, ammonium, chromium and lead values were significantly different upstream and downstream of the outfall. Of these, only pH negatively impacted on the incoming stream of wastewater at the WWTW, lowering the mean from pH 6.69 to 5.98. However, pH 5.98 was still within the specification limits for discharge into the WWTW (Table 1.2).

Although Table 2.5 shows a significant difference in sulphate concentration between the effluent from the site and at the outfall, there was no significant difference between the concentrations of sulphate upstream and downstream of the effluent outfall at the WWTW. Sulphate concentration and pH levels in the wastewater from the industrial site

however were not within the limits required for discharge into the WWTW. High sulphate concentration in wastewaters can cause corrosion, which could be detrimental to the structure of the launder at the WWTW.

Table 2.6 Mean values of constituents at the WWTW upstream and downstream the final effluent outfall.

Component	WWTW upstream	WWTW downstream	% Change	t-value
pH	6.70	6.0	10.61	**4.10**
Settleable solids (mL/L)	11	15	-32.14	-0.92
Suspended solids (mg/L)	156	240	-54.92	-1.76
Total solids (%)	0.21	0.26	-23.80	-0.97
Total dissolved solids (mg/L)	1.21	1.6	-35.54	-1.36
COD (mg/L)	4200	3700	10.96	0.33
BOD (mg/L)	3400	3100	7.64	0.25
Sulphate (mg/L)	0.00	21	-	-1.83
Sulphide (mg/L)	3.2	3.0	7.50	0.98
Nitrate (mg/L)	280	290	-12.6	-0.28
Ammonium (mg/L)	19	11.0	42.84	**2.52**
Colour (Hazen)	190	950	-400	-6.22
Turbidity (FAU)	58	160	-175.86	-3.04
Cyanide (mg/L)	0.053	0.069	-30.19	-1.59
Cadmium (mg/L)	0.016	0.009	43.75	1.45
Copper (mg/L)	0.015	0.000	100	2.06
Chromium (mg/L)	0.36	0.29	19.44	**3.76**
Lead (mg/L)	1.08	0.67	37.96	**6.40**
Nickel (mg/L)	0.00	0.00	0	0.00
Zinc (mg/L)	0.61	0.60	1.64	0.72

Microbial analyses performed on the six wastewater samples showed little evidence of bacterial presence and no evidence of SRP in any of the wastewaters (photographs not shown). This was not an entirely unexpected result, as the wastewaters sampled are all exclusively of industrial origin and were not expected to contain much active biological matter.

The biofilm samples taken from the sewer wall downstream of the effluent outfall contained large numbers of bacteria, indicated by a general 4',6-diamidino-2-phenylindole (DAPI) stain (Figure 2.9). A small proportion of the bacteria present were shown by the probe for sulphate-reducing prokaryotes (SRP) to be sulphate-reducers

(Figure 2.10), although there are minor false positives from particulate material visible. This indicates a risk of corrosion of the concrete wall of the sewer network.

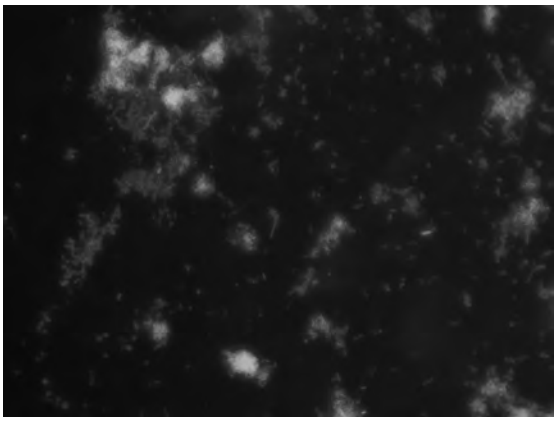

Figure 2.9 DAPI stain of sewer wall biofilm sample from WWTW downstream of outfall.

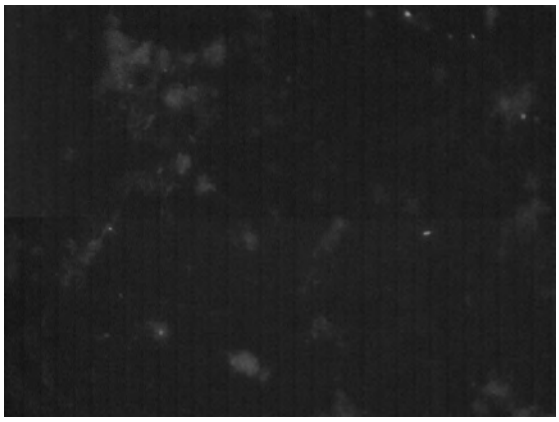

Figure 2.10 Fluorescent stain (probe 385 for general SRP) of sample from WWTW downstream outfall.

The corrosion of cement due to sulphate (in the presence of SRP) occurs as follows. The reduction of sulphate generates hydrogen sulphide (eq. 1).

$$SO_4^{2-} + \text{organic carbon} \rightarrow HCO_3^- \text{(carbon dioxide)} + H_2S \quad (1)$$

When sulphide is released as hydrogen sulphide gas it adsorbs to moist surfaces (e.g. concrete). Aerobic bacteria then carry out the first transformation (eq. 2). Even though the hydrogen sulphide can corrode, it is the formation of sulphuric acid that does the most damage (eq. 3).

$$H_2S + 2O_2 \rightarrow H_2SO_4 \quad (2)$$

$$H_2SO_4 + \underset{\text{(cement)}}{CaCO_3} \rightarrow H_2O + CO_2 + \underset{\text{(gypsum)}}{CaSO_4} \quad (3)$$

The rate of sulphuric acid production determines the extent of the damage that can occur in terms of concrete corrosion. If the rate of sulphuric acid production is high, then some of the acid is washed away before a reaction can take place. However, if the rate of production is low, almost all the sulphuric acid produced will react with concrete (Hvited-Jacobsen and Halkjær Nielsen, 2000). Concurrent engineering studies performed

at UCT found that cement corrosion was occurring at the WWTW, with more rapid corrosion of concrete coupons placed downstream of the final effluent outfall than upstream. Using standard concrete mix coupons, the researchers recorded a loss of 3.2 % in mean mass of coupons placed downstream of the outfall in comparison to less than 0.5 % loss for the coupons upstream. It was also noted that control coupons placed in the domestic sewer line had no significant difference to the ones placed upstream of the outfall (Appendix I).

The potential for the formation of sulphide gas from the high sulphate levels in the final effluent of the ethanol production process was the concern and not necessarily the sulphide content, which was above the specifications of 1 mg/L but nevertheless still low enough not to produce a high concentration of sulphuric acid to the extent that excessive corrosion could occur. Since sulphate was a major concern for the WWTW and the levels of sulphate in all the wastewater streams from the site are high there was no need to treat the streams separately. Although the final wastewater from the site entering the WWTW has high concentrations or values for most of the components measured, these concentrations did not seem to greatly affect the mixed wastewater at the WWTW.

The main components of concern were settleable solids, pH and sulphate concentration. The settleable solids concentrations leaving the site could be decreased by stabilizing the erratic hydraulic retention times used in operation of the settler. The analytical procedure showed that the remaining solids are easily settled and it is reasonable to conclude that their intermittent escape into the sewer is owing to the occasions on which the retention times in the settler are too short for the process to complete. The low pH and high sulphate concentrations posed more of a problem. Previous research has shown that use of SRP in anaerobic reactors can improve high sulphate strength wastewaters and increase pH simultaneously (Silva *et al*., 2002; Maree *et al*., 2004). Growth and activity of SRP at low pH is usually inhibited. However their activity results in pH increments at their immediate environment. During sulphate reduction bicarbonate ions are formed which react with protons to form carbon dioxide and water to remove acidity from solutions as carbon dioxide gas:

$$HCO_3^- + H^+ \rightarrow CO_2 + H_2O \quad (4)$$

The ability of these microorganisms to use bicarbonate ions to buffer the waters pH is the reason they are used to treat low pH wastewaters and are especially well known to treat acid mine drainage, which characteristically has low pH (Bratcova *et al*., 2002). Since SRP are anaerobes and are able to treat high sulphate wastewater this was deemed to be the best option to use for treatment of the ethanol distillery wastewater. Also, the COD:BOD ratio (< 10) indicated that the wastewater should be amenable to biological treatment. A potential disadvantage of using this as a treatment method was the production of hydrogen sulphide gas, which causes chemical problems such as corrosion, odour, and increase of effluent COD and biological problems such as toxicity and inhibition. These can cause process failure (José Vela *et al*., 2002). This was to be avoided by using a two step process involving the downstream conversion of sulphide to elemental sulphur or by treating the sulphide gas through CO_2 stripping (Maree *et al*., 2004).

2.4 Interim summary and conclusions

From these studies it can be concluded that there were two main problem components in the wastewater that required treatment, namely pH and sulphate. The pH had a negative effect on the downstream wastewater discharged at the WWTW. Sulphate did not have an effect in comparison to the upstream level of sulphate entering the WWTW, however, the sulphate concentration was high and needed to be reduced so as to prevent corrosion at the WWTW due to the possible presence of SRP in the sewer wall biofilm. The other components tested for in the final wastewater were not of great concern to the municipality, as the addition of final wastewater from the site into the WWTW did not significantly affect the mixed wastewater. Those that did differ significantly had a positive effect on the upstream wastewater. The low COD:BOD ratio suggested that the wastewater could be biologically treated and there have been cases in literature that have successfully treated wastewater with similar characteristics. The treatment strategy chosen was a sulphidogenic anaerobic process with a second step for aerobic oxidation of any sulphide generated during sulphate reduction to elemental sulphur.

Chapter 3
Bioreactor Design and Operation

3.1 Choice of treatment system

After characterisation of the wastewater (Chapter 2) it was found that the most important factors of concern with regards to this wastewater were sulphate concentration and pH. Other components such as settleable solids, nitrate, COD and BOD concentrations also needed to be addressed, but sulphate was of the highest priority for the ethanol production plant. The reason is because the WWTW was concerned about corrosion of the cement launder. Methods were investigated for ways of treating high sulphate-containing wastewaters with subsequent or additional removal or reduction of some of the other components present in the wastewater.

Biological treatment has been developed over decades and is capable of competing with physicochemical ways for treatment of sulphate-rich wastewaters at full-scale. Some methods used are aerobic or anaerobic classical methods, trickling filters, lagoons, and evaporation-condensation with or without combustion or direct application onto soil as fertilizer. However these methods are often costly and create other pollutants or by-products, which are potentially hazardous (Jiménez *et al*., 2003). Anaerobic biological sulphate removal has been used to treat industrial effluent from the mining and the food and fermentation industries. Methods used to reduce sulphate in wastewater often also result in metal removal and neutralization of the wastewater being treated (Maree *et al*., 2004).

The problem with anaerobic digestion of sulphate-rich wastewaters is the dissimilatory sulphate reduction by SRP whereby use of sulphate as a terminal electron acceptor for degradation of organic compounds and hydrogen generates sulphide. Sulphide is potentially toxic to other bacteria involved in anaerobic digestion. However, the occurrence of this sulphate reduction reaction can be used as a biological treatment

strategy if a sulphide removal step is also employed within the system design (Lens *et al*., 2000).

Maree *et al*. (2004) developed an integrated system for the treatment of acid and sulphate-rich effluents, using a combination of chemical and biological processes to obtain the highest removal of sulphate possible. The basis of the biological steps used in their process was adapted to the needs of this particular project, especially since Maree and Strydom (1985) showed that sulphate could be reduced in an anaerobic packed-bed reactor using molasses as a carbon and energy source. Further, Maree and Hill (1989) found similar results in a three-stage process using molasses as a carbon and energy source for sulphate removal. They found that when using molasses either the fermented or unfermented form could be used as a carbon and energy source, where in the fermented form acetate was the main carbon source used by the SRP and when using the sterile unfermented form, sucrose was the main carbon source for the SRP with acetate being the metabolic end product (Maree *et al*., 1991).

Using the biological design from Maree *et al*. (2004) a sulphur biofilm can be recovered due to the conversion of sulphide to elemental sulphur by sulphide-oxidizing bacteria (SOB), after the sulphate reduction by SRP in the reactor. If a process can produce a significant amount of elemental sulphur from sulphide under heterotrophic conditions it can contribute significantly to integrated biological systems using sulphate removal processes to treat high sulphate wastewaters, which are produced in large quantities in South Africa (Molwantwa *et al*., 2004).

3.2 Materials and methods

The experimental set up was an anaerobic contact process, shown in Figure 3.1. The system comprised a continuously stirred tank reactor (CSTR) with a working capacity of 30 L. The contents of the reactor were stirred from the centre for even mixing. The contents of the reactor overflowed into a 30 L clarifier, entering approximately halfway down the clarifier. The CSTR was closed and placed under positive pressure to prevent

any odourous gas from escaping into the atmosphere, with a pipe leading from the CSTR into a dead-ended silicone tube (gas permeable) immersed in the clarifier as a precautionary measure to use any foul air that might be produced in the CSTR to aerate the clarifer. The clarifier was to act as a sulphide-oxidizing unit. The CSTR was inoculated with both activated sludge and digested sludge obtained from the Amanzimtoti wastewater treatment works. Final effluent from a holding tank was pumped into the CSTR. Settled sludge from the bottom of the clarifier was recycled back into the top of the CSTR. Start-up of the system was one week, to allow initial acclimatisation of the bacteria. During this time the CSTR was fed batchwise with a combination of settled sewage from the same WWTW and wastewater (settled final effluent) from the ethanol production site. The proportion of sewage used was decreased stepwise until the influent comprised 100 % site wastewater. The hydraulic retention time (HRT) was 36 hours. Recycle pump flow rate and feed flow rate were equal during the acclimatisation period. The recycle flow was later increased to twice the flow rate of the feed pump to resolve biomass blockages in the recycle tubes.

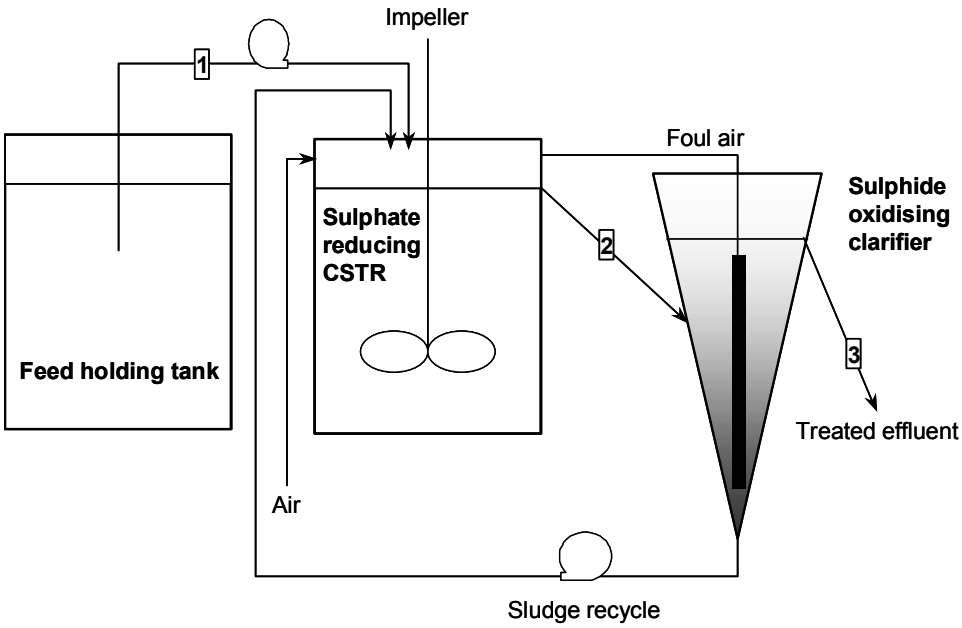

Figure 3.1 Schematic diagram of sulphate reducing anaerobic contact process.

The system was run continuously and samples were taken from day 0 three times a week from three sampling points: 1 influent (from holding tank) 2 mixed liquor overflow from CSTR and 3 effluent (to drain). See appendix E1 for a photograph of the on-site anaerobic contact process (ACP). The following parameters were monitored: COD, sulphate, sulphide, pH and both suspended solids and mixed liquor suspended solid (MLSS). Chemical oxygen demand, sulphate and sulphide concentrations were measured with photometric test kits as described in Chapter 2. The pH was measured using an inoLab desktop pH meter. Suspended solids and MLSS were performed according to Chapter 2.2. All test methods were based on Standard Methods (APHA *et al*., 1998). Calculation methods for sludge age and HRT can be found in appendix D.

3.3 Results

The results of the tests performed during the 20-week period of bioreactor operation are documented below. Figure 3.2 represents the total sulphate removal efficiency trend during the trial. Appendix I illustrates actual chemical concentrations of components measured.

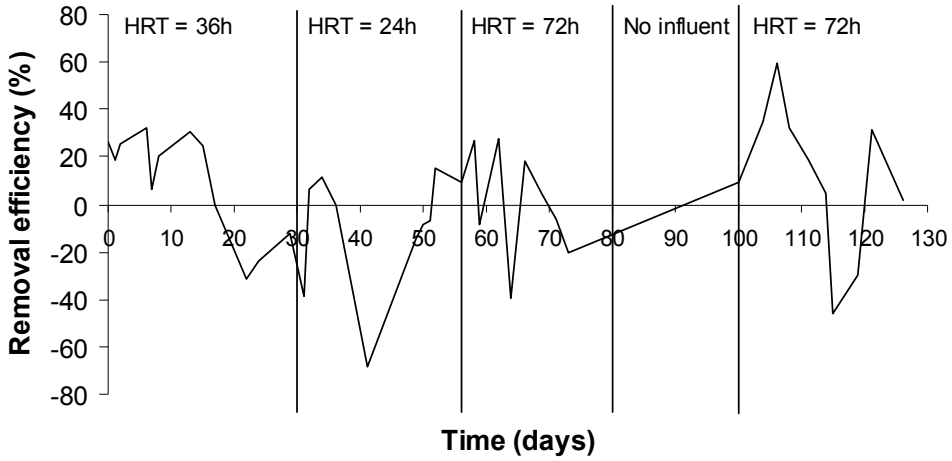

Figure 3.2 Sulphate removal efficiency from the final effluent during the 20-week trial.

This figure shows the overall sulphate removal as monitored during the trial. Since the trends did not follow the theory, i.e. reduction in sulphate from the wastewater to below

250 mg/L, as observed by Maree *et al*. (2004) and increase in pH of the wastewater, steps were taken to adjust conditions to enhance the SRP activity, as it was thought that the other microorganisms in the inoculum were able to dominate the SRP population by competition with substrate availability. These adjustments included changing HRT, addition of more concentrated SRP inoculum, pH adjustment and sulphide dosing.

The following results are reported according to each HRT used during the trial. Figure 3.3 shows the removal efficiency of sulphate from the wastewater in the bioreactor system during the initial HRT (36 hours). It can be seen that from day 0 to day 15 there was sulphate removal, with the highest removal being 32.25 % on day 6. This trend of increasing sulphate removal was expected to continue. However, on day 7 the trend was reversed. The least amount of sulphate removed between days 0 to 15 was on day 7 with only 6.33 % removal.

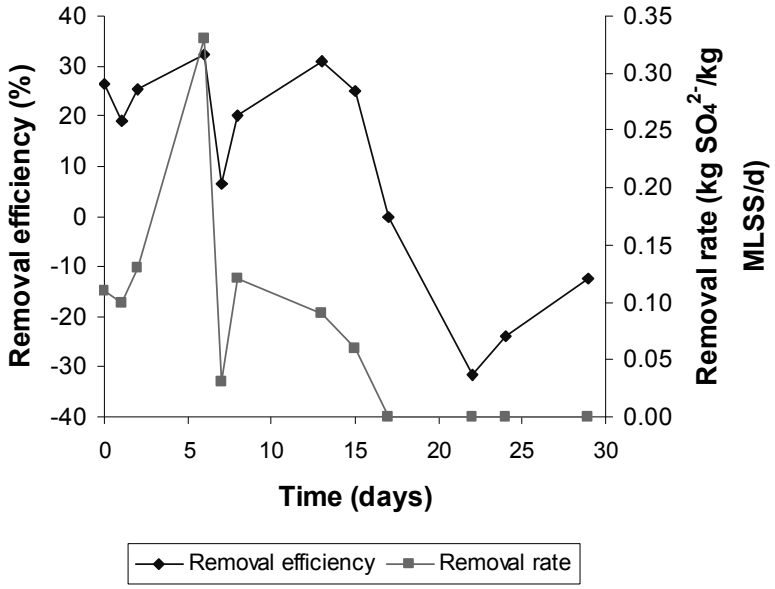

Figure 3.3 Sulphate removal efficiency from the final effluent at 36 hours HRT.

The reason for such a fluctuation in the removal of sulphate in those first few days could be explained, as the microorganisms need time to reach steady state. However, it can be seen in Figure 3.3 that from day 15 to 29 there was no sulphate removal. Although the sulphate removal efficiency seemed to be good in the first few days, the removal rate was fairly low with a steady decrease from day 8 until no sulphate was being removed for the

rest of that HRT period. The HRT was decreased in order to stress the heterotrophic bacteria which appeared to be outcompeting the SRP, in line with previous work in which Maree *et al*. (2004) found that decreasing HRT (from 48 to 5.5 hours) corresponded with an increasing sulphate reduction rate (from 0.2 to 12.4 g SO_4/L/d). The decrease in the HRT of the system to 24 hours did not improve the sulphate reduction, in fact the sulphate removal worsened. Figure 3.4 shows the removal efficiency of sulphate during the 24 hour HRT. When the HRT was decreased to 24 hours the sludge age was also adjusted from eight to six days by increasing sludge wastage. Results showed that sulphate removal only occurred on two days during the entire run time at 24 hours HRT, with the removal efficiency only being 5 and 10 %, on day 32 and 34 respectively. Thereafter no sulphate reduction occurred.

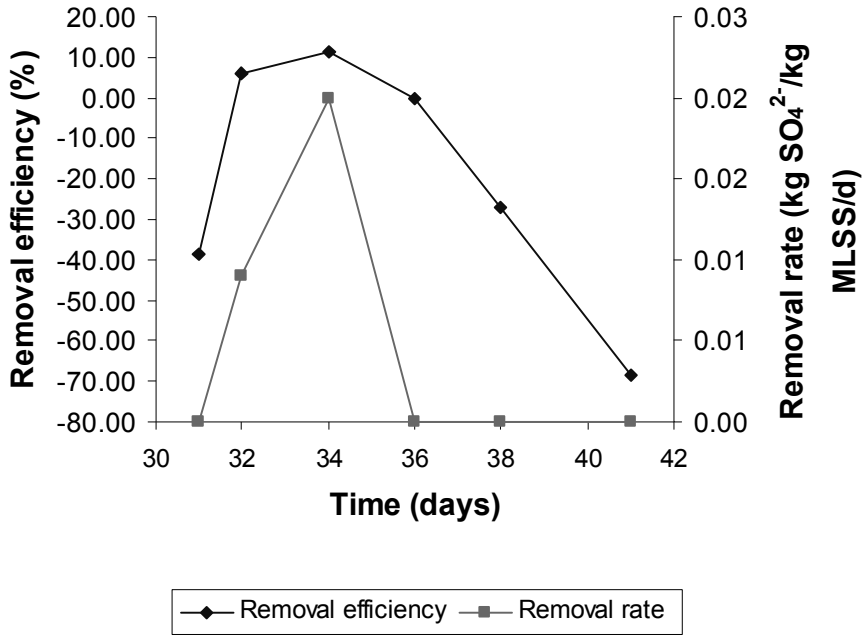

Figure 3.4 Sulphate removal efficiency from the final effluent at 24 hours HRT.

Since wastewater is a major problem for industries in terms of disposal, effective treatment is essential for discharge and trials are done with the intention of scale up to full-scale plants. Due to the fact that large amounts of wastewater are generated by industrial production companies, often more than the product itself, short HRTs are desired in order to cope with large volumes. Hence the aim was to start at a high HRT to

allow the microorganisms to acclimatise to the wastewater and then decrease it stepwise to as low as possible. However, the results from the decrease in HRT from 36 to 24 hours indicated that the reactor was performing better at the higher HRT. Therefore the decision was made to further increase the HRT to 72 hours to determine if improved reactor performance could be achieved. Prior to the change in HRT to 72 hours more concentrated SRP from Grootvlei mine, Gauteng, maintained at Rhodes University, Grahamstown, South Africa were added to the CSTR on day 42 to augment the consortium from the original sludge inoculum. The feed and recycle pumps were also turned off for a more anaerobic environment for six days. Samples were taken from the CSTR to monitor progress, those results (Table 3.1) indicated that even with the dosing of a more concentrated culture of SRP under less aerobic conditions sulphate reduction was still unstable. There was a steady decrease in total concentration from day 43 to 45 but a great increase on day 48. Still there seemed to be dominance of other microorganisms in the consortium outcompeting the SRP.

Table 3.1 Sulphate concentration of mixed liquor in the CSTR after bioaugmentation.

Day	Sulphate concentration (mg/L)
42	3800
43	5400
44	5200
45	4000
48	8600

Figure 3.5 shows the sulphate removal after the pumps were switched back on from day 48 until the end of the trial. During this time the only HRT used was 72 hours although other operational changes were made.

During the trial at both 36 and 24 hour HRT it was noticed that the pH remained fairly unchanged (Table 3.2). Therefore, in conjunction with the increase in HRT to 72 hours the influent pH in the holding tank was adjusted with sodium hydroxide (20 % solution) to pH 6.5 before feeding the reactor. Also the headspace air supply was turned off as it was thought that the excess oxygen content in the reactor might have been contributing to

the failure of the SRP (as sulphate reduction is anaerobic). The only oxygen content was that from the atmosphere through a small opening in the lid of the reactor.

It took three days to gradually adjust to the change in flow rate and thereafter began the process of sulphate reduction. However there was a fluctuation between days on which sulphate reduction occurred, and when there was no reduction taking place. Figure 3.5 shows that there was a steady decease in the removal efficiency of sulphate, from 17.82 % to below the 0 % mark, indicating a net gain in sulphate, possibly due to reoxidation from sulphide. Although there seemed to be a greater increase in sulphate removal in comparison to the 36 hour HRT, the removal rate was low at less than 0.04 kg/day for that period (data not shown).

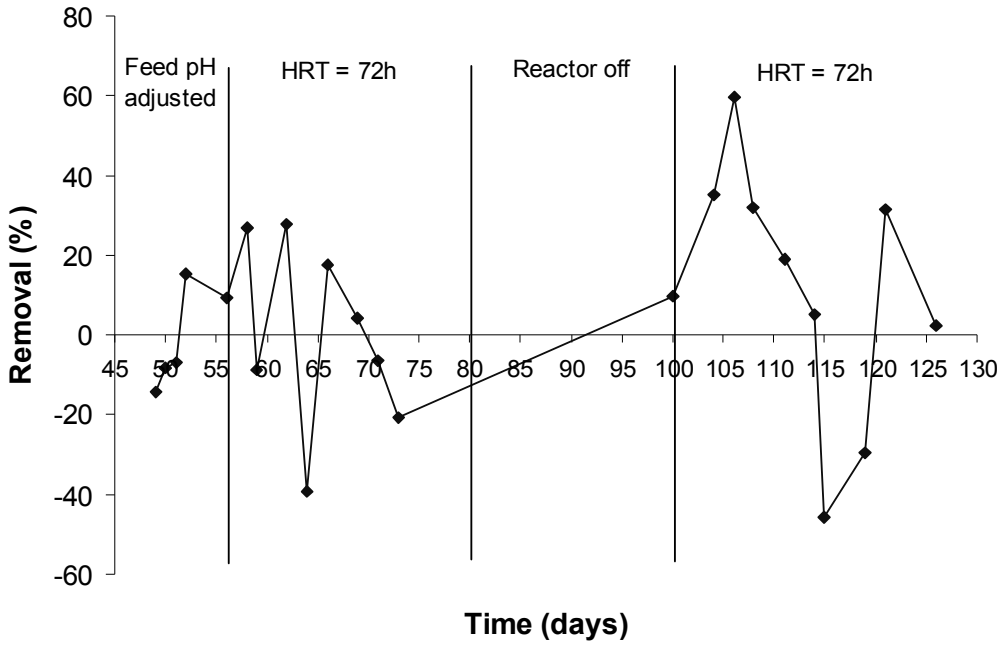

Figure 3.5 Sulphate removal efficiency at 72 hours HRT.

These results led to the decision to turn off the reactor completely and keep it sealed until day 100. During this time the reactor was also dosed with 100 mg/L of sulphide, as high sulphide concentrations are known to inhibit the methanogenic bacteria that can compete with SRP for substrates (Percheron *et al*., 1997).

During the first few days after restarting the reactor the sulphate removal seemed to steadily improve resulting in the highest removal efficiency recorded during the trial, approximately 60 %. However, there was no subsequent removal after that day with the removal efficiency gradually decreasing until the end of the trial, with the exception of day 121 when removal reached 30 %.

Sulphide was measured throughout the trial (Figure 3.6). It was expected that the concentration in the CSTR would increase from sulphate reduction by SRP and decrease in the clarifier to below the influent level, owing to SOB converting sulphide into elemental sulphur, which was expected to appear as a film on the clarifier liquid surface.

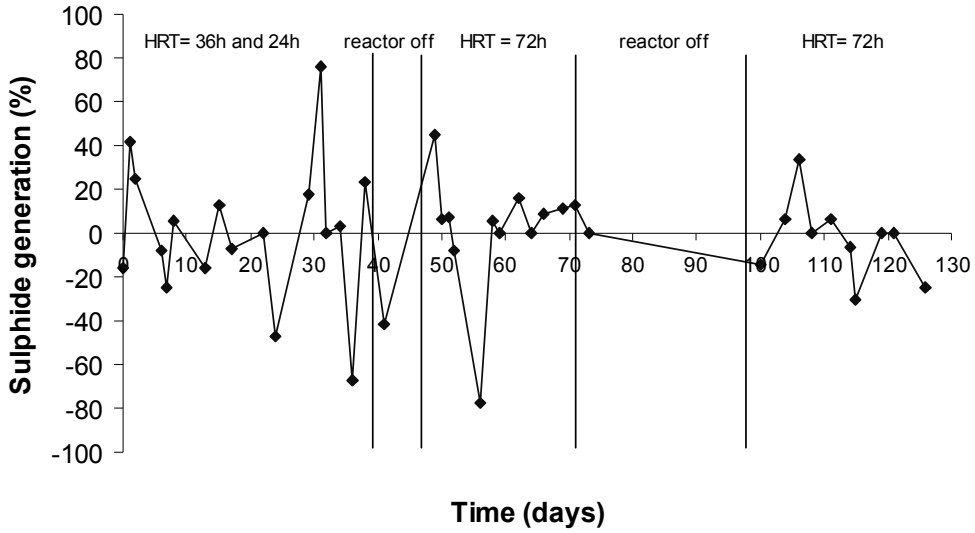

Figure 3.6 Sulphide generation in the CSTR during the 20-week trial.

It appeared that the differing HRT had no effect on the sulphide production in the CSTR during the trial, which only peaked at varying points. The sulphide generation coincides with sulphate reduction at each change of HRT. At the beginning of the trial sulphate removal was noted and Figure 3.6 shows that within the first few days there was up to 40 % sulphide production, but as with the sulphate, removal sulphide generation decreased and remained low until the change in HRT to 24 hours. At the beginning of the 24 hour HRT period there was sulphate removal and again sulphide generation in the CSTR, in fact the sulphide generated here was the highest throughout the period of the trial. The

same trend is noticed for the 72 hour HRT change although the generation of sulphide is low for the first change to 72 hours HRT (day 56 to 73) it is slightly higher when switched on after the reactor was off during days 73 and 100.

Sulphide removal from the clarifier was mostly good during the 36 and 24 hour HRT periods, during which there was no sulphide removal on very few days (Figure 3.7). Removal was also fairly good after the reactor was off from day 73 to day 100. The sulphide removal from the system as a whole was better than the sulphate removal from the system, which was the principal aim. Sulphide was low to begin with and therefore the decrease in sulphide concentration resulted in even lower values, which were closer to the permitted discharge limits. Even though the sulphide levels showed some trends and removal was high in most cases there was no consistent removal of sulphide from the wastewater or generation of sulphide arising from sulphate reduction seen in the reactor.

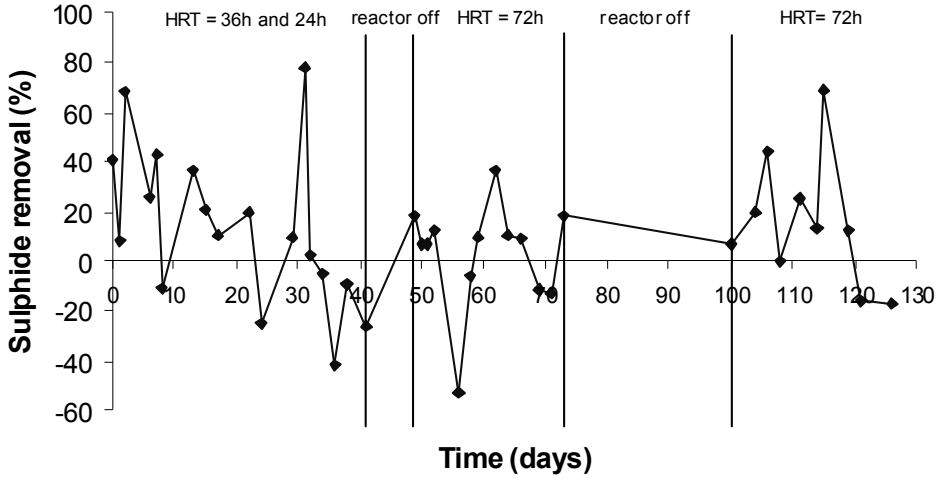

Figure 3.7 Sulphide removal efficiency from the clarifier during the 20-week trial.

The pH of the mixed liquor in the CSTR did not change throughout the trial as indicated by the low standard deviations from the data set (Table 3.2). It can be seen that the only time there was a slight increase in the pH value was when the pH of the feed tank was adjusted using NaOH. Even though the pH was adjusted to 6.5, which is above the specification limit of 5.5, the average pH measured was 5.09 in the holding tank 24 hours after adjustment and pH 4.81 in the effluent during that time period, which was still below the specification. This suggests that the wastewater was strongly buffered. Dosing

with NaOH did not maintain the adjusted higher pH in order for the SRP to survive as hoped, as their optimum pH is approximately 6.6. Also, the volume of NaOH added was large and it would not have been economically viable to continuously dose with NaOH due to the high cost, storage and handling issues of chemicals. The rest of the pH values were all below 4.5.

Table 3.2 pH values of the ACP during its running time.

HRT (hours)		Influent	CSTR	Effluent
36	Max	4.91	4.48	4.49
	Min	4.22	4.21	4.2
24	Max	4.53	4.48	4.47
	Min	4.25	1.28	4.29
24 Feed adjusted to pH 6.5	Max	5.55	5.27	5.13
	Min	4.71	4.67	4.52
72	Max	4.71	4.72	4.78
	Min	3.98	3.98	3.98

Chemical oxygen demand showed the best results in terms of removal efficiency (Figure 3.8). Although there was little COD removal during the first stage of the experiment, while running at both 24 and 72 hour HRT the COD removal was good at times, although not consistent. The removal of COD shows that the consortium was active. However, the COD-utilizing bacteria were not SRP, indicated by the fact that the sulphate was not being reduced while the carbon was being utilised. The removal rate of COD was much higher than the removal rate of sulphate, with COD removal rates as high as 19 kg/day. In comparison, maximum removal rates of sulphate did not even reach 0.5 kg/day.

The poor performance results led to a focus on the sulphur compound parameters measured after the reactor had been completely switched off with anaerobic conditions and additional sulphide dosing, which took place from day 100 to day 126. The CSTR showed signs of recovering, as the sulphate removal increased to 60 %, but a decrease in removal was shown thereafter, except on day 121 when there was 30 % removal of sulphate (Figure 3.9). Total sulphide removal however did not increase and remained low, except for the peak on day 115. It was interesting to note that on day 115, when

there was an increase in COD removal, (Figure 3.10) there was no sulphate removal and on day 121, when there was no COD removal from the wastewater there was sulphate removal. If the SRP had been utilizing the carbon then simultaneous removal of both sulphate and COD from the system should have been observed. This could suggest that for some reason, perhaps changes in wastewater composition, something caused the competing bacteria to be suppressed, allowing the SRP to dominate on day 121 only.

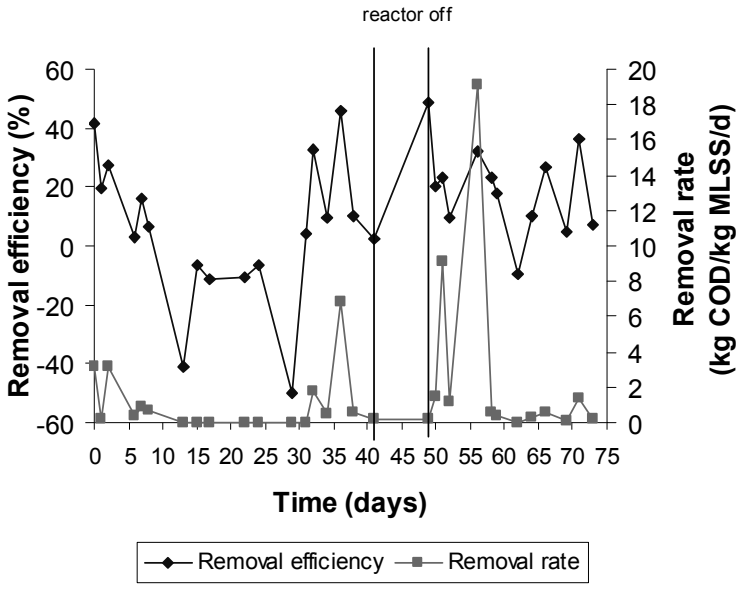

Figure 3.8 COD removal from the final effluent from day 0 to 73.

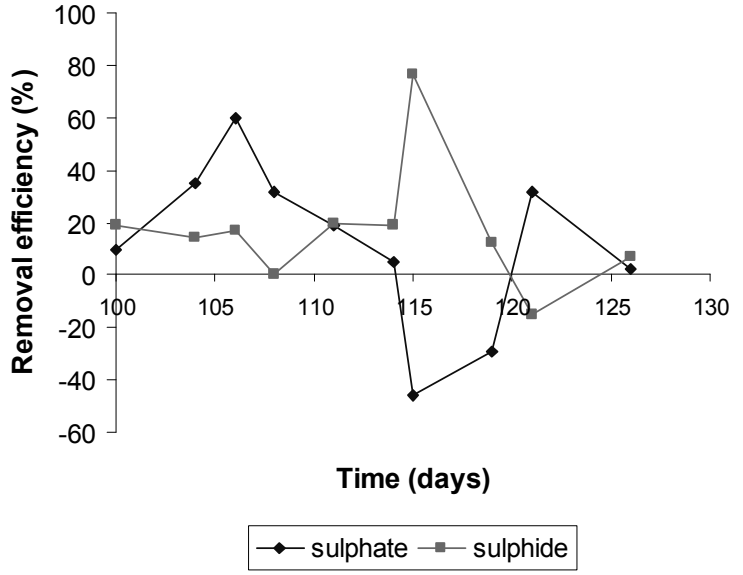

Figure 3.9 Sulphate and sulphide removal efficiency after restarting the system.

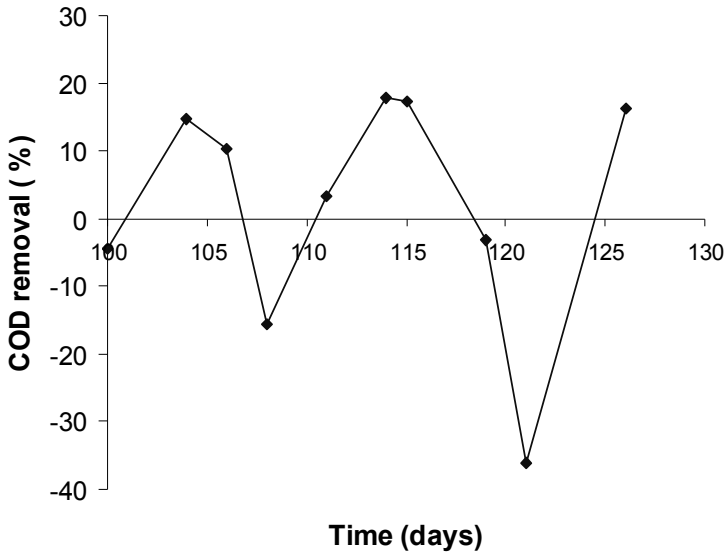

Figure 3.10 Chemical oxygen demand removal efficiency after restarting the bioreactor.

The pH values for all three sample points did not differ from the results previously discussed, with the average being approximately pH 4.2 (data not shown).

Volatile fatty acids were determined using a spectrophotometric method from the Standing Committee of Analysts (1979) (Appendix E). Five sets of samples were taken and the results showed that the total VFA concentration of the wastewater was very high in comparison to the maximum 0.3 g/L necessary for successful anaerobic digestion (Table 3.3). Usually, concentrations above this result in digester failure (SCA, 1979). The holding tank, which contained the wastewater, also had a high concentration of VFAs, although this was not a representation of the VFAs in the wastewater discharged from the site, as there was biomass growth on the surface of the wastewater in the feed holding tank which may have resulted in VFA production. The mean VFA concentration in the CSTR was substantially higher than the wastewater in the feed tank and treated effluent. This suggested that the acidogenic bacteria were active in the CSTR.

Table 3.3 Volatile fatty acid concentrations of samples from the bioreactor (g acetic acid/L).

	Feed Tank	CSTR	Effluent
Day 114	11.898	14.319	14.624
Day 115	10.928	11.335	13.460
Day 119	13.018	16.555	13.979
Day 121	9.869	13.014	10.925
Day 126	10.248	12.263	13.195
Average	**11.192**	**13.497**	**13.236**
Standard deviation	**1.279**	**2.028**	**1.402**

Given the poor process performance data, the FISH protocol used in Chapter 2 to investigate the corrosion potential of the wastewater was utilised to determine whether SRP were present in the ACP in sufficient numbers. The results (Figures 3.11 to 3.14) were compared to results obtained using the same protocol on a sample from a sulphate-reducing laboratory anaerobic digester (Figures 3.15 and 3.16).

The results of DAPI staining showed that the ACP contained comparable quantities of bacterial matter to the laboratory digester (Figures 3.11, 3.13 and 3.15). Using specific probes for *Desulfotomaculum* spp. on samples from the ACP (Figures 3.12 and 3.14) resulted in a number of false positives from the presence of particulate matter, but comparison with a general probe for SRP applied to the laboratory digester sample (Figure 3.16) indicated that sufficient numbers of SRP were present to call the ACP a SRP-containing system. The results indicated that the bioaugmentation had resulted in the presence of sulphate-reducers in the ACP consortium, and that the failure of the sulphate reduction process was not due to a straightforward lack of SRP.

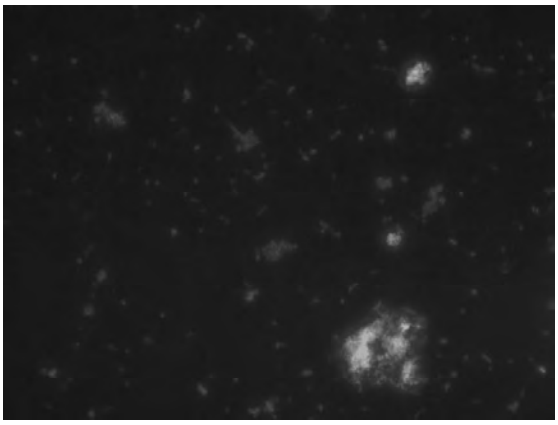

Figure 3.11 DAPI stain of CSTR sample

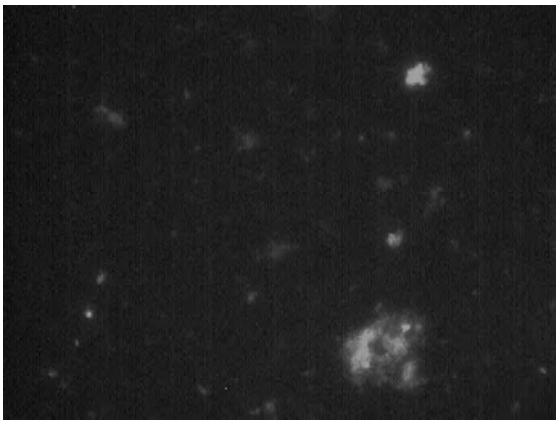

Figure 3.12 Fluorescent stain (probe 228 for *Desulfotomaculum*) of CSTR sample

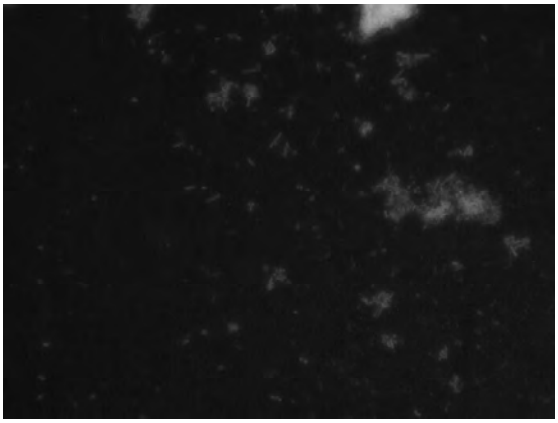

Figure 3.13 DAPI stain of CSTR sample

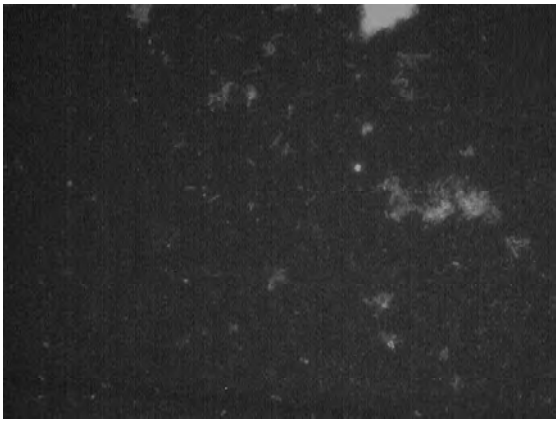

Figure 3.14 Fluorescent stain (probe 687 for *Desulfotomaculum*) of CSTR sample

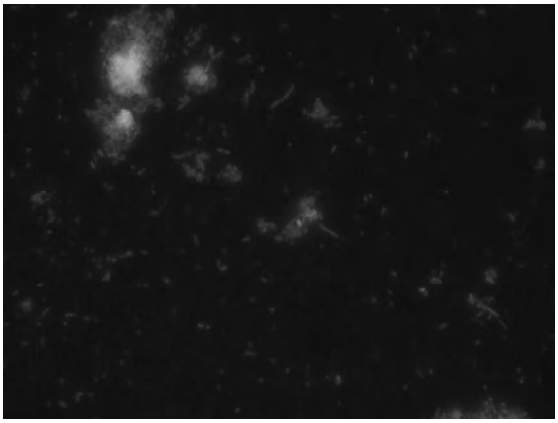

Figure 3.15 DAPI stain of mixed SRP culture maintained in the laboratory on Postgate media

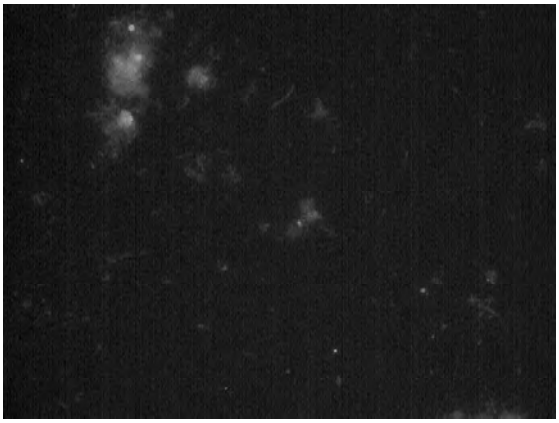

Figure 3.16 Fluorescent stain (probe 385 for SRP) of mixed SRP culture maintained in the laboratory on Postgate media

3.4 Discussion

The principal aim of the project was to reduce sulphate by employing the SRP present in a consortium during anaerobic digestion, in which SRP use sulphate as the terminal electron acceptor, thus reducing it to sulphide. Sulphide oxidisers present in the aerobic clarifier of the system oxidise sulphide to elemental sulphur as a biofilm on the surface of the liquid. The biological reduction of sulphate also usually results in the degradation of organic compounds and an increase in pH due to proton consumption and the production of alkalinity. For this sulphate reduction to occur the SRP need an electron donor, which can be in the form of simple compounds (e.g. H_2 gas) or a complex combination of compounds (e.g. solid waste). Since SRP may not be able to use some complex organic matter it needs to broken down anaerobically first, to more simple compounds (Hansford *et al*., 2004).

When treating a high sulphate wastewater in an anaerobic system using insoluble sludge as a carbon source there are many reactions that can take place. Figure 3.17 is an overview of these reactions.

Menert *et al*., (2004) also describes the five sub-processes during the process of anaerobic digestion of high sulphate wastewater: hydrolysis, fermentation, acetogenesis, reduction of sulphates and methanogenesis.

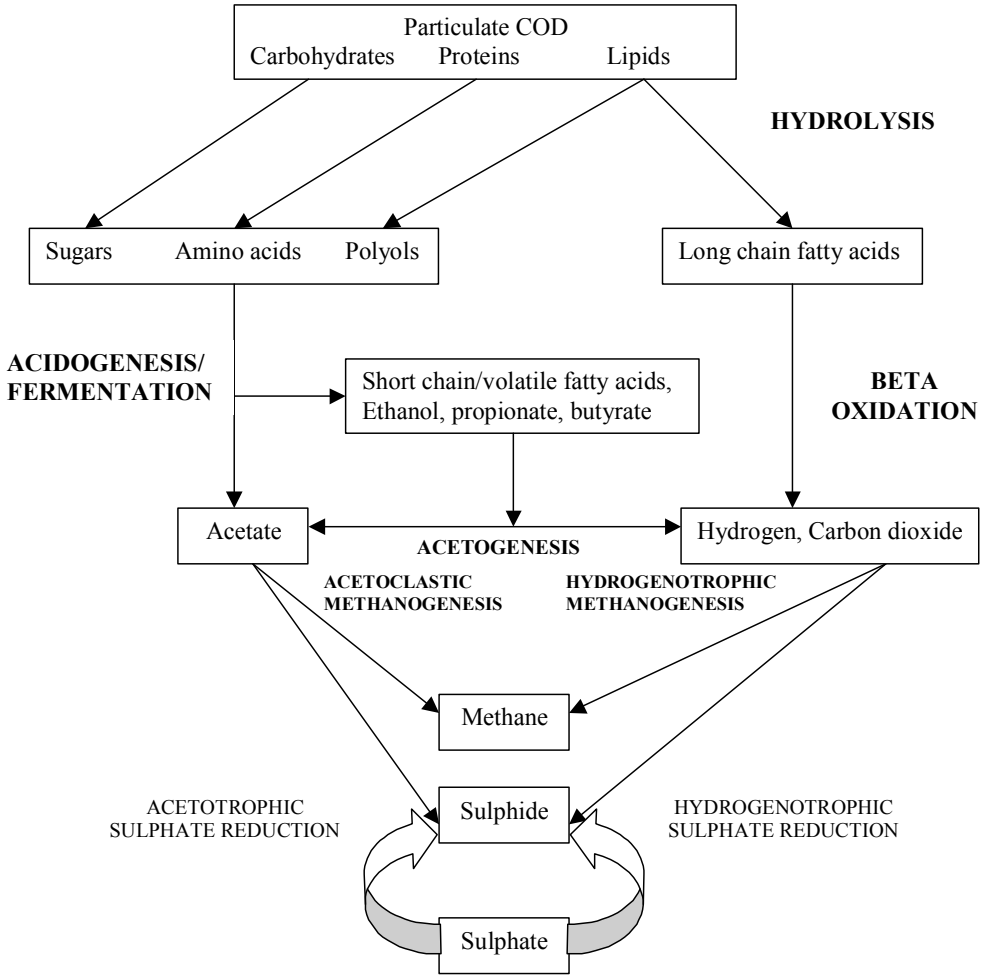

Figure 3.17 Diagram of the major substrate flows in an anaerobic system.
(Source: Hansford *et al*., 2004)

In most cases of anaerobic digestion, sulphate reduction to sulphide is unwanted due to the adverse effects of hydrogen sulphide gas production. However in this case sulphate reduction was the desired process. Since sulphate reduction to sulphide almost always takes place in anaerobic digestion unless operational changes are made and controlled this should not have proved to be a difficult feat. The sulphide production, although often a problem for many researchers, can be rectified by a number of methods, such as dilution of the sulphate wastewater, pH increase of the reactor, chemical precipitation, oxidation and stripping (Menert *et al*., 2004). Biological methods can also be used, which is what was intended in this case, in which colourless sulphur bacteria can remove sulphide by the conversion to elemental sulphur. The conversion proceeds as follows:

$$2HS^- + O_2 \rightarrow 2S^0 + 2OH^- \quad (5)$$

These bacteria were expected to be in the clarifier as growth of these bacteria need microaerophilic conditions (Menert *et al*., 2004).

During the biological removal of sulphate from high sulphate wastewater Maree *et al*. (2004) found that volumetric sulphate reduction from 0.2-12.4 g SO_4/L/d was due to an increase in biomass concentration with time and adaptation to the wastewater, while the specific sulphate reduction rate increased due to the improved performance of the microorganisms due to their improved adaptation to the surroundings. The finding of the bioreactor set up in this case did not show similar results, with no trend of increasing sulphate removal. Maree *et al*. (2004) also found that sulphate was removed to below 250 mg/L by day 37 and remained stable until the end of the trial. Despite using a similar set up to the one described by Maree *et al*. (2004), during this trial the sulphate concentration never reached less than 2000 mg/L. The reason for this could be owing to the fact that real wastewater was used in this case, unlike Maree *et al*. (2004) who used synthetic sulphate-rich wastewater to determine sulphate removal capabilities. When using real wastewater many other factors play a role in determining the outcome of reactor performance such as possible presence of inhibitors, and most probably in this case, competition for substrates by other bacteria in the sludge inoculum. This would not have been accounted for in the other experiment, as nutrients specific for SRP would have been used. Also the correct carbon source was used to ensure reactor success, whereas the spent molasses present in the wastewater was to provide carbon in this study.

Maree *et al*. (2004) stated that this process has a distinct characteristic for its stability. Although this may have been true in their case, the present study found that with this reactor there was no stability noted. They also stated that any plant failure would only occur from deterioration in the quality of effluent, which was what was observed when sludge was lost, and HRT was changed. This may partly explain failure of this reactor, as wastewater composition is likely to change, due to the molasses used for the fermentation process, as molasses composition is affected by seasonal variation in the sugar cane. Also the wastewater treated in this case was final mixed wastewater from the whole site, which

is not only the wastewater produced from fermentation but also all the other wastewaters generated around the industrial site. The lactulose production plant on site does not send out wastewater regularly as it is a batch process, so wastewater from that production plant may or may not be present in the final mixed wastewater at any given time. Even so there should have been some steady sulphate reduction at some parts during the trial and only changes indicated on certain days (e.g. when HRT was adjusted). But the sulphate removal profile displays no consistent trend in sulphate reduction.

The process performance results indicated and the FISH results confirmed that the sulphate reducers were present in the system and at times reducing sulphate, however they seemed to be competing poorly with other bacteria during anaerobic digestion, hence the constant troughs and peaks in the profile. The most common competition was that between SRP and MPB. The outcome of this competition was important, because unwanted MPB were competing with wanted SRP for hydrogen and acetate. It was desirable that the system comprised mostly dominating SRP. Methane producing bacteria did however have some important roles. These hydrogen-utilising bacteria have a relationship with fermenting bacteria, which need low H_2 partial pressure for successful fermentation. Also, these bacteria were required to produce large biomass flocs via their production of extracellular polymeric substance (EPS) in which both SRP and MPB are retained (Hansford *et al*., 2004).

Previous studies of competition between SRP and MPB have showed that under low substrate conditions SRP have the advantage due to the half velocity constants being an order of magnitude less than the MPB especially in a CSTR (Hansford *et al*., 2004). However for a UASB with better floc formation MPB have the advantage. Also a shorter sludge age allows for a decrease in the time taken for SRP to dominate. Other factors include the sensitivities of each to inhibitors such as sulphide and pH. Greater inhibition of sulphide has been reported for H_2 and acetate utilising SRP in comparison to H_2 and acetate utilising MPB. Optimal pH ranges for both microorganisms are similar however in a mixed culture MPB have better growth rates below pH 6.9 than SRP and above pH 8.5 SRP have the advantage (Hansford *et al*., 2004).

Some of these could have been factors affecting the performance of the bioreactor, hence the changes made to steer competition towards SRP. Omil *et al*. (1997) discuss that adjustment of operational parameters is able to steer the competition between SRP and MPB, such as temperature shocks and influent composition and that application of certain parameters or short-term shocks are able to modify the competition between these microorganisms. This shock treatment would create an unfavourable environment for a short time period allowing one population to dominate or outcompete the other if their sensitivities to the shock are different. The first change to the ACP operation was the decrease in HRT to 24 hours, which followed the procedure of Maree *et al*. (2004) where the gradual decrease in HRT from 48 to 5.5 hours yielded high sulphate removal. Conversely, these results showed that during the 24 hour HRT the reactor performance declined.

Subsequently a more concentrated culture of SRP was added to boost the population in order for domination of the consortium. Omil *et al.* (1997) also mentions that the addition of pure cultures to the system may be able to alter the competition between these two acetate-utilising microorganisms, hence the addition of a more concentrated culture of SRP to the CSTR. Although not pure, the bioaugmentation inoculum was taken from a thriving, stable sulphate-reducing digester. This in conjunction with switching off the pumps to allow the SRP to dominate the population did not satisfactorily reduce the sulphate levels in the treated wastewater in the long term. This is in accordance with the findings of Omil *et al*. (1997) who later showed that addition of 1 L of a pure culture of acetate-degrading SRP to a UASB reactor did not lead to sustained sulphidogenic acetate-removal capacity of the biomass. Also, as SRP are anaerobes it was thought that the air used to create the slight positive pressure in the headspace of the CSTR caused too few anoxic microniches within the flocs for the SRP to thrive, and the air supply was removed in the hope that the more anaerobic conditions would steer the competition toward the SRP in conjunction with the extra inoculum added. Omil *et al*. (1997) however found that short-term air exposure did decrease the COD removal capacity but both groups of microorganisms were equally affected by the air exposure. The acetate-utilising SRP did slightly recover and removed more COD. This agrees with previous findings that SRP are

tolerant to molecular oxygen (Fukuii and Takii, 1990). Omil *et al*. (1997) stated that the reactor performance on the whole declined after the first day of oxygen exposure suggesting that the process is a fragile one. This assertion, while contradicted by Maree *et al.* (2004) is supported by the present study.

The methods used above to improve the ACP performance proved to be unsuccessful. Since it was noticed that the reactor performed better at the higher HRT 36 hours (Figures 3.3 and 3.4) the HRT was increased to 72 hours. Initially the change in HRT seemed to select for sulphate reduction, but was unable to be maintained and there is a steady decline of sulphate removal. Perhaps the dosing of the influent with NaOH (20 % solution) to pH 6.5 aided in the initial sulphate reduction seen at the beginning of the change to 72 hours HRT. As the pH did not remain at the adjusted value, the sulphide possibly could have been reoxidised to sulphate resulting in the net gain in sulphate for the first phase at 72 hours HRT. This is because the unit was not airtight (i.e. not anoxic) and it was possible that the hydrogen sulphide would oxidise back to sulphate, thus apparently 'gaining' sulphate. However when the unit was sealed the apparent sulphate 'gain' was reversed and sulphate removal was observed. After the reactor was completely sealed off (from days 73 and 100) and dosed with sulphide (Figure 3.5) it can be seen that this had a significant effect on the sulphate reduction rate increasing the removal to approximately 60 %, but thereafter removal efficiency began to decline. This indicated that it is perhaps possible to remove sulphate from this wastewater using biological treatment, but perhaps more stringent control in operational parameters needed to be in place for success.

Sulphide generation due to dissimlatory sulphate reduction by SRP in anaerobic digestion generally poses a threat to anaerobic treatment strategies, as sulphide is corrosive and can be toxic to other bacterial populations involved in anaerobic digestion (Lens *et al*., 2000). However, in this case the generation of sulphide was desired to allow the SRP population to dominate the consortium and remove sulphate. Also the sulphide generation problem was adjusted for in the design by employing an anaerobic and part aerobic step in which SOB were to convert the sulphide into a useful floating sulphur film. The results showed

that at each change in HRT the sulphide generation corresponded with the sulphate removal indicating the SRP were able to dominate the consortium at times, possibly due to the shock treatment of the operational changes. Although there was no steady sulphide generation, probably because the sulphate reduction was also not steady, the sulphide production in the CSTR was due to sulphate reduction. The overall aim was removal of as much sulphide as possible by conversion into elemental sulphur from consumption of oxygen by SOB. The sulphide removal was better than the sulphide generation, with the best sulphide removal occurring during the 36 hour HRT. As with the generation of sulphide in the CSTR there was no trend in terms of sulphide removal in the clarifier, indicating that the sulphide removal was not due to the SOB. There was also no sulphur film formed as expected on top of the clarifier however, there was biofilm growth on top of the clarifier, but this was fungal and not bacterial. (See appendix E.2 for photograph of the biofilm). In addition, the level of sulphide produced was low, even after sulphide dosing. It was possible that the sulphide was released from the CSTR as gas, but this should have been accounted for by the silicone tube submerged into the clarifier to dissolve any H_2S gas that might escape. The pH of the bulk liquid was low and the form of sulphide present in the environment is dependant on pH. Hydrogen sulphide gas predominates at pH lower than 7, which is why it was expected to be present. Above pH 7 HS^- and S^{2-} are present and the pKa value for H_2S is 7. At no time was a smell of hydrogen sulphide detected at the ACP site. Even in the effluent samples the sulphide was very low, and since there was no elemental sulphur film formed the low sulphide in the treated effluent cannot be attributed to oxidation of sulphides to elemental sulphur.

The pH of the system remained very stable throughout the 126-day trial (Table 3.2). The only change in pH during the trial was when the influent pH was artificially increased using NaOH (20 % solution). Although there was a change in the pH after adjustment, it did not remain at the adjusted level but dropped within 24 hours, proving that the wastewater is highly buffered. Also perhaps the NaOH was not a good agent to use especially since a large amount was needed for the slight increase in pH to occur. It was discussed earlier that there was a possibility that MPB may have been dominating the SRP, especially since efforts to steer the competition in the favour of SRP to achieve

sulphate reduction were unsuccessful. However the low pH in the reactor may suggest otherwise. A study of the pH ranges of a number of MPB and SRB (pure cultures) showed that growth rates for MPB were similar over a the pH range 6.8-9.5 with the optimum between pH 7.0 and 7.5 and pH optima for growth rates of SRP cultures were between pH 7.5 and 8.0 (O'Flaherty *et al.*, 1998). This suggested that none of these bacteria were thriving in the wastewater since the pH was low. Even though methanogens and sulphidogens compete for the same substrates, inhibition of methanogens can also be caused from organic overload where the rate of acetogenesis exceeds methanogenesis, which leads to a pH decrease and digester failure (Nedwell and Reynolds, 1995). Volatile fatty acids are important intermediates during the metabolic pathway of anaerobic digestion, however if these are not broken down and accumulation occurs this can cause microbial stress on the system resulting in a pH decrease and ultimately digester failure (Buyukkamaci and Filibeli, 2004). This could explain why the pH was not being increased and furthermore, since SRP were not stable in their environment and reducing sulphate as expected, they were unable to create alkalinity in the wastewater from the production of carbonate. Acidogens present in the anaerobic digester, responsible for the production of acetate and other volatile fatty acids, were most suited to the environment and no pH changes were seen.

Since presence or production of high concentrations of VFA in anaerobic digesters is known to cause digester failure, during the last few days of the trial total VFA concentrations were determined (Table 3.3). Hill *et al*. (1987) suggested that when acetic acid levels exceed 0.8 g/L it indicates digester failure and the levels of total VFAs detected in the reactor were not below 9.869 g acetic acid/L and reached levels as high as 14.624 g acetic acid/L. These high concentrations could have been the reason for the digester failure, as the SRP and MPB would not be able to tolerate such high levels of VFAs. The average concentrations of VFAs in the CSTR and effluent were higher than in the holding tank, which suggests that the VFAs were being produced and did not originate in the wastewater.

Of all the parameters measured in the trial the COD showed the most consistent removal particularly after day 30. This showed that the consortium in the reactor was active as the carbonaceous material was removed, however the SRP were not responsible for the COD removal, as there was no concurrent sulphate reduction. In fact Figures 3.9 and 3.10 show that where there were indications of sulphate removal, the COD removal was low or 0 %, and vice versa.

3.5 Interim summary and conclusions

The SRP in the bioreactor were unable to increase the pH from 4.4 or to consistently reduce the sulphate in the wastewater, possibly due to competition with MPB or domination of acidophiles in the consortium which produced acetate and lead to production of other VFAs known to cause reactor failure when found in high concentrations as was noticed here The sulphide generation corresponded with sulphate reduction at certain points during the trial in the CSTR, but no steady sulphate reduction or sulphide generation was maintained. Sulphide concentrations were generally low throughout the trial, even though there was slight generation in the CSTR, which was often removed in the clarifier. Sulphate-reducing prokaryotes were present but struggled to continuously remove sulphate from the wastewater. It is thought that they were outcompeted by other microorganisms present the consortium used for inoculation of the bioreactor, indicated by COD removal results which showed consumption of carbon but not by SRP because the COD removal did not correspond with sulphate reduction. It can be concluded that while SRP were present in the ACP they were not able to reduce sulphate sufficiently for discharge into the environment despite changes to operational parameters such manual pH adjustment, change in HRT, sulphide dosing and decrease in oxygen exposure.

Since the bioreactor started showing signs of recovery immediately after sulphide dosing and complete closure of the system it was concluded that the SRP might be able to treat the wastewater under controlled conditions. A lab-scale experiment was designed in order to determine whether the wastewater was treatable under ideal conditions.

Chapter 4
Sulphate-reducing bioreactor: batch study

4.1 Introduction

Sulphate-reducing prokaryotes are able to reduce the sulphate concentration in high strength wastewaters and increase the alkalinity of these wastewaters, provided they are not outcompeted by other bacteria which are often found in the consortia used for inoculation when treating wastewater. Chapter 3 described the problems associated with the ACP used to treat the distillery wastewater. A batch test was designed to determine if the wastewater was treatable under ideal conditions. The set-up was to employ specific conditions suited to SRP alone, with the aim that only sulphate reduction should take place. This differs from the system described in Chapter 3, which was designed for both sulphate reduction and sulphide oxidation. Results from this batch test should show whether the SRP have the capability of reducing the sulphate in this particular wastewater, and if the pH can be increased to the specifications set for discharge to the sewer. Also, if sulphate reduction does take place this would provide an estimate of the HRT needed for maximum sulphate reduction. This reactor should show a decrease in sulphate concentration and an increase in sulphide concentration. The pH levels are expected to increase to within the range of suitable conditions for SRP. This experiment is to be performed without adjustment to the wastewater as adjusting parameters could prove to be too costly when scale-up is considered, even if the process was to be successful. The principal aim of this batch trial is to determine whether the SRP can adapt to treat the ethanol production wastewater given ideal conditions.

4.2 Materials and methods

Figure 4.1 shows a schematic of the bioreactor design. It consisted of a 5 L stirred reactor containing 30 % SRP inoculum obtained from Rhodes University, Grahamstown, South Africa (initially isolated from Grootvlei mine, Gauteng) and 70 % wastewater from the ethanol production plant. The reactor was completely anaerobic, with a line taking

offgas into a 7 % zinc acetate trap and a sample port to monitor activity. A sealable port was available for nitrogen gas sparging during sample collection to prevent oxygen from entering the system.

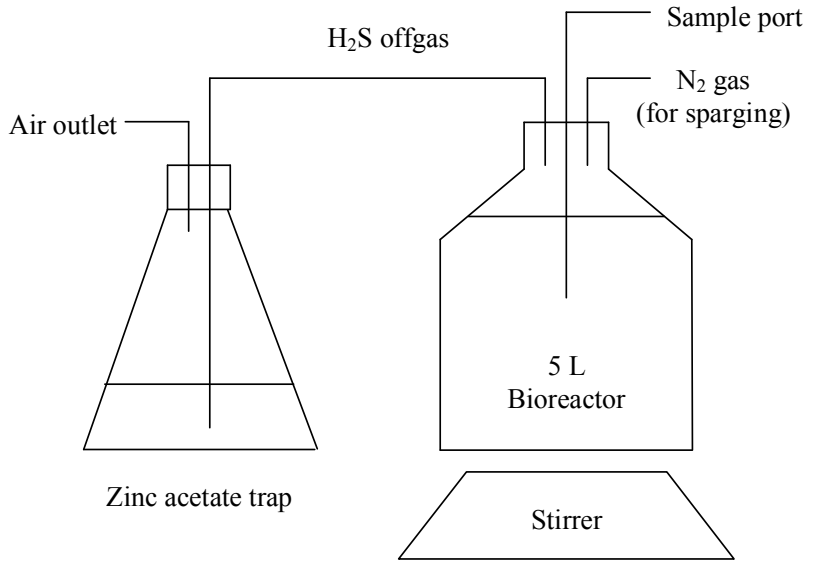

Figure 4.1 Schematic diagram of the laboratory sulphate-reducing digester.

Daily samples were extracted from the digester from day 0 to day 3 and thereafter samples were taken three times weekly until day 30. The samples were tested for pH, sulphate and sulphide concentration as described in Chapter 2.

4.3 Results

Day 0 represents the values of the wastewater only, before start up of the experiment. From days 1 to 20 the sulphide and sulphate followed the same trend. From day 20 to the end of the trial the sulphate gradually decreased to approximately 1760 mg/L. Sulphide on the other hand followed no distinct pattern. After an increase in sulphide concentration of ~ 7 mg/L between days 0 and 1 there was a steady decrease until day 6, when the sulphide showed peaks and troughs throughout the rest of the trial (Figure 4.2).

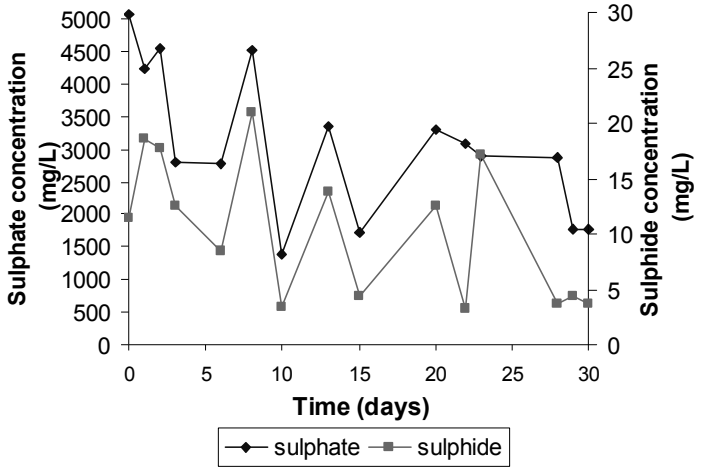

Figure 4.2 Sulphate and sulphide concentration during the laboratory trial.

These results were not expected. By comparison, the laboratory digester from which the inoculum had been taken was continually providing results such as those shown in Figure 4.3. The results were expected to differ to a certain extent, as this digester is fed on modified Postgate media, which is specific for the nutritional needs of the genus *Desulfovibrio*. Lactate, pyruvate and malate belonging to the carbon 3 and carbon 4 fatty acids are used as carbon sources and ammonium ions serve as the nitrogen source for the growth of the organism (Postgate, 1984).

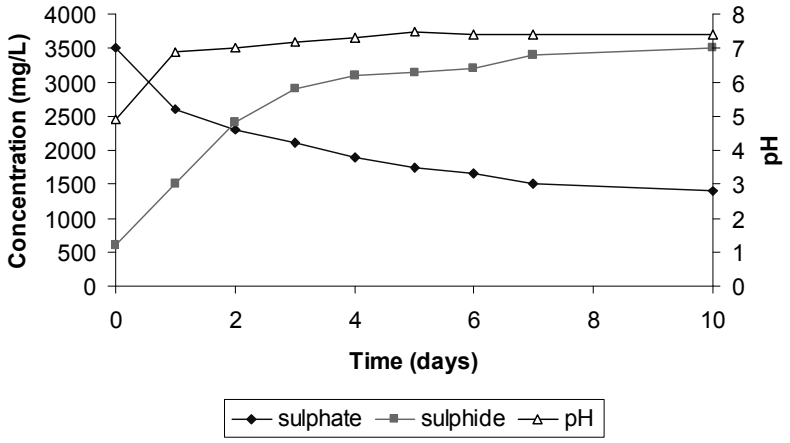

Figure 4.3 Typical sulphate and sulphide profile for a lab-scale digester operating well.

A less marked increase in sulphide concentration than shown in Figure 4.3 was expected, as the laboratory bioreactor used in the present study was connected to a zinc acetate trap

to prevent pressure build-up during the longer than usual running time (30 days compared to 10) and to prevent the escape of H_2S gas into the air of the laboratory, whereas the example digester did not vent to a trap and hence could be expected to accumulate more dissolved sulphide.

On day 1 both the sulphate decrease and sulphide increase was as expected, because this was due to the mixing of the inoculum and the wastewater. The SRP inoculum had a high concentration of sulphide. The SRP started to reduce some of the sulphate present. The sulphide concentration in this experiment is also similar to the sulphide trend in the continuous reactor, in that the concentrations were low. Although the levels did not meet the requirements for discharge (1 mg/L) the values were always low and did not increase as expected from sulphate reduction to sulphide. However there was some reduction in sulphate for various samples, with the last sample having a sulphate concentration of 1760 mg/L. The overall reduction of sulphate from the initial concentration found in the wastewater and the final concentration after 30 days was approximately 65 %. However 1670 mg/L was not the lowest sulphate concentration; this was 1380 mg/L recorded on day 10. From these results it seemed that the reduction in sulphate did not correlate to the sulphide concentration in the digester. The constant peaks and troughs during the trial suggested that the SRP, although present and trying to metabolise the sulphate, were being inhibited by components in the wastewater itself.

Figure 4.4 shows the pH profile of the reactor during the trial. Again day 0 is the pH of the wastewater before inoculation and day 1 represents the mixed inoculum and wastewater. The batch-fed digester which was the source of the inoculum regularly increases the pH of new media from 4.9 to a steady 7.3 ± 0.3 (Figure 4.3). The pH of the lab digester fed distillery wastewater showed initial promise with a slight increase to pH 5.2 on day 1, but thereafter steadily decreased by small amounts and reached a steady state at pH 4.4.

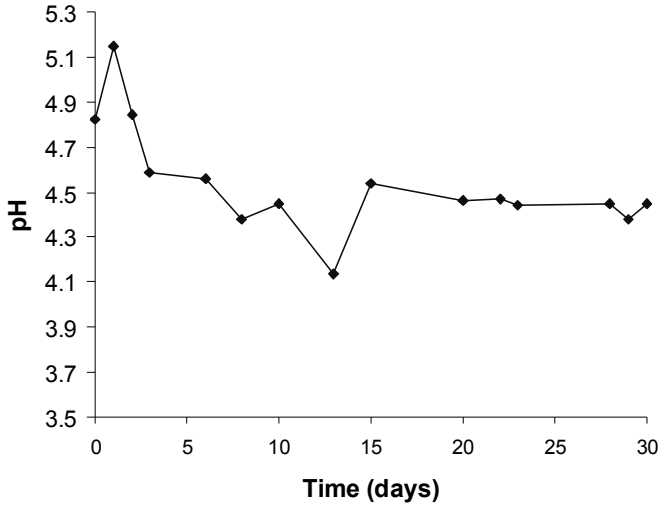

Figure 4.4 pH of the laboratory bioreactor during the 30 day trial.

4.4 Discussion

Although anaerobic digestion has been gaining wider popularity in treating high sulphate wastewater it has its disadvantages, such as corrosion, low methane production, need for H_2S gas removal and reduced COD removal efficiency due to the presence of H_2S gas. The biggest problem with anaerobic treatment however is the competition of acidogenic, acetogenic and methanogenic bacteria with SRP for the substrates present in the wastewater. The outcome of the competition is important because it will determine the amount of end products produced from these organisms. Sulphate-reducing prokaryotes should outcompete the methanogenic bacteria, as SRP consume hydrogen below a minimum threshold for hydrogen metabolism in comparison to MPB. Also, the release of sulphide from the sulphate consumption of the SRP is toxic and permeates through the cell membrane of the methanogenic bacteria, and denaturing the native proteins in the cytoplasm. If the MPB are outcompeted then sulphate reduction should theoretically become dominant in the process of anaerobic digestion when treating sulphate rich wastewaters (Percheron *et al*., 1997; Lens *et al*., 2000). However, this seemed to not be the case in this reactor, as no steady sulphate reduction took place and there was very little detectable sulphide production, leading to the assumption that the MPB were not being dominated. Lens *et al*. (2000) suggest that besides growth kinetics there are other

factors that may affect the competition between SRP and MPB such as type of seed sludge. In addition, Percheron *et al*. (1997) stated that in order for successful anaerobic digestion the start-up period is crucial and needs to be carefully monitored, because treating molasses wastewater using a chemostat and even with low organic loading rates is very seldom successful. They found that an essential part of the start-up was the use of the correct sludge. When comparing wine distillery wastewater sludge to acclimatised molasses wastewater sludge it was found that the behaviour was different and that use of wine distillery wastewater sludge for treatment of the beet molasses wastewater reduced the most amount of total organic carbon (TOC) without accumulation of acetate. On the other hand treatment of the wastewater with the molasses wastewater sludge showed a significant rise in acetate production. Percheron *et al*. (1997) study concluded that with the use of the correct sludge it was possible to treat the molasses wastewater and that perhaps there were essential nutrients in the wine distillery wastewater sludge that were not present in the molasses wastewater sludge.

Hydraulic retention time is also a major factor for the success of a bioprocess. One of the aims of this trial was to determine what the optimum HRT would be for maximum sulphate reduction with as short a wastewater processing time as practicable. However, since the concentrations of the sulphate and sulphide monitored varied throughout the trial this was not determined. The final HRT used in the process from Chapter 3 was 72 hours. Other researchers treating similar wastewater under anaerobic conditions have used both shorter and much longer HRTs. Jiménez *et al*. (2003) performed studies under different HRTs and organic loading rates which showed that for the anaerobic digestion of beet molasses wastewater an HRT of 53.5 days maintained a pH of 8 and COD removal efficiency of 93.7 % with the least amount of volatile acidity. Any lower HRT had only a 68.6 % COD removal with volatile acidity reaching 9.3 g acetic acid/L. Nedwell and Reynolds (1995) used methanogenic and sulphate-reducing digesters to treat landfill leachate under anaerobic conditions, the initial HRT for start up of their digester was 5 days (120 hours), reduced to 60 hours on day 265. Maree *et al*. (2004) used an HRT of 5.5 hours to achieve 92 % removal of sulphate (from an initial concentration of 1672 mg/L).

Researchers performing many different configurations of anaerobic digestion have often regulated the temperature of the reactor to 35 °C (Percheron *et al.*, 1997; Jiménez *et al*., 2003; Blonskaja *et al*., 2003; Jiménez *et al*., 2004). In the present study the digester was kept at room temperature (25 ± 5 °C) throughout the experiment, which would partly account for the slower reaction rates.

The pH of a reactor is a crucial parameter for determining reactor success or failure. The increase in pH of the reactor on day one was due to the mixing of the acidic wastewater with the high alkalinity of the SRP inoculum, especially since the volume of the inoculum used was 30 % of the total volume of the reactor. The pH should have increased throughout the trial until it was at optimum for growth and activity of the SRP. However, this did not occur and the day 1 pH value of 5.15 was the highest throughout the trial. The pH subsequently dropped until reaching approximately pH 4.4 and remained stable throughout the rest of the trial. These pH results are similar to those found for the on-site ACP described in Chapter 3, with the average pH for all three ACP samples collected also being approximately 4.4 unless artificially adjusted (Figure 4.5).

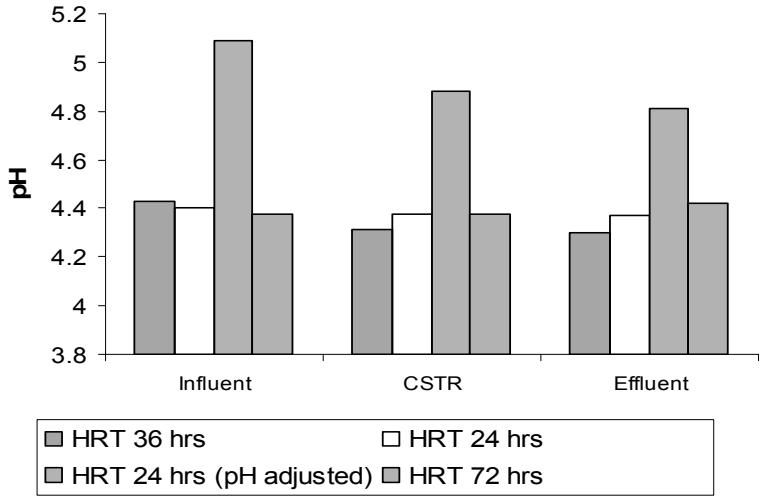

Figure 4.5 pH values for the samples taken during the continuous sulphate reducing bioreactor study at different HRT.

Sulphate-reducing prokaryotes, methanogens and syntrophs have varying pH optima and growth ranges and therefore the pH of the reactor will play an important role in the outcome of competition of the organisms. It has been found that in the case of acetate utilising methanogens and SRP, the SRP have a higher pH optimum and range for growth and therefore should have dominated the consortium (O'Flaherty *et al*., 1998). The reactor system pH did not favour sulphate reduction by SRP despite the fact that domination is almost always the unwanted result for methanogenic anaerobic digestion when there is an excess of sulphate present (Lens *et al*., 2000).

This drop in pH and maintenance thereof in the reactor suggested that acidogens present were living in ideal conditions which in turn lead to postulating that this particular wastewater is highly buffered. Since the SRP were known to be present in high numbers and yet were unable to increase the pH, acidophiles were able to dominate the microbial population. With these organisms dominating there is increased production of volatile fatty acids, which result in digester failure if produced in concentrations exceeding 300 mg/L (SCA, 1979). Production of VFAs also leads to a decrease in pH, which further inhibits SRP.

4.5 Interim summary and conclusions

It was concluded that despite treating the final on-site wastewater with a more concentrated inoculum of SRP and under strict anaerobic conditions, the results still did not show a significant sulphate reduction. In addition, the pH of the batch reactor followed similar trends to that of the on-site ACP by remaining low at approximately the same pH as the wastewater generated by the production plant on a regular basis. These results indicated that the wastewater itself was inhibitory to SRP, possibly due to chemical inhibitors present in the wastewater or the lack of an assimilable carbon source.

Chapter 5
Investigation of possible inhibitory effects on sulphate reducing prokaryote activity

5.1 Inhibition test design

Since sulphate reduction almost always takes place during anaerobic digestion and both the on-site contact process and the lab-scale digester did not support this it was probable that there were inhibitors present affecting the growth and activity of the SRP.

Cane molasses is the by-product of the sugar refinery process and the sugars in cane molasses can be broken down into sucrose, 26 - 31 %, reducing sugars (glucose and fructose), 4-12 % (Curtin, 1983) and a small amount of unfermentable sugars. It may be possible that some of these unfermentable sugars are inhibitory or simply unavailable to the SRP present in the system, as it is necessary for the SRP to have a suitable carbon source for growth and activity. When wastewaters do not contain the necessary electron donors or carbon source for complete sulphate reduction it is necessary to add the appropriate donors, such as acetate, ethanol and methanol. These are often preferred over complex wastes such as molasses (Lens *et al*., 2000). Acetate degradation can occur without sulphate reduction and acetate can be an energetically poor substrate. Lactate is a common intermediate from complex organic degradation and has been used successfully as an organic substrate, which supports the growth of bacteria (Dill *et al*., 2001). Hence the use of a suitable carbon source affects the outcome of anaerobic digestion. When there are economic constraints influencing the carbon source used, use of waste carbon sources such as sewage sludge (Nedwell and Reynolds, 1995) and tannery effluent (Boshoff *et al*., 2004) can be successful, although Dill *et al*. (2001) reported that the majority of successful experiments using complex carbon sources such as molasses or sawdust for wastewater treatment were mostly carried out at laboratory or pilot scale. This trial was designed to assess the effects of the provision of different carbon sources found in the wastewater on sulphate reduction and to determine whether adjustment of the wastewater pH would render it digestable.

5.2 Materials and methods

Two sets of flask tests were set up in a similar way to the lab digester. The first set consisted of flasks to investigate the effects of five different initial pH values, the other a set to investigate the use of three different non-fermentable sugars as carbon sources. The working volume of each flask was 250 mL. Each flask was sealed with a butyl rubber stopper through which passed two tubes, one a liquid sampling port, the other entered the headspace of the flask and was attached to a 7% zinc acetate trap to prevent H_2S gas from escaping into the atmosphere. The flasks were covered with metal foil to prevent photosynthetic activity from producing oxygen. The flasks were inoculated with 10 % inoculum of SRP isolated from Grootvlei mine, Gauteng, and maintained at Rhodes University, Grahamstown.

The set of four 'carbon' tests had a control which contained modified Postgate medium (Appendix F). The test flasks each contained a different sugar source in place of the lactate and yeast extract used in the control. The sugars used were galactose, xylose and raffinose.

The other set of five 'pH' tests contained settled final effluent. The control flask pH was unadjusted and the test flasks were each adjusted to a different pH using slaked lime (CaO). The initial pH values of the inoculated flasks were 4.51 (control), 5.0, 5.5, 6.5 and 7.0.

Samples were taken from all flasks daily for 10 days and tested for pH, sulphide and sulphate concentration using the methods described in Chapter 2. After inoculation and during sampling the flasks were sparged with nitrogen gas to maintain strictly anaerobic conditions.

5.3 Results and discussion: different carbon sources

In the control flask (Postgate media) the pH remained stable at approximately 7.0 throughout the trial, showing that the SRP were growing and surviving well (Figure 5.1). Failure often results in a pH change due to other microorganisms becoming dominant from competition for substrates. Replacement of the carbon source with xylose and raffinose caused the pH to remain stable for the first few days after which it steadily decreased to approximately pH 5.0. The pH in the galactose flask on the other hand dropped within the first few days but slowly recovered to reach a final pH of 6.6. This could mean that the SRP took a while to adjust to the environment and then slowly recovered, resulting in the pH increase observed.

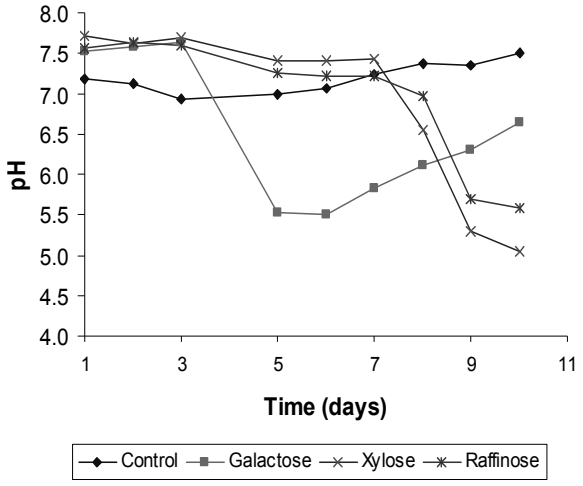

Figure 5.1 pH values of each flask during the 10-day experiment.

The sulphate and sulphide concentrations observed were again not as expected. The data was normalised by making all the initial concentrations (which ranged from 760 mg/L to 940 mg/L) equal to 100 % in order to better equate the trends for all the tests flasks. Figures 5.2 and 5.3 depict their trends during the 10-day experiment. The sulphate concentrations were expected to decrease steadily, but this was not observed. Sulphate concentrations for all flasks seemed to follow a similar trend. The reaction fluctuated at the beginning of the trial and after day 6 sulphate concentrations slowly decreased. The control had a steady decrease from day 5. Raffinose had the best net removal of sulphate

while galactose was very unstable and by the end of the experiment no net sulphate removal was achieved.

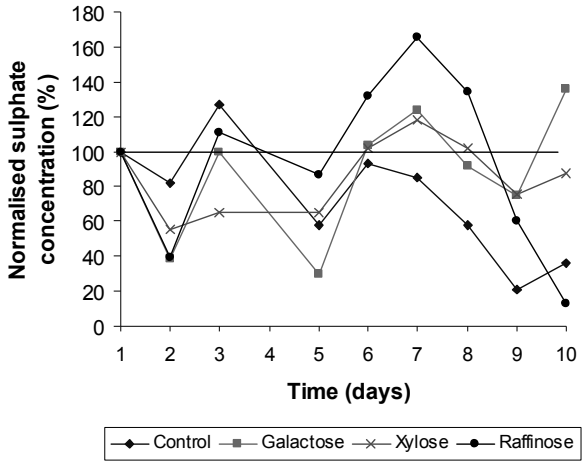

Figure 5.2 Normalised [sulphate] using different carbon sources.

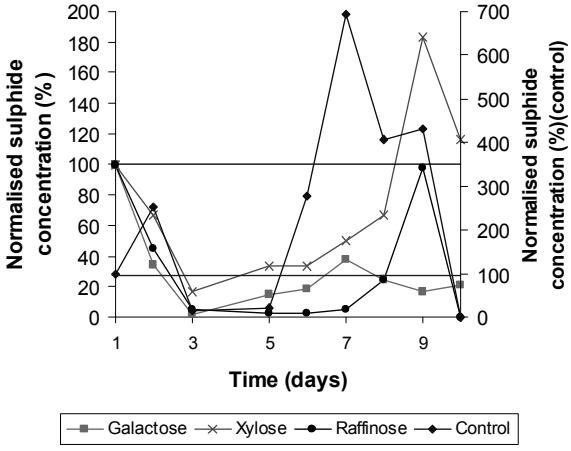

Figure 5.3 Normalised [sulphide] using different carbon sources.

Error bars omitted for clarity.

Sulphide production was expected in at least the control flasks. No sulphide production occurred from day 1 to 3 for all the test flasks. The flasks fed with galactose and xylose in place of lactate then started increasing in sulphide concentration until day 6. Thereafter the sulphide concentration in the galactose flasks decreased again and remained below the starting concentration. The concentration of sulphide in the xylose flasks continued increasing with a drop in concentration on day 10, but the concentration still remained higher than the sulphide starting concentration, indicating a net gain in sulphide in that flask. Raffinose showed no significant increase in sulphide levels and resulted in a net loss of sulphide. The level of sulphide in the control showed significant increases in concentration during the experiment maintaining substantial gain in sulphide, however on day 10 the sulphide concentration was very low with the end result being a net loss in sulphide. The sulphide accumulation was not expected to equal that of the model digester referred to in Figure 4.3 owing to the use of the zinc acetate traps, but it was expected to increase until saturation and evolution of the gas.

When comparing the sulphate reduction and sulphide generation it can be seen that at some points on the graphs these measurements do correlate. For example, in the raffinose

test on day 3 the sulphate concentration had increased and there was very little sulphide produced. Figure 5.2 shows that sulphate concentration started decreasing from day 7 to day 10, while the sulphide level increased at that time (Figure 5.3), indicating that the sulphide was generated from the reduction of sulphate. This trend is also shown in the xylose test for days 7 to 9 inclusive.

The pH of the control during the tests using different sugars as carbon sources remained mostly unchanged and at a suitable level for SRP to thrive, indicating that the medium used had a good carbon source available. However, sulphate reduction in the control was not good during the first few days with continuous fluctuation possibly due to the water used for making up the media not being deoxygenated beforehand, so while the system was anaerobic it was not anoxic. The peaks could therefore be due to reoxidation of the sulphide to sulphate. The sulphate levels in the control did start decreasing after day 6 and showed a net reduction in sulphate by day 10. The pH values for raffinose and xylose were similar to that of the control until day 8, when both started decreasing, perhaps because the media had been exhausted since the flask contained only 250 mL total working volume. It is also possible that the SRP were dying off and there was accumulation of VFAs, which would result in a pH decrease. None of the sugars used resulted in good sulphate reduction, suggesting that their presence in wastewater does not equivalate to good provision of carbon for growth of SRP. Previous studies performed by Dill *et al*. (2001) using defined carbon sources for SRP for treatment of pH adjusted acid mine drainage (AMD) found that lactic acid and butyric acid had the best sulphate reduction with proprionic acid and methanol following. Ethanol and pyruvate had lower sulphate reduction. Their graphs also showed fluctuation in sulphate concentrations during the first few days of operations and then more steady sulphate reduction later in the trial. The average sulphate reduction in that case was much greater than was found in this study. The length of their trial was longer than the 10 days used in this case and the working volume was higher (900 mL), so it is possible that acclimatisation of the inoclula may have allowed sulphate reduction to take place.

5.4 Results and discussion: pH

The second set of flask tests was carried out to determine the effect of different initial wastewater pH values on the growth and activity on SRP, since the pH of both the on-site process (Table 3.2) and the lab digester (Figure 4.3) remained mostly unchanged during the experiments. Figure 5.4 shows that on day one all the flasks had higher pH values than their original starting pH (day 0), including the control, which was fed with unadjusted wastewater. After day one the pH in each test flask decreased until day 5, when they remained stable. The decrease in pH of the tests in which the original pH was high 6.5 and 7.0 was greater than in the other two tests, where the initial pH was not much higher than the control. The control seemed to remain fairly stable throughout the experiment. Even though the pH levels decreased in all the flasks, the ones at pH 6.5 and pH 7.0 initially remained above the wastewater specification limit of 5.5, stabilising at pH 5.74 and 5.81 respectively.

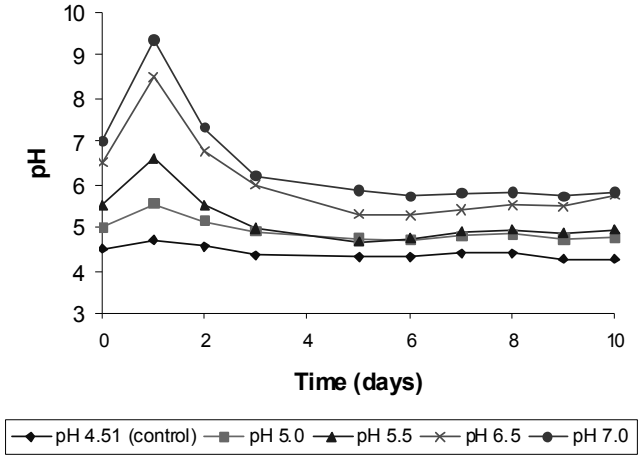

Figure 5.4 pH values of each flask during the pH adjustment experiment.

The pH was probably higher on day 1 than day 0 due to the mixing of the alkaline inoculum with the wastewater. The control flask did not fluctuate much during the following nine days and remained at a similar pH to the starting pH. This was in keeping with the pH trend noticed for both the on-site ACP and the lab bioreactor, in which little change occurred. For the flasks with initial pH 5.0 and 5.5, pH values for both flasks were almost exactly the same except for the initial pH, then the pH 5.5 flasks dropped to

the same pH levels as the pH 5.0 flasks. The other flasks had a steeper drop in pH than those mentioned above, finally averaging pH 5.5-5.8 which falls within the specification range. However this drop in pH for all the flasks demonstrated that initial adjustment of pH will not help maintain a pH more suitable for SRP growth and activity in the ACP. Since the pH in the media flasks even with changing carbon sources remained fairly stable at approximately pH 7.0 suggests that the change in pH is due to the final effluent characteristics and not the SRP culture used.

The normalised data for sulphate and sulphide concentrations within the test flasks at different pH values adjusted with slaked lime (CaO) (Figure 5.5) showed slight sulphate reduction in all the flasks for the first few days. Thereafter the concentrations fluctuated with no steady trends until the end of the trial. However, the net sulphate removal for pH 5.5, 6.5 and 7.0 was better than the control, in which the sulphate at day 10 was higher than the starting concentration (net gain in sulphate). At pH 5.0, although reduction seemed to initially take place within the wastewater, the net sulphate removal was negligible. Final sulphate concentrations for pH 5.5, 6.5, and 7.0 flasks were 3180, 2960 and 3000 mg/L, respectively. Even though there was net sulphate removal the actual concentrations were still high and did not meet the desired specification, which was 250 mg/L.

There was little to no sulphide generation in these flasks, with low sulphide concentrations found for most samples. Unlike with the ‘carbon’ flasks, changes in sulphide generation in Figure 5.6 did not correlate with changes in sulphate removal. These flasks resulted in a net loss of sulphide instead of a net gain, which was not expected, since three of the five flasks also showed a net sulphate loss. Since there was fluctuation in both sulphate removal and sulphide generation throughout the experiment, the results are discussed as net gain and loss of sulphate and sulphide over the 10-day period.

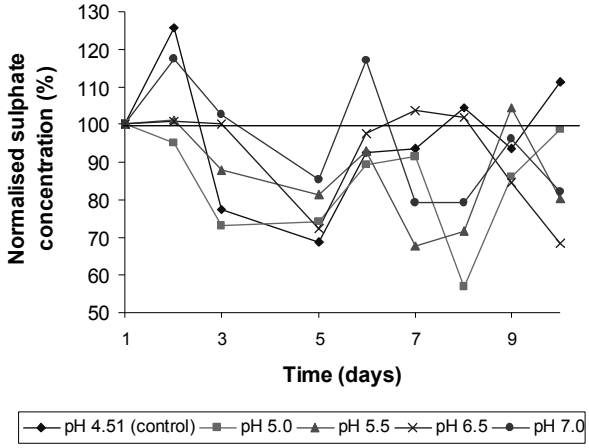

Figure 5.5 Normalised [sulphate] of each pH test.

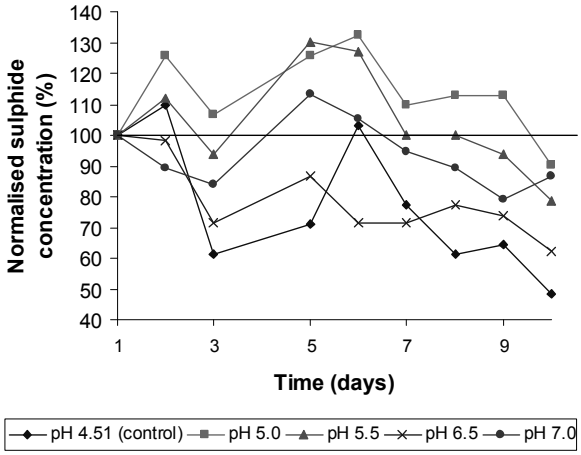

Figure 5.6 Normalised [sulphide] of each pH test.

Error bars omitted for clarity.

The control showed a net sulphate gain with a net loss in sulphide, these results are similar to some of the data shown in the on-site ACP and lab digester. There was sulphate loss for the flasks at pH 5.5, 6.5 and 7.0. However for those flasks there was no net sulphide generation. There was no net sulphide generation for all flasks indicating that sulphate loss did not result in sulphide gain. It was expected that the flasks with starting pH 6.5 and 7.0 would be successful, since SRP grow best above pH 6.0 and the other test flasks (pH 4.51, 5.0 and 5.5) would fail. Although these flasks were better than the other test flasks, the final sulphate concentrations were still too high and did not meet the specification requirements. This means that the pH of the wastewater was not the only factor causing failure. Again these results imply that the wastewater itself was causing process failure. This was surprising, since molasses was expected to be a good carbon source. Dill *et al*. (2001) performed experiments using undefined carbon sources to determine sulphate reduction and found that molasses was a good source, reducing sulphate at a rate of 52.6 mg/L/d. Maree *et al*. (1991) also found molasses in unfermented or fermented form to be a good carbon and energy source for anaerobic digestion. The wastewater used for the experiments, although a mixture of other on-site wastewaters consisted mostly of dunder, the wastewater produced after molasses fermentation with yeast to produce alcohol. Therefore it was expected that the SRP would grow actively under anaerobic conditions to reduce the sulphate in the wastewater.

5.5 Results and discussion: phenols

Literature has mentioned that the presence of phenolic compounds can cause inhibition of biological processes (Jiménez *et al*., 2004). Sugar cane-based fermentation wastewater has been found to contain poorly biodegradable compounds such as phenolics (Harada *et al*., 1996; Wilkie *et al*., 2000 and Guimarães *et al*., 2004), and due to the intense colour of the wastewater a sample from the industrial site was tested for total phenol (Appendix G).

It was found that the total phenol concentration was 3.3 ± 0.3 g/L. In comparison, the total phenol content of beet molasses wastewater is 0.45 g/L (Jiménez *et al*., 2003), which is considered high. After a 24 hour treatment with the enzyme laccase (known mainly for lignin degrading capabilities, however also capable of polymerising phenolic compounds (Yaver *et al*., 1996)) at 1000 units/L the total phenol concentration was reduced to 1.9 g/L. Many phenolic compounds are known to be toxic and interfere with the activity of bacteria involved in anaerobic digestion, especially methanogenic bacteria (Jiménez *et al*., 2003). This could help explain the build up of VFAs seen in the on-site ACP (Chapter 3), since methanogens are responsible for VFA breakdown. The presence of high phenolic content in wastewater slows down the anaerobic digestion process and retards removal of some of the organic material in the wastewater, which in turn results in the necessity to use long HRT (Jiménez *et al*., 2003). Jiménez *et al*. (2003) found that using a two stage aerobic-anaerobic system where the first step treated the phenolic content, colour and the initial COD removal using fungi and the anaerobic digestion step removed the remainder of the organic content was successful. They were able to reduce the colour intensity, remove 70 % of the phenols 90 - 96.5 % of the COD. This suggests that fungal pre-treatment to remove phenols may be necessary for sulphate reduction to be successful for this wastewater. This is supported by the fact that there was 42 % removal of total phenol from this wastewater after treatment with laccase within 24 hours.

Another factor possibly relating to digester failure is that the wastewater used was undiluted. Jiménez *et al*. (2003) diluted their beet molasses wastewater to 50 %. FitzGibbon *et al*. (1998) performed studies to determine the effect molasses spent wash

concentration had on growth rates of different strains of fungi. They found that at high concentrations of molasses spent wash fungal growth was inhibited due in part to inhibitory phenolic compounds (vanillic and gallic acid). Therefore dilution of the wastewater was necessary to obtain good removal of colour and subsequently phenols. They found that the fungus *Coriolus veriscolor* achieved the best colour removal at 12.5 % v/v molasses spent wash (decrease of 0.43 units) in comparison to the decrease of 0.065 units at 25 % v/v molasses spent wash for the same strain (FitzGibbon *et al*., 1998). Perhaps wastewater dilution also needs to be a future consideration when attempting to treat this wastewater.

5.6 Interim summary and conclusions

The SRP were partially able to use the alternative sugar source in the media since there was sulphate reduction at times during the experiment, which coincided with sulphide generation. Also the pH levels of the flasks remained fairly unchanged during the experiment. Therefore it can be concluded that the unfermentable sugars in molasses waste were not inhibitory to SRP growth and activity, even though the sulphate removal was not steady. Inhibitory effects may only occur when the sugars were used as a sole carbon source.

Adjustment of pH to levels more acceptable for wastewater discharge and more suitable for growth and activity of SRP did not result in better sulphate removal from the wastewater. The flasks starting at pH 6.5 and 7.0 were better than the flasks at the lower pH's but there was no significant improvement to the SRP performance. Therefore pH is not the principal factor affecting the success of the anaerobic digester.

The high content of phenols in the wastewaters leads to the conclusion that the method of biological treatment used in this instance was not suitable for this wastewater, as in general only fungi are able to degrade phenolic compounds. The presence of the phenols was inhibitory to the microorganisms used in anaerobic digestion, and can be deemed to have resulted in digester failure.

Chapter 6
Final Conclusions and Recommendations

6.1 Summary and discussion

The wastewater characterisation of all the wastewaters on site showed that sulphate and pH were the main problem components. Sulphate is found in many industrial wastewaters as sulphuric acid is used for many of the industrial processes especially in the food and fermentation industry (Lens *et al*., 2000). The use of sulphuric acid results in the presence of high concentrations of sulphate in wastewater produced. Sulphate itself is non-toxic but the presence of high concentrations of sulphate upsets the sulphur cycle (Silva *et al*., 2002) and in the presence of SRP sulphate is converted into hydrogen sulphide gas (Lens *et al*., 2000). When the gas escapes into the atmosphere it reacts with oxygen to produce sulphuric acid (Hvitved-Jacobsen and Halkjær Nielsen, 2000), which was thought to be the corrosion causing agent of the cement launder at the WWTW.

Tests performed using FISH techniques to determine the microbial populations in the wastewater samples found no evidence of SRP presence in any of the wastewaters tested, i.e. onsite and at the WWTW. This evidence showed that the problem could not be ameliorated by removal of microorganisms from the wastewater. The high sulphate concentration in the wastewater (2770.43 mg/L) was not within the specification limits for discharge (250 mg/L) and required a treatment strategy for removal.

The pH of the wastewater leaving the industrial site had a negative impact on the receiving wastewater stream at the WWTW, where there was a significant difference between the pH measured upstream and downstream of the wastewater outfall. The low pH probably arose from the use of acid in the lactulose production process (the lactulose production facility discharged a highly acidic wastewater, ~ pH 2.5), and from the use of vast amounts of sulphuric acid, which is combined with the dunder (~ pH 4.1). Although the results showed that the addition of the lactulose production wastewater did not reduce

the acidity of the dunder further, the combination of these two wastewaters results in a highly acidic final effluent. This is because the dunder was very highly buffered; even when pH of the wastewater is physically adjusted it fails to remain at the adjusted pH. This was of concern because the pH of water plays a very important role in the downstream effects after discharge into aquatic systems. Industrial activities usually cause an increase in acidity levels rather than an increase in alkalinity (DWAF, 1996). Addition of water with differing pH to that of the receiving stream can severely impact on biota of that stream if the pH changes. When there is a gradual adjustment of pH to more acidic levels it can result in a change in community structure where acid-tolerant organisms replace less tolerant ones. However, when acid wastes are discharged into waters with bicarbonate alkalinity it results in the formation of free carbon dioxide. Free carbon dioxide can be liberated when the water is alkaline and this causes toxicity to fish, even if the pH does not decrease to a level normally considered toxic (DWAF, 1996). So despite the level of pH in the downstream wastewater (5.98) being within the specified discharge limits (5.5-9.5), it can still impact on the aquatic environment, and a higher pH would be preferable, to prevent damage to the aquatic ecosystem.

The wastewater was treated using an anaerobic contact process (ACP) utilising SRP to reduce sulphate of the wastewater to more acceptable discharge levels. This method of treatment was unsuccessful with no steady sulphate removal, and that which was removed was only approximately 20-30 %. In order for the concentration to reach levels acceptable for discharge into the sewer network, 95 % removal would have been necessary. Also, the sulphate reduction was probably not due to the SRP as the sulphide generation as most times did not correlate with the sulphate reduction, which is what occurs when SRP are active. The biological reduction of sulphate also usually results in the degradation of organic compounds and an increase in pH due to proton consumption and the production of alkalinity (Hansford *et al*., 2004). There were peaks and troughs in sulphate removal throughout the ACP trial. Changes made to the operational parameters of the ACP did not result in a change in the process performance. The cause of system failure appeared to be competition with other microorganisms present in the inoculum, such as MPB (which compete for the same substrates although also do not survive well at

low pH) and acidophiles (which may have been more suited to the wastewater environment and adapted quicker than the others, therefore dominating the consortium). Since the wastewater was so diverse, with many different components and the variability in the combination of wastewaters that makes up the final mixed wastewater, it is highly possible that some of those intermittent components were inhibitory to the SRP, preventing successful sulphate reduction with increasing pH. The use of an undefined carbon source i.e. fermented molasses, could have aided in the ACP failure. Suitable carbon sources are necessary for microorganisms to survive and in turn degrade organic compounds, therefore removing them from the wastewater, resulting in cleaner water for discharge. However, in this case the wastewater composition was hardly adjusted by treatment with SRP. Use of this treatment was not effective in sulphate reduction and pH improvement and the wastewater should not be discharged as such into natural water courses.

Although results showed poor removal of sulphate, there was some limited COD removal from the wastewater. This implied that the carbonaceous material was being used, but not by SRP, since sulphate reduction was not increasing. This was not meant to be the case, so although the COD was being removed the principal aim of sulphate removal was not being achieved. Microbial community analysis showed that SRP were present in the ACP, they were simply not functioning.

Investigation into the failure of the ACP resulted in the discovery of high VFA concentrations (~ 13 mg/L). Generation of VFA, especially acetate, often occurs during anaerobic digestion from the fermentation process carried out by acidophiles. Methanogens are responsible for the breakdown of the VFAs, however if conditions are not suitable for methanogens e.g. incorrect pH, such as in this case, VFAs accumulate. High concentrations of VFAs are toxic to SRP and MPB, and increasing concentrations of VFAs in a digester indicate process failure even before the pH is affected (Hill *et al*., 1987).

Studies performed to investigate other inhibitors showed that unfermentable carbon sources possibly present in the wastewater were not inhibitory to the SRP and initial chemical treatment of the wastewater to a higher pH did not greatly improve the sulphate reduction. The SRP were able to use the unfermentable carbon sources to survive. Growth in the wastewater at the higher pH values did not differ from the results obtained during the ACP because the wastewater is highly buffered. This was proven since many adjustments could not greatly affect the composition of the wastewater. These results meant that use of SRP for treatment of the wastewater was not feasible and perhaps this wastewater was not even amenable to biological treatment. Perhaps a combination of chemical and biological treatment is necessary for successful treatment, even if this means that the cost would be greater than biological treatment alone. Part of the aim was for cost-effective treatment, however this may not be possible unless more extensive research is carried out.

The existence of phenols in wastewater is not uncommon and their presence is known to cause inhibitory effects (Jiménez *et al*., 2003). Phenols are by-products usually where organic chemicals are used and in South Africa phenolic wastes develop during distillation of wood and coal (DWAF, 1996). Since phenols were found in high concentrations in the wastewater and high concentrations of VFAs were being produced, digester failure was inevitable. Phenols are toxic and interfere with the activity of bacteria involved in anaerobic digestion, especially methanogens. This explains why there was an accumulation of VFAs, since methanogens are responsible for VFA breakdown. Phenols in high concentrations tend to slow down anaerobic digestion and can retard organic content removal from wastewater, which in turn means high HRTs are necessary (Jiménez *et al*., 2003). Phenols can undergo many transformations and reactions, which could result in lethal concentrations of polyphenols. Some of the factors affecting these interactions are change in temperature which increases resistance in fish, decrease in dissolved oxygen which increases lethality of the phenol and an increase in salinity increases the sensitivity of phenol (DWAF, 1996). Phenol is thought to be a neurotoxin which affects fish and ultimately results in death. Fish that do survive have effects such as necrosis of tissue and inflammation due to long term exposure (DWAF, 1996). The

acute effect value for phenol in freshwater set down by DWAF, (1996) is 500 µg/L with the target being 30 µg/L, however the concentration of phenol found in this wastewater was 3300 mg/L. This level of phenol not only affects the microorganisms in the treatment process resulting failure but will also affect the receiving water (even though it is not fresh water), as the water introduced into these streams has extremely high concentrations of phenol, lethal to fish.

Due to the fact that there were extreme amounts of phenols found in the wastewater, use of bacteria to treat the wastewater is not feasible because fungi are usually the only microorganisms that can breakdown these compounds (very few bacteria have been isolated that are capable of phenol degradation (Boopathy, 1995)). Therefore future work should include methods of treating phenolic components first, as they seem to be the barrier to sulphate reduction in the wastewater.

A novel treatment for both phenol removal and sulphate reduction is use of a sulphate-reducing bacterium isolated by Boopathy, (1995) from swine manure. The bacterium was able to use phenol as its sole source of carbon and energy and sulphate as the electron acceptor under anaerobic conditions. The degradation product of the phenol metabolism was acetic acid, which could not be further metabolized to CO_2. Although during their trial the acetic acid was accumulated as an end product, in a real ecosystem this problem can be eliminated by the presence of acetate-utilising methanogens e.g. the *Methanosarcina* species. Use of this bacterium is also practical since the start-up inoculation for an industrial phenolic wastewater treatment process could be swine manure, which is easily available and low cost (Boopathy, 1995).

More promisingly, Jiménez *et al*. (2003) showed that a combination of aerobic and anaerobic processes could achieve a higher percentage COD removal, a shorter HRT and greater decolourisation of beet molasses wastewater in comparison to using an anaerobic process only. The aerobic treatment consisted of shaking flasks containing a 50 % dilution of the wastewater and inoculated with spores from different strains of fungi. Air input was added to the flasks at 3 L/h per L of molasses. Since fungi grow well at low pH

(4-5) adjustment to the wastewater pH was not necessary. The anaerobic treatment consisted of stirred reactors placed in a thermostatic chamber. They were inoculated with biomass from a methanogenic anaerobic digester. After initial use of synthetic medium the reactor were acclimatised by batch feedings of diluted wastewater. Two sets of experiments were conducted, one with unfermented molasses and one with previously fermented molasses. It was found that the fungi were able to decolourise the wastewater starting on day one and improving by days four and five. This rapid decolourisation may have been due to degradation and/or adsorption of tannins and possibly some phenolic compounds onto the fungal mycelium. The phenol removal by *Penicillium decumbens* was good with a 74 % removal after three days of treatment. In addition the COD removal was up to 50.7 % with *P. decumbens* and similar for the other fungal species used. The subsequent anaerobic treatment showed that the higher HRTs (for treatment with both fermented and unfermented molasses) maintained a high pH and the best COD removal (%) with low volatile acidity detected.

This treatment method achieved great results and seems to be ideal for what the present study was trying to accomplish. The only component not mentioned by Jiménez *et al.* (2003) was sulphate, which was of principal concern for this experiment, however if such successful removal of phenols with simultaneous colour and COD removal and high pH can be achieved, the barrier to sulphate reduction is removed and then sulphate removal may be addressed. Since beet molasses wastewater and cane sugar molasses wastewater have similar characteristics, it is possible that similar results can be achieved if this treatment strategy is used.

6.2 Final conclusions and recommendations

From the experimental results of this study the following conclusions can be drawn:

1. Characterisation of the wastewater produced by ethanol production showed that the sulphate concentration was likely to be the source of downstream sulphide. Quantifiable concentrations of SRP were not detectable in any of the site wastewater streams i.e. dunder, lactulose production wastewater, mixed wastewater on-site, wastewater discharged at outfall, wastewater upstream outfall and wastewater downstream outfall.

2. Significant in-sewer microbial corrosion by SRP was possibly taking place as SRP were present in the sewer wall biofilm.

3. The wastewater sulphate concentration needed to be reduced and the pH increased.

4. The COD and BOD concentrations were comparable to measurements recorded for similar wastewaters and the COD:BOD ratio indicated that the wastewater was amenable to biodegradation.

5. The anaerobic contact process employed to treat the wastewater was not successful in reducing the sulphate concentration to levels within the discharge specification limits. The process used was not suitable for this wastewater. The lab-scale digester and bench scale flask tests demonstrated that the wastewater itself was inhibitory to biological treatment using SRP.

The concentration of total phenols was high enough to entirely account for process failure. Based on the work conducted in this study, the following recommendations are made:

1. Wastewater characterisation is very important. Only the components that were mentioned on the list of discharge limits were determined before deciding on a treatment strategy. Some other basic components were measured such as those related to solids content of the wastewater, COD and BOD to determine oxygen availability for biological treatment. In future a more extensive and thorough characterisation made over a longer time period is necessary to avoid problems, such as those which arose during treatment. Research into the effects of the combinations of the different components need to be addressed. For example, phenol determination done at the beginning of the study would have affected the treatment strategy used. Since the quality of substrate for ethanol fermentation i.e. molasses, can vary according to season changes, in terms of the sugar cane produced, characterisation of the wastewater should be carried out over 12 months to determine whether this seasonal variation affects the wastewater quality. This is important in terms of treatment process design as changes in the results from treatment may be attributable to the changing wastewater composition and not process failure.

2. Once extensive characterisation has been established, correct methods can be looked into on ways of successfully treating the wastewater. Batch studies need to be conducted first to determine optimum conditions under which the treatment process operates and then scale-up options can be investigated.

3. Since it was discovered that the VFA and phenolic concentrations in the wastewater were high, it was concluded that these were the major factors affecting the process failure. Therefore future work should include methods of removing these components first, as they seem to be the barrier for sulphate reduction in the wastewater. The extremely high amounts of phenols found in the wastewater make the use of bacteria to treat the wastewater unfeasible. Fungi are usually the only microorganisms that can breakdown these compounds (although bacteria have been isolated that are capable of phenol degradation) and it is recommended that fungal pre-treatment be investigated more extensively.

APPENDIX A
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APPENDIX B
Standards concentration for metals

Table B.1 Metals standard concentrations used for metals detection in the wastewater samples analysed on the AAS GBC 909AA

Metal	Cadmium	Chromium	Copper	Lead	Nickel	Zinc
Standards concentrations	0.2 mg/L	2 mg/L	1 mg/L	2.5 mg/L	1.8 mg/L	0.4 mg/L
	0.4 mg/L	6 mg/L	2 mg/L	5 mg/L	2 mg/L	0.6 mg/L
	0.6 mg/L	10 mg/L	3 mg/L	10 mg/L	4 mg/L	0.8 mg/L
	0.8 mg/L	12 mg/L	4 mg/L	15 mg/L	6 mg/L	1.0 mg/L
	1 mg/L	15 mg/L	5 mg/L	20 mg/L	8 mg/L	1.2 mg/L
	1.4 mg/L					1.5 mg/L
	1.8 mg/L					

APPENDIX C
FISH Protocol for SRP

Sample fixation

- Add three volumes of fixate (1.5 mL) to one volume (0.5 mL) sample, and maintain at 4 ˚C for 1-3 hours.
- Centrifuge cells at 10 000 rpm for 5 minutes and remove fixate.
- Wash cells in 1 × phosphate buffered saline (PBS) (1.5 mL), centrifuge at 10 000 rpm for five minutes and resuspend in 0.75 mL 1 × PBS to give $10^8 – 10^9$ cells/mL.
- Add one volume (0.75 mL) of ice cold ethanol (100 %) and vortex.
- Fixed cells can be spotted onto glass slides or stored at -20 ˚C, but a minimal time between fixations and hybridization is preferable.

Slide preparation

- Briefly vortex fixed cells to resuspend settled material.
- Apply 5 µL of sample to the slide.
- Air dry (or oven) thoroughly to prevent cells detaching in subsequent steps.
- Dehydrate slides in an ethanol series (3 minutes each in 50, 80 and 90 %).
- Slides can be stored at -20˚C, but minimal time between fixation and hybridization is preferable.

Hybridisation

- Pre-warm hybridisation oven to 46 ˚C (depending on probe).
- Prepare fresh hybridisation buffer in a 2 mL microcentrifuge tube in the following order:
    - 360 µL of 5 M NaCl (final concentration 0.9 M)
    - 40 µL of 1M Tris HCl (final concentration 20mM, pH 7.2)
    - x µL of 100 % formamide (final concentration 0.01 %)*
    - 2 µL of 10 % SDS (final concentration 0.01 %)
    - make up to 2 mL with autoclaved ddH_2O

  *where x depends on the specific probe

- non	- 0 µL
- 338, 129, 221, 687, 808	- 200 µL
- 660	- 600 µL
- 228	- 1000 µL

- Add 8 µL of hybridisation buffer to each slide containing sample.
- Pour remaining hybridisation buffer into a 10 mL polypropylene tube.
- Add 0.5 mL of each probe (and competitor probe if required) at the working concentration of 50 ng/µL.
- Place a coverslip on top, being careful to avoid air bubbles. This ensures the entire sample is exposed to hybridisation mix.
- Place slides in 50 mL tube containing excess hybridisation buffer.
- Screw on cap and lay tube horizontally in hybridisation oven for 1-2 hours (4 hours to overnight also possible).

Washing

- During hybridisation, prepare 50 mL of wash buffer in a 50 mL polypropylene tube. Add reagents in the following order:
  - x mL 5 M NaCl*
  - 1 mL 1M Tris HCl (final concentration 20 mM, pH7.2)
  - 50 µL of 10 % SDS (final concentration 0.01 %)
  - make up to 50 mL with autoclaved ddH_2O

  *where x depends on specific probe

- Non	- 9000 µl
- 338, 129, 221, 687, 804	- 4500 µl
- 660	- 1020 µl
- 228	- 180 µl
- Pre-warm to 48 ˚C in water bath.
- Following hybridisation, rinse wells immediately with wash buffer (48 ˚C) into hybridisation tube, using pipette.
- Carefully remove slide from hybridisation tube.
- Place into wash buffer tube and maintain at 48 ˚C for 10-15 minutes.
- Remove slides from wash buffer.
- Rinse briefly in a beaker of ice cold dH_2O.
- Dry slides in the dark. Rapid transfer of slides during these steps in important due to light sensitivity.
- (Optional) for counterstaining with 4',6-diamidino-2-phenylindole (DAPI), add 20 µL of 0.1 µg/mL DAPI to smear. Make sure all the wells are covered and incubate (in cuvette box) for 5 minutes then rinse briefly with ddH_2O and dry.
- Add Citofluor (anti-fading solution) in a thin film to the slide and cover with a large coverslip. Press coverslip down gently to remove excess Citofluor.

Microscopy

- Observe slide using an epifluorescence or confocal laser scanning microscope starting with the lowest formamide concentration and working upwards.

APPENDIX D
Calculation procedures

Sludge age (for on-site anaerobic contact process)

Sludge age = Total volume of reactor (L) / Total volume of sludge removed (L/day)
= Days

Hydraulic Retention Time (HRT)

HRT = Volume of reactor (L) / Flow rate (L/hr)
= Hours

APPENDIX E
Photographs of bioreactor and fungal biofilm

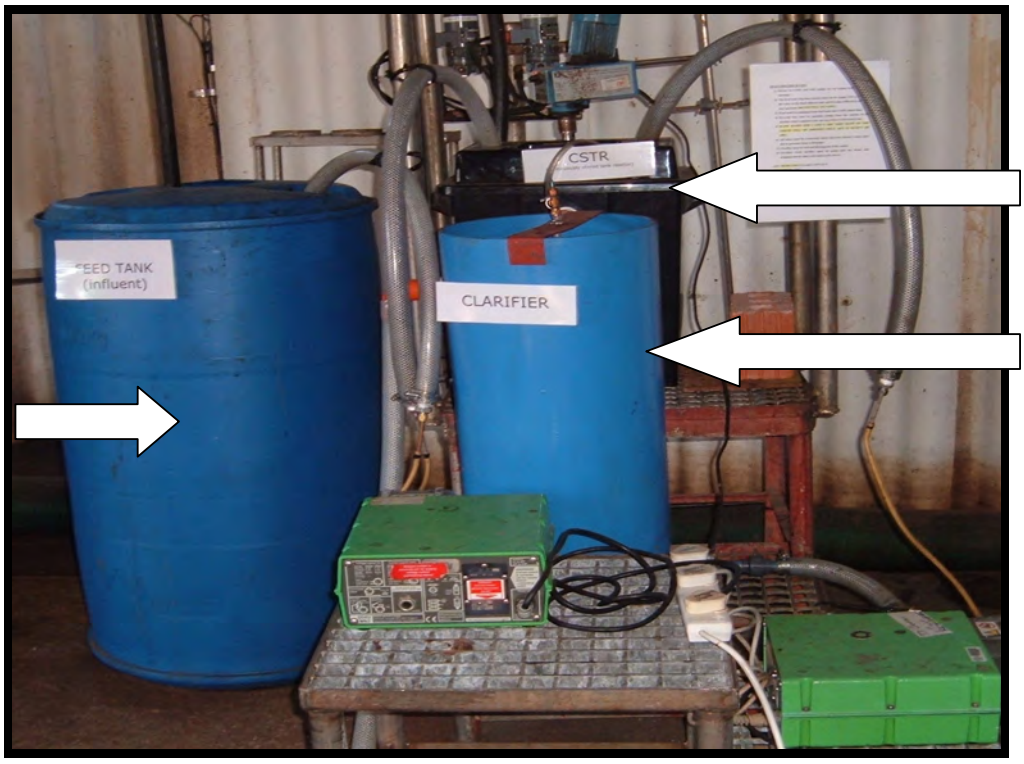

Figure E.1 Photograph of the on-site contact digester treating ethanol production wastewater.

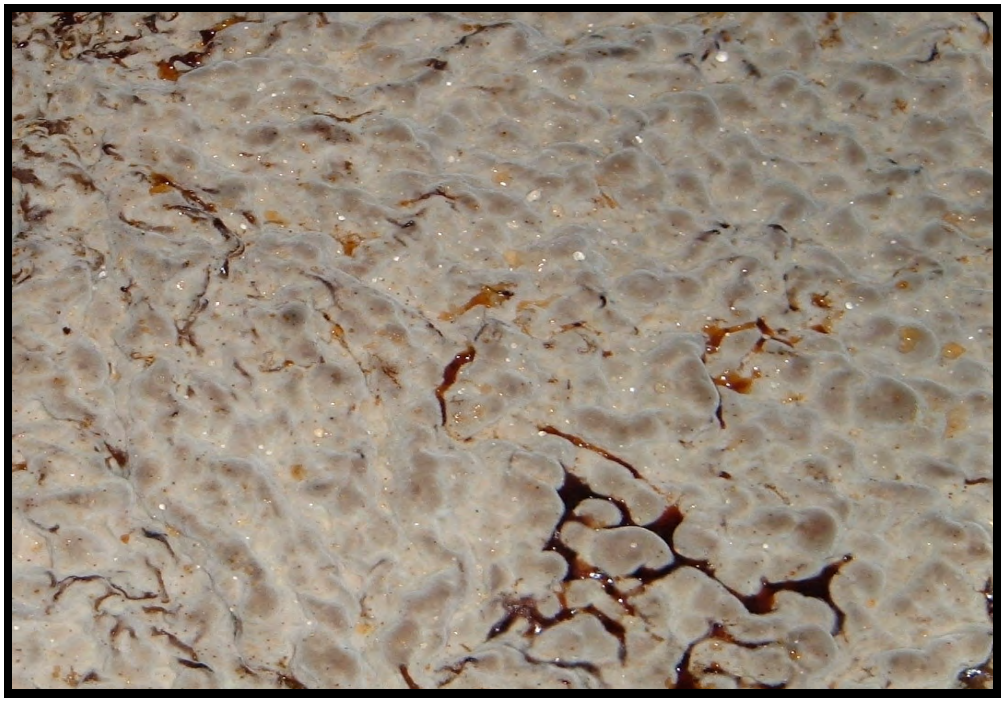

Figure E.2 Photograph of the biofilm growth on the surface of the clarifier during the experiment.

The biofilm growing on the surface was a fungus growing symbiotically with a bacterium

APPENDIX F
Volatile Fatty Acid Determination

The method for volatile fatty acid determination needed the linearity of the calibration curve to be checked before application of the method to samples, as linearity of the curve may depend on the instrument used. Below is the curve generated using the PharmaSpec UV-1700 Shimadzu spectrophotometer.

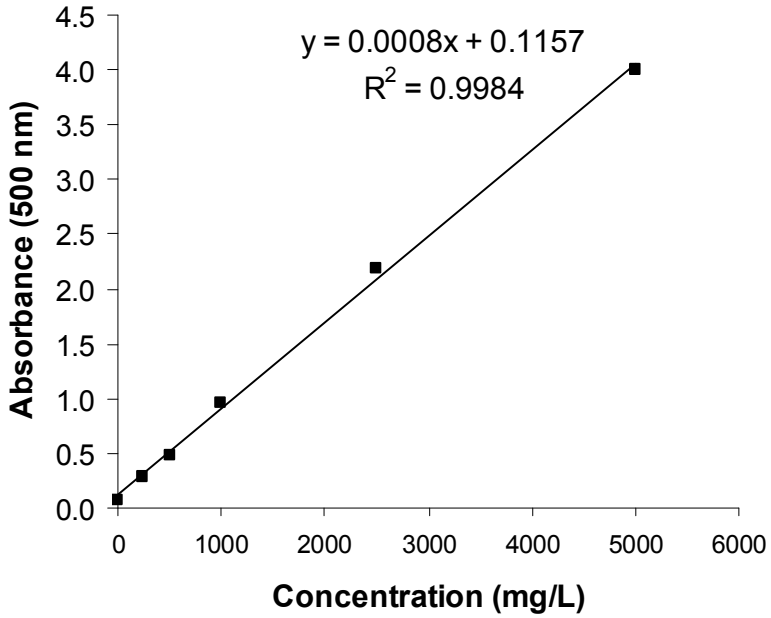

Figure F.1 Calibration curve

APPENDIX G
Postgate media

Modified Postgate Media (Atlas, 1946)

Solution 1 (990 mL)

Sodium lactate (70 % solution)	3.5 g
$MgSO_4.7\ H_2O$	2.0 g
NH_4Cl	1.0 g
Na_2SO_4	1.0 g
Yeast Extract	1.0 g
K_2HPO_4	0.5 g
$CaCl_2.\ 2\ H_2O$	0.1 g

Add components to distilled deionised water, bring volume to 990 mL. Mix and autoclave for 15 minutes. Allow to cool.

Solution 2 (10 mL)

Ascorbic acid	0.1 g
Sodium thioglycollate	0.1 g

Once solution 1 is cool, add solution 2 to solution 1 and mix.

APPENDIX H
Phenol assay

To 1.6 mL dH_2O add 100 µL sample.

Add 250 µL Folin Ciocalteu's reagent and mix well for 30 seconds.

Add 1.5 mL $NaCO_3$ (100 g/L) and mix well. Leave for 1 minute.

Add 6.55 mL dH_2O.

Pipette 300 µL samples into a 96-well plate and measure the absorbance at 765 nm using a PowerWave (Biotek Instruments Inc.) multi well plate reader.

The total phenol concentration was 3 300 mg/L based on phenol for the standard curve.

After a 24 hour-treatment with laccase at 1 000 units/L the total phenol concentration was reduced to 1 900 mg/L.

APPENDIX I
Methods for concrete coupon studies

The work report here is incomplete, however the methods used are outlined here, which established the results reported in Chapter 2.

Visual and analytical assessment of cement structures

An inspection of the WWTW site was conducted at the beginning of the study to assess the current degree of corrosion and identify potential high-risk areas. The preliminary assessment suggested that the degree of corrosion observed was not significantly greater than what would be expected for a 30 year-old structure. The observed corrosion appeared to be due to non-specific surface leaching. Minimal invasive testing was to be carried out to confirm the visual observations.

Submerged coupon tests

A number of concrete coupons (75mm diameter), cast from components commonly utilised in the study area, were submerged in five effluent streams at the site. The coupons were routinely analysed to quantify the corrosion rate.

Laboratory corrosion tests

Based on the effluent characterisation analyses, a number of important components (specific organic acids etc.) may be identified which warrant further study. In this case, the corrosion of concrete samples by pure solutions and defined mixtures of the identified components will be quantified to determine which of those components may play a significant role in corrosion by the mixed effluent (van Hille, pers. comm., 2004).

APPENDIX J
Primary data

Table J.1 Table of results for experiments performed on-site for wastewater characterisation.

Dunder	Settleable solids mg/L	Suspended solids mg/L	pH	TDS g/L	Total solids %
9-Feb	17	13258	4.55	25.90	13.74
11-Feb	6	13598	4.30	25.40	12.32
13-Feb	1	15322	4.34	25.70	13.56
16-Feb	16	13718	4.52	13.54	13.54
18-Feb	33	7112	4.51	13.61	13.61
20-Feb	8	8350	4.52	27.80	13.60
23-Feb	100	7934	4.83	27.60	11.08
25-Feb	8	5966	4.36	26.20	14.05
27-Feb	0	7376	4.64	26.70	13.26
1-Mar	0.5	7546	4.58	27.00	13.88
AVE	**18.95**	**10018.00**	**4.52**	**23.95**	**13.26**
STD DEV	**30.20**	**3499.46**	**0.16**	**5.52**	**0.90**
Lactulose production wastewater					
9-Feb	0	176	2.50	17.40	1.69
11-Feb	0	14	1.94	17.00	2.04
13-Feb	0	124	1.65	19.80	1.75
16-Feb	0	28	6.95	4.66	0.65
18-Feb	0	154	3.05	6.89	0.44
20-Feb	0	162	1.34	21.60	2.04
23-Feb	0	178	2.63	16.60	1.56
25-Feb	0	226	1.55	21.10	2.45
27-Feb	0	104	3.35	3.90	0.76
1-Mar	0	104	1.72	8.37	1.29
AVE	**0**	**127.00**	**2.67**	**13.73**	**1.47**
STD DEV	**0**	**66.99**	**1.65**	**6.99**	**0.67**
Final wastewater					
9-Feb	125.0	6158	4.15	16.70	6.02
11-Feb	45.0	3740	4.05	17.95	6.53
13-Feb	22.0	4636	4.13	18.60	7.27
16-Feb	12.0	2974	4.18	18.40	6.86
18-Feb	11.0	3962	3.97	19.00	7.15
20-Feb	5.0	2712	4.00	18.70	5.62
23-Feb	4.0	3124	4.35	18.40	5.79
25-Feb	2.0	3010	4.00	19.30	9.17
27-Feb	0.5	3866	4.15	17.60	6.43
1-Mar	0.2	3680	4.09	18.8	7.441
AVE	**22.67**	**3786.20**	**4.11**	**18.35**	**6.83**
STD DEV	**38.45**	**1013.95**	**0.11**	**0.76**	**1.03**

Wastewater outfall					
9-Feb	24.0	4482	4.11	18.20	6.59
11-Feb	15.0	648	4.05	18.50	6.75
13-Feb	18.0	4070	4.08	18.60	6.89
16-Feb	6.0	2320	3.96	16.90	6.23
18-Feb	30.0	3280	3.96	17.70	6.52
20-Feb	12.0	2898	3.96	18.70	6.60
23-Feb	1.3	3800	4.33	18.90	4.57
25-Feb	1.6	2432	3.87	18.20	6.42
27-Feb	5.0	3304	4.11	16.90	6.16
1-Mar	1.7	4156	4.05	18.20	7.23
AVE	**11.46**	**3139.00**	**4.05**	**18.08**	**6.40**
STD DEV	**10.13**	**1136.39**	**0.13**	**0.71**	**0.71**
Downstream outfall					
9-Feb	1.3	90	6.08	1.91	0.29
11-Feb	3.5	0	6.05	2.07	0.31
13-Feb	5.0	140	5.73	1.32	0.20
16-Feb	5.0	500	5.28	1.32	0.22
18-Feb	16.0	432	6.16	1.83	0.32
20-Feb	8.0	292	6.62	1.81	0.33
23-Feb	35.0	154	5.36	1.62	0.26
25-Feb	25.0	314	5.99	1.76	0.22
27-Feb	38.0	382	6.50	1.51	0.21
1-Mar	5.0	116	6.06	1.25	0.22
AVE	**14.18**	**242.00**	**5.98**	**1.64**	**0.26**
STD DEV	**13.71**	**165.21**	**0.43**	**0.28**	**0.05**
Upstream outfall					
9-Feb	0	36	7.81	2.630	0.26
11-Feb	5	0	6.80	1.008	0.11
13-Feb	15	138	6.87	0.086	0.12
16-Feb	6	60	5.78	0.882	0.54
18-Feb	24	226	8.12	1.083	0.14
20-Feb	10	340	8.71	1.320	0.20
23-Feb	15	106	5.56	0.990	0.15
25-Feb	10	110	6.79	1.330	0.14
27-Feb	23	416	6.34	2.850	0.33
1-Mar	4	130	6.78	0.850	0.11
AVE	**11.20**	**156.20**	**6.96**	**1.30**	**0.21**
STD DEV	**8.01**	**133.30**	**1.00**	**0.83**	**0.14**

Table J.2 Nitrate concentration (mg/L) of the different wastewater streams.

	Dunder	Final wastewater	Lactulose production wastewater	Wastewater outfall	Upstream outfall	Downstream outfall
	4340	4940	208	6800	244	304
	6140	4180	408	5740	346	348
	5700	3100	616	4440	476	218
	4040	3160	300	6720	338	292
	4340	3660	454	4240	182	258
	6000	3020	448	4200	184	198
	6180	1960	324	3700	366	232
	6000	1800	390	4360	130	420
	6540	2600	420	5760	322	354
	7640	2520	572		216	306
Average	5692.00	3094.00	414.00	5106.67	280.40	293.00
Std dev.	1131.73	969.03	121.43	1164.69	106.46	68.93

Table J.3 Sulphate concentration (mg/L) of the different wastewater streams.

	Dunder	Final wastewater	Lactulose production wastewater	Wastewater outfall	Upstream outfall	Downstream outfall
	1381.88	3229.20	2453.61	2988.40	0.00	11.45
	3764.69	3654.47	2227.08	3328.20	0.00	0.00
	1701.08	3139.85	2243.81	1985.32	0.00	0.00
	2727.23	3147.36	1474.88	3189.83	0.00	103.37
	3632.59	3173.64	1972.45	2691.51	0.00	73.76
	1777.68	3027.44	2374.88	1675.27	0.00	18.95
	4340.71	3074.21	2335.84	3358.55	0.00	0.00
	3852.04	3342.89	2506.38	3255.15	0.00	0.00
	3980.00	3345.14	1325.68	2461.66	0.00	4.80
	3557.41	3117.22	1585.03		0.00	0.00
Average	3071.53	3225.14	2049.96	2770.43	0.00	21.23
Std dev.	1085.38	183.21	435.25	615.70	0.00	36.71

Table J.4 Sulphide concentration (mg/L) of the different wastewaters.

	Dunder	Final wastewater	Lactulose production wastewater	Wastewater outfall	Upstream outfall	Downstream outfall
	11.80	7.20	1.80	10.00	3.40	3.40
	13.40	7.00	2.40	8.00	2.00	2.20
	12.60	7.40	2.00	8.40	3.40	4.20
	8.00	5.80	3.20	8.40	3.40	2.00
	7.60	7.00	3.20	5.60	3.40	3.60
	11.80	5.60	1.80	6.20	3.40	1.80
	13.00	5.20	1.60	4.40	2.00	2.00
	10.20	7.60	3.00	5.80	4.00	3.20
	9.00	5.40	2.40	6.00	3.80	3.80
	12.80	8.20	3.60		3.20	3.40
Average	11.02	6.64	2.50	6.98	3.20	2.96
Std dev.	2.16	1.05	0.71	1.79	0.67	0.87

Table J.5 Ammonium concentration (mg/L) of the different wastewaters.

	Dunder	Final wastewater	Lactulose production wastewater	Wastewater outfall	Upstream outfall	Downstream outfall
	5.60	3.60	16.20	3.60	21.40	30.20
	28.20	4.20	0.80	3.20	13.80	12.00
	4.80	2.40	1.20	3.20	0.40	2.20
	5.40	3.60	1.60	2.40	18.40	12.60
	4.60	3.20	1.60	2.80	25.20	1.00
	5.00	3.40	2.80	3.00	32.80	11.60
	6.40	2.60	4.20	2.00	19.40	16.80
	5.20	2.40	2.80	2.60	20.80	7.80
	4.20	3.40	2.60	3.20	26.20	11.20
	5.20	9.60	2.40		14.40	4.80
Average	7.46	3.84	3.62	2.89	19.28	11.02
Std Dev	7.31	2.11	4.53	0.49	8.72	8.38

Table J.6 Biological oxygen demand concentration (mg/L) of the different wastewaters.

	Dunder	Final wastewater	Lactulose production wastewater	Wastewater outfall	Upstream outfall	Downstream outfall
	44500	30000	722	31700	4898	1932
	53000	35900	1915	30900	2997	4134
	48000	30800	3020	26600	2999	5219
	52000	30800	8791	26700	2657	3506
	37500	30000	4495	30400	3345	813
Average	47000	31500	3788.6	29260	3379.2	3120.8
Std dev.	6294.84	2491.99	3123.63	2427.55	883.19	1754.75

Table J.7 Chemical oxygen demand concentration (mg/L) of the different wastewaters.

	Dunder	Final wastewater	Lactulose production wastewater	Wastewater outfall	Upstream outfall	Downstream outfall
	56785	36685	1140	41385	6285	2385
	67985	38685	4085	40685	3985	4085
	64085	43385	2885	39885	3785	4985
	54285	41185	3085	51585	11185	785
	61685	37585	4685	37485	3485	7085
	85585	36685	7235	39685	1015	2685
	69385	35485	3585	32585	3185	7185
	66685	37585	14085	36085	3685	4385
	59585	36385	2245	38185	785	2585
	49285	37985	7485		4285	945
Average	63535	38165	5051.5	39729.4	4168	3711
Std dev.	10020.34	2405.46	3760.17	5195.94	2924.34	2259.81

Table J.8 Colour measurements (Hazen units) of different the wastewaters.

	Dunder	Final wastewater	Lactulose production wastewater	Wastewater outfall	Upstream outfall	Downstream outfall
	86000	33000	0.1	44000	200	1600
	72000	37000	<0.5	37000	100	1100
	76000	31000	2.8	37000	100	800
	58000	31000	<0.5	39000	100	1000
	62000	31000	<0.6	35000	300	1500
	72000	31000	<0.7	32000	200	500
	72000	29000	<0.8	19000	300	1100
	64000	34000	0.1	29000	300	500
	60000	29000	0.1	36000	200	600
	74000	38000	0.1		100	800
Average	69600	32400	0.64	34222	190	950
Std dev.	8579.04	3098.39	1.21	7084.80	87.56	386.58

Table J.9 Turbidity measurements (FAU) of the different wastewaters.

	Dunder	Final wastewater	Lactulose production wastewater	Wastewater outfall	Upstream outfall	Downstream outfall
	9400	2800	2	6600	60	300
	7000	3800	<1	3400	20	220
	7400	2400	18	3400	20	220
	2000	2000	<1	4000	20	120
	4000	2000	<1	2800	100	240
	6800	2600	<1	2400	40	20
	6800	1600	<1	1200	140	180
	4400	2400	<1	2000	80	20
	4400	2400	<1	3000	80	80
	6800	3800	<1		20	200
Average	5900	2580	10	3200	58	160
Std dev.	2146.31	726.94	11.31	1523.15	41.58	95.68

Table J.10 Cyanide concentration (mg/L) of the different wastewaters.

	Dunder	Final wastewater	Lactulose production wastewater	Wastewater outfall	Upstream outfall	Downstream outfall
	1.800	0.660	0.003	0.860	0.056	0.073
	2.270	0.980	0.009	0.820	0.073	0.059
	1.950	0.590	0.006	0.710	0.076	0.052
	1.350	0.590	0.013	0.800	0.010	0.071
	2.410	0.680	0.003	0.690	0.051	0.093
			0.008		0.053	0.081
			0.004		0.075	0.079
			0.082		0.076	0.043
			0.004		0.014	0.047
			0.011		0.048	0.093
Average	1.956	0.700	0.014	0.776	0.053	0.069
Std dev.	0.417	0.162	0.024	0.073	0.024	0.018

Table J.11 The following table shows the metals concentrations found in the different wastewaters

Lead (Pb) mg/L

	Dunder	Lactulose production wastewater	Final wastewater	Downstream outfall	Upstream outfall	Wastewater outfall
	3.502	1.163	0.659	0.573	0.858	1.561
	0	1.356	1.317	0.751	1.023	1.443
	0	1.408	1.616	0.661	1.019	1.49
	0.193	1.263	2.672	0.706	1.551	1.387
	0.144	1.089	3.002	0.574	1.206	1.61
	0.067	1.027	4.513	0.679	1.092	1.634
	0.285	1.06	0.54	0.635	1.127	1.65
	0.134	1.382	0.408	0.693	0.91	1.837
	0.297	1.172	0.479	0.683	0.984	1.931
	0.451	1.471		0.754	0.993	1.954
Average	0.51	1.24	1.69	0.67	1.08	1.65
Std dev.	1.06	0.16	1.43	0.06	0.19	0.20

Nickel (Ni) mg/L

	Dunder	Lactulose production wastewater	Final wastewater	Downstream outfall	Upstream outfall	Wasteater outfall
	0.344	0	0.038	0	0	0
	0.278	0	0.04	0	0	0
	0.407	0	0.028	0	0	0
	0.229	0	0.068	0	0	0
	0.169	0	0.077	0	0	0
	0	0	0.077	0	0	0
	0	0	0.087	0	0	0
	0.179	0	0.082	0	0	0
	0.132	0	0.042	0	0	0
	0.153	0		0	0	0
Average	0.1891	0	0.0599	0	0	0
Std dev.	0.132311	0	0.022602	0	0	0

Chromium (Cr) mg/L

	Dunder	Lactulose production wastewater	Final wastewater	Downstream outfall	Upstream outfall	Wasteater outfall
	0	0.134	0.213	0.256	0.295	0.387
	0	0.181	0.152	0.249	0.32	0.421
	0	0.148	0.206	0.31	0.354	0.397
	0	0.151	0.208	0.288	0.379	0.449
	0	0.15	0.2	0.274	0.343	0.433
	0	0.134	0.228	0.268	0.361	0.407
	0	0.146	0.247	0.402	0.343	0.456
	0	0.202	0.246	0.265	0.413	0.502
	0	0.175	0.264	0.32	0.369	0.513
	0	0.21		0.286	0.378	0.57
Average	0.00	0.16	0.22	0.29	0.36	0.45
Std dev.	0.00	0.03	0.03	0.04	0.03	0.06

Cadmium (Cd) mg/L

	Dunder	Lactulose production wastewater	Final wastewater	Downstream outfall	Upstream outfall	Wasteater outfall
	0.084	0.042	0.118	0.084	0.127	0.058
	0.004	0	0	0.008	0.034	0.003
	0	0	0	0	0	0.004
	0	0	0	0	0	0.002
	0	0	0	0	0	0.003
	0	0	0	0	0	0.001
	0	0	0	0	0	0
	0	0	0	0	0	0
	0	0	0	0	0	0
	0	0		0	0	0.001
Average	0.009	0.004	0.013	0.009	0.016	0.007
Std dev.	0.026	0.013	0.039	0.026	0.040	0.018

Copper (Cu) mg/L

	Dunder	Lactulose production wastewater	Final wastewater	Downstream outfall	Upstream outfall	Wasteater outfall
	0.288	0	0.853	0	0.071	0.44
	0.16	0.095	0.606	0	0.025	0.258
	0.14	0.001	0.643	0	0.007	0.182
	0.33	0.035	0.569	0	0.007	0.203
	0.126	0	0.721	0	0.034	0.174
	0.05	0	0.387	0	0.005	0.114
	0.034	0	0.569	0	0	0.06
	0.01	0	0.337	0	0	0.087
	0.097	0	0.955	0	0	0.086
	0.057	0	0	0	0	0.201
Average	0.129	0.013	0.564	0.000	0.015	0.181
Std dev.	0.107	0.031	0.273	0.000	0.023	0.111

Zinc (Zn) mg/L

	Dunder	Lactulose production wastewater	Final wastewater	Downstream outfall	Upstream outfall	Wasteater outfall
	1.538	0.308	1.072	0.529	0.658	1.388
	1.406	0.121	1.081	0.382	0.791	1.146
	1.139	0.267	1.137	1.008	0.367	1.088
	1.018	0.327	0.979	0.479	0.336	0.922
	0.877	0.215	0.954	0.529	0.527	0.929
	>1.5	0.275	1.037	0.55	0.447	0.917
	0.965	0.277	0.951	0.45	1.5	0.845
	1.251	0.169	0.88	0.85	0.688	0.826
	1.055	0.363	1.115	0.866	1.007	0.826
	1.456	0.577		0.323	0.657	0.998
Average	1.189	0.290	1.023	0.596	0.608	0.988
Std dev.	0.235	0.124	0.086	0.229	0.347	0.177

Table J.12 pH of the samples taken from the contact process developed on-site for wastewater treatment.

HRT 36 hours				
Day	**Influent**	**CSTR**	**Effluent**	**Comments**
0	4.74	4.36	4.40	
1	4.91	4.37	4.37	
2	4.64	4.48	4.49	
6	4.27	4.21	4.20	
7	4.22	4.21	4.21	
8	4.49	4.34	4.28	
13	4.27	4.32	4.23	
15	4.31	4.25	4.25	
17	4.38	4.32	4.31	
22	4.31	4.30	4.31	
24	4.38	4.34	4.35	
29	4.23	4.24	4.24	
HRT 24 hours				
31	4.34	4.33	4.32	
32	4.53	4.48	4.47	
34	4.48	4.43	4.42	
36	4.25	4.28	4.29	

38	4.36	4.34	4.33	
41	4.41	4.39	4.38	
42		**4.31**		No influent/addition of more SRP
43		**4.24**		
44		**4.40**		
45		**4.22**		
48		**4.32**		
49	4.92	4.67	4.52	pH adjusted influent in feed tank/pumps on
50	5.16	4.88	4.88	
51	5.55	5.27	5.13	
52	4.71	4.69	4.72	
HRT 72 hours				raised stirrer
56	4.59	4.66	4.76	
58	4.71	4.72	4.78	
59	4.44	4.45	4.61	
62	4.40	4.37	4.38	
64	4.31	4.34	4.39	
66	4.37	4.28	4.27	
69	4.31	4.28	4.28	
71	4.34	4.34	4.32	
73	3.98	3.98	3.98	
80		4.29		samples while ACP was completely off
87		4.42		
90		4.02		
94		4.47		after sulphide addition (100mg/L)
97		4.46		
100	4.52	4.39	4.35	after clarifier was drained
104	4.56	4.41	4.41	
106	4.32	4.36	4.44	
108	4.29	4.49	4.53	
111	4.25	4.24	4.25	
114	4.15	4.18	4.16	
115	4.15	4.19	4.19	
119	4.16	4.16	4.17	
121	4.04	4.04	4.05	
126	4.00	4.03	4.03	

Table J.13 Sulphate concentration (mg/L) of the samples taken from the contact process developed on-site for wastewater treatment.

Day	Influent	CSTR	Effluent	Removal (%)	Comments	Removal/MLSS	
HRT 36 hours							
0	5900	3900	4340	**26.44**		0.11	kg/day
1	5300	4900	4300	**18.87**		0.10	kg/day
2	6300	5300	4700	**25.40**		0.13	kg/day
6	6200	4700	4200	**32.26**		0.33	kg/day
7	7900	7100	7400	**6.33**		0.03	kg/day
8	6500	5000	5200	**20.00**		0.12	kg/day
13	5200	5200	3600	**30.77**		0.09	kg/day
15	5600	5000	4200	**25.00**		0.06	kg/day
17	4800	4600	4800	**0.00**		0.00	kg/day
22	3800	4400	5000	**-31.58**		0.00	kg/day
24	4200	4800	5200	**-23.81**		0.00	kg/day
29	3200	4600	3600	**-12.50**		0.00	kg/day
HRT 24 hours							
31	2600	11000	3600	**-38.46**		0.00	kg/day
32	3200	2800	3000	**6.25**		0.01	kg/day
34	3600	/	3200	**11.11**		0.02	kg/day
36	6200	5200	6200	**0.00**		0.00	kg/day
38	4400	5400	5600	**-27.27**		0.00	kg/day
41	3800	4600	6400	**-68.42**		0.00	kg/day
42		**3800**			No influent/add more SRP		
43		**5400**					
44		**5200**					
45		**4000**					
48		**8600**			pH adj feed/pumps on		
49	4200	5000	4800	**-14.29**		0.00	kg/day
50	4800	4600	5200	**-8.33**		0.00	kg/day
51	5800	5600	6200	**-6.90**		0.00	kg/day
52	7800	6400	6600	**15.38**	raised stirrer	0.07	kg/day
HRT 72 hours							
56	6600	6200	6000	**9.09**		0.21	kg/day
58	7400	5800	5400	**27.03**		0.04	kg/day
59	4600	5000	5000	**-8.70**		0.00	kg/day
62	5960	6260	4300	**27.85**		0.03	kg/day
64	4340	4780	6040	**-39.17**		0.00	kg/day
66	5500	6340	4520	**17.82**		0.02	kg/day
69	5060	4540	4840	**4.35**		0.00	kg/day
71	4280	4820	4560	**-6.54**		0.00	kg/day
73	4000	4560	4820	**-20.50**		0.00	kg/day
80		3880			samples while rig was off		
87		3260					
90		3100					

94		3740			after sulphide addition (100mg/L)		
100	3140	3740	2840	**9.55**	after clarifier was drained		
104	3580	3760	2320	**35.20**			
106	5000	4800	2020	**59.60**			
108	6080	4320	4140	**31.91**			
111	6720	6320	5440	**19.05**			
114	5960	4440	5660	**5.03**			
115	3800	6500	5540	**-45.79**			
119	4000	5500	5180	**-29.50**			
121	6880	5620	4720	**31.40**			
126	5520	6200	5400	**2.17**			

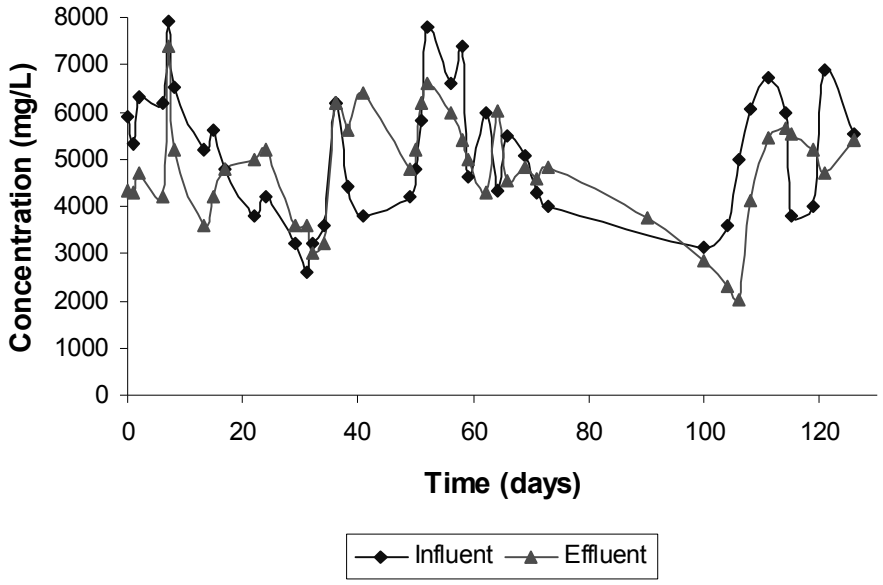

Figure J.1 Actual sulphate concentrations of influent and effluent measured during the bioreactor experiment.

Table J.14 Sulphide concentration (mg/L) of the samples taken from the contact process developed on-site for wastewater treatment.

Day	Influent	CSTR	Effluent	Removal (%)	Comments	Removal/MLSS	
HRT 36 hours							
0	15.9	13.7	8.1	**49.06**		0.00060	kg/day
1	6.8	11.6	10.6	**-55.88**		0.00000	kg/day
2	20.8	27.8	8.8	**57.69**		0.00100	kg/day
6	10.4	9.6	7.1	**31.73**		0.00055	kg/day
7	21.6	17.3	9.9	**54.17**		0.00060	kg/day
8	10.3	10.9	12.1	**-17.48**		0.00000	kg/day

13	4.4	3.8	2.4	**45.45**		0.00010	kg/day
15	4.2	4.8	3.8	**9.52**		0.00002	kg/day
17	6.2	5.8	5.2	**16.13**		0.00005	kg/day
22	6.0	6.0	4.8	**20.00**		0.00009	kg/day
24	9.4	6.4	8.0	**14.89**		0.00005	kg/day
29	8.6	10.4	9.4	**-9.30**		0.00000	kg/day
HRT 24 hours							
31	17.2	72.0	15.8	**8.14**		0.00007	kg/day
32	7.2	7.2	7.0	**2.78**		0.00001	kg/day
34	11.2	11.6	12.2	**-8.93**		0.00000	kg/day
36	18.4	11.0	15.6	**15.22**		0.00016	kg/day
38	11.8	15.4	16.8	**-42.37**		0.00000	kg/day
41	15.0	10.6	13.4	**10.67**		0.00008	kg/day
42		**7.4**			No influent/add more SRP		
43		**15.8**					
44		**12.0**					
45		**3.6**					
48		**25.4**					
49	7.8	14.2	11.6	-48.72	pH adj feed/pumps on	0.00000	kg/day
50	2.8	3.0	2.8	0.00		0.00000	kg/day
51	2.6	2.8	2.6	0.00		0.00000	kg/day
52	5.2	4.8	4.2	19.23	raised stirrer	0.00006	kg/day
HRT 72 hrs							
56	12.8	7.2	11.0	14.06		0.00062	kg/day
58	3.2	3.4	3.6	-12.50		0.00000	kg/day
59	4.0	4.0	3.6	10.00		0.00001	kg/day
62	11.6	13.8	8.8	24.14		0.00006	kg/day
64	3.8	3.8	3.4	10.53		0.00001	kg/day
66	4.0	4.4	4.0	0.00		0.00000	kg/day
69	3.2	3.6	4.0	-25.00		0.00000	kg/day
71	2.8	3.2	3.6	-28.57		0.00000	kg/day
73	11.0	11.0	9.0	18.18		0.00005	kg/day
80		7.4			samples while rig was		
87		4.8			off		
90		5.8	5.8				
94		4.4			after sulphide addition (100mg/L)		
100	3.2	2.8	2.6	18.75	after clarifier was drained		
104	2.8	3.0	2.4	14.29			
106	3.6	5.4	3.0	16.67			
108	3.0	3.0	3.0	0.00			
111	3.0	3.2	2.4	20.00			
114	3.2	3.0	2.6	18.75			
115	3.4	2.6	0.8	76.47			
119	3.2	3.2	2.8	12.50			
121	2.6	2.6	3.0	-15.38			
126	3.0	2.4	2.8	6.67			

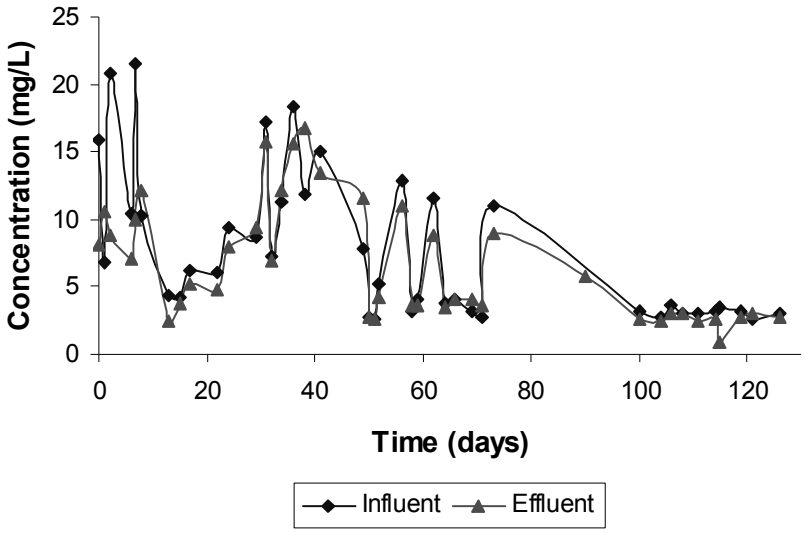

Figure J.2 Actual sulphide concentrations of influent and effluent measured during the bioreactor experiment.

Table J.15 Table showing the removal and generation of sulphide (mg/L) during the contact process.

Day	Sulphide generation in CSTR	Sulphide removal in clarifier
HRT 36 hours		
0	-16.06	40.88
1	41.38	8.62
2	25.18	68.35
6	-8.33	26.04
7	-24.86	42.77
8	5.50	-11.01
13	-15.79	36.84
15	12.50	20.83
17	-6.90	10.34
22	0.00	20.00
24	-46.88	-25.00
29	17.31	9.62
HRT 24 hours		
31	76.11	78.06
32	0.00	2.78
34	3.45	-5.17
36	-67.27	-41.82

38	23.38	-9.09
41	-41.51	-26.42
49	45.07	18.31
50	6.67	6.67
51	7.14	7.14
52	-8.33	12.50
HRT 72 hrs		
56	-77.78	-52.78
58	5.88	-5.88
59	0.00	10.00
62	15.94	36.23
64	0.00	10.53
66	9.09	9.09
69	11.11	-11.11
71	12.50	-12.50
73	0.00	18.18
100	-14.29	7.14
104	6.67	20.00
106	33.33	44.44
108	0.00	0.00
111	6.25	25.00
114	-6.67	13.33
115	-30.77	69.23
119	0.00	12.50
121	0.00	-15.38
126	-25.00	-16.67

Table J.16 COD concentration (mg/L) of the samples taken from the contact process developed on-site for wastewater treatment.

Day	Influent	CSTR	Effluent	Removal (%)	Comments	Removal/MLSS	unit
HRT 36 hours							
0	107150	70350	62895	**41.30**		3.16	kg/day
1	118135	103235	95235	**19.38**		0.23	kg/day
2	138900	110700	100900	**27.36**		3.17	kg/day
6	89480	91780	86880	**2.91**		0.42	kg/day
7	112810	103710	94610	**16.13**		0.94	kg/day
8	113400	130000	105900	**6.61**		0.70	kg/day
13	89840	93040	126640	**-40.96**		0.00	kg/day
15	106640	103240	113440	**-6.38**		0.00	kg/day
17	132040	123640	147040	**-11.36**		0.00	kg/day
22	109240	102040	120840	**-10.62**		0.00	kg/day
24	123040	122440	131440	**-6.83**		0.00	kg/day
29	89660	139060	134660	**-50.19**		0.00	kg/day

HRT 24 hours							
31	10000	108200	9600	**4.00**		0.02	kg/day
32	125640	89440	84240	**32.95**		1.80	kg/day
34	101440	113640	92040	**9.27**		0.52	kg/day
36	256840	135440	139040	**45.87**		6.80	kg/day
38	115050	126450	103650	**9.91**		0.59	kg/day
41	115020	108420	112020	**2.61**		0.15	kg/day
42		**130870**			No influent/add more SRP		
43		**116270**					
44		**134250**					
45		**106350**					
48		**111670**					
49	207050	126450	106250	48.68	pH adj feed/pumps on	0.22	kg/day
50	120860	106660	96260	20.35		1.45	kg/day
51	117730	87930	90130	23.44		9.07	kg/day
52	212720	105520	193120	9.21	raised stirrer	1.19	kg/day
HRT 72 hrs							
56	174500	126100	118900	31.86		19.11	kg/day
58	120620	178020	92620	23.21		0.58	kg/day
59	115600	99360	94940	17.87		0.41	kg/day
62	109760	107060	120500	-9.78		0.00	kg/day
64	109700	102660	98740	9.99		0.27	kg/day
66	141680	119220	103680	26.82		0.62	kg/day
69	98600	127600	93780	4.89		0.11	kg/day
71	163960	88020	104220	36.44		1.35	kg/day
73	100220	100140	93240	6.96		0.16	kg/day
100	95440	106600	99840	-4.61	Clarifier drained and pumps		
104	107480	92640	91640	14.74	turned back on after being off for 3 wks		
106	99500	88400	89360	10.19			
108	84740	104920	97900	-15.53			
111	93800	92980	90680	3.33			
114	113880	86540	93640	17.77			
115	109320	82800	90480	17.23			
119	87960	87980	90760	-3.18			
121	94040	88620	128040	-36.15			
126	97320	85480	81580	16.17			

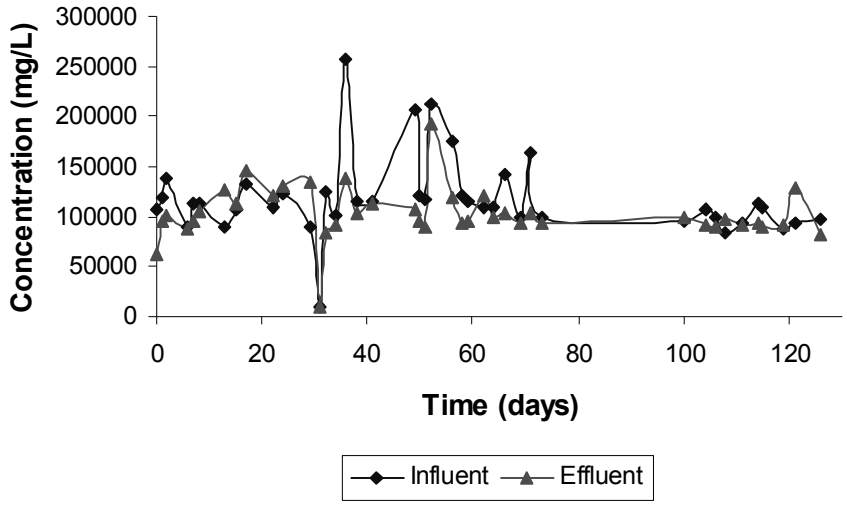

Figure J.3 Actual COD concentrations of influent and effluent measured during the bioreactor experiment.

Table J.17 MLSS concentration (mg/L) of the samples taken from the contact process developed on-site for wastewater treatment.

Day	**Influent**	**CSTR**	**Effluent**	**Comments**
HRT 36 hours				
0		18668		
1		13340		
2		15876		
6		8032		
7		25756		
8		14368		
13	7672	24884	4424	
15	6252	29820	5744	
17	10932	28232	6912	
22	62224	17256	8452	
24	17976	35120	10816	
29	4736	3836	4248	
HRT 24 hours				
31	3896	41192	5952	
32	7384.4	46107.6	5284	
34	4516	36028	4020	
36	8596	34640	6328	
38	9206.66	38233.33	4866.67	
41	8432	41380	5940	
42		**28904**		No influent/addition of more

				SRP
43		**31704**		
44		**28595**		
45		**27784**		
48		**39044**		
49	4044	15388	4632	pH adjusted influent in feed tank/pumps on
50	6468	32532	3256	
51	3072	6084	2056	
52	5400	32992	3424	raised stirrer
HRT 72 hours				
56	6768	1940	2492	
58	4112	32248	3476	
59	5288	33848	2852	
60	6420	33320	3508	
64	7136	27328	18864	
66	4820	40600	3928	
69	6976	29496	5936	
71	7060	29604	5652	
73	5376	28916	4360	

The following tables represent the statistical results for each of the components measured during the characterisation of the different wastewater samples using a paired t-test for two sample means.

Table J.18 Sulphate statistics table.

	Upstream outfall	**Downstream outfall**	**Wastewater on site**	**Wastewater at outfall**
Mean	0	21.232	3237.131389	2770.430833
Variance	0	1347.825411	36144.56754	379086.3839
Observations	10	10	9	9
Pearson Correlation	#DIV/0!		0.455471201	
Hypothesized Mean Difference	0		0	
df	9		8	
t Stat	-1.828833151		2.520352628	
P(T<=t) one-tail	0.050340986		0.017894558	
t Critical one-tail	1.833113856		1.85954832	
P(T<=t) two-tail	0.100681972		0.035789116	
t Critical two-tail	2.262158887		2.306005626	

Table J.19 Sulphide statistics table.

	Upstream outfall	Downstream outfall	Wastewater on site	Wastewater at outfall
Mean	3.2	2.96	6.466666667	6.977777778
Variance	0.453333333	0.762666667	0.9	3.214444444
Observations	10	10	9	9
Pearson Correlation	0.529101889		0.444867199	
Hypothesized Mean Difference	0		0	
df	9		8	
t Stat	0.984916179		-0.950729839	
P(T<=t) one-tail	0.175200456		0.18478818	
t Critical one-tail	1.833113856		1.85954832	
P(T<=t) two-tail	0.350400913		0.369576359	
t Critical two-tail	2.262158887		2.306005626	

Table J.20 Nitrate statistics table.

	Upstream outfall	Downstream outfall	Wastewater on site	Wastewater at outfall
Mean	280.4	293	3157.777778	5106.666667
Variance	11334.04444	4751.333333	1010644.444	1356500
Observations	10	10	9	9
Pearson Correlation	-0.326139187		0.611866372	
Hypothesized Mean Difference	0		0	
df	9		8	
t Stat	-0.275796055		-6.048707414	
P(T<=t) one-tail	0.394466333		0.000153177	
t Critical one-tail	1.833113856		1.85954832	
P(T<=t) two-tail	0.788932667		0.000306353	
t Critical two-tail	2.262158887		2.306005626	

Table J.21 BOD statistics table.

	Upstream outfall	Downstream outfall	Wastewater on site	Wastewater at outfall
Mean	3379.2	3120.8	31500	29260
Variance	780031.2	3079145.7	6210000	5893000
Observations	5	5	5	5
Pearson Correlation	0.514554816		-	
Hypothesized Mean Difference	0		0	
df	4		4	
t Stat	0.24740985		0.232189064	
P(T<=t) one-tail	0.408385755		0.413893405	
t Critical one-tail	2.131846486		2.131846486	
P(T<=t) two-tail	0.81677151		0.82778681	
t Critical two-tail	2.776450856		2.776450856	

Table J.22 COD statistics table.

	Upstream outfall	Downstream outfall	Wastewater on site	Wastewater at outfall
Mean	4168	3711	38185	39729.44444
Variance	8551778.889	5106760	6505000	26997777.78
Observations	10	10	9	9
Pearson Correlation	-0.390400976		0.566321484	
Hypothesized Mean Difference	0		0	
df	9		8	
t Stat	0.333138117		-1.07743845	
P(T<=t) one-tail	0.373329746		0.156349082	
t Critical one-tail	1.833113856		1.85954832	
P(T<=t) two-tail	0.746659493		0.312698164	
t Critical two-tail	2.262158887		2.306005626	

Table J.23 Colour statistics table.

	Upstream outfall	Downstream outfall	Wastewater on site	Wastewater at outfall
Mean	190	950	31777.77778	34222.22222
Variance	7666.666667	149444.4444	6444444.444	50194444.44
Observations	10	10	9	9
Pearson Correlation	0.114890034		0.329742412	
Hypothesized Mean Difference	0		0	
df	9		8	
t Stat	-6.219209872		-1.095898059	
P(T<=t) one-tail	7.76239E-05		0.15250732	
t Critical one-tail	1.833113856		1.85954832	
P(T<=t) two-tail	0.000155248		0.30501464	
t Critical two-tail	2.262158887		2.306005626	

Table J.24 Turbidity statistics table.

	Upstream outfall	Downstream outfall	Wastewater on site	Wastewater at outfall
Mean	58	160	2444.444444	34222.22222
Variance	1728.888889	9155.555556	387777.7778	50194444.44
Observations	10	10	9	9
Pearson Correlation	-0.044683952		0.490473641	
Hypothesized Mean Difference	0		0	
df	9		8	
t Stat	-3.04240493		-14.01741863	
P(T<=t) one-tail	0.006981726		3.25408E-07	
t Critical one-tail	1.833113856		1.85954832	
P(T<=t) two-tail	0.013963451		6.50816E-07	
t Critical two-tail	2.262158887		2.306005626	

Table J.25 Ammonium statistics table.

	Upstream outfall	Downstream outfall	Wastewater on site	Wastewater at outfall
Mean	19.28	11.02	3.2	2.888888889
Variance	75.95733333	70.26177778	0.38	0.241111111
Observations	10	10	9	9
Pearson Correlation	0.268209253		0.412961536	
Hypothesized Mean Difference	0		0	
df	9		8	
t Stat	2.524783415		1.532091834	
P(T<=t) one-tail	0.016256234		0.082018436	
t Critical one-tail	1.833113856		1.85954832	
P(T<=t) two-tail	0.032512468		0.164036871	
t Critical two-tail	2.262158887		2.306005626	

Table J.26 Cyanide statistics table.

	Upstream outfall	Downstream outfall	Wastewater on site	Wastewater at outfall
Mean	0.0532	0.0691	0.7	0.776
Variance	0.0005944	0.000329433	0.02615	0.00533
Observations	10	10	5	5
Pearson Correlation	-0.088685989		0.323990285	
Hypothesized Mean Difference	0		0	
df	9		4	
t Stat	-1.588157043		-1.10087136	
P(T<=t) one-tail	0.073356696		0.166373137	
t Critical one-tail	1.833113856		2.131846486	
P(T<=t) two-tail	0.146713393		0.332746273	
t Critical two-tail	2.262158887		2.776450856	

Table J.27 Settleable solids statistics table.

	Upstream outfall	Downstream outfall	Wastewater on site	Wastewater at outfall
Mean	11.2	14.18	22.67	11.46
Variance	64.17777778	188.024	1478.333444	102.6471111
Observations	10	10	10	10
Pearson Correlation	0.675002943		0.555134649	
Hypothesized Mean Difference	0		0	
df	9		9	
t Stat	-0.924485557		1.046029964	
P(T<=t) one-tail	0.189676854		0.161413229	
t Critical one-tail	1.833113856		1.833113856	
P(T<=t) two-tail	0.379353708		0.322826458	
t Critical two-tail	2.262158887		2.262158887	

Table J.28 Suspended solids statistics table.

	Upstream outfall	Downstream outfall	Wastewater on site	Wastewater at outfall
Mean	156.2	242	3786.2	3139
Variance	17769.28889	27293.33333	1028094.622	1291388.667
Observations	10	10	10	10
Pearson Correlation	0.485991514		0.487613862	
Hypothesized Mean Difference	0		0	
df	9		9	
t Stat	-1.764025266		1.871598082	
P(T<=t) one-tail	0.05577622		0.047029309	
t Critical one-tail	1.833113856		1.833113856	
P(T<=t) two-tail	0.111552439		0.094058617	
t Critical two-tail	2.262158887		2.262158887	

Table J.29 pH statistics table.

	Upstream outfall	Downstream outfall	Wastewater on site	Wastewater at outfall
Mean	6.956	5.983	4.107	4.048
Variance	0.995804444	0.185623333	0.012645556	0.015906667
Observations	10	10	10	10
Pearson Correlation	0.718640084		0.850332466	
Hypothesized Mean Difference	0		0	
df	9		9	
t Stat	4.098922751		2.802471781	
P(T<=t) one-tail	0.00134051		0.010314583	
t Critical one-tail	1.833113856		1.833113856	
P(T<=t) two-tail	0.00268102		0.020629167	
t Critical two-tail	2.262158887		2.262158887	

Table J.30 Total dissolved solids statistics table.

	Upstream outfall	Downstream outfall	Wastewater on site	Wastewater at outfall
Mean	1.30286	1.6404	18.345	18.08
Variance	0.694784729	0.0787616	0.573583333	0.497333333
Observations	10	10	10	10
Pearson Correlation	0.332578236		0.15997856	
Hypothesized Mean Difference	0		0	
df	9		9	
t Stat	-1.357842641		0.883319702	
P(T<=t) one-tail	0.103788711		0.200021548	
t Critical one-tail	1.833113856		1.833113856	
P(T<=t) two-tail	0.207577423		0.400043096	
t Critical two-tail	2.262158887		2.262158887	

Table J.31 Total solids statistics table.

	Upstream outfall	Downstream outfall	Wastewater on site	Wastewater at outfall
Mean	0.2102	0.2582	6.8282	6.3959
Variance	0.018661067	0.002609956	1.066918844	0.508441433
Observations	10	10	10	10
Pearson Correlation	-0.215004721		0.347045964	
Hypothesized Mean Difference	0		0	
df	9		9	
t Stat	-0.974290473		1.325209896	
P(T<=t) one-tail	0.177684888		0.108878477	
t Critical one-tail	1.833113856		1.833113856	
P(T<=t) two-tail	0.355369776		0.217756954	
t Critical two-tail	2.262158887		2.262158887	

Table J. 32 Metals statistics tables.

Lead		
	Wastewater on site	Wastewater at outfall
Mean	1.689555556	1.615888889
Variance	2.036888278	0.031356611
Observations	9	9
Pearson Correlation	-0.373560943	
Hypothesized Mean Difference	0	
df	8	
t Stat	0.147102686	
P(T<=t) one-tail	0.44334531	
t Critical one-tail	1.85954832	
P(T<=t) two-tail	0.88669062	
t Critical two-tail	2.306005626	

Nickel		
	Wastewater on site	Wastewater at outfall
Mean	0.059888889	0
Variance	0.000510861	0
Observations	9	9
Pearson Correlation	#DIV/0!	
Hypothesized Mean Difference	0	
df	8	
t Stat	7.94906574	
P(T<=t) one-tail	2.28608E-05	
t Critical one-tail	1.85954832	
P(T<=t) two-tail	4.57215E-05	
t Critical two-tail	2.306005626	

Chromium		
	Wastewater on site	**Wastewater at outfall**
Mean	0.218222222	0.440555556
Variance	0.001098694	0.001958028
Observations	9	9
Pearson Correlation	0.621955584	
Hypothesized Mean Difference	0	
df	8	
t Stat	-19.00099836	
P(T<=t) one-tail	3.04583E-08	
t Critical one-tail	1.85954832	
P(T<=t) two-tail	6.09165E-08	
t Critical two-tail	2.306005626	

Cadmium		
	Wastewater on site	**Wastewater at outfall**
Mean	0.013111111	0.007888889
Variance	0.001547111	0.000355361
Observations	9	9
Pearson Correlation	0.996851234	
Hypothesized Mean Difference	0	
df	8	
t Stat	0.760666193	
P(T<=t) one-tail	0.234340402	
t Critical one-tail	1.85954832	
P(T<=t) two-tail	0.468680804	
t Critical two-tail	2.306005626	

Copper		
	Wastewater on site	**Wastewater at outfall**
Mean	0.564	0.1805
Variance	0.074451111	0.012325833
Observations	10	10
Pearson Correlation	0.224994388	
Hypothesized Mean Difference	0	
df	9	
t Stat	4.48406492	
P(T<=t) one-tail	0.000761739	
t Critical one-tail	1.833113856	
P(T<=t) two-tail	0.001523478	
t Critical two-tail	2.262158887	

Zinc		
	Wastewater on site	**Wastewater at outfall**
Mean	1.022888889	0.987444444
Variance	0.007468861	0.035097028
Observations	9	9
Pearson Correlation	0.499537781	
Hypothesized Mean Difference	0	
df	8	
t Stat	0.654556964	
P(T<=t) one-tail	0.265558456	
t Critical one-tail	1.85954832	
P(T<=t) two-tail	0.531116912	
t Critical two-tail	2.306005626	

Table J.33 Results of the 5 L laboratory bioreactor (Chapter 4).

Day	Sulphate (mg/L)	Sulphide (mg/L)	pH
0	5080	11.4	4.82
1	4240	18.6	5.15
2	4560	17.8	4.84
3	2800	12.6	4.59
6	2780	8.4	4.56
8	4520	21.0	4.38
10	1380	3.4	4.45
13	3360	13.8	4.14
15	1720	4.4	4.54
20	3300	12.6	4.46
22	3100	3.2	4.47
23	2900	17.2	4.44
28	2880	3.6	4.45
29	1780	4.4	4.38
30	1760	3.6	4.45

The tables below are the results obtained from the flask reactors to determine SRP inhibition.

Table J.34 The pH recorded for the flask tests determining optimum pH.

Day	pH 4.51 (control)	pH 5.0	pH 5.5	pH 6.5	pH 7.0
1	4.69	5.56	6.6	8.52	9.35
2	4.58	5.15	5.53	6.76	7.33
3	4.36	4.91	4.96	5.98	6.19
5	4.33	4.73	4.68	5.3	5.84
6	4.32	4.69	4.72	5.29	5.73
7	4.39	4.81	4.89	5.4	5.78
8	4.39	4.83	4.95	5.53	5.82
9	4.25	4.72	4.86	5.47	5.73
10	4.27	4.78	4.92	5.74	5.81

Sulphate and normalised sulphate concentrations measured for the pH optimum test

Table J.35 Sulphate concentration (mg/L) for pH flask tests.

Day	pH 4.51 (control)	pH 5.0	pH 5.5	pH 6.5	pH 7.0
1	3960	4740	3960	4320	3660
2	4980	4500	4000	4360	4300
3	3060	3460	3480	4320	3760
5	2720	3520	3220	3120	3120
6	3660	4240	3680	4220	4280
7	3700	4340	2680	4480	2900
8	4140	2700	2840	4400	2900
9	3700	4080	4140	3660	3520
10	4400	4680	3180	2960	3000

Table J.36 Normalised sulphate concentration (%) for pH flask tests.

Day	pH 4.51 (control)	pH 5.0	pH 5.5	pH 6.5	pH 7.0
1	100.000	100.000	100.000	100.000	100.000
2	125.758	94.937	101.010	100.926	117.486
3	77.273	72.996	87.879	100.000	102.732
5	68.687	74.262	81.313	72.222	85.246
6	92.424	89.451	92.929	97.685	116.940
7	93.434	91.561	67.677	103.704	79.235
8	104.545	56.962	71.717	101.852	79.235
9	93.434	86.076	104.545	84.722	96.175
10	111.111	98.734	80.303	68.519	81.967

Sulphide and normalised sulphide concentrations measured for the pH optimum test

Table J.37 Sulphide concentration (mg/L) for pH flask tests.

Day	pH 4.51 (control)	pH 5.0	pH 5.5	pH 6.5	pH 7.0
1	6.2	6.2	6.6	10.6	7.6
2	6.8	7.8	7.4	10.4	6.8
3	3.8	6.6	6.2	7.6	6.4
5	4.4	7.8	8.6	9.2	8.6
6	6.4	8.2	8.4	7.6	8
7	4.8	6.8	6.6	7.6	7.2
8	3.8	7	6.6	8.2	6.8
9	4	7	6.2	7.8	6
10	3	5.6	5.2	6.6	6.6

Table J.38 Normalised sulphide concentration (%) for pH flask tests.

Day	pH 4.51 (control)	pH 5.0	pH 5.5	pH 6.5	pH 7.0
1	100.000	100.000	100.000	100.000	100.000
2	109.677	125.806	112.121	98.113	89.474
3	61.290	106.452	93.939	71.698	84.211
5	70.968	125.806	130.303	86.792	113.158
6	103.226	132.258	127.273	71.698	105.263
7	77.419	109.677	100.000	71.698	94.737
8	61.290	112.903	100.000	77.358	89.474
9	64.516	112.903	93.939	73.585	78.947
10	48.387	90.323	78.788	62.264	86.842

Table J.39 The pH recorded for the flask tests determining inhibition of SRP using different carbon.

Day	Control	Galactose	Glucose	Xylose	Raffinose
1	7.19	7.53	7.52	7.72	7.57
2	7.12	7.58	7.58	7.62	7.63
3	6.93	7.64	7.54	7.7	7.6
5	6.99	5.53	7.28	7.41	7.25
6	7.07	5.51	7.08	7.4	7.21
7	7.24	5.83	6.22	7.42	7.21
8	7.38	6.12	5.03	6.56	6.97
9	7.36	6.3	4.66	5.29	5.7
10	7.5	6.65	4.6	5.05	5.59

Sulphate and normalised sulphate concentrations measured for the flask test determining the inhibition of different carbon sources

Table J.40 Sulphate concentration (mg/L) for carbon source flask tests.

Day	Control	Galactose	Glucose	Xylose	Raffinose
1	660	940	760	980	760
2	540	360	620	540	300
3	840	940	720	640	840
5	380	280	620	640	660
6	615	972	949	1000	1000
7	560	1160	1060	1160	1260
8	380	860	960	1000	1020
9	140	700	620	740	460
10	240	1280	1120	860	100

Table J.41 Normalised sulphate concentration (%) carbon source flask tests.

Day	Control	Galactose	Glucose	Xylose	Raffinose
1	100.000	100.000	100.000	100.000	100.000
2	81.818	38.298	81.579	55.102	39.474
3	127.273	100.000	94.737	65.306	110.526
5	57.576	29.787	81.579	65.306	86.842
6	93.182	103.404	124.868	102.041	131.579
7	84.848	123.404	139.474	118.367	165.789
8	57.576	91.489	126.316	102.041	134.211
9	21.212	74.468	81.579	75.510	60.526
10	36.364	136.170	147.368	87.755	13.158

Sulphide and normalised sulphide concentrations measured for the flask test determining the inhibition of different carbon sources

Table J.42 Sulphide concentration (mg/L) carbon source flask tests.

Day	Control	Galactose	Glucose	Xylose	Raffinose
1	2.6	10.6	5.6	1.2	8
2	6.6	3.6	0.2	0.8	3.6
3	0.4	0.2	1.2	0.2	0.4
5	0.6	1.6	0.2	0.4	0.2
6	7.2	2	0.3	0.4	0.2
7	18	4	3.2	0.6	0.4
8	10.6	2.6	3.8	0.8	2
9	11.2	1.8	3.2	2.2	7.8
10	0.03	2.2	2	1.4	0

Table J.43 Normalised sulphide concentration (%) carbon source flask tests.

Day	Control	Galactose	Glucose	Xylose	Raffinose
1	100.000	100.000	100.000	100.000	100.000
2	253.846	33.962	3.571	66.667	45.000
3	15.385	1.887	21.429	16.667	5.000
5	23.077	15.094	3.571	33.333	2.500
6	276.923	18.868	5.357	33.333	2.500
7	692.308	37.736	57.143	50.000	5.000
8	407.692	24.528	67.857	66.667	25.000
9	430.769	16.981	57.143	183.333	97.500
10	1.154	20.755	35.714	116.667	0.000