SULPHATE REDUCTION UTILIZING HYDROLYSIS OF COMPLEX CARBON SOURCES

THESIS

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ABSTRACT

Due to environmental pollution caused by acid mine drainage (AMD), the Department of Water Affairs has developed a National Water Bill for managing and controlling the water environment to prevent AMD pollution. The application of sulphate reducing bacteria have been demonstrated for the treatment of AMD. However, the scale-up application of this technology ultimately depends on the cost and availability of a carbon source. This study evaluated the use of sewage sludge to provide a carbon source for sulphate reduction in synthetic drainage wastewaters. The demonstration of this process in a laboratory-scale reactor proved that sewage sludge could provide a useful model and viable carbon source for evaluation of sulphate reduction as a process for treating AMD.

Since sewage sludge is a complex carbon source, hydrolysis reactions controlling the anaerobic digestion of particulate substrate from this medium were optimized by evaluating the effect of pH on hydrolysis. Controlled and uncontrolled pH studies were conducted using a three stage mixed anaerobic reactor. Analysis of the degradation behaviour of the three important organic classes (carbohydrate, proteins and lipids) revealed that each class followed an individual trend with respect to pH changes. In addition, the solubilization of organic particulate carbon was also shown to be a function of pH. The hydrolysis pattern of organic substrate and COD solubilization was induced at pH 6.5 rather than at high pH values (7.5 and 8.5).

The biodegradation activity of sewage sludge was characterized by the API-ZYM^{TN} test system to provide rapid semiquantitative information on the activity of hydrolytic enzymes associated with the degradation of carbohydrate, lipids, proteins and nucleic acids. A wide range of enzyme activities with phosphatases, aminopeptidases, and glucosyl hydralases dominating were displayed. The pattern of substrate hydrolysis correlated to the degradation efficiency of each organic class as a function of pH. The evaluation of scale-up application for sulphate reduction utilizing sewage sludge as a carbon source demonstrated that large water volume flows could possibly be treated with this cost-effective technology. Generation of alkalinity and sulphide in this medium was shown to be successful in the removal of heavy metals by precipitation.

The use of this technology coupled to reduced cost involved showed that biological sulphate reduction utilizing hydrolysates of complex organic particulate from sewage sludge as a carbon source has a potential scale-up application for the treatment of AMD.

CHAPTER ONE

GENERAL INTRODUCTION

1.1 WATER BALANCE IN SOUTH AFRICA

Water plays an extremely important role in all spheres of the community. Not only is it an essential dietary constituent, but it has a fundamental role to play in industry and is a valuable resource for recreational purposes. The Republic of South Africa's water resources are limited in respect of quantity and usable quality. Approximately 8.6% of total annual rainfall (on average 52.5 milliard m^3) reaches the rivers. It is envisaged that, with the improved planning currently being applied it would be possible to utilise advantageously approximately 60% of the mean annual run-off. This together with the groundwater resources will yield a reasonably assured supply of 31.5 milliard m^3 water per year (Department of Water and Forestry, 1986).

Due to a greater portion of the population concentrated in towns and cities, the expected water requirements of these areas including, industries, mining and power generation are 16.7 milliard m^3 per year. The demand for agriculture, mainly irrigation farming, is estimated at 12.8 milliard m^3 per year. The total annual demand for distributable water in all consumer sectors will thus be 29.5 milliard m^3 i.e only 2.0 milliard m^3 less than the total estimated distributable water of 31.5

milliard m^3 - 2.0 milliard m^3 being less than two year's increment at this current rate of growth in water consumption (Henzen & Pieterse,1978; Department of Water & Forestry, 1986). At present there are regional water imbalances between supply demand. The Pretoria-Witwatersrand-Vereeniging-Sasolburg (PWVS) complex is the most notable as it relies heavily on imported water from neighbouring catchments such as the Orange, Tugela, Usutu and Komati rivers. Construction of the Lesotho Highlands Water Scheme is progressing to provide additional water. The Vaal Catchment receives 8% of the mean annual run-off in the country, yet has the highest concentration of urban, industrial, mining and power generation development in South Africa. Pressure on rivers, streams and impoundments leads to water being recycled on a large-scale (Braune & Rogers, 1987).

However, it is clear that South Africa is rapidly approaching a crisis point where available water resource will no longer be able to meet the demand. In addition, it should be realised that agricultural, urban and industrial development will place increasing pressures on the quality of water availability, thereby threatening the usability of these supplies. In this effort, effluents resulting from industrial and agricultural activities should also receive the necessary attention. In addition, effort must be made to limit the use and wastage of water. Where possible, water must be re-used without damage or sacrificing the quality of the end product in industrial applications or health aspects for domestic purposes (Henzen & Pieterse, 1978; Trusler *et al.*, 1989).

1.2 THE NATIONAL WATER POLICY

A general review of South Africa's Water Law has been under way since 1995. As part of the process, a White paper on a National Water Policy was published in April 1997. The department of Water and Forestry is currently publishing a new National Water Bill for public comment and acceptance before the approval by the parliament in late 1998, and anticipated promulgation in early 1999.

The Policy sets out principles on which the New Water Law is to be based and formulates the government's approach to future water governance. The National Water Policy is firmly founded on the concept of *" integrated water resource management on a catchment basis"*, that is, a process and implementation strategy to achieve equitable access to, and sustainable use of water resources by all stakeholders at catchment, regional and international levels, while maintaining the characteristics and integrity of water resources at the catchment scale within agreed limits. It also provides for the development of a registry of water use authorisation to manage and co-ordinate all licences and to ensure effective financial control over water use levies. Moreover, the Policy binds the Department of Water and Forestry to play a distinctive and pioneering role in promoting and facilitating the establishment of statutory-directed catchment management in fulfilment of the above approach (Viljoen *et al.*, 1998).

1.2.1 Purpose of the Water Act

The purpose of the Act is to ensure that the nation's resources are protected, used, developed, conserved, managed and controlled to meet the basic human needs of present and future generations, to address the result of past racial and gender discrimination, and to promote the equitable access to water of the people. To provide for the needs of the future generation, the Government promotes the efficient, sustainable and beneficial use of water. This means that pollution and the degradation of water resources must be reduced or prevented, thereby protecting aquatic and associated ecosystems and their biological diversity.

In order to prevent water pollution, and in the particular situation where pollution of water resource occurs, or might occur, as a result of activities on land, the person who owns, control, occupies or uses the land in question is responsible for taking measures to prevent pollution of water resources. If these measures are not taken, the catchment management agency concerned may itself do whatever is necessary to prevent the pollution or to remedy its effects, and to recover all reasonable costs from the persons responsible for the pollution. Under emergency situations such as the spilling of a harmful substance that finds or might find its way into a water resource, the responsibility for remedying the situation rests with the person responsible for the incident or the substance involved. The effective management of water resources will facilitate the achievement of these fundamental principles as well as to ensure social and economic development and adherence to international obligations in accordance with the Constitutional mandate for water reform.

If there is a failure to act, the relevant catchment management agency may take the necessary steps and recover the costs from every responsible person (Perkins, 1998). Being empowered to act on behalf of the nation, the Minister has ultimate responsibility to ensure that water is allocated equitably and used beneficially in the public interest, while promoting environmental values. As this Act is founded on the principle that the National Government has overall responsibility for and authority over water resource management, including the equitable allocation and beneficial use of water in the public interest, a person can only be entitled to use water if the use is permissible under the Act (Perkins, 1998; Viljoen *et al.*, 1998).

1.3 ACID MINE DRAINAGE - The Problem

South Africa is the world's major producer of precious metals and gem diamonds. (Henzen & Pieterse, 1978; Thompson, 1980). In addition, the metal processing, plating and finishing industries produce metals such as: copper, zinc, cadmium, lead, nickel, silver and mercury (Peters & Ku, 1985).

In the mining of each of these mineral resources, a pollution problem may be created in one way or the other with its attendant adverse effects on the available water supply sources. The environmental pollution by mine waters can also be caused by sulphur compounds which occur in the liquid, solid and gas phases. For example, sulphate rich solutions are produced bacteriologically from pyrite during mining operations, or from spent sulphuric acid. Acid mine drainage (AMD) waters contain high concentrations of dissolved heavy metals and sulphate, and

can have pH values as low as 2.5. Although concern over sulphates as pollutants has been moderate, it has been recognised that the introduction of sulphates into the aquatic environments disrupts the ecological balance and creates difficulty for other water users. Turbidity, taste, odour, toxicity problems and increased biocorrosion of pipelines and heat exchangers are representative of these effects (King *et al.*, 1975).

Mining effluents contribute to mineralisation of receiving water and may prove to be toxic to man, animals and plants due to unacceptably high concentrations of heavy metals and cyanide. Solid waste in the form of gypsum is produced by the fertilizer industry when phosphoric acid is leached from calcium phosphate rock with sulphuric acid, or by the mining industry when sulphuric acid containing effluents are neutralised with lime. Gaseous sulphur dioxide is produced by power stations when electricity is generated from coal and hydrogen sulphide gas by the coal-to-fuel industry (Maree *et al.*, 1991).

It is estimated that 200 Ml/day of mining effluent containing high levels of calcium sulphate, is discharged into the public streams of GAUTENG area (Maree *et al.*, 1988). This presents a sulphate load of 73 000t/annum. In a study conducted by the Department of Water Affairs (1986), the Eastern Transvaal was shown to contain sulphate content in the runoff water from the areas with the high mining activities which varied between 200 and 2000mg/L, while in the areas of low mining activities it varied only between 10 and 55mg/L. The sulphate load in this area amounts to 12 000t/annum.

Treatment of the problem may be considered as chemical or biological processes.

1.3.1 Chemical Treatment Processes

A range of chemical processes have been applied to the treatment of AMD. These include :

1.3.1.1 Precipitation with Barium Salts

Barium has been extensively used in the treatment of boiler feed water for the removal of sulphates. Barium sulphate is a very insoluble compound, and one way of removing sulphate from water is its precipitation as the barium sulphate salt. The source of barium is usually barium carbonate. The removal of sulphate using barium salts consist of two stage barium carbonate and four stage barium sulphide processes (Maree *et al.*, 1989). The removal of sulphate and calcium from water by means of barium carbonate ($BaCO_3$) and barium sulphide (BaS) can be presented by the following reactions respectively :

$$Ca^{2+} + SO_4^{2-} + BaCO_3(s) \rightarrow BaSO_4\,(s) + CaCO_3 \quad .....(1)$$

$$Ca^{2+} + SO_4^{2-} + BaS \rightarrow BaSO_4(s) + CaS^{2-} \quad .....(2)$$

In both processes, sulphate is precipitated as barium sulphates, calcium as calcium carbonate, magnesium as magnesium hydroxides and heavy metals as either metal hydroxides or metal sulphide as show in equation 3 below.

$$BaCO_3 + MgSO_4 + Ca(OH)_2 \rightarrow BaSO_4 + Mg(OH)_2 + CaCO_3 \quad \text{..... (3)}$$

$$\mathbf{MS}\,(OH)^{2+} + H_2O \leftrightarrow \mathbf{MS} + 2H_2O \quad \text{..... (4)}$$

where **M** represents heavy metals such as iron, zinc, copper . The raw materials, barium sulphide or barium carbonate, are recovered by thermal process from the precipitated barium sulphate, which has been used for treatment of various industrial effluents, or neutralized process water from slimes dam recovery, acid mine drainage, gold plant filter wash water and power station cooling water. By-products such as sulphur, sodium bisulphide and heavy metals can be recovered (King *et al.*, 1975).

1.3.1.2 Neutralisation with Limestone

Limestone ($CaCO_3$) can be used as an alternative to lime as it is cheaper and it occurs naturally in a pure state as limestone and with magnesium as dolomitic limestone. Another advantage of neutralisation with calcium carbonate is the production of smaller sludge volumes than those produced from neutralisation with lime (Henzen & Pieterse, 1978).

Several researchers have reported the use of $CaCO_3$ as a neutralizing agent for acid waters (Thompson, 1980; Barnes & Romberger, 1986).

In recent studies, Maree *et al.*, (1992) investigated the practicality of using cheaper limestone instead of lime for the treatment of acidic effluents investigated. From this study, it was determined that in case of lime treatment, the rate of neutralisation is fast when stiochiometric dosages of lime are applied. Partial removal of sulphate is achieved if sufficient crystallisation time is provided and complete removal of heavy metals, depending on the pH of the treated water. With limestone processes, the rate of $CaCO_3$ neutralisation is directly related to the dosage of $CaCO_3$ and the particle size. Aeration marginally accelerated the rate of $CaCO_3$ neutralisation as a result of CO_2 stripping. The partial sulphate removal is achieved during $CaCO_3$ neutralisation as a result of $CaSO_4$ precipitation. Iron (III) and aluminium are effectively removed during $CaCO_3$ neutralisation. The rate of neutralisation is retarded by the presence of iron (II) in solution. The capital costs for lime and limestone neutralisation are similar, but the chemical cost in case of limestone neutralisation amounts to only 29% of that of lime (Maree *et al.*, 1992).

1.3.1.3 Slurry Precipitation Recycle Reverse Osmosis (SPARRO)

The SPARRO process is a novel design developed in South Africa by the Chamber of Mines, from a seeded RO concept originated from USA (Chamber of Mines Research Organisation, 1988). Two of the main advantages of the process are that it produces a high quality solid gypsum by-product which could be sold and can operate at very high recovery ratios, which

reduces the quantity for brine disposal. The quality of the product water is related to the overall water recovery and this would be adjusted to each raw mine water treated. The process was successfully tested at pilot scale on scaling mine water from ERPM Hercules Shaft. However, the disadvantage of this process is that it entails high capital, maintenance and operational costs, and membrane fouling can occur.

1.3.1.4 Disadvantages of Chemical Treatment Processes

Generally, chemical treatment has the following disadvantages: production of large volumes of unstable metal hydroxides mixed with gypsum which are costly to dispose of, especially when toxic metals content classifies it as hazardous waste (Rowley *et al.*, 1994). The sludge may precipitate in the reactor, which may cause problems of plugging, abrasion and toxicity. The consideration of the possible scheme for recovery and reuse of the chemicals would be the key for not only developing a satisfactory solution to the problem of ultimate sludge disposal, but also would lead to the development of a process wherein superior economic advantages could be demonstrated.

The need for an economically viable alternative treatment to AMD has led to the investigation of many processes to determine the most feasible one. Thus, the biological anaerobic treatment of industrial wastewater and acid mine water in particular, utilising sulphate reduction by sulphate reducing bacteria (SRB) has become a major topic of interest.

1.3.2 Biological Treatment Processes

The role and use of sulphate reducing bacteria have attracted the attention of Biotechnologists due to its fundamental properties and possible residual water treatment process application in AMD remediation (Herrera *et al.*, 1991; Barton, 1995). The use of wetlands for the treatment of AMD, i.e, passive treatment technology has been developed recently for the treatment of AMD (Robinson & Robb, 1995; Van Zyl, 1996; Younger *et al.*, 1997). The operation of the process uses both SRB and acidophilic iron bacteria (Hendin *et al.*, 1989). The advantage of the process is that it provides a low potential cost approach to long term management of the problem. However, drawbacks include the large surface area requirement for higher AMD flows and concerns relating to diffuse spread and long term stability of the metals deposited.

The "active" biological sulphate reduction based on the growth of sulphate reducing bacteria (SRB) has been identified as a potentially valuable process for removing contaminant metals from coal and metal mine drainage (Postgate, 1984; Widdel & Hansen, 1992; Barton, 1995). Sulphate reduction in acid mine may result in the generation of alkalinity which neutralizes the acidity and also in the precipitation of heavy metals as metal sulphide. The economic advantage of such processes from pollution point view and market value of sulphur and heavy metals is that these products can be recovered (Barnes *et al.*, 1991; Herrera *et al.*, 1991).

The overall reaction for the metal sulphide formation may be represented as follows:

$$\text{Metal Sulphate} + \text{Carbon Substrate} \rightarrow \text{Metal Sulphide} + CO_2 + H_2O + \text{Cells} \quad \text{.....(4)}$$

Under anaerobic conditions, SRB oxidize simple organic compounds such as lactic acid with the sulphate and thereby generate hydrogen sulphide and bicarbonate ions:

$$3SO_4^{2-} + \text{lactic acid} \rightarrow 3H_2S + 6HCO^{3-} \quad \text{..... (5)}$$

hydrogen sulphide in turn reacts with many contaminant metals to remove them from solution as insoluble metal sulphides :

$$H_2S + M^{2+} \rightarrow MS\ (s) + 2H^+ \quad \text{.....(6)}$$

where M include metals such as Cd, Fe, Pb, Ni, and Zn. Bicarbonate ions react with protons to form CO_2 and water and remove acidity from the solution as CO_2 gas :

$$HCO^{3-} + H^+ \rightarrow CO_2\ (g) + H_2O \quad \text{..... (7)}$$

The H_2S and HCO^{3-} formed during sulphate reduction equilibrate into a mixture of H_2S, HS^-, S^{2-}, CO_2, HCO^{3-} and CO_3^{2-}. If sufficient sulphate reduction occurs, this mixture will buffer the solution

pH to a particular value, typically in a range of 6-7. Raising the pH of acidic water will cause most metals to precipitate as insoluble hydroxides or carbonates (Dvorak *et al.*, 1992).

1.3.2.1 Sulphate Reducing Bacteria (SRB) : Their Metabolic Potential

SRB are obligatory anaerobic bacteria comprising the genera of Gram negative : *Desulfovibrio, Desulfobulbus, Desulfotomaculum, Desulfobacter, Desulfococcus, Desulfosarcina*, and Gram positive *Desulfonema.* These bacteria are very varied in morphology. They include rods with various sizes and shapes (from lemon-like to fusiform), vibroid, and filamentous forms (Postgate, 1984; Radchenko & Tashirev, 1991).

All SRB share a common ability to dissimilate sulphate for energy (Widdel, 1988). The initial step in the biochemical pathway is the transport of exogenous sulphate across the bacterial cell membrane into the cell, and proceeds by the action of ATP sulphurylase which combines sulphate with ATP to produce adenosine phosphosulphate (APS), as well as pyrophosphate. The cleavage of APS yields inorganic phosphate, and is then converted to sulphite (SO^{3-}) by cytoplasmic enzyme APS reductase. The subsequent reduction for the formation of hydrogen sulphide (H_2S) then proceeds readily (Postgate, 1984). The production of H_2S, which, being a strong reducing agent, can inhibit the growth of certain aerobic micro-organisms (Widdel, 1988), and conversely plays an important role in the natural environment in that it functions as an electron donor for the growth of sulphur bacteria (Gibson, 1990).

SRB occupy ecological niches where access of oxygen is limited : water logged soils, bottom marine and fresh water sediments, polluted environment such as sour whey digesters, spoiled foods, anaerobic purification plants, and sewage plants (Postgate, 1984; Ueki *et al.*, 1988). These bacteria grow over a broad temperature range, from 0 to 98°C (Widdel & Hansen, 1992). They also prefer an environment with a pH range of between 7.0 to 8.0 (Widdel, 1988), and a redox potential ranging from -70 to -110mV (Vainshtein & Gogotova, 1987; Barnes *et al.*, 1991).

SRB are known to use a broad spectrum of organic compounds as energy substrates summarized in Table1.

Table 1. Compounds used as Energy Substrates for SRB (Hansen, 1988).

Inorganic	Hydrogen, carbon monoxide
Monocarboxylic Acids	Formate, acetate, propionate, isobutyrate, higher fatty acids up to C18, lactate
Dicarboxylic acids	Succinate, fumarate, malate, oxalate, maleinate, glutarate, pimelate
Alcohols	Methanol, ethanol, propanol, ethylenegycols, glycerol
Amino acids	Glycine, serine cycteine, valine, leucine, aspartate, glutamate, phenylalamine
Miscellaneous	Fructose, choline, benzoate, nicotinic acid, phenol.

These bacteria usually depend on the activities of other bacteria for the supply of energy sources (Hansen, 1988). In natural environments, the electron donors oxidized by SRB are always low-molecular weight compounds, which are fermentation products from the anaerobic bacterial degradation of carbohydrates, proteins and lipids, and acidogenesis products from degradation of long chain fatty acids (Ueki *et al.*, 1988). However, during anaerobic treatment of sulphate containing wastewaters, SRB especially of the *Desulfovibrio* species, and methane producing bacteria (MPB) can compete for hydrogen and acetate as common primary substrates. The higher affinity of SRB for these substrates than MPB is considered to be an important factor which explains why sulphate reducers outcompete hydrogenotrophic methanogens in sulphate-sufficient environments (Bhattacharya *et al.*, 1996).

1.3.2.2 Biological Sulphate Reduction : Process Studies

The biological sulphate removal process has been considerably researched in the past years. Middelton & Lawrence (1977), determined the kinetics of microbial sulphate reduction in completely mixed reactors using acetic acid as carbon source and observed a sulphate reduction rate of 0.29g SO_4/L.d. Cork & Cusanovich (1978) developed a continuous purge system, using an inert carrier gas (75% argon and 25% CO_2), to feed sulphide removed from actively growing cultures of *Desulfovibrio desulphuricans* to cultures of *Chlorobium thiosulfatophilum* for oxidation of sulphur. Sulphate reduction rate of 6.3g SO_4/L.d was observed in a completely mixed reactor containing 12 600mg/L lactic acid, at pH 6.5 and temperature 30°C. Some 91% of the hydrogen.

sulphide produced was swept off into a sulphide oxidizing chamber containing *Chlorobium sp.* where 88% was converted to sulphur, which presents an overall sulphur yield of 80%.

Maree & Strydom (1985), studied sulphate reduction and heavy metal removal in an upflow packed bed reactor to establish well developed microbial biofilms for sulphate removal from mine water using sugar, sewage sludge and pulp mill effluent as energy sources. They concluded that 1.6g sugar, 16.7ml sulphite liquor were necessary to remove 1 800mg/L sulphate. Over-saturated calcium carbonate levels and unutilized carbonaceous material prevented water from being reused directly after anaerobic treatment.

To overcome this problem , an integrated process for biological sulphate removal from gold and uranium plant effluents (Maree *et al.*, 1986) and from gypsum waste (Maree *et al.*, 1987) was investigated in an anaerobic packed bed reactor using molasses as a carbon source. This process comprise three stage processes, that is primary anaerobic, aerobic, and secondary anaerobic treatment processes. During primary anaerobic treatment approximately 90% of the influent sulphate was removed. In addition 450mg/L sulphides, elemental sulphur and metal sulphides were produced. Heavy metals were efficiently removed in this stage while calcium carbonate reached over-saturation levels. The end-products occurring in anaerobically treated water, namely soluble organic carbon, residual hydrogen sulphide, and calcium carbonate, were successfully removed in a subsequent aerobic stage. In this stage COD was removed from 1 100 to 300mg/L, hydrogen sulphide to 20mg/L, and calcium carbonate crystallised out.

Weakly biodegradable organic components originating from the molasses, as well as heavy metals were removed below detectable levels. The attractive features of this process are that sulphur may be recovered as a by-product from effluent purification, and also reusable water is produced from mining effluents. The success of these processes hinge, however, on the removal and downstream processing of the H_2S which is produced in the primary anaerobic stage.

Maree & Hill (1989), reported the development of another process aimed at the feasibility of stripping H_2S from the primary anaerobic stage and subsequently converting it to elemental sulphur using molasses as a carbon source. During anaerobic treatment, influent sulphate at 1 600mg/L was converted to 700mg/L sulphide, 700mg/L elemental sulphur as SO_4^{2-} and small quantities of metal sulphide. Hydrogen sulphide produced in the anaerobic stage was subsequently stripped off in a closed system with either CO_2 or nitrogen as carrier gas to low levels of 10 and 30mg/L sulphide respectively. The H_2S was in turn transported to the sulphur production stage where it was oxidised to elemental sulphur when brought into contact with a ferric solution.

The end products occurring in the effluent of the stripping stage, namely, soluble organic carbon, and calcium carbonate were successfully removed in a subsequent aerobic stage. In this stage, COD was removed to 360mg/L while 780mg/L calcium carbonate crystallized out. A C:N:P ratio of 300:10:1 was found to be effective for the effective functioning of the aerobic bacteria. Complete removal of strontium was also obtained in this stage. The success of this study proved that the biological sulphate process is a viable option for treatment of sulphate wastes and for the

production of reusable water and/or the recovery of valuable by products such as elemental sulphur, sodium bisulphide and heavy metals. The disadvantage of the treatment processes using molasses as a carbon source, is that molasses is expensive and may not be available in sufficient quantities in the future, as it is used in other industrial processes.

Reis *et al.*, (1988) investigated the acidogenic phase of a two-stage anaerobic digestion process using distillery molasses slops from ethanol production containing high sulphate concentrations of 4.25g/L. Removal of sulphate was studied at pH 5.8, 6.2, 6.6 in a continuously stirred tank reactor and pH ranging from 7.7-7.9 in an upflow fixed bed reactor (test system). Biological sulphate removal increased with pH, so did acetic acid production from fermentative bacteria and SRB. Sulphate reduction was more efficient in the fixed film reactor than in stirred tank reactor. The soluble sulphides from the sulphate reduction present in the acidogenic reactor effluent were precipitated before the methanogenic phase to avoid biogas contamination and methanogenic bacteria inhibition, under such conditions, sulphide concentrations in the biogas produced was very low and high methane production was achieved.

Maree *et al.*, (1989) described a process whereby sulphuric acid-rich water, rich in heavy metals, can be treated biologically for sulphate removal, without prior neutralisation with an alkali such as lime. This is possible because alkalinity produced in the biological sulphate process. By contacting metal-contaminated water with H_2S gas stripped from the anaerobic reactor, heavy metals can be precipitated as metal sulphides. The heavy metals can in addition be precipitated

selectively through pH control. The pH of the feed water can be raised by recirculation of a side stream of the highly buffered water from the anaerobic reactor. The principle of selective recovery of heavy metals was also demonstrated by Hammack & Edenborn (1992) who showed that copper, zinc and iron can be separated selectively by bubbling H_2S gas through three reactors, connected in series, at pH values of 1.6, 3.8, and 6.2.

A major disadvantage when sulphate is removed biologically from an effluent with organic carbon source as a substrate, is the high cost and the residual organic carbon content, which requires downstream treatment after the anaerobic reactor. This disadvantage was overcome by replacing organic carbon sources with producer gas (du Preez & Maree, 1994). In this study, the rate of sulphate and nitrate removals were demonstrated at pilot scale using producer gas as an energy source. Producer gas, also called synthesis gas, is generated from any material containing carbon and hydrogen. Some industrial sources are : from steam and methane, by the partial oxidation of fuel oil, or by coal gasification. In this study the resultant mixture of H_2, CO, CO_2 and N_2 were used as energy source for SRB in an anaerobic packed-bed system. Sulphate was reduced to sulphide at a rate of 1.2g SO_4/L.d with H_2 and CO as substrates, and 2.4g SO_4/L.d with only CO as energy source. Nitrate was converted to nitrogen gas in the anaerobic reactor. Sulphide was removed from 2 000 mg/L (as SO_4) to less than 90mg/L. Producer gas is considered to be highly attractive as a source of energy for SRB for the following reasons, no organic compounds are added to the water which then need to be removed in separate stages. Producer gas is produced from coal which it is widely available. Low-grade coal, containing sulphur deposits can safely be used, as sulphur compounds would be treated by the system.

The feasibility of biological sulphate reduction in a gas lift reactor using hydrogen and carbon monoxide as energy sources was investigated (van Houten *et al.*, 1994). Attention was paid to biofilm formation, sulphide toxicity, sulphate conversion rate optimisation, and gas liquid mass transfer. The study demonstrated that SRB are able to form biofilms under turbulent flow conditions, and their growth was still possible at free H_2S concentrations up to 450mg/L. When free H_2S concentrations were kept below 450mg/L, a maximum conversion rate of 30g SO_4^{2-}/L.d was achieved after 10 days of operation.

Scale-up applications of active AMD treatment technologies have been limited, but the successful operation of biological sulphate reduction has been applied at the Buldelco zinc refinery, Netherlands (Scheerem *et al.*, 1993). The Paques Co. has been engaged for the past ten years in the development and installation of the treatment systems based on biotechnological processes to remove sulphur compounds from water, air and gaseous streams. The Paques sulphate and metal removal technology, marketed under the name THIOPAQ, provides an efficient, biological means of bioremediating zinc refinery waste waters. However, the overall cost of the technology and the carbon source is substantial.

The development of an Algal Sulphate Reducing Process (ASPAM) utilizing the basic reactor design for the treatment of AMD for scale-up application in South Africa has been reported (Rose *et al.*, 1997). The process was based on a development of High Rate Algal Pond (HRAP) for the treatment of tannery effluent (Rose *et al.*, 1996), and the use of algal biomass as a carbon source for sulphate reduction (Boshoff *et al.*, 1996; van Hille & Duncan, 1996).

The process involved the settling of metal sulphide in the anaerobic compartment of a facultative pond treating some source of organic waste material in a co-disposal function. In addition to providing algal biomass as an independent carbon source, and for the final polishing of metals from the waste stream, the HRAP may also be used to provide control of sulphide release with oxidation of the surface of the facultative pond. This approach could achieve a potentially high level of control in the pre-settlement of heavy metal loads from large wastewater flows.

Since overall costs of the technology depend mainly on the carbon source used, in South Africa the process of biological sulphate reduction has been considerably researched but has not been applied on any significant scale-up mainly because the cost estimations indicated that a suitable source of organic carbon for the bacteria would make the process uneconomical. Various organic carbon sources have been studied for biological sulphate reduction including sewage sludge (Butlin *et al.*, 1956; Pipes, 1960; Conradie & Grutz, 1973), sauerkraut filtrate, peach peelings, and brewery wort (Corrick *et al.*, 1970), ethanol and methanol (Postgate, 1984; Swezyk & Pfenning, 1987), cattle waste (Ueki *et al.*, 1988), molasses (Maree & Hill, 1989), lactate and cheese whey (Olezkiwicz & Hilton, 1986; Herrera *et al.*, 1991), producer gas (du Preeze *et al.*, 1992), microalgal biomass and tannery waste (Boshoff *et al.*, 1996). These waste sources all proved to be cost effective, with varying degrees of efficiency. The economical significance of such processes is that the technology of sulphur and heavy metal recovery can be developed. Sludge handling cost can be minimized or eliminated.

1.4 RESEARCH HYPOTHESIS

Municipal sewage sludge presents a potential low-cost, readily available feedstock of complex particulate organic matter as a potential organic carbon source for sulphate reduction. SRB are unable to utilize complex substrates directly. Instead, they use the fermentation products of these substrates. Therefore, optimization of the hydrolysis reactions controlling the anaerobic digestion of particulate substrates from sewage sludge could provide for a useful source of biodegradable carbon for efficient biological sulphate reduction. Furthermore, generation of sulphide and alkalinity in this process could be applied to the removal and precipitation of heavy metals contained in AMD and thus reduce the toxicity of these wastewaters on the environment.

1.5 RESEARCH OBJECTIVES

In order to explore the hypothesis laid out above, the following objectives were identified:

1. To evaluate the feasibility of sewage sludge as a carbon source for sulphate reduction.

2. To evaluate effect of pH on hydrolysis of organic particulate from sewage sludge.

3. To characterise enzymes present in sewage sludge and to determine the enzyme activities as a function of pH.

4. To examine the scale-up application of sulphate reduction utilizing sewage sludge as a carbon source for treatment of mine drainage waste.

5. To examine the metal removal capacity of sulphide-rich effluent from pilot-scale sulphate reducing anaerobic reactor fed sewage sludge.

CHAPTER TWO

SEWAGE SLUDGE AS A CARBON SOURCE FOR BIOLOGICAL SULPHATE REDUCTION IN MINE DRAINAGE WASTEWATERS

2.1 INTRODUCTION

Acid Mine Drainage (AMD) is one of the most serious environmental problems facing the coal and metal mining industries. It is directly responsible for mineralization of the receiving water when discharged in excess amounts. It constitutes an even greater indirect problem through salinity-associated corrosion, taste impairment of drinking water and serving as a substrate for organisms implicated in bio-corrosion (du Preeze *et al.*, 1992). The long-term nature of the problem, and the large volumes of AMD requiring treatment, present particular technological challenges for the design of remedial interventions.

Biological sulphate reduction has been identified as a potentially valuable process for removing sulphates and heavy metals from industrial effluents. The application of the process has been demonstrated in active treatment systems for AMD remediation at laboratory and pilot scale tests in various reactor designs, including packed bed anaerobic reactors (Riviera, 1983; Maree *et al.*, 1987), fluidised bed systems (Umita *et al.*, 1988; van Houten *et al.*, 1994), anaerobic filters (De

Walle *et al.*, 1979), and the baffle reactor (Grobiki & Stuckey, 1992). The cost and availability of the carbon and electron donor source is a major factor which constrains further process development. The presence of short chain fatty acids, especially acetate, carbohydrates, and proteins contained in sewage sludge, could provide a low-cost, easily available feedstock for biological sulphate reduction. Laboratory-scale studies were undertaken to investigate the feasibility of sewage sludge used as a carbon source and electron donor for sulphate reduction in a synthetic mine drainage wastewaters.

2.2 MATERIALS AND METHODS

2.2.1 Reactor Design and Operational Procedures

The laboratory-scale continuous stirred tank reactor (CSTR) used for studies of sulphate reduction is shown in figure 2.1. The reactor consisted of a sulphate reducing anaerobic reactor with a working volume of 5L, a 2L stirred flask as a feed reactor, and a 1L flasks collecting overflow. The reactors were sealed with rubber stoppers and operated without introducing air into the system. A gas trap containing zinc acetate was used to collect H_2S escaping from the anaerobic reactor.

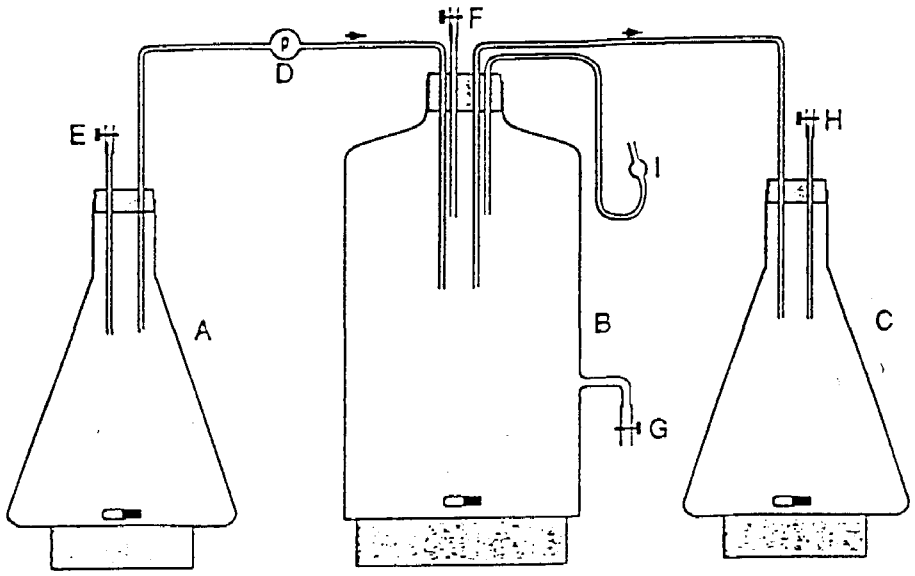


Fig 2.1 Schematic diagram of the laboratory-scale reactor used for monitoring biological sulphate reduction in synthetic mine drainage wastewater

A : Feed Vessel, B : Anaerobic Reactor, C : Overflow Vessel, D : Peristaltic Pump, E : Influent Port , F : Port 1, G: Port 2 and H : Overflow Port, I : Gas Trap Device

2.2.2 Feed Source and Seeding

The sewage sludge, used as a carbon source in this study, was obtained from the Grahamstown Municipal Works. The sludge was collected from the underflow lines of the primary clarifiers and macerated to break down the solids which may cause the blockage of tubing The sludge was stored in closed containers in the laboratory cold room at 4°C for a maximum period of 5days. Sulphate was added to the sewage to simulate a blend producing sulphate and COD content of between 800 to 1 130 and 2 000 to 2 500mg/L respectively. The reactor was seeded with an active mixed culture of sewage sludge sourced from the Grahamstown works anaerobic digester.

A synthetic mine water of sulphate solution (described in Appendix I) mixed with sewage sludge was fed into the anaerobic reactor using a peristaltic pump at a rate giving a hydraulic retention time (HRT) in the reactor of 5 days, and a feeding dilution rate of 0.2 per day. The reactor was operated at room temperature for a period of 38 days. Equations for calculating HRT and dilution rate are provided in Appendix II.

2.2.3 Analytical Procedures

Samples were drawn daily and analysed for pH, total sulphate, total sulphide, dissolved sulphide and chemical oxygen demand (COD). Both experimental and control samples were analysed in triplicate and the results averaged. The control samples used deionised water instead of effluent. Determinations of sulphate, sulphide and COD were carried out according to analytical procedures described in Standard Methods (APHA, 1985), calorimetric method using *NN* Diethyl-*p*-phenylenediamine (Rees *et al.*, 1971), and the Merck Spectroquant^{TN} system respectively. Samples of dissolved sulphide were prepared by centrifugation for 15minutes at 5000rpm. Details for analytical tests and reagents used are provided in Appendix 1 and II.

2.3 RESULTS AND DISCUSSION

The chemical composition of the industrial effluent before and after treatment in the anaerobic reactor is given in table 2.1.

Table 2.1 Water quality of synthetic mine effluent mixed with sewage sludge before and after treatment. (Guideline for aquatic life, Kiestra & Eggers 1986).

Water quality parameter	Guideline for aquatic life	Before treatment	After treatment
pH	6.5 - 9.0	3.5	7.0 - 7.8
Sulphate (mg/L)	1 400	950 - 1 150 (sd = 99.833)	187 - 217 (sd = 19.311)
Sulphide (mg/L)	-	0	87
COD (mg/L)	-	2 100 - 2 500 (sd = 165.20)	1 000 - 1 240 (sd = 98.995)

During the anaerobic treatment stage, complex organic substrates from sewage sludge supplied the energy requirement for SRB to create carbonaceous elements suitable for new cell synthesis. The net effect of these reactions is a decrease in biodegradable chemical oxygen demand (COD). COD removal is reported in figure 2.2 and 2.3 with the reactor showing an improved performance over time. At steady state operation, the COD concentration was reduced from 2 400 to1 240mg/L on day 30 representing the average of 50% removal. During this period, the initial SO_4: COD ratio was 996 : 2 400mg/L or 1: 2.5 and was reduced to 250mg/L SO_4 and 1 240 mg/L COD or 1:5. This represents a COD : SO_4 utilization ratio of 1:4 assuming no electron flow to methanogenesis.

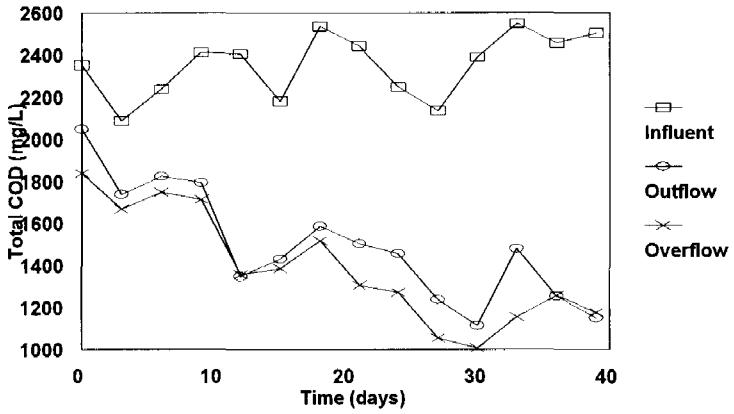


Figure 2.2 Total COD reduction in a laboratory-scale sulphate reducing STR fed sewage sludge as a carbon source

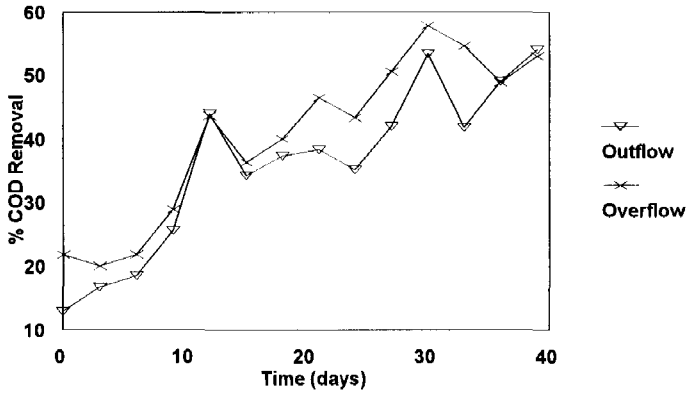


Figure 2.3 Percentage removal of COD in a laboratory-scale sulphate reducing STR fed sewage sludge as a carbon source

The results for sulphate removal over a period of 38 days are shown in figure 2.4 and 2.5. During the start-up period, the sulphate concentration decreased from 1136 to 330 mg/L representing a maximum removal efficiency of 70%.

After the adaptation period, sulphate reduction improved, resulting in a decrease in sulphate from 1 130 to 217mg/L on day 34. This represented an average sulphate removal of 80% in the anaerobic reactor. However, no further improvement in sulphate removal was observed from day 30 to day 38. Limitations in complete sulphate removal by biological sulphate reducing systems has been previously noted (Maree *et al.*, 1986; Sarner, 1990).

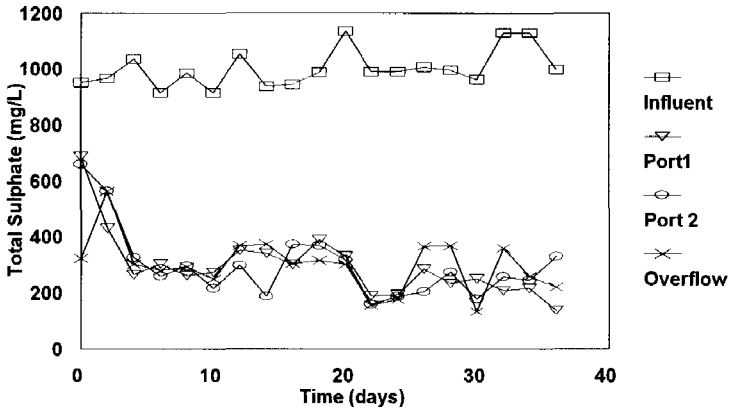


Figure 2.4 Sulphate removal in a laboratory-scale sulphate reducing STR fed sewage sludge as a carbon source

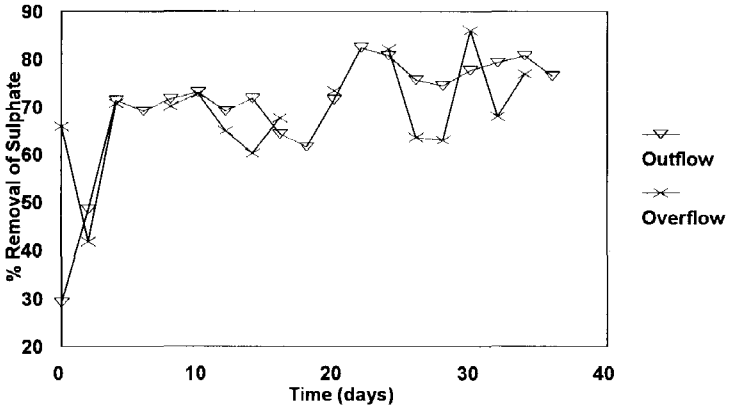


Figure 2.5 Percentage removal of sulphate in a laboratory-scale sulphate reducing STR fed sewage sludge as carbon source

The results pertaining to total sulphide production are shown in figure 2.6. Sulphide was produced slowly for the first 8 days with gradual increase observed up to 20 days of operation after which sulphide production stabilized . The sulphide concentration increased to 87mg/L after steady state operation had been achieved.

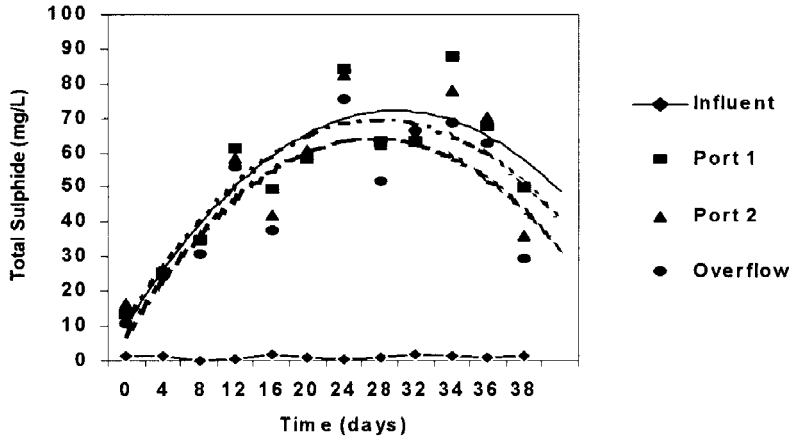


Figure 2.6 Total sulphide production in a laboratory-scale sulphate reducing STR fed sewage sludge as a carbon source (—Port 1, ---- Port 2, — — — Overflow)

Measurement of dissolved sulphide levels reported in figure 2.7 shows a similar trend to that reported for total sulphide production.

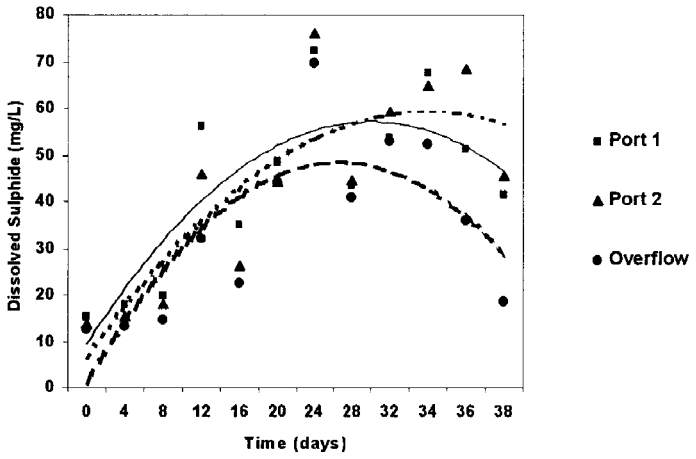


Figure 2.7 Dissolved sulphide production in a laboratory-scale STR fed sewage sludge as a carbon source (zero influent sulphide, —Port 1, --- Port 2, — — — Overflow)

Heavy metals in the effluent precipitated as metal sulphides and collected in the overflow vessel as shown in figure 2.8. The concentration of metal sulphide precipitates was lower during the start-up of the experiment but increased when sulphate reduction was established, resulting in a maximum concentration of 20 and 10mg/L in the anaerobic reactor and overflow vessel respectively. These results indicate that the total concentration of heavy metals which can be tolerated is directly related to the quantity of sulphides produced.

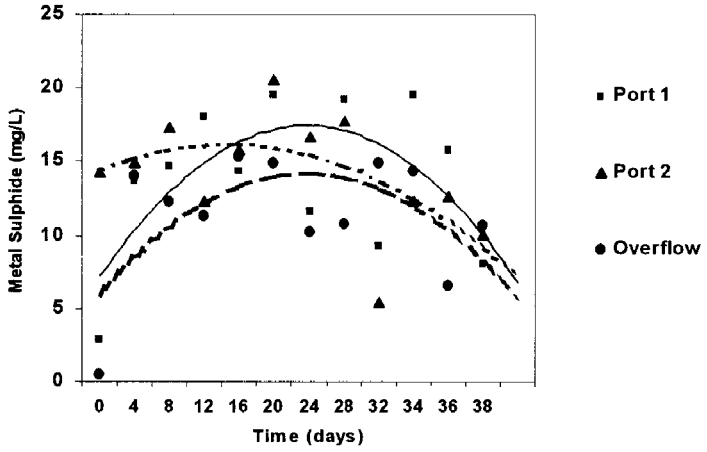


Figure 2.8 Metal sulphide precipitates in a laboratory-scale sulphate reducing STR fed sewage sludge as a carbon source (–Port 1, ---Port 2, — — — Overflow)

As shown in figure 2.9, the generation of alkalinity in the sulphate reducing reactor was evident in the elevation of acid water pH from 6.0 to 7.8.

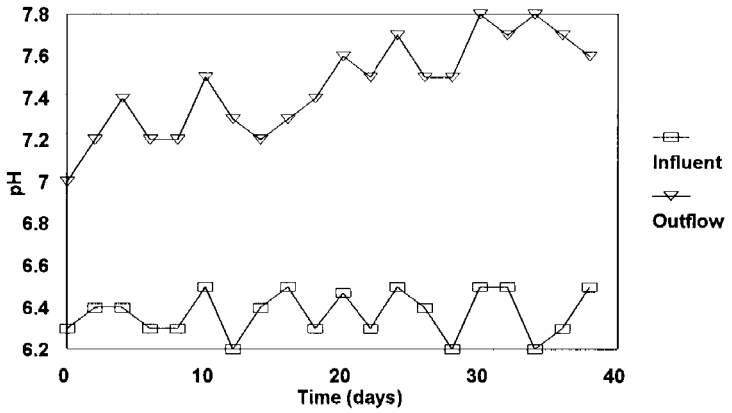


Figure 2.9 The pH profiles in a laboratory-scale sulphate reducing STR fed sewage sludge as a carbon source

2.4 CONCLUSION

The laboratory-scale reactor results showed that sewage sludge may be used as a carbon source for bacterial sulphate reduction when added directly to AMD. During anaerobic treatment of the synthetic AMD, influent sulphate concentration was reduced by 80%, with sulphide production of 87mg/L and COD reduction of 55%. Sewage sludge showed a significant capacity to act as an alkaline reagent by raising the pH of the acidic effluent from 3.5 to 6.5 prior to treatment. The pH was increased further to 7.8 by sulphate reduction. Furthermore, the precipitation of heavy metals as metal sulphides by this process was demonstrated.

It is therefore concluded that sewage sludge provides a useful model and a viable carbon source for the evaluation of sulphate reduction as a process for treating AMD. The success of the laboratory-scale study of sewage as a carbon source for sulphate reduction provided the basis for scale-up development of biological sulphate reduction process at pilot-scale.

CHAPTER THREE

INFLUENCE OF pH ON BIOLOGICAL HYDROLYSIS OF SEWAGE SLUDGE

3.1 INTRODUCTION

Due to the great quantities of organic residuals generated in urban and industrial areas, the anaerobic digestion process has been used for the degradation and stabilization of large fractions of organic matter (Novaes, 1986). Different steps involved in anaerobic digestion shown in figure 3.1 have been described by Brinch *et al.*, (1994). In the first stage (1) hydrolysis, complex organic substrates such as carbohydrates, proteins and lipids are hydrolysed through extracellular enzymes to produce volatile fatty acids and other low molecular weight soluble carbon compounds. In the acidogenic phase (2) the products from hydrolysis are fermented into volatile acids and in the subsequent acetogenic phase (3) high molecular fatty acids as well as volatile acids (except for acetate) are decomposed through β-oxidation. Finally, methane is produced in the last stage.

Since bacteria are unable to assimilate particulate organic material, it first has to be broken down into soluble polymers or monomers. Thus, hydrolysis is the first step required for the microbial utilization of complex biopolymers. Therefore, it is the hydrolysis rate that determines the overall rate of particulate organic degradation (Eliosov & Argaman, 1995).

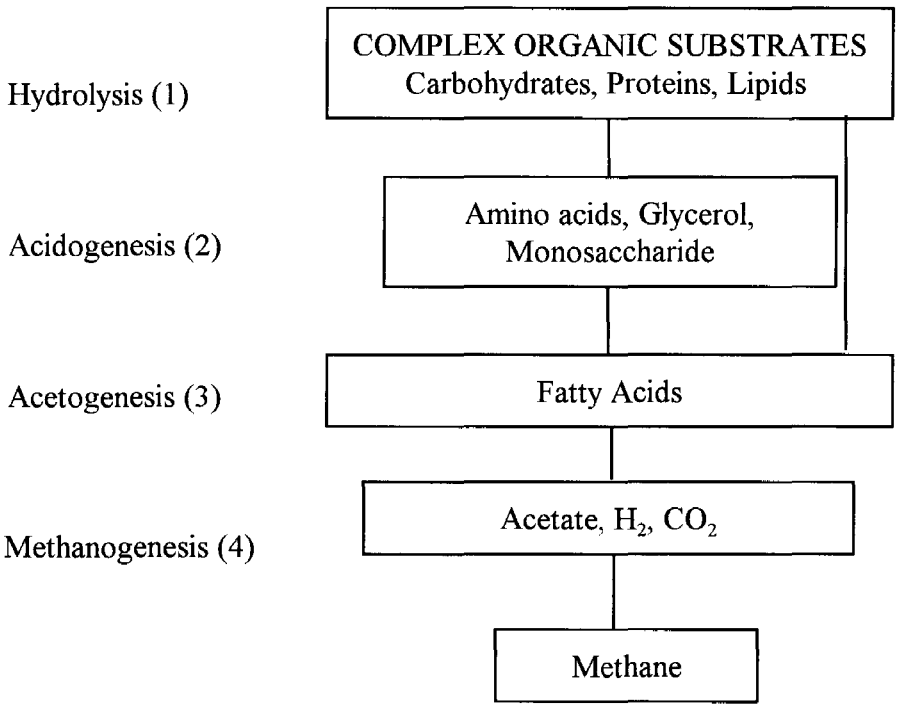


Figure 3.1 Illustration of metabolic steps involved in anaerobic digestion of complex organic substrates (Brinch, *et al.*, 1994)

During the acid phase stage, including both hydrolysis and fermentation, fermentation products of carbohydrate metabolism, (i.e soluble C1 and C4 end products such as organic acids : formic and lactic acids), alcohols (ethanol, butanol, propanol) and products of lipid metabolism glycerol, ketones and aldehydes may be generated (Gottschalk, 1986). The acid phase process increases the concentration of soluble organics suitable for use as energy sources for bacteria carrying out other processes such as phosphorus removal, and two-stage biological denitrification (Elefsiniotis & Oldham, 1994).

Hydrolysis of particulate organic matter is considered a function of factors such as pH, temperature, microbial biomass (the source of hydrolytic enzymes), the type of particulate substrate, particle size and the remaining concentration of degradable particulate matter (Eastman & Ferguson, 1981; Perot *et al.*, 1988). By controlling the residence time and temperature, it is possible to stop the anaerobic digestion at the hydrolysis step (i.e process 1, 2 and 3) (Eastman & Ferguson, 1981). The optimal operating conditions for hydrolysis process with respect to the production of soluble carbon are a residence time of 2-3 days, and a process temperature of 25°C. At these process conditions the pH value will range between 5.3 and 6.0. At lower process temperature the yield of dissolved easily degradable carbon will drop considerably and at a high process temperature the yield will increase slightly (Brinch *et al.*, 1994).

Much information available on the effect of pH on the acidogenic digestion process has been obtained from studies using simple carbon substrates such as glucose, sucrose or lactose (Zoetemeyer *et al.*, 1982; Joubert & Britz, 1986; Hsu & Yang, 1991). Relatively few studies are reported for hydrolysis of complex substrates such as sewage (Eastman & Ferguson, 1981; Perot *et al.*, 1988). Since little is known regarding the influence of pH on the anaerobic digestion of municipal waste, the objective of this study was to investigate the effect of low pH (acid production) and high pH (alkalization) on substrate degradation, and the extent of soluble organic carbon production without methanogenesis during hydrolysis of complex particulate substrate.

3.2 MATERIALS AND METHODS

3.2.1 Feed Source and Characteristics

The sewage sludge used in this study was obtained from the Grahamstown Municipal Works. The sludge was collected in one batch of 4L from the primary clarifiers of the sewage treatment plant. This sludge was mixed using a macerator to break large solids particles that may cause blockage of the laboratory pump lines and then stored in a laboratory cold room at 4°C. When needed, the total COD content of the sludge was determined and then adjusted by dilution with tap water to a concentration of between 1 000 and 1 500 mg/L prior feeding. This was to ensure uniform feed characteristics for the entire experimental program. Classification of the organic composition of the feed shown in table 3.1, reveals that lipids and carbohydrate fractions were predominant components in the feed, while the contribution of the proteins was small.

Table 3.1 Organic Composition of the Feed

Organic Class	Content (mg/L)	Mean
Lipids	700 - 1 200	950
Carbohydrates	200 - 300	250
Proteins	30 -80	55
Total		1 255

3.2.2 Experimental Design and Operation

The experimental apparatus used in this study is shown in figure 3.2

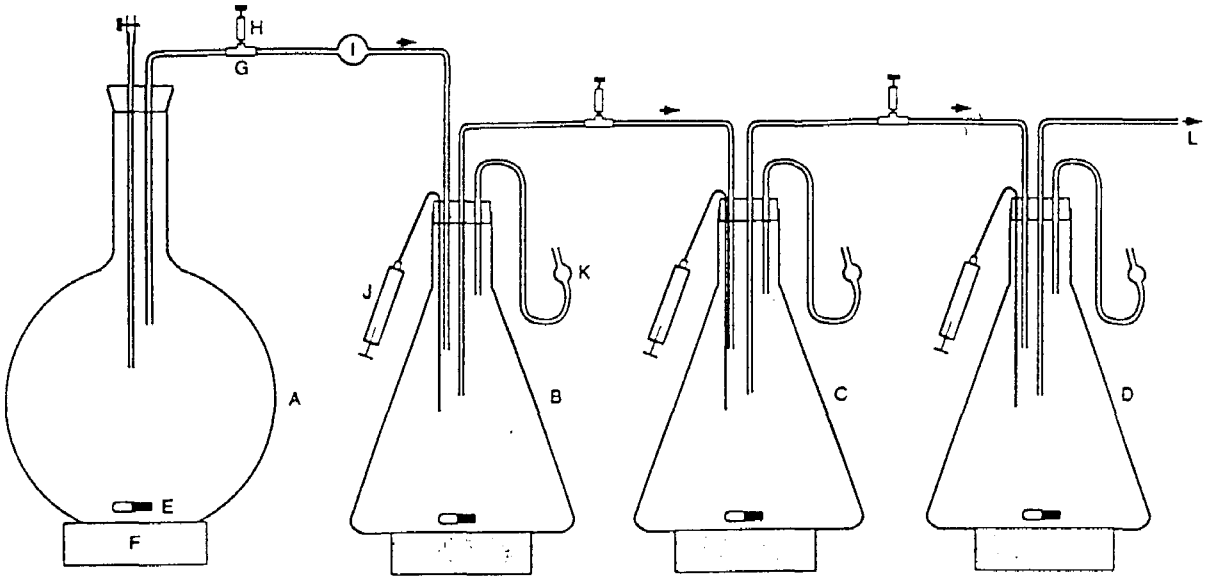


Figure 3.2 Schematic experimental apparatus of a three stage anaerobic reactor for hydrolysis of sewage sludge

A - Feed Vessel, B, C and D - Anaerobic Reactors (Reactor 1, 2 and 3 respectively), E - Stirrer bar for mixing, F - Magnetic Stirrer Plate, G - Hungate Tube, H - 5ml Syringe for addition of either NaOH or HCl , I - Peristaltic Pump, J - 20ml syringe for sampling out, K -Gas Trap Device containing Zinc Acetate, L -Effluent

The experimental apparatus consisted of three stirred 1L flasks as anaerobic reactors for degradation of sewage sludge, and a 5L stirred round-bottom flask for the feed. The inlets of the reactors were closed with rubber stoppers sealed with silicon sealant to keep them anaerobic. A gas trap device containing zinc acetate was used to prevent air entrapment. The inlets of the reactors were modified for the insertion of glass tubing which were extended with silicon rubber tubing to allow the flow of digested sludge from one reactor to the other. A peristaltic pump was used to feed the reactors. Thin tubing was inserted into each of the three reactors (R1, R2, and R3) with a 20ml syringe connected to the exterior of the tubing for sampling the effluent from each reactor. A hungate tube was connected to the tubing between each reactor for the addition of NaOH or HCl to adjust the pH of the reactors to the required value.

The system was fed continuously and was considered to be at steady state after a week of uninterrupted operation. In each experiment six days were allowed for return to steady state. This was based on three full volume changes for the reactor system which had a hydraulic retention time of 2 days and a feed dilution rate of 0.5 per day. The pH of the reactors was continuously monitored and adjusted by addition of dilute aqueous solutions of 0.1M NaOH and 0.1M HCl for controlled pH studies. The frequency of sampling for all chemical analysis was carried out every second day, except pH which was recorded and monitored on a daily basis. Equations used for calculating HRT and dilution rate are provided in Appendix I

3.2.3 Analytical Methods

Samples were taken from the feed reactor, and three semi-continuous flow anaerobic reactors and analysed for total solids, suspended solids, carbohydrates, proteins, lipids, COD, and dissolved COD and solids. COD concentrations were measured using the Merck Spectroquant^{NT} System. Proteins were determined by the Lowry method using bovine serum albumin as a standard (Lowry *et al.*, 1951). Carbohydrate content was estimated by the phenol-sulphuric acid method using glucose as a standard (Dubios *et al.*, 1956). Lipids were extracted with chloroform-methanol solution and determined by weight (Christie, 1992). Total solids and suspended solids were determined according to the method described by Standard methods (APHA, 1985). Dissolved samples of COD and solids were centrifuged at 5 000rpm for 15 minutes to remove large solid materials and filtered through GF/C microfibre glass filters prior to analysis. Details for all analytical tests and reagents used are provided in Appendix 1 and II.

3.3 RESULTS AND DISCUSSION

3.3.1 Hydrolysis of Sewage Sludge Without pH Control.

The pH profiles of the feed reactor, reactor 1 (R1), reactor 2 (R2), and reactor 3 (R3) for hydrolysis of sewage sludge under standard conditions are shown in table 3.2. During this study, the pH was not controlled but remained between 7.0 and 7.2 in the feed, while the pH of the R1, and R2, but not R3, showed progressive acidification for a period of six days.

Table 3.2 Profiles of pH values measured for reactor operation without pH control.

Time (days)	Feed	Reactor 1	Reactor 2	Reactor 3
0	7.2	7.3	7.0	7.2
2	7.2	7.2	6.9	7.2
4	7.2	7.0	7.1	7.2
6	7.0	6.8	6.8	6.9

The profiles of COD reduction in anaerobic reactors is shown in figure 3.3. Initially, the concentration of COD was reduced from 1 190 to 1 035mg/L (12%) in R1 and to 830mg/L in R2. The reduction efficiency increased to 30 and 20% in these reactors by day six when the pH dropped to 6.8. However, in R3 lower COD reduction efficiency of 10% was observed probably due to low hydrolysis rate of organic particulate matter. A rapid reduction in COD content observed in R1 and R2 indicates the degradation of readily biodegradable organic carbon in these reactors (Kim *et al.*, 1997).

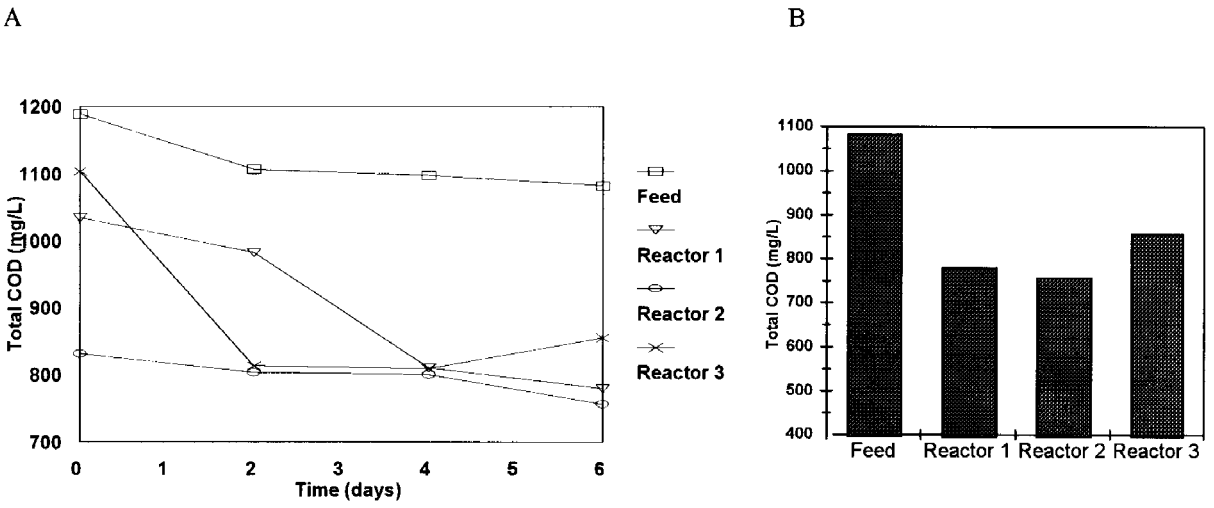


Figure 3.3 Total COD reduction in anaerobic reactors during (A) steady state operation and (B) on day six without pH control

As shown in figure 3.4, the production of soluble COD increased for a period of 4 days with acid production in R1 and R2 . However, in R3 the production of soluble COD was not enhanced due to accumulation of unsolubilized particulate matter. Solubilization in R1 and R2 could be attributed to the higher rate of substrates (lipids, carbohydrates and proteins) hydrolysis occurring at pH 6.8. Increased concentration of soluble organics during acidification has been previously noted (Eastman & Ferguson, 1981).

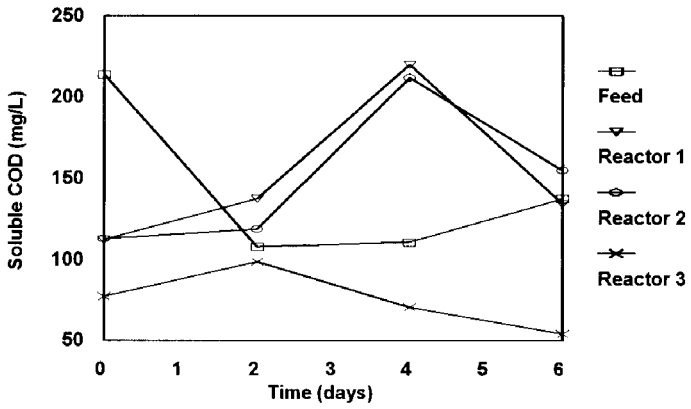


Figure 3.4 Production of soluble COD in anaerobic reactors without pH control

The hydrolysis of solids reported in figure 3.5 shows the maximum total solid degradation of 60% in R2 at pH 6.8, and 40% in R1 at pH 7.2, with no degradation observed in R3. This could suggest the presence of small particles hydrolysed at a faster rate in R1 and R2 than the large particles with low-surface-to volume ratios hydrolysed at a slower rate in R3 (Eastman & Ferguson, 1981).

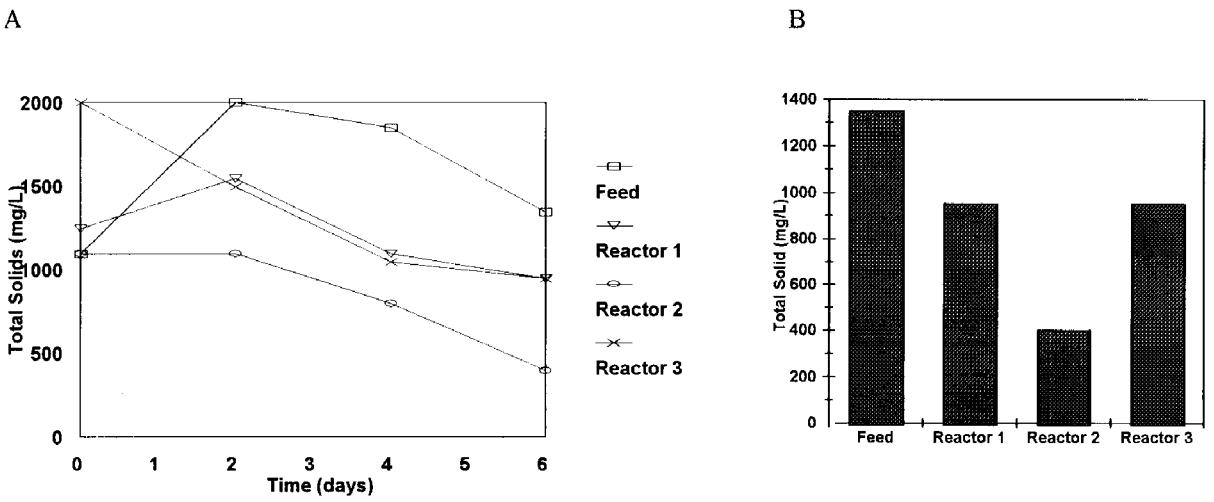


Figure 3.5 Total solids content in anaerobic reactors during (A) steady state and (B) on day six without pH control

The production of total dissolved solids (soluble solids) in anaerobic reactors reported in figure 3.6 shows induced production of soluble solids similar to that reported on COD solubilization.

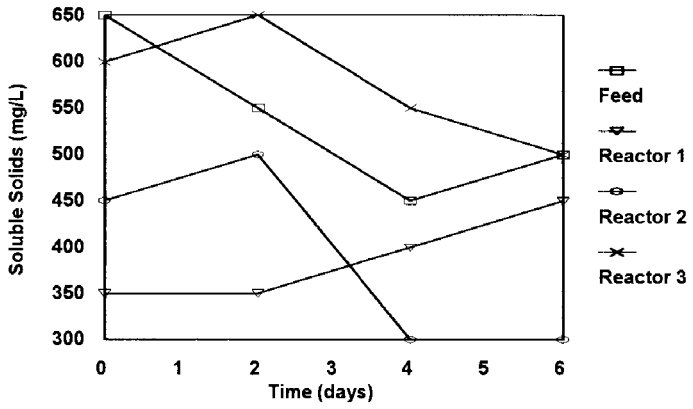


Figure 3.6 Production of soluble solids in anaerobic reactors without pH control

The result pertaining to the reduction of total suspended solids (TSS) is shown in figure 3.7. In all the reactors (R1, R2 and R3) the concentration of TSS decreased for a period of 6 days irrespective of pH changes. This could be expected as the result of reduction of solids observed in these reactors, indicating hydrolysis.

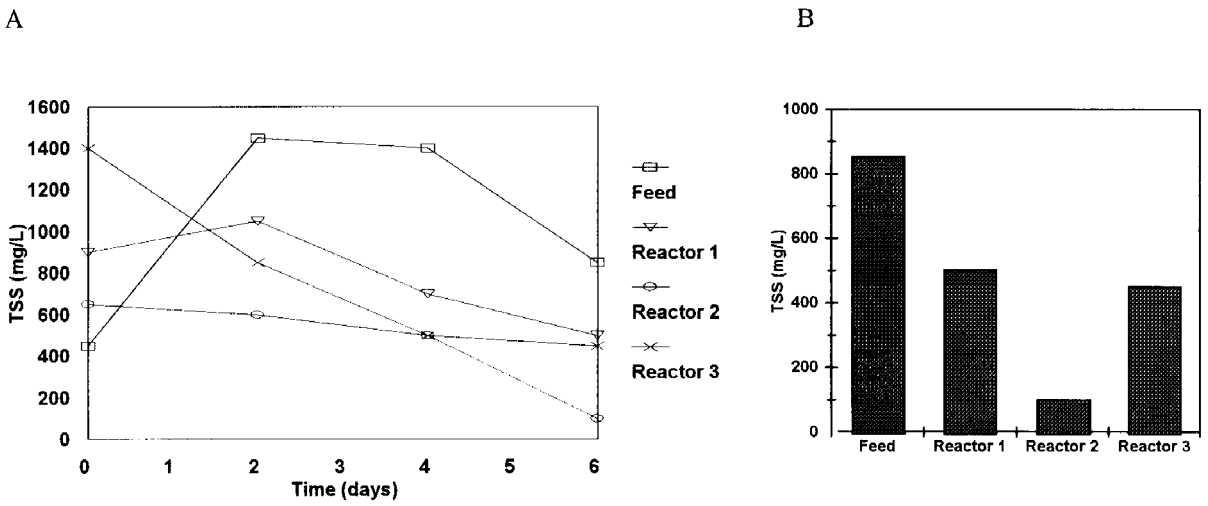


Figure 3.7 Total Suspended Solids in anaerobic reactor during (A) steady state operation and (B) on day six without pH control

The profiles of carbohydrate degradation is shown in figure 3.8. During start-up operation, the concentration of carbohydrates was reduced from 300 to 217mg/L (27%) in R1 and to 180mg/L (15%) in R2. The degradation efficiency increased to 45% by day 6 in R1 and R2 at pH 6.8. However, no carbohydrate degradation was observed in R3 due to accumulation of unsolubilized particulate matter.

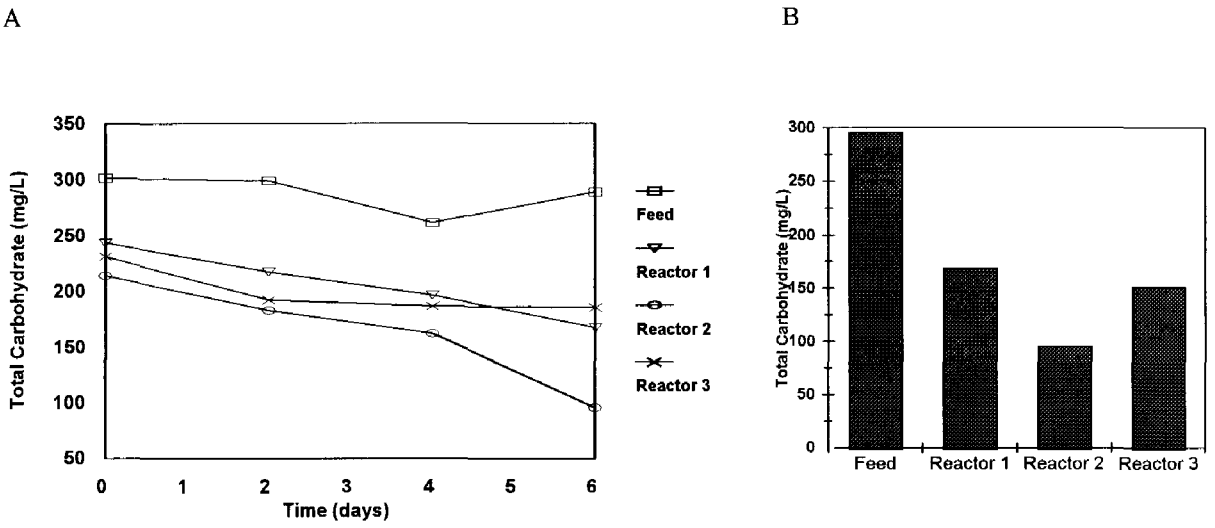


Figure 3.8 Carbohydrates degradation in anaerobic reactors during (A) steady state operation and (B) on day six without pH control

The results of protein degradation shown in figure 3.9 shows a similar pattern to that reported in figure 3.8 on carbohydrate degradation in anaerobic reactors. The concentration of proteins was reduced from 50 to 35mg/L in R1 and R2 representing the maximum degradation efficiencies of 30% by day 6 at pH 6.8. The extensive degradation of both carbohydrates and proteins observed with acid production indicated the fermentation of these substrates during the acid phase.

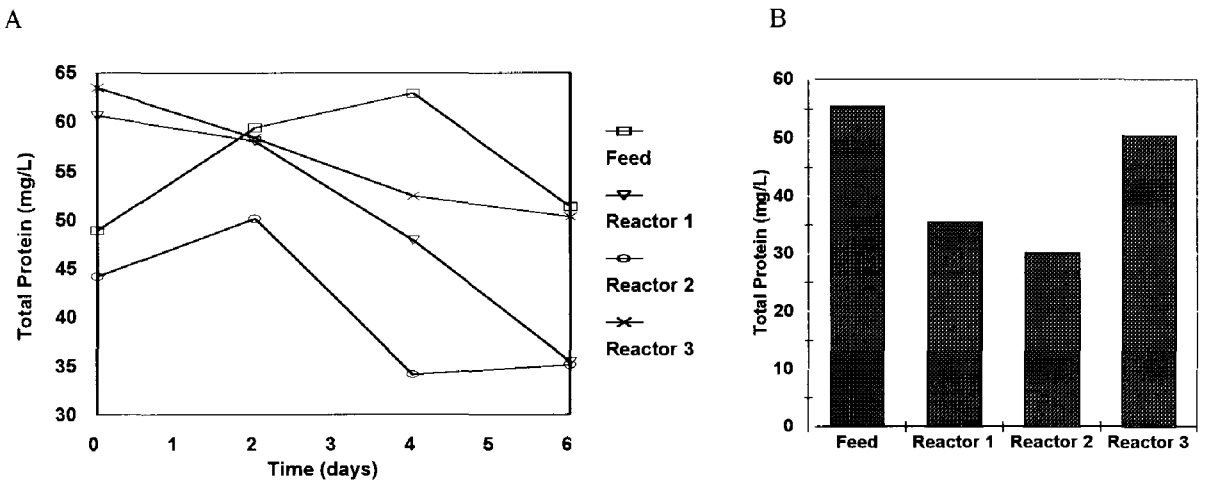


Figure 3.9 Protein degradation in anaerobic reactors during (A) steady state operation and (B) on day six without pH control

The degradation of lipids is shown in figure 3.10. Initially the reduction in concentration of lipids from 1 200 to 400mg/L (65%) was observed at pH 7.3. The pH decrease to 6.8 resulted in a decrease in reduction efficiency to 15% in R1. In R2, lipid concentration was reduced from 400 to 300mg/L (25%). Hydrolysis of lipids was observed in R3, where only about 6% of the lipids were degraded on day 6. These results indicate that acid production could have affected the initial step of lipid hydrolysis. Hanaki *et al.*, (1987) also showed that lipids were scarcely degraded at pH 6.0.

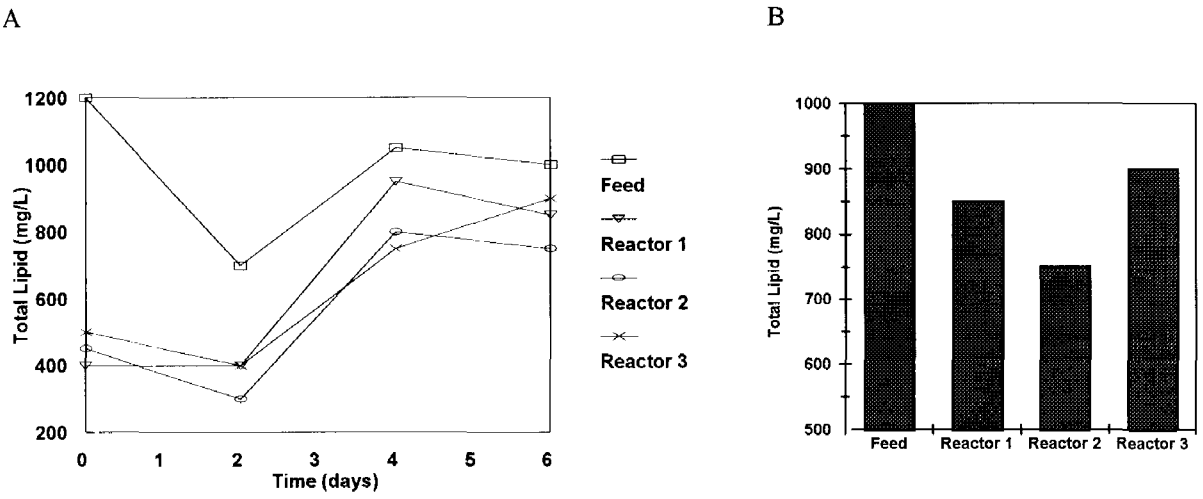


Figure 3.10 Lipid degradation in anaerobic reactor during (A) steady state operation and (B) on day six without pH control

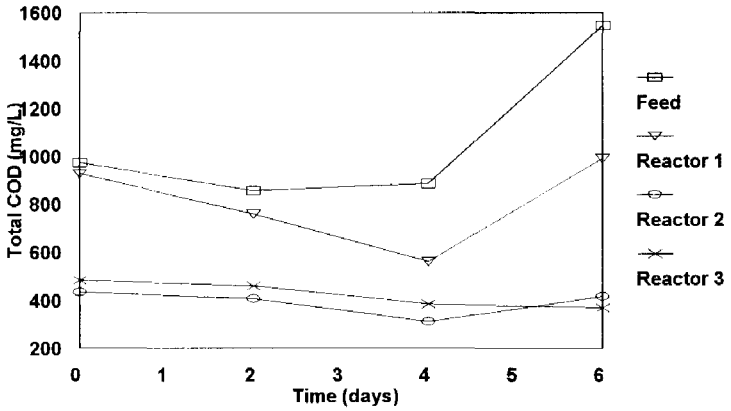
These results suggest that in order to maximize substrate degradation and organic carbon solublilization during hydrolysis, the pH of the system must be controlled and/or optimized.

3.3.2 The Effect of pH on Sewage Sludge Hydrolysis

The degradation experiments were operated with pH control at pH 6.5, 7.5, and 8.5 to determine the effect of pH on the reactions of hydrolysis. The COD reduction in anaerobic reactors reported in figure 3.11, 3.12 and 3.13 show improved reduction over time. At pH 6.5, COD content was reduced from1 500 to 995mg/L and to 420mg/L in R2 representing the maximum reduction efficiencies of 35 and 60% by day six respectively.

However, only 10% of COD was reduced in R3 as a result of unsolubilized organic particulate accumulation.

A



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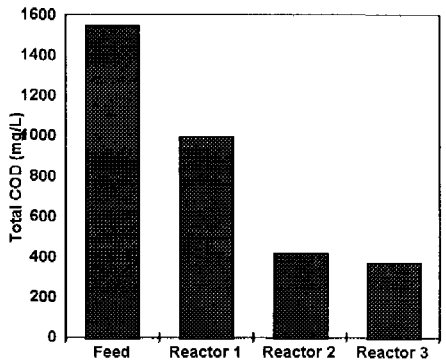
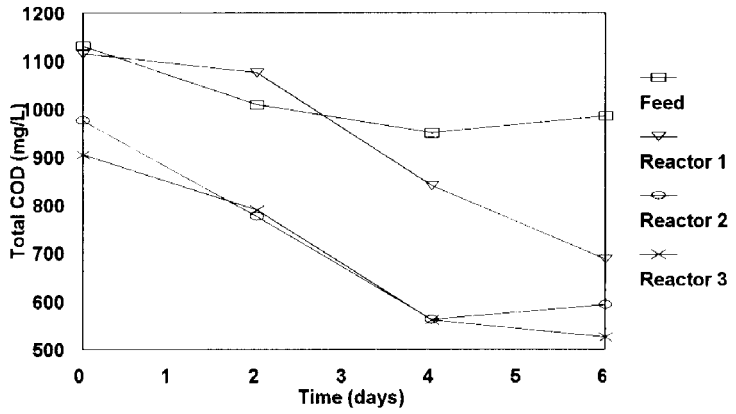


Figure 3.11 Total COD reduction in anaerobic reactors during (A) steady state operation and (B) on day six at pH 6.5

Increasing the pH of the reactors to 7.5 and 8.5, lowered the COD reduction efficiencies to a maximum of 30% in R1 and R2. Although the COD reductions were lowered at these pH values, the COD reduction of 17% was obtained in R3 at pH 8.5.The high COD reduction efficiency obtained at pH 6.5 compared to pH 7.5 and 8.5 as shown in figure 3.14, is an indication of induced hydrolysis at pH 6.5.

A



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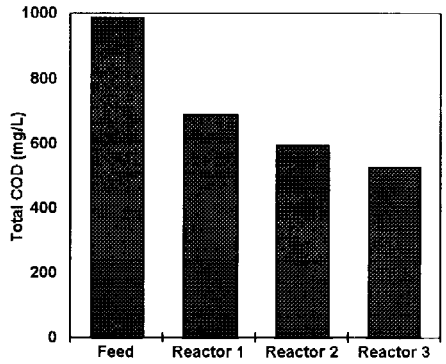
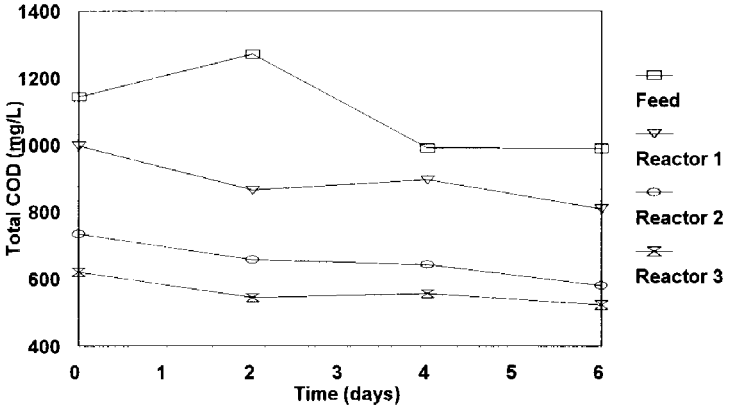


Figure 3.12 Total COD reduction in anaerobic reactors during (A) steady state operation and (B) on day six at pH 7.5

A



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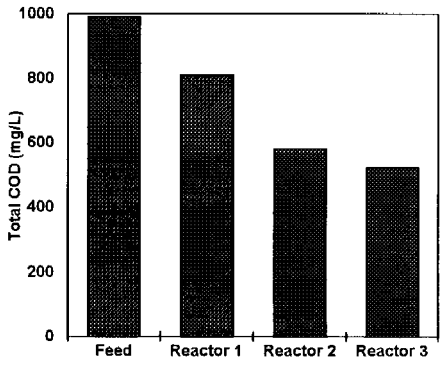


Figure 3.13 Total COD reduction in anaerobic reactors during (A)steady state operation and (B) on day six at pH 8.5

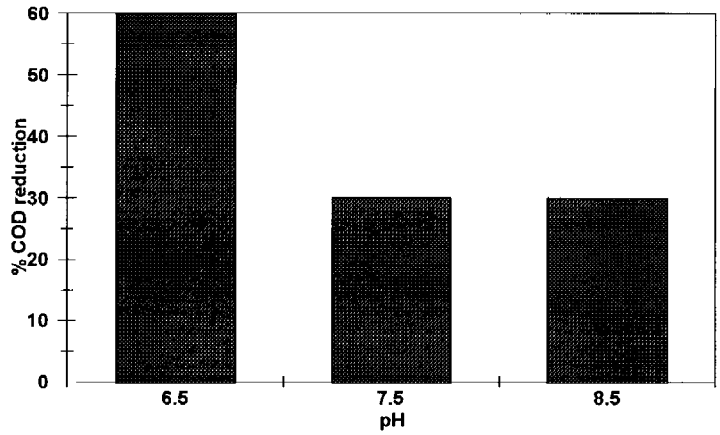


Figure 3.14 Comparison of COD reduction efficiency at steady state as a function of pH during hydrolysis of sewage sludge

The results pertaining to COD solubilization at pH values of 6.5, 7.5 and 8.5 are shown in figure 3.15, 3.16, and 3.17 respectively. At pH 6.5, the increase in soluble COD content was observed from day 2 to day 6. This could be attributed to favoured hydrolysis of organic particulates at this pH.

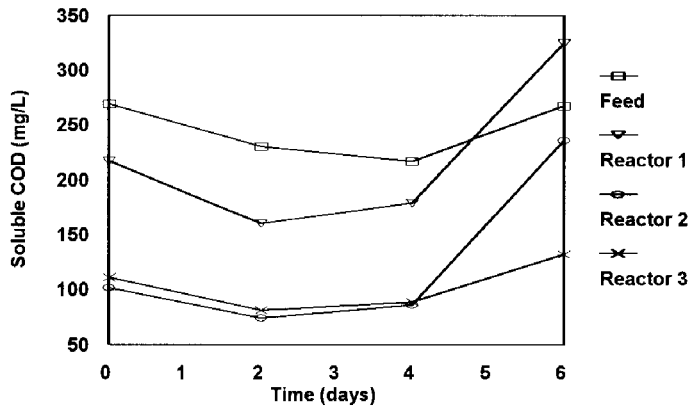


Figure 3.15 Production of soluble COD in anaerobic reactors at pH 6.5

The production of soluble COD at pH 7.5 and 8.5 did not show a linear pattern in all reactors as expected, probably due to lower COD reduction efficiencies obtained and/or low hydrolysis rate at these pH values.

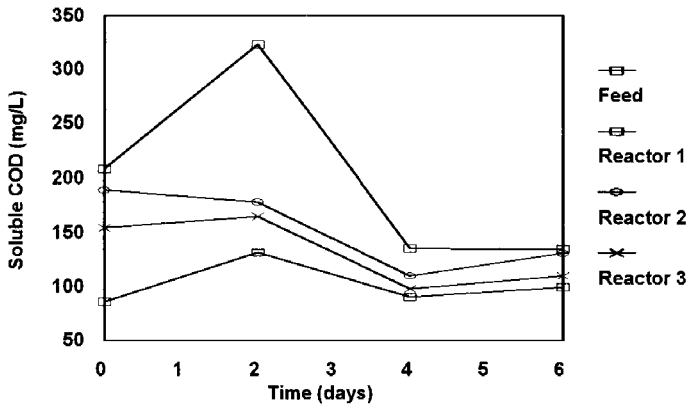


Figure 3.16 Production of soluble COD in anaerobic reactors at pH 7.5

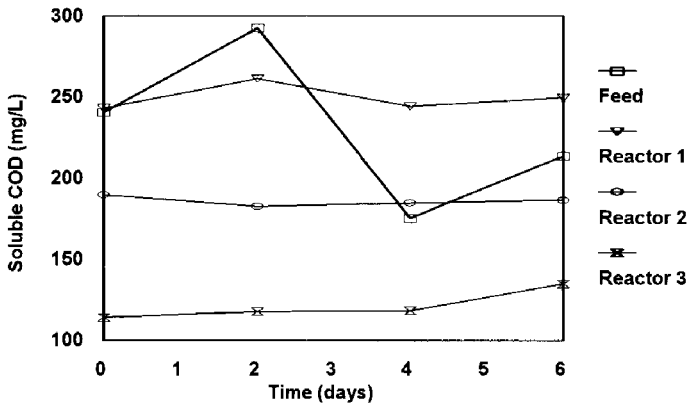
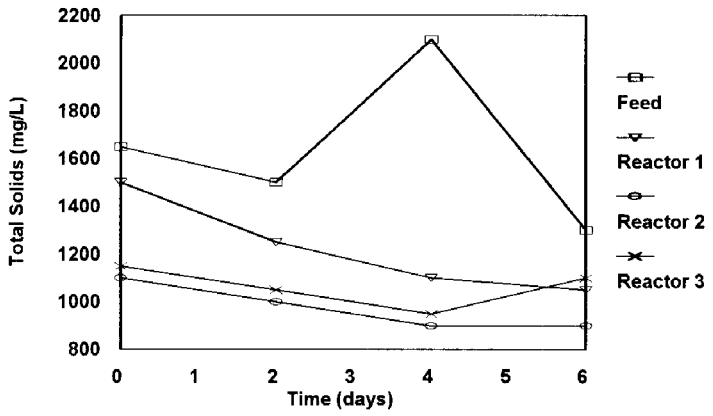


Figure 3.17 Production of soluble COD in anaerobic reactors at pH 8.5

The profiles of solids reduction in anaerobic reactors is shown in figure 3.18, 3.19, and 3.20.At pH 6.5, the concentration of solids was reduced from 2 100 to 1 100mg/L (50%) in R1 and to 900mg/L in R2 (20%) by day 6. When the pH was increased to 7.5, the solid reduction efficiency increased to 60% in R2. Due to accumulation of unsolubilized organic matter in R3, solid reduction would not be expected in R3.

A



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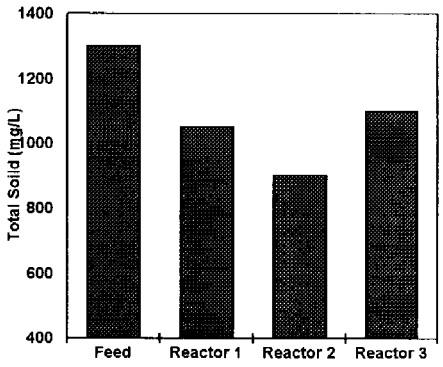


Figure 3.18 Reduction of solids in anaerobic reactors during (A) steady state operation and (B) on day six at pH 6.5

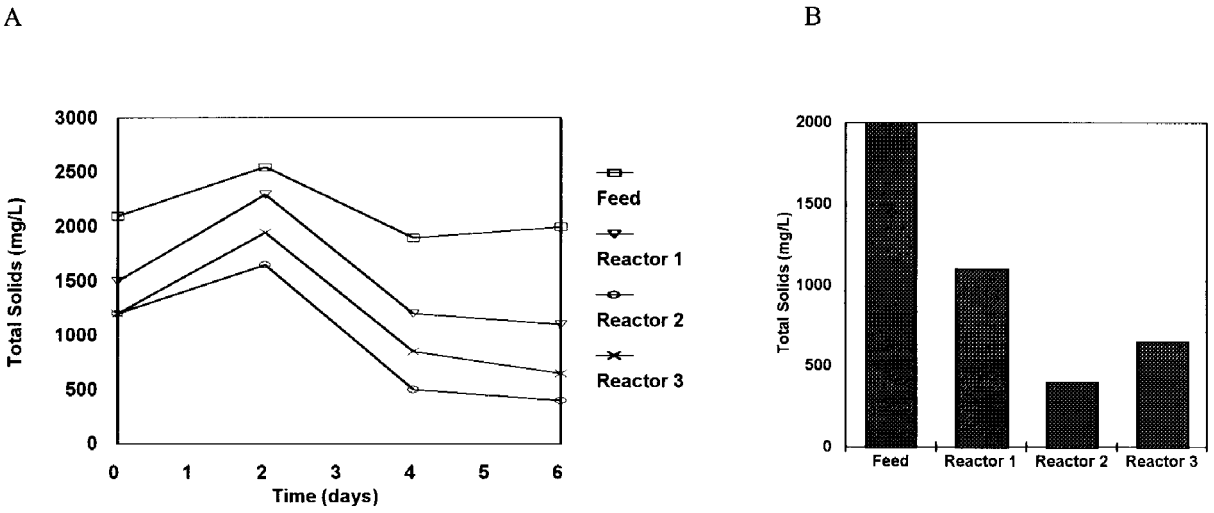


Figure 3.19 Reduction of solids in anaerobic reactors during (A) steady state operation and (B) on day six at pH 7.5

At pH 8.5, solid reduction efficiency in R1 was similar to that reported at pH 7.5, and decreased slightly to 55% in R2. Furthermore, reduction of solids from 1 600 to 1 350 (20%) was observed in R3 on day 4.

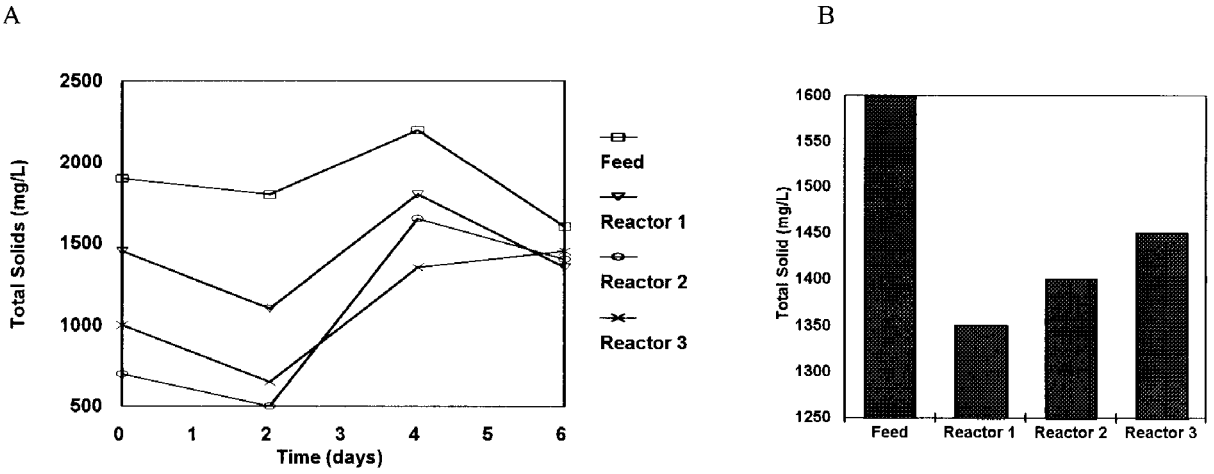


Figure 3.20 Reduction of solids in anaerobic reactor during (A) steady state operation and (B) on day six at pH 8.5

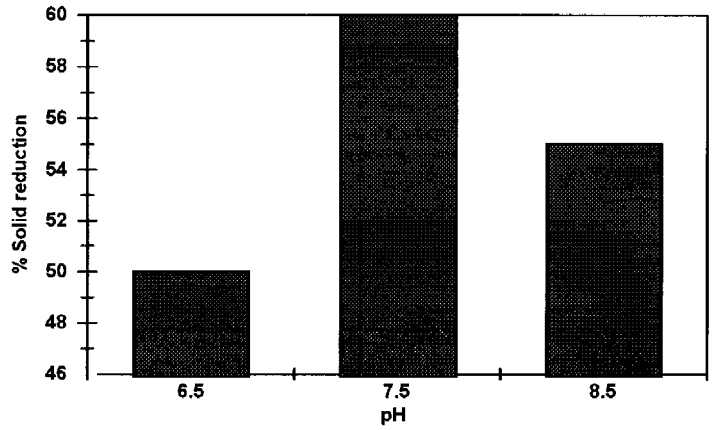


Figure 3.21 Comparison of solid reduction efficiency at steady state as a function of pH during hydrolysis of sewage sludge.

A linear trend in production of soluble solids was obtained at pH 6.5 in anaerobic reactors as shown in figure 3.22.

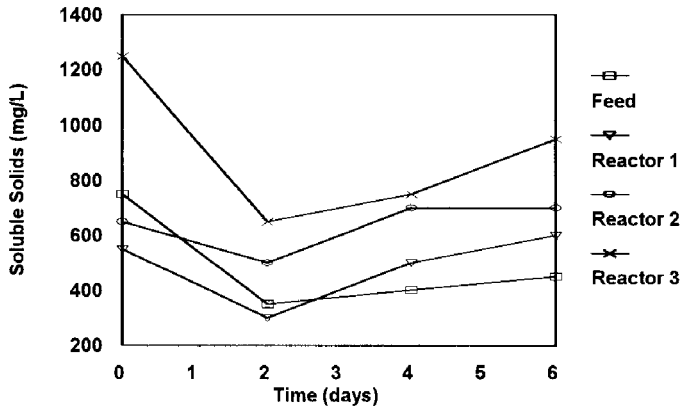


Figure 3.22 Production of soluble solids in anaerobic reactors at pH 6.5

The production of total dissolved solids (soluble solids) did not show a linear pattern in all reactors at higher pH values reported in figure 3.23, and 3.24. Since the rate of hydrolysis is dependent on the physical characteristics of particles, increased solubilization of solids at pH 6.5 could be due to the presence of non-settleable particles, which were relatively smaller and solubilized faster than those at pH 7.5 and 8.5.

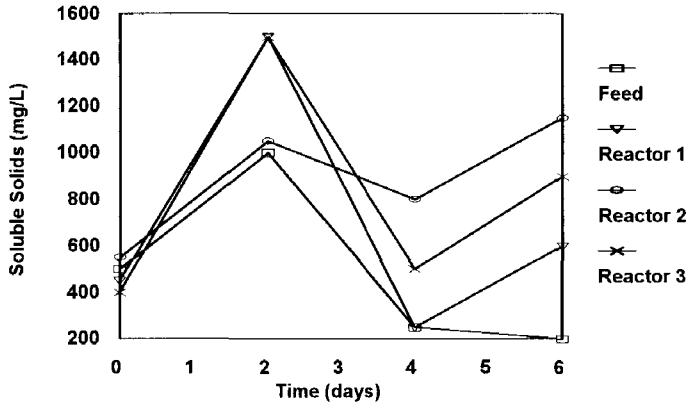


Figure 3.23 Production of soluble solids in anaerobic reactors at pH 7.5

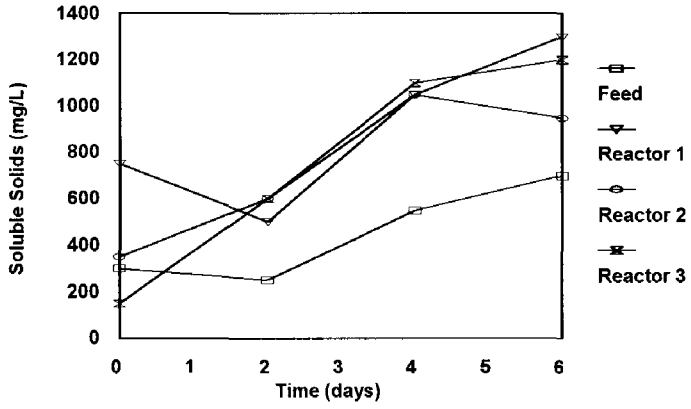


Figure 3.24 Production of soluble of solids in anaerobic reactors at pH 8.5

The reduction of total suspended solids at pH 6.5 reported in figure 3.25 shows the decrease in TSS from day 2 to 6 in R1, and only to day 4 in R2 and R3. In R1, the concentration of TSS was reduced from 1 700 to 600mg/L and to 200mg/L in R2 by day 4. This represents the maximum reduction efficiency of 65% in these reactors. However, in R3 the concentration of TSS was reduced from 200 to 150mg/L by day 6 representing the reduction efficiency of 25%.

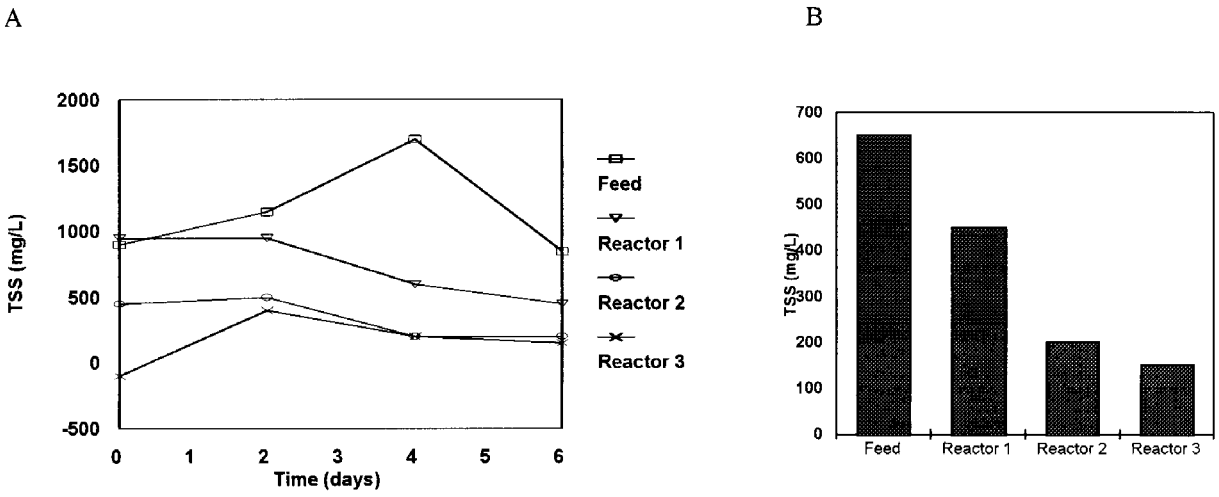


Figure 3.25 Reduction of total suspended solids (TSS) in anaerobic reactors during (A) steady state operation and (B) on day six at pH 6.5

At pH to 7.5 and 8.5, the reduction efficiency of TSS decreased by 20% in R1 and 40 and 50% in R2 and R3 respectively. However, this could be expected as a result of lower solid reduction and solubilization reported at these pH values.

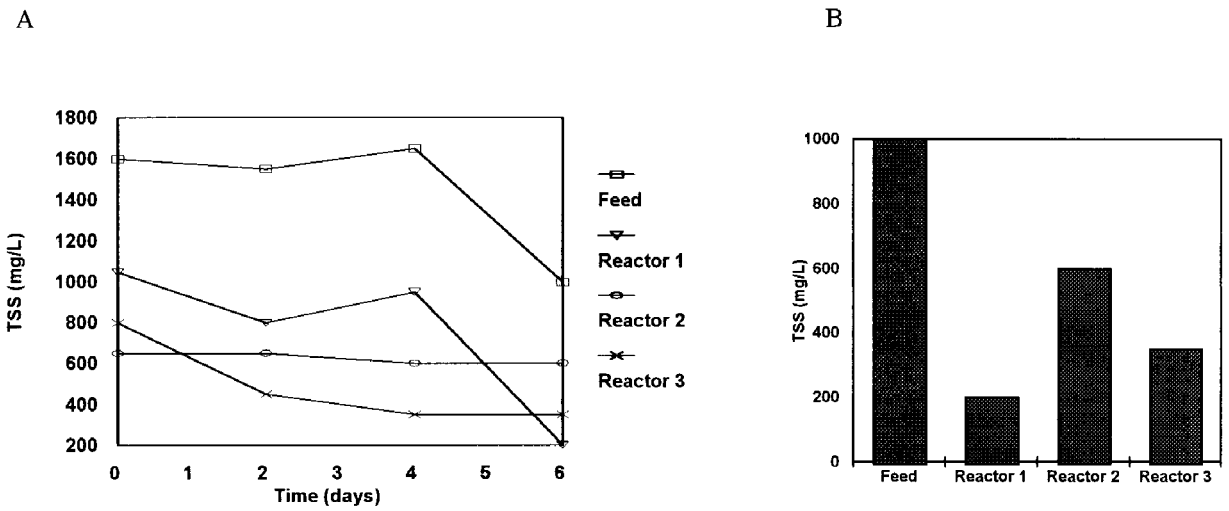


Figure 3.26 Reduction of total suspended solids (TSS) in anaerobic reactors during (A) steady state operation and (B) on day six at pH 7.5

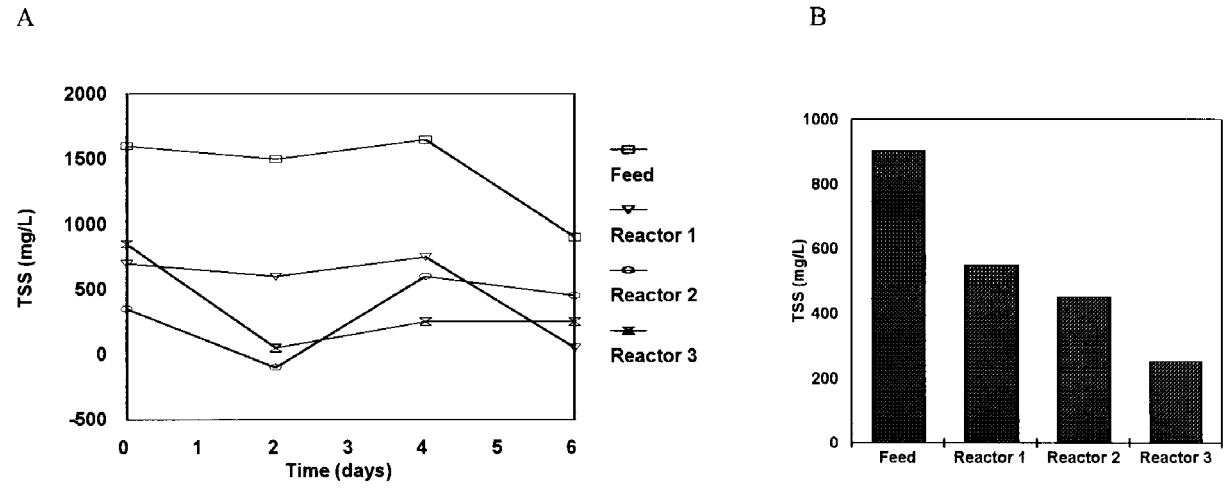


Figure 3.27 Reduction of total suspended solids (TSS) in anaerobic reactors during (A) steady state operation and (B) on day six at pH 8.5

The profiles of carbohydrates degradation at pH values of 6.5, 7.5 and 8.5 are shown in figure 3.28, 3.29 and 3.30 respectively. At pH 6.5, the concentration of carbohydrate in R1 decreased over time for a period of six days, while in R2 and R3 decreased for a period of four days. During this period carbohydrate concentration was reduced from 203 to 95mg/L (50%) in R1 and from 115 to 35mg/L (65%) in R2. However, the degradation was not greatly enhanced in R3, only 2% of carbohydrates was degraded. These results could suggest that the growth of bacterial population responsible for carbohydrates degradation was favoured and the release of extracellular enzymes was induced at pH 6.5.

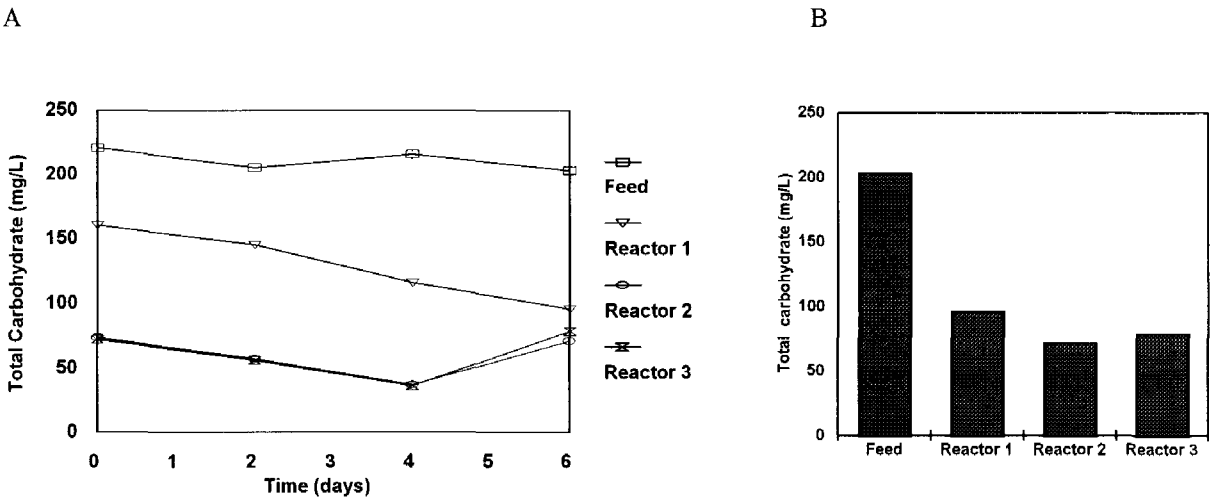
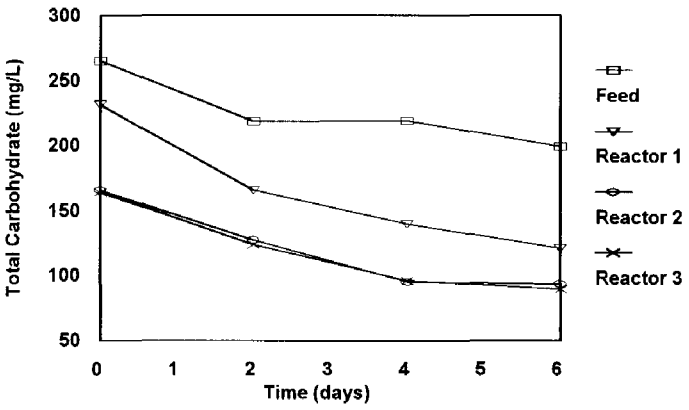


Figure 3.28 Carbohydrate degradation in anaerobic reactors during (A) steady state operation and (B) on day six at pH 6.5

When the pH was increased to 7.5, the concentration of carbohydrates was reduced from 200 to 121mg/L on day 6 in R1 and 140 to 95mg/L on day 4 in R2. These results show the decrease in degradation efficiency to a maximum value of 40 and 30% in these reactors.

A



B

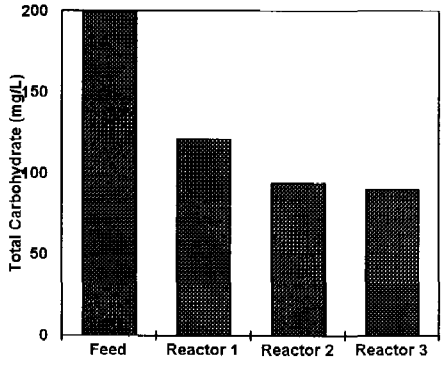


Figure 3.29 Carbohydrate degradation in anaerobic reactors during (A) steady state operation and (B) on day six at pH 7.5

A further increase in pH to 8.5 resulted in degradation efficiencies of 50% in R1 and 40% in R2, and the degradation of 30% was obtained in R3. Eastman & Ferguson (1981) reported the maximum hydrolysis rate of complex carbohydrate at pH value between 6.0 and 6.5 . This could suggest that the activity of the enzymes involved increases at pH 6.5 other than other pH values tested.

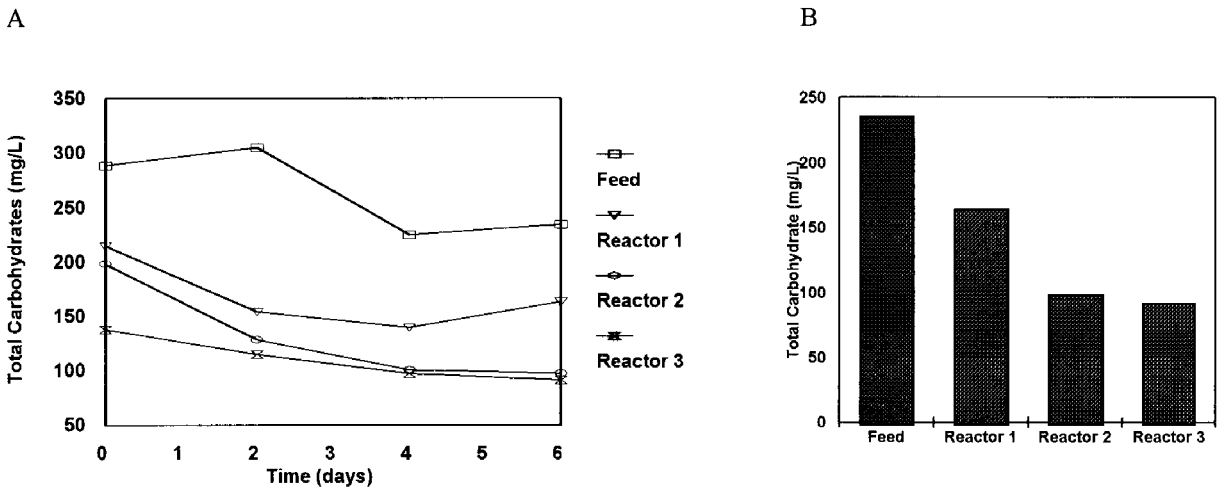


Figure 3.30 Carbohydrate degradation in anaerobic reactors during (A) steady state operation and (B) on day six at pH 8.5

The high carbohydrate degradation efficiency at pH 6.5 compared to pH 7.5 and 8.5 as illustrated below in figure 3.31 indicates that acid generation at pH 6.5 induced carbohydrate fermentation during hydrolysis.

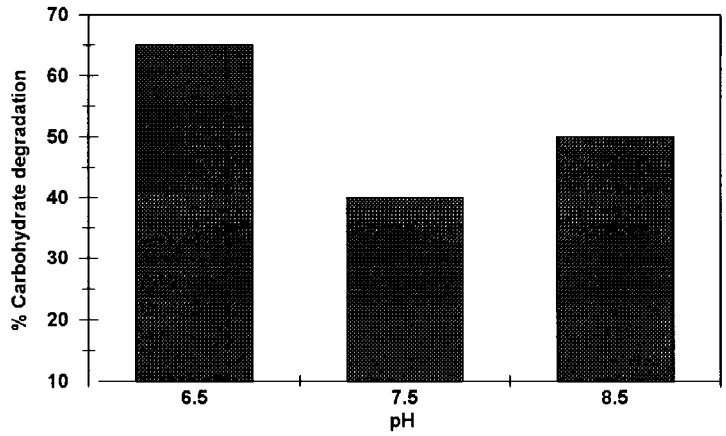


Figure 3.31 Comparison of carbohydrate degradation efficiency at steady state as a function of pH during hydrolysis of sewage sludge

The results pertaining to protein degradation are shown in figure 3.32, 3.33, and 3.34 at pH values of 6.5, 7.5, and 8.5 respectively. The maximum degradation efficiency of 40% was observed at pH 6.5 in R1 and R2.

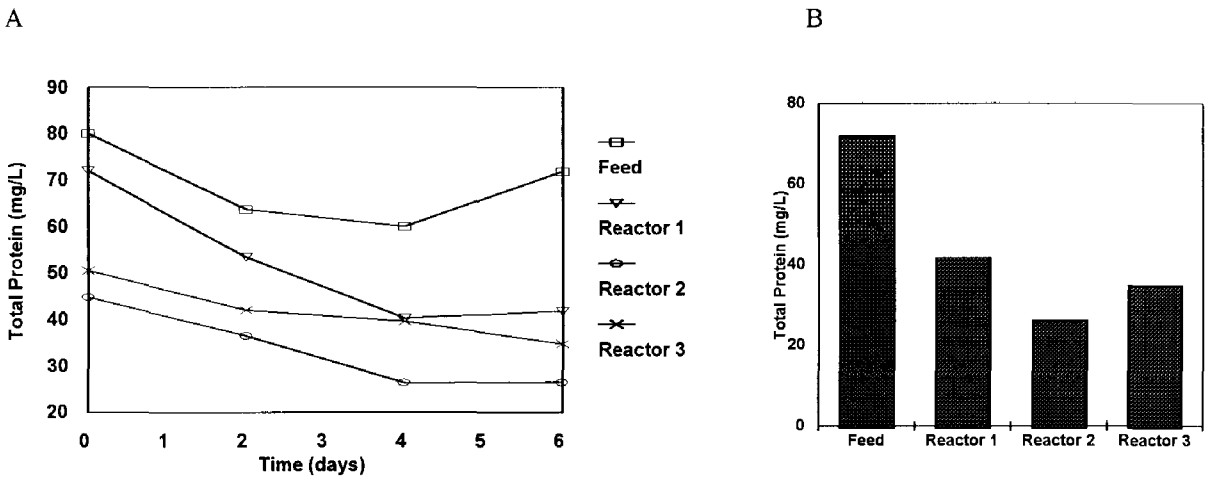
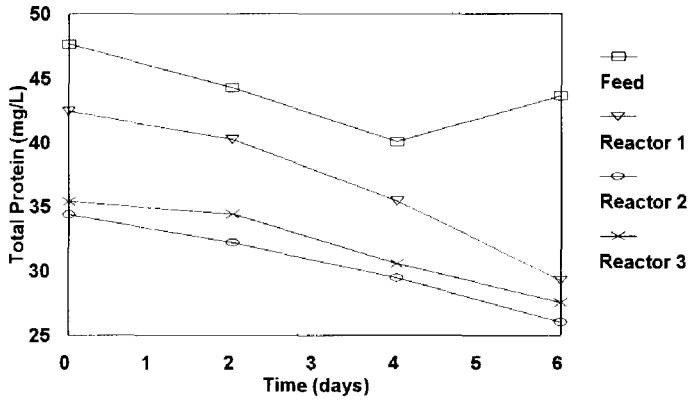


Figure 3.32 Protein degradation in anaerobic reactors during (A) steady state operation and (B) on day six at pH 6.5

An increase in pH value to 7.5 and 8.5 did not increase the rate of protein degradation. At pH 7.5 the concentration of proteins was reduced from 45 to 30mg/L (30%) in R1 by day 6. While in R2 only 10 % of protein was degraded by day 6.

A



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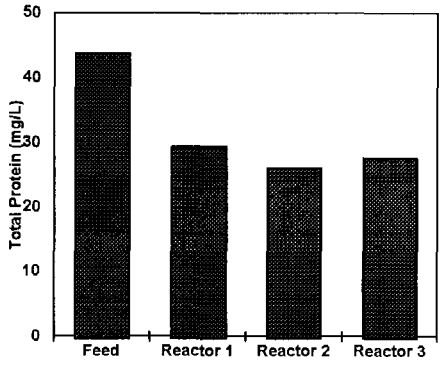
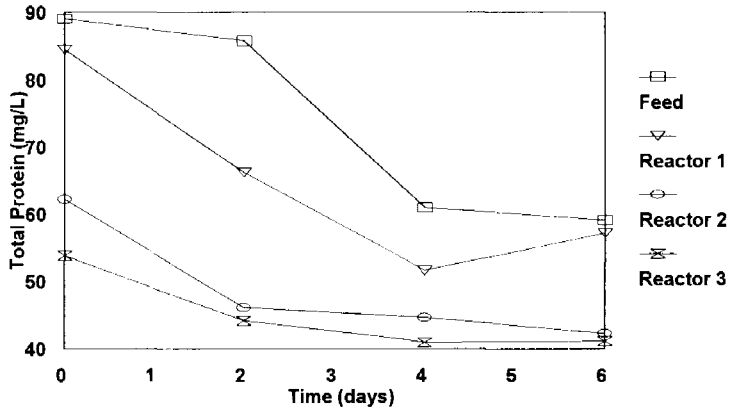


Figure 3.33 Protein degradation in anaerobic reactors during (A) steady state operation and (B) on day six at pH 7.5

A further increase in pH to 8.5 resulted in degradation efficiencies of 20 and 30 % in R1 and R3 on day 2. At pH 6.5 and 7.5 no protein degradation was observed in R3 whereas at pH 8.5 about 30% protein was degraded. Although many proteolytic organisms prefer a neutral pH environment, proteolytic enzymes may exhibit their maximum activity at different pH values ranging from 2 to 10 (Bailey & Ollis 1977).

A



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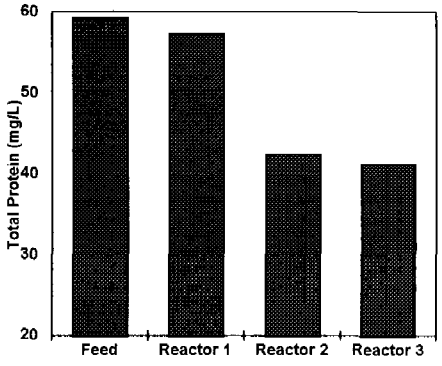


Figure 3.34 Protein degradation in anaerobic reactors during (A) steady state operation and (B) on day six at pH 8.5

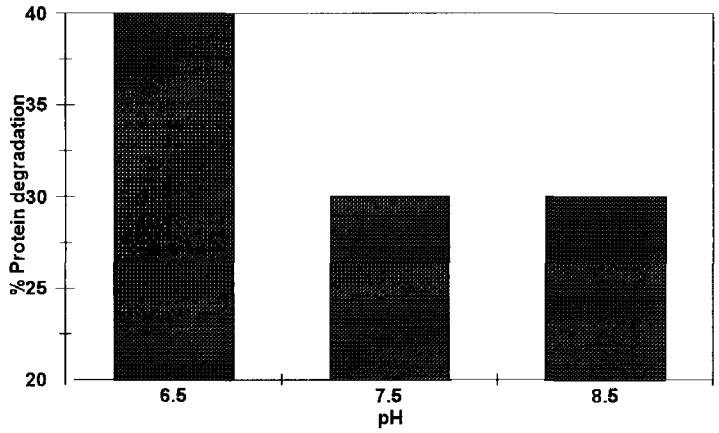
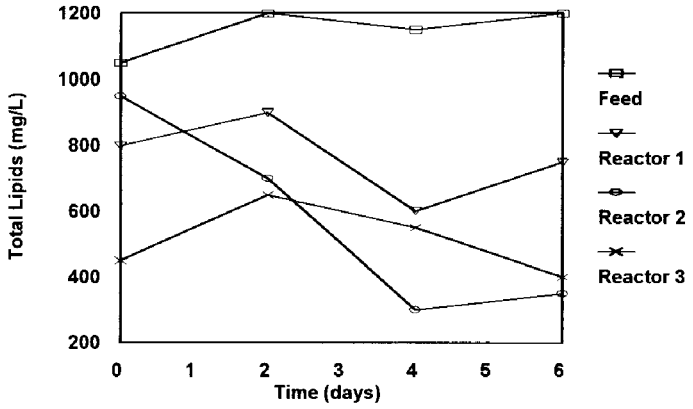


Figure 3.35 Comparison of protein degradation efficiency at steady state as a function of pH during hydrolysis of sewage sludge

The degradation profiles of lipids at pH values of 6.5, 7.5 and 8.5 are shown in figure 3.36, 3.37, and 3.38 respectively. At pH 6.5 the concentration of lipids was reduced from 1 150 to 600mg/L in R1 and to 300mg/L in R2 representing the maximum degradation of 50% by day 6. An increase in pH to 7.5 increased the rate of degradation to 60% and 70% in R1 and R2 respectively.

A



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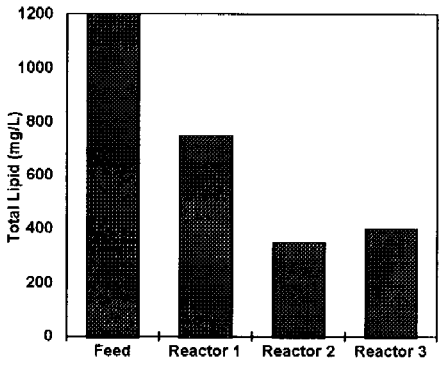
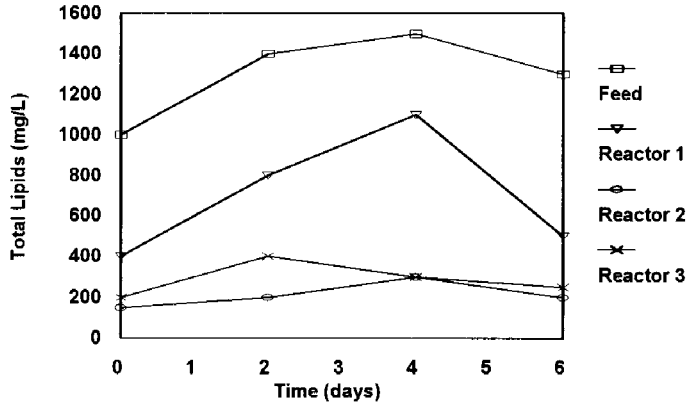


Figure 3.36 Lipid degradation in anaerobic reactors during (A) steady state operation and (B) on day six at pH 6.5

A



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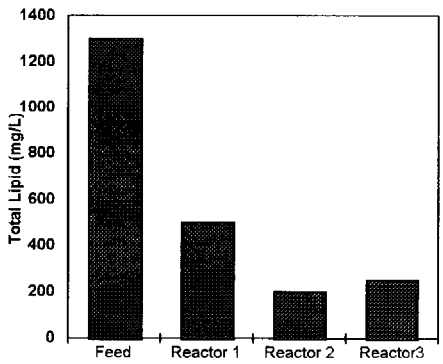


Figure 3.37 Lipid degradation in anaerobic reactors during (A) steady state operation and (B) on day six at pH 7.5

When the pH was increased further to 8.5, degradation dropped to 45%. In all reactors no lipid degradation was observed. These results indicate that pH 7.5 was the optimal value for lipids hydrolysis in this study, which suggest that pH 6.5 and 8.5 did not induce the release of lipolytic enzymes. Elefsiniotis & Oldham (1994) reported reduced lipolytic activity at pH 6.1, and lower lipid degradation rate at less than 6 have been previously reported (Hanaki *et al.*, 1987).

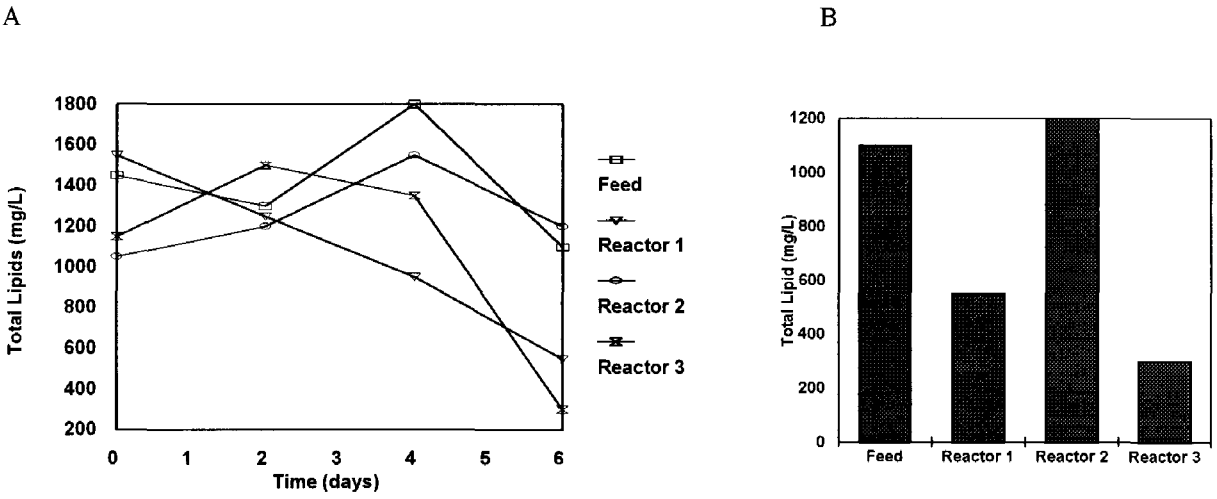


Figure 3.38 Lipid degradation in anaerobic reactors during (A) steady state operation and (B) on day six at pH 8.5

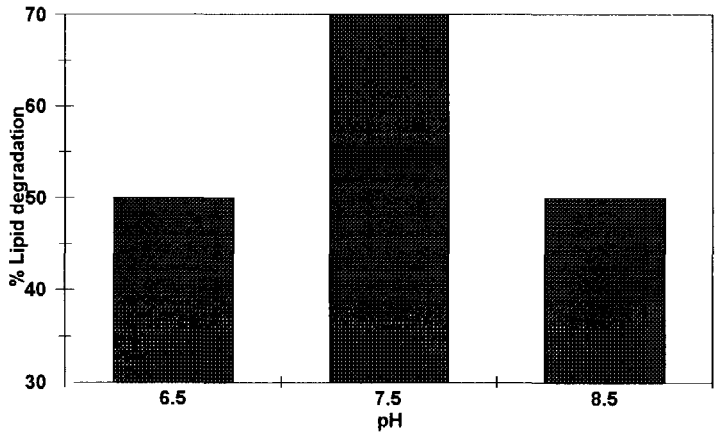


Figure 3.39 Comparison of lipid degradation efficiency at steady state as a function of pH during hydrolysis of sewage sludge

3.4 CONCLUSION

During hydrolysis of sewage sludge without pH control, the pH in anaerobic reactors decreased over time as a result of acid production due to fermentation products of carbohydrate , protein and lipid metabolism. This indicated progressive hydrolysis of complex organic particulate from sewage sludge. The production of soluble organic particulates did not show a linear trend due to pH changes that could have interfered with the activity of hydrolytic enzymes since they are pH dependent. Substrate degradation as a function of pH resulted in extensive lipids degradation (66%) at neutral pH, while carbohydrates (43%) and protein were extensively degraded at more acidic pH values.

Since hydrolysis is the limiting step of the overall anaerobic digestion process, it was, therefore, clear that the optimization of the operational conditions (i.e pH of hydrolysis) was necessary in order to optimize COD solubilization and the degradation of particulate organic matter. Studies on sewage sludge hydrolysis with pH control showed that the process of hydrolysis is pH dependent. During this study, the COD content of the effluent was reduced to 60% at pH 6.5, while at pH 7.5 and 8.5 only a 30% COD reduction occurred. This indicated the more effective degradation of organic matter at pH 6.5 other than at other pH values.

The production of soluble organic carbon and soluble solids was greatly enhanced at pH 6.5 other than pH 7.5 and 8.5. This can be attributed to the higher efficiencies of carbohydrate and protein degradation and probably the fermentation products from metabolism of these substrate at pH 6.5 compared to other pH values tested. Furthermore, major differences were found in the extent to which particulate fractions were hydrolysed. The degradation of each component followed an individual behaviour pattern with pH changes. Lipids were extensively degraded (70%) at pH 7.5, carbohydrates (65%) and proteins (53%) at pH 6.5. These results clearly showed that the activity of hydrolytic enzymes and the growth of bacteria carrying out the process of hydrolysis were pH dependent. However, this could also suggest that the favourable conditions for the bacterial growth must be provided because hydrolysis is accomplished with enzymes released by the organisms

In general, differences in the extent to which organic particulates were hydrolysed were found in each of the anaerobic reactors (R1, R2, and R3) during both studies with and without pH control. Hydrolysis was greatly enhanced in R1 and R2 but not in R3. This indicated the accumulation of unsolublilized organic matter in R3 and the feasibility of a two stage anaerobic digestion of organic particulates from sewage sludge.

It therefore appears that the hydrolysis of sewage sludge can potentially be optimized. This could in turn have a considerable benefit for its use as a hydrolysed carbon source for sulphate reduction.

CHAPTER FOUR

ENZYME ACTIVITY IN SEWAGE SLUDGE HYDROLYSIS

4.1 INTRODUCTION

In the first step of sewage sludge hydrolysis process, complex organic compounds, mainly carbohydrates, proteins and lipids are broken down into simpler low-molecular weight compounds in the presence of extracellular enzymes (Novaes,1986). Therefore it would be expected that hydrolysed sludge would have a characteristic enzyme profile reflecting the relative activity of enzymes in the extracellular hydrolysis (Nybroe *et al.*, 1992).

The extracellular enzymes in activated sludges originate from the influent sewage or from the sludge itself by cell autolysis or as actively excreted exoenzymes (Frolund *et al.*, 1995). The enzymes are either free in solution or associated with the cell surface of the producer or adsorbed on surfaces other than that of their producer (Chrost, 1991). Several different types of enzymatic activities have been reported in activated sludge (Nybroe *et al.*, 1992; Boczar *et al.*, 1992). These are enzymes responsible for hydrolysis of proteins (aminopeptidases, proteases and esterases), lipids (lipases, esterases), nucleic acids (phosphatases) and carbohydrates (glucosyl hydrolases).

The enzyme activity and isolation of specific enzymes have been used to characterize and identify metabolic activities of bacterial populations and to have been related to waste removal efficiency (Richards *et al.*, 1984; Teuber & Brodish, 1977; Lotter & Van der Merwe, 1987). In addition to the use of enzymes in hydrolysis of organic waste, enzyme activities have received particular attention for a number of other reasons. Characterization of enzyme activities can provide insight into biochemical factors controlling the treatability of xenobiotic chemicals, patterns of enzyme activities can be useful in identifying microbial populations, they can be used as a monitor of active biomass, and as an indicator of specific biological processes such as COD and phosphorus removal. Enzymes can also be added exogenously to treatment processes to improve overall treatment efficiencies (Boczar *et al.*, 1992).

However, due to the complexity of the microbial systems in sludge digestion, and the lack of reliable rapid quantitative enzyme assay methodologies, there is little information on the effect of pH on concentrations, activities and specifities of enzymes in sewage sludge. The objective of this study was to characterize and compare the effect of pH on the profiles of hydrolytic enzymes in sewage sludge using the semiquantitative API-ZYM^{TN} system.

The API-ZYM^{TN} system provides a rapid, semiquantitative colorimetric assay and relative activity of specific hydrolytic enzymes including phosphateses, esterases, lipases, proteases, and glucosyl hydrolases. This system has been used to examine enzymes of anaerobes (Tharagonnet, 1977), and for the determination of extracellular cell-associated hydrolases of *Pseudomonas flourenscens* species (McKeller, 1986).

4.2 MATERIALS AND METHODS

4.2.1 Experimental Design and Operational Procedures

The same laboratory experimental apparatus, operational procedures, feed source used in Chapter 3 were used in this study. Enzyme activity at pH 6.5, 7.5 and 8.5 was compared to evaluate the effect of pH on hydrolytic enzymes from sewage sludge.

4.2.2 Determination of Enzyme Profiles

The API-ZYM^{TN} test kit was obtained from BioMerieux Company and used as described by the manufacturer. About 65*ul* (microliters) of samples from each reactor, i.e Feed, Reactor 1 (R1), Reactor 2 (R2) and Reactor (R3) was added to each of cupule. The test strips were incubated at 37°C for 4 hours in a humid atmosphere, which was provided by dispensing 5ml of sterile water into the incubation tray. To visualize the enzymatic activity, a drop of each reagent, ZYM A and ZYM B solutions was added to each microcupule and the reaction was allowed to develop under bright light for 7minutes. Appropriate levels of enzyme product were scored on a scale of 0 to 5 using the API-ZYM^{TN} colour chart provided by the manufacturer. A score of 1 represented 5 nanomoles (nM) of enzyme product with 2, 3, 4 and 5 representing 10, 20, 30 and 40nM, respectively. The API-ZYM^{TN} enzymes assayed are given in table 5.1.

Table 4.1 Enzymes assayed and Substrate utilized by the API-ZYM^{TN} system

API no.	Enzyme Assayed	Substrate Utilized
1	Control - Temoin	-
2	Phosphatase alkaline	2-naphthyl phosphate
3	Phosphatase acid	2-naphthyl phosphate
4	Naphthol phosphohydorylase	Naphthol-AS-BI-phosphate
5	Esterase (C4)	2-naphthyl butyrate
6	Esterase Lipase (C8)	2-naphthylcaprylate
7	Lipase (C14)	2-naphthylmyristate
8	Leucine arylamidase	L-leucyl-naphthylamide
9	Valine arylamidase	L-valyl-2-naphthylamide
10	Cystine arylamidase	L-cystyl-2-naphthylamide
11	Trypsin	N-benzoyl-DL-arinine-2-naphthylamide
12	Chymotrypsin	N-glutaryl-phenylalanine-2-naphthlamide
13	alpha-galactosidase	6-Br-2-naphthyl-alpha-D-galactopyranoside
14	beta-galactosidase	2-naphthyl-beta-D-galactopyranoside
15	beta-glucuronidase	Naphthyl-AS-BI-beta-B-glucuronide
16	alpha-glucosidase	2-naphthyl-alpha-D-glucopyranoside
17	beta-glucosidase	6-Br-2-naphthyl-beta-Dglucopyranoside
18	glucosaminidase	1-naphthyl-N-acetyl-D-glucosamine
19	alpha-mannosidase	6-Br-2-naphthyl-alpha-D-mannopyranoside
20	alpha-fucosidase	2-naphthyl-alpha-L-fucopyranoside

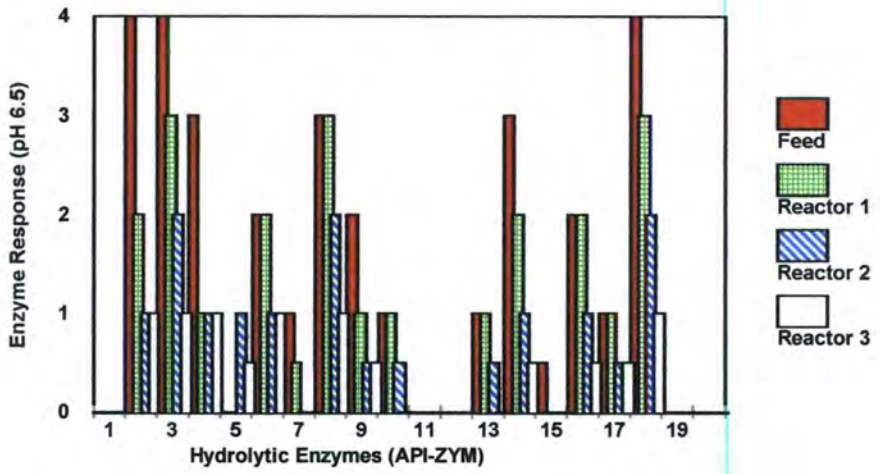
4.3 RESULTS AND DISCUSSION

4.3.1 Enzyme Characterization.

The wide range of enzymes characterized from sewage sludge reported in figure 4.1 A, B and C showed that sewage sludge contains variety of enzymes to degrade a wide range of organic matter. Since the enzyme assays were performed on non-filtered samples, it could be suggested that the enzymes assayed could include cell associated and/or particle associated (detritus or dead cells) enzymes, free enzymes and intracellular enzymes that pass through the cell membrane. Also included would be enzymes not adsorbed or bound to organic matter, enzymes accessible in dead but not lysed cells (Boczar *et al.*, 1992). Among the enzymes assayed, glucosyl hydrolases (13-20), phophatases (2-3), and aminopeptidases (8-10) were present in high concentrations at all pH values tested (figure 4.1). These results were comparable to enzymes assayed in activated sludge reported by Boczar *et al.*, (1992) indicating high concentrations of enzymes from sewage sludge capable of hydrolysing proteins and carbohydrate.

In general, the amount of enzymes scored in figure 4.1 shows that the enzymes assayed from sewage sludge are relatively higher in R1 and R2 compared to R3, indicating the availability of enzymes to substrates in R1 and R2. These results would be expected since higher degradation efficiencies were obtained in these reactors during the study of sewage sludge hydrolysis reported in Chapter 3.

A



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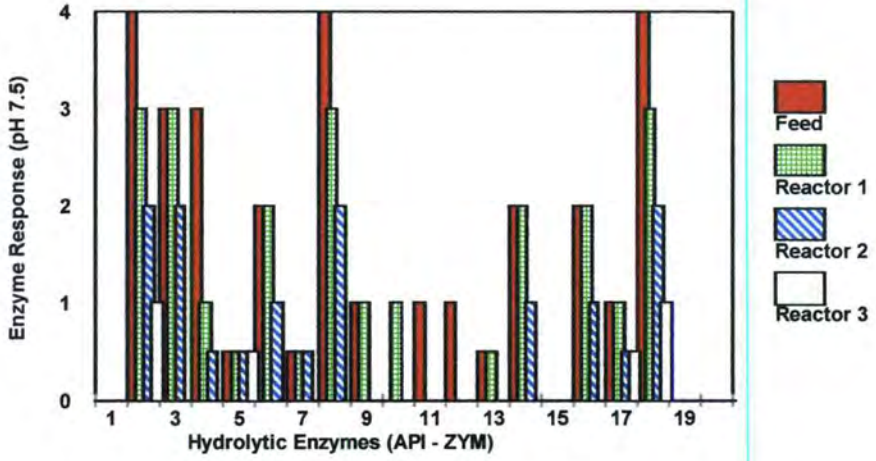

C

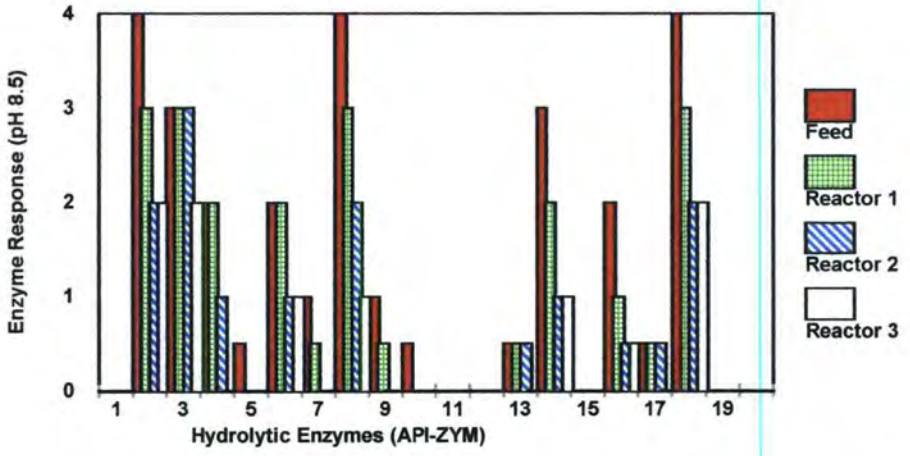

Figure 4.1 API-ZYM^{TN} enzyme profiles from sewage sludge at different pH values (A: pH 6.5, B: 7.5, C: 8.5)

1 : Control, 2 & 3 : Phosphatases, 4 : Phosphorylases, 5 & 6 : Esterases, 7 : Lipases, 8 - 10: Aminopeptidases, 11 & 12 : Proteases, 13 - 20: Glucosyl hydrolases

Among the enzymes assayed, proteases (11&12) and glucosyl hydrolases of alpha-mannosidase (19), and alpha-fucosidase (20) reflected a negative response in all anaerobic reactors and at all pH values tested. This could be attributed by the fact that the substrates catalysed by these enzymes were not present in sewage sludge. The short chain esterases (C4 - C8) exhibited higher activities than the long chain lipases (C14). Limitation of esterases activity to carbon chain-length greater than C10 by cell permeability or transport considerations has been previously noted (Boczar *et al.*, 1992).

4.3.2 The Effect of pH on Enzyme Activity.

The activity of aminopeptidases was higher than the protease activity at all pH values tested, suggesting that it was the aminopeptidases that were largely responsible for hydrolysis of proteins within the sludge samples. Figure 4.2 shows the activity of aminopeptidases as a function of pH with maximum enzyme activity at pH 6.5 in all stages of the reactors, with equal activity at pH 7.5 in R1 and R2. An increase in pH to 8.5 resulted in decreased activity of aminopeptidases in R1 and R2. These results show that the protease enzymes exhibited the maximum activity at neutral pH, and supports the findings of maximum protein degradation efficiencies at pH 7.5 in R1 and R2 as reported in Chapter 3. Thus even though aminopeptidases and protease activities have been shown to be responsible for protein degradation in activated sludges, the protease activity alone might not accurately reflect protein catabolism in all systems (Richard *et al.*, 1984).

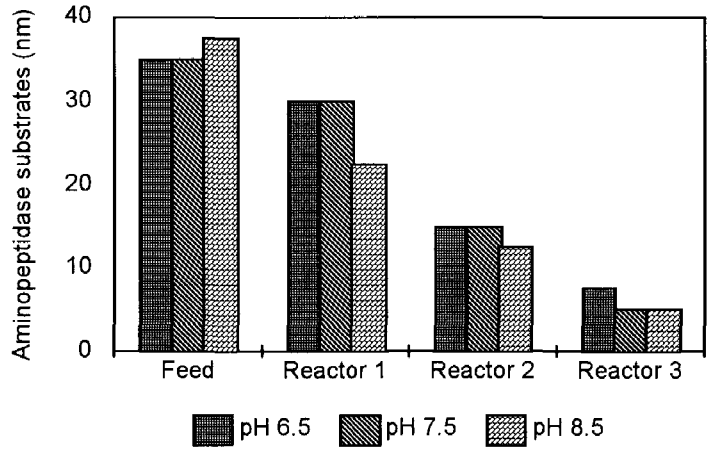

Figure 4.2 Activity of aminopeptidases during sewage sludge hydrolysis in anaerobic reactors as a function of pH

The phosphatases exhibited higher activity than phosphorylases indicating that the phosphatases were largely responsible for degradation of nucleic acids phosphatases during sewage sludge hydrolysis. The phosphatase activity (figure 4.3) shows that these enzymes exhibited their maximum activity at pH 8.5 in all reactors. However, a decrease in pH to 7.5 decreased the enzyme activity only in R2 and R3, while a further decrease in pH to 6.5 resulted in lower activity in all stages of reactors.

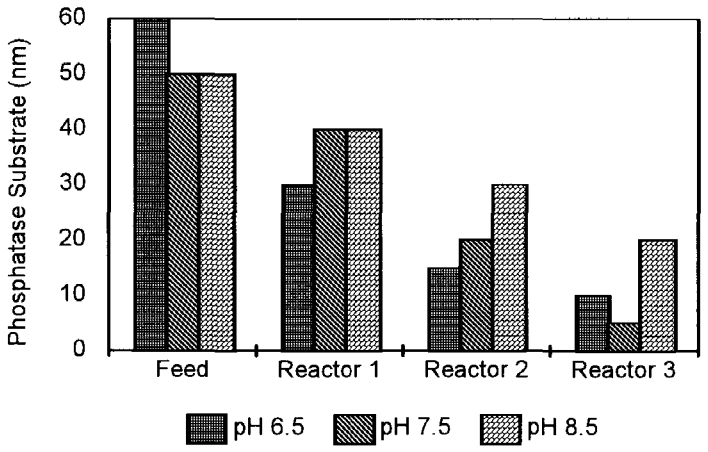

Figure 4.3 Activity of phosphatases during sewage sludge hydrolysis in anaerobic reactors as a function of pH

The activity of phosphorylases (figure 4.4) shows high activity at pH 8.5 in R1 and R2 and no activity in R3. At pH 6.5 the enzyme activity was the same in all reactors, and lower in R2 with no activity in R3 at pH 7.5. These results suggest that these enzymes exhibit their maximum activity at alkaline pH. The acid phosphatases reflected higher activity at pH 6.5 and alkaline phosphatase at pH 7.5 and 8.5 as expected indicating that the activity of these enzymes is strongly affected by pH (figure 4.1).

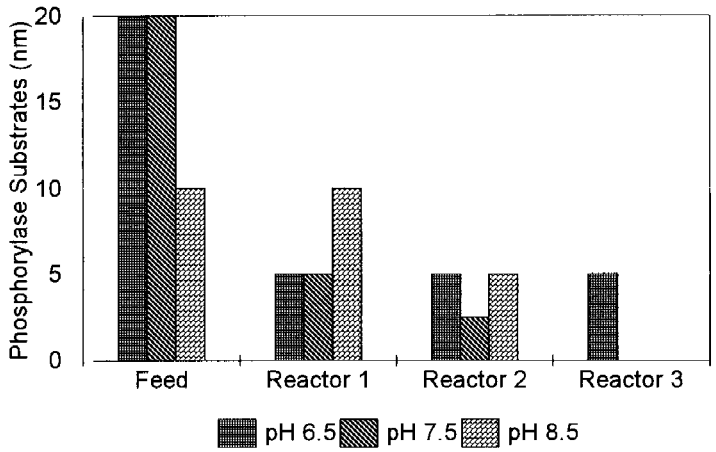

Figure 4.4 Activity of phosphorylase during sewage sludge hydrolysis in anaerobic reactors as a function of pH

Measurement of glucosyl hydrolases (figure 4.5) shows high activity at pH 6.5 in R1 and R2. An increase in pH to 7.5 decreased the enzyme activity in all reactors, and at pH 8.5 in R1 and R2. These results correlate to high degradation efficiencies of carbohydrate at pH 6.5 reported in Chapter 3. The mean glucosyl hydrolases activity was higher than all other enzyme groups at all pH values tested in anaerobic reactors, indicating that it is primarily the carbohydrates fraction of

the sludge that is solubilized during the process of sewage sludge hydrolysis (Gujer & Zehnder, 1983). Similarly, Nybroe *et al.*, (1992) found high glucosyl hydrolases activity in activated sludge with a higher proportion of carbohydrate than other organic classes. This may have reflected an active population of carbohydrate utilizing bacteria.

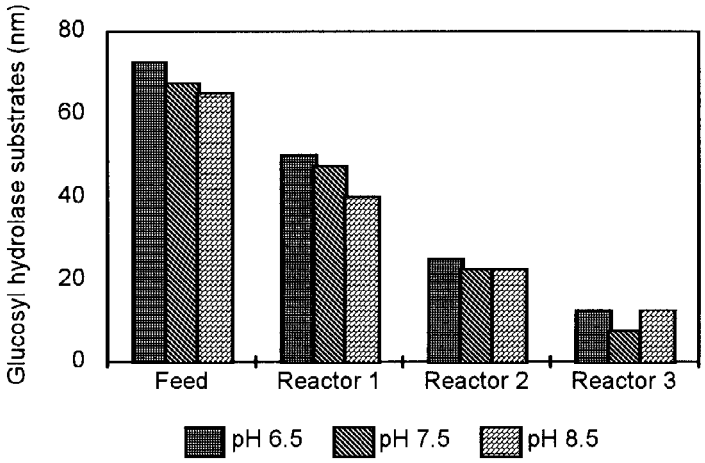

Figure 4.5 Activity of glucosyl hydrolases during sewage sludge hydrolysis in anaerobic reactors as a function of pH

The activity of esterases as a function of pH is reported in figure 4.6. The enzyme activity was high in R1 at all pH values tested, with slightly higher at pH 7.5. These enzymes exhibited the highest activity at pH 6.5 in R2 and R3. This result shows that the esterases exhibit maximum activity at lower pH values.

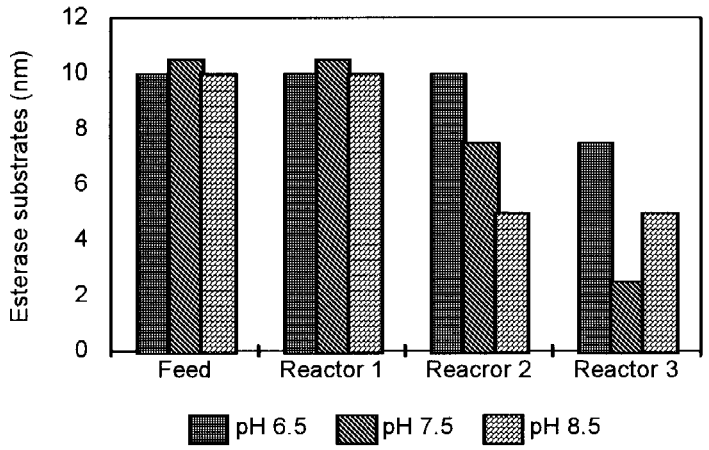

Figure 4.6 Activity of esterases during sewage sludge hydrolysis in anaerobic reactors as a function of pH

The lipase activity reported in figure 4.7 shows similar maximum enzyme activity at all pH values tested in R1, but no activity in R3. In addition, at pH 6.5 and 8.5 no enzyme activity was observed in R2, but only at pH 7.5. In general, these findings correlate with the high lipid degradation efficiency at pH 7.5 reported in Chapter 3, indicating increased lipase activity at neutral pH. The higher activity of esterases than lipases indicate that it is largely the short chain fatty acids which are degraded during sewage sludge hydrolysis.

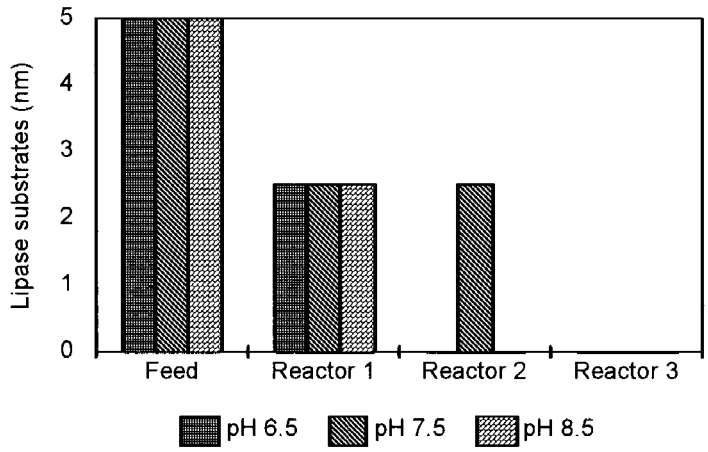

Figure 4.7 Activity of lipases during sewage sludge hydrolysis in anaerobic reactors as a function of pH

4.4 CONCLUSION

The enzyme assay provided a reproducible profile of hydrolytic enzyme activities in sewage sludge. The high activity of glucosyl hydrolases, phosphatases, and aminopeptidases indicated the groups of organics which are likely to be hydrolysed during anaerobic digestion of sewage sludge. High levels of enzyme activities in R1 and R2 supported finding of extensive hydrolysis in these reactors reported in Chapter 3. High esterase than lipase activity suggest that the short chain fatty acids degraded faster than the long chain fatty acids during lipid hydrolysis. In general, the enzyme activities as a function of pH correlated to the degradation of each organic class (carbohydrate, proteins and lipids) during hydrolysis of sewage sludge reported in Chapter 3.

The effect of pH on enzyme activity did not make much difference to the enzyme activity in R1, but a significant difference was observed in R2 and R3. This result could probably be due to accumulation of organic particulate matter in these reactors. The gradual wash out of enzymes from the reactor system, especially in R2 and R3 provide an explanation of the presence of unhydrolyzed organic substrates as reported in Chapter 3. Therefore the addition of hydrolytic enzymes to the system could improve the reactor performance.

The API-ZYM^{TN} system provided a rapid, semiquantitative enzyme assay system and could be useful in characterizing and monitoring biodegradative activity of sewage sludge, assessment of changes in the treatment efficiency due to toxic loads, and to correlate biodegradation activity with the type and levels of enzymes present (Boczar *et al.*, 1992).

CHAPTER FIVE

SCALE-UP EVALUATION STUDIES OF SEWAGE SLUDGE AS A CARBON SOURCE FOR SULPHATE REDUCTION IN SYNTHETIC MINE DRAINAGE WASTEWATERS

5.1 INTRODUCTION

Previous studies on sulphate reduction at a laboratory-scale have proved that hydrolysate of sewage sludge could provide a carbon source and electron donor for sulphate removal and sulphide production when added directly in synthetic drainage wastewaters. As a result of this work, the scale-up application of the process was undertaken at pilot-scale. The feasibility of tannery effluent as a carbon source for sulphate reduction had been previously demonstrated (Boshoff *et al.*, 1996). The application of anaerobic sulphide production in this medium, to the precipitation and removal of metal sulphides from mine and zinc refinery wastewaters, provided a useful model for the evaluation of the co-disposal of organic waste products to provide a carbon source for biological sulphate reduction. In addition, the feasibility of sulphate reduction at pilot-scale has been previously demonstrated for the treatment of AMD (Dvorak *et al.*, 1992; du Preeze & Maree, 1994).

The objectives of this study was to optimize and compare sulphate reduction at pilot-scale utilizing sewage sludge as a carbon source with laboratory-scale sulphate reduction, and to determine the effect of solid recycle on the reactor performance.

5.2 MATERIALS AND METHODS

5.2.1 Pilot Plant Description

The schematic diagram of the pilot-scale sulphate reducing stirred tank reactor (STR) is illustrated in figure 5.1. The pilot-scale reactor consisted of a $1m^3$ stirred feed tank for making up the feedstock, sulphate reducing anaerobic STR with three sampling ports, and a 400L settling tank for recycling solids into the anaerobic reactor. The reactors were designed and obtained from Sinvac Plastics in Pretoria.

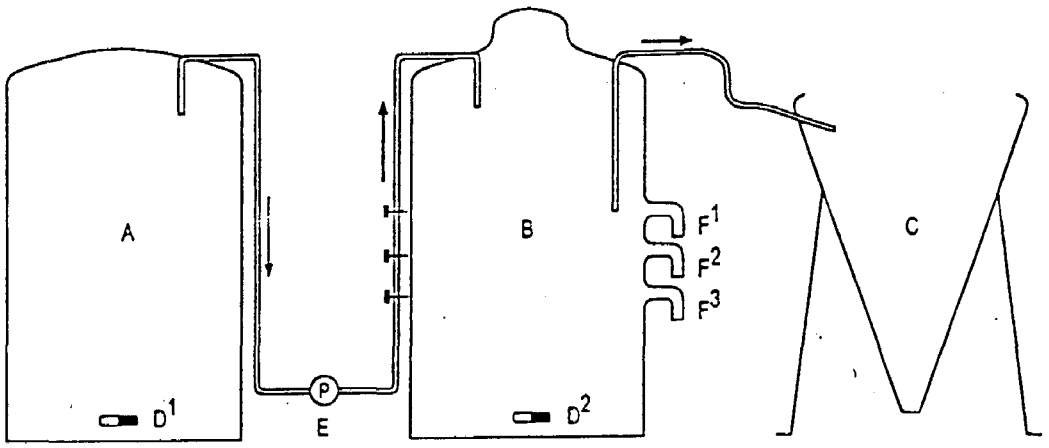

Figure 5.1 Schematic diagram of a pilot-scale reactor used for monitoring sulphate reduction in synthetic drainage wastewaters

A : Feed Tank, B: Anaerobic Digester, C: Settling Tank, D1 and 2 : Submersible Pump, E : Feeding Pump, F1, 2, 3: Port 1, 2 and 3 respectively.

5.2.2 Operational Procedures

The continuous stirred tank (CST) pilot plant reactor utilizing sewage sludge as a carbon source was operated at the Grahamstown Municipal Works. The seed culture was obtained from the same reactor previously fed tannery effluent as a carbon source for sulphate reduction (Boshoff *et al.*, 1996). The reactor was fed sewage sludge from an anaerobic digester at the sewage treatment plant and mixed with sulphate solution.

The system was operated in a continuous mode during the course of the study. Submersible pumps were used for mixing the contents in the anaerobic reactor and feed tank. The sulphate concentration of the synthetic mine water mixed with feedstock was maintained between the range of 900 and 1 400mg/L. Fresh feedstock was prepared every second day and dosed as a mixture at 5 days hydraulic retention time and dilution rate of 0.2 per day. A volume of 1L of sulphide- rich solid from settling tank was recycled into the sulphate reducing anaerobic reactor to evaluate the effect of solids recycle on sulphate reduction. Equations used for calculating HRT and dilution rate are provided in Appendix II.

5.2.3 Analytical Procedures

Samples were taken daily as port 1, 2 and 3. Analysis for total sulphate, total and dissolved sulphide, chemical oxygen demand (COD) and pH were carried out according to the procedures used in Chapter 2 under Materials and Methods. Total solids were determined according to standard methods (APHA, 1985). The sulphate reduction efficiencies represent the mean results from the three sampling ports. Details for preparation of analytical reagents and procedures is shown in Appendix 1 and II.

5.3 RESULTS AND DISCUSSION

5.3.1 Optimization of Pilot- plant for Sulphate Reduction

The biological sulphate reduction in a pilot-scale STR was monitored over a period of 48 days and compared to laboratory-scale. The results pertaining to COD and sulphate removals reported in figure 5.2 and 5.3 show improved reactor performance similar to that reported in a laboratory-scale reactor. During the start-up operation, COD and sulphate concentrations were reduced from 2 465 to 1 170mg/L and 1 140 to 600mg/L respectively. After steady state had been achieved, COD was reduced from 2 300 to 775mg/L and sulphate from 1 130 to 160mg/L. During this period, the SO_4: COD ratio of 1:2.2 was reduced to 1:4 representing a COD: SO_4 utilization ratio of 1:2.

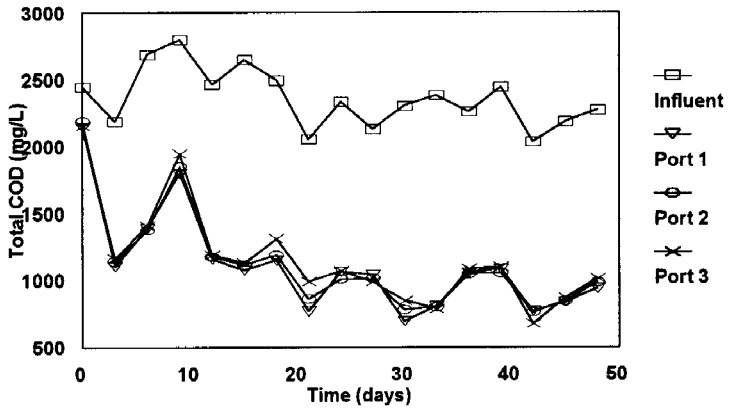

Figure 5.2 Total COD reduction in a pilot-scale sulphate reducing STR fed sewage sludge as a carbon source

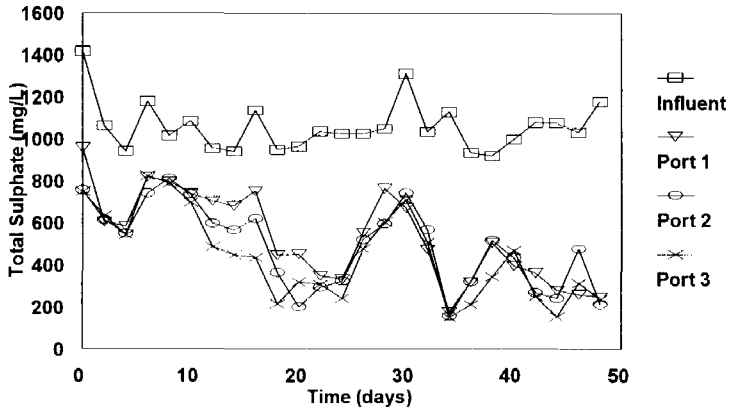

Figure 5.3 Sulphate removal in a pilot-scale sulphate reducing STR fed sewage sludge as a carbon source

As shown in figure 5.4, the COD and sulphate removal efficiency was 50 and 45% during the start-up operation, and 65 and 85% respectively after steady stated were achieved. These results show improved COD and sulphate removals compared to those obtained in a laboratory-scale studies. This could be attributed by improved substrate degradation and/or substrate diffusion in a pilot-scale reactor as a result of increased surface area.

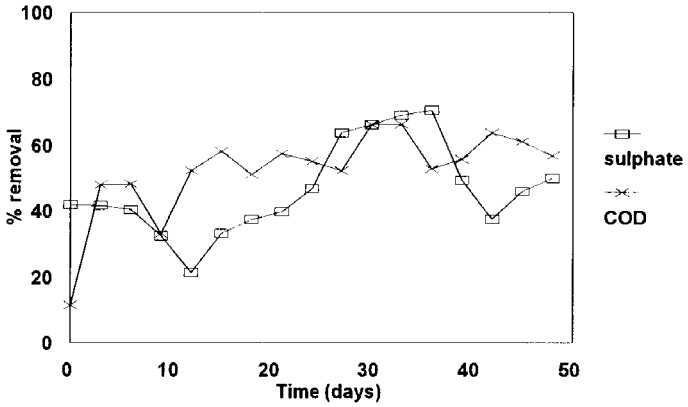

Figure 5.4 Percentage removal of COD and sulphate in a pilot-scale sulphate reducing STR fed sewage sludge as a carbon source

The profiles of total and dissolved sulphide production shown in figure 5.5 and 5.6 indicate improved reactor performance over time similar to that reported on COD and sulphate removals in figure 5.2 and 5.3. The optimum achievement of 85mg/L total sulphide and 68mg/L dissolved sulphide were comparable to that obtained in a laboratory scale sulphate reducing reactor.

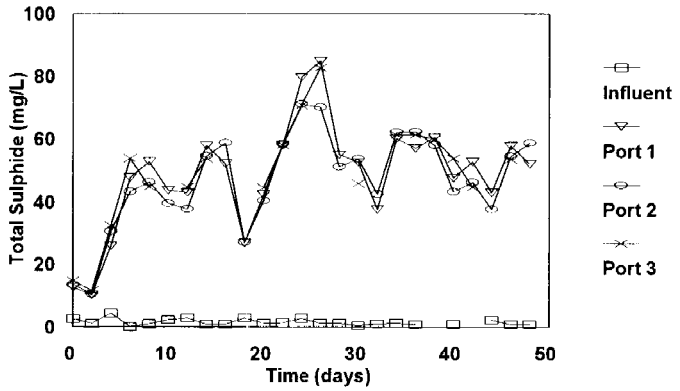

Figure 5.5 Total sulphide production in a pilot-scale sulphate reducing STR fed sewage sludge as a carbon source

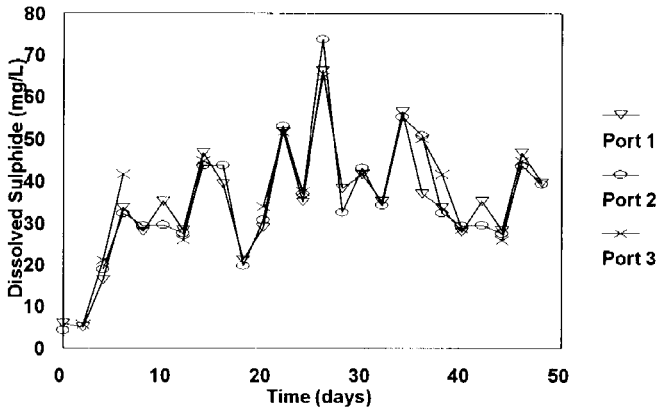

Figure 5.6 Dissolved sulphide in a pilot-scale sulphate reducing STR fed sewage sludge as a carbon source (zero influent sulphide)

Measurement of metal sulphide precipitates reported in figure 5.7 follow a similar trend to that reported on sulphide production. Similar to sulphate reduction in laboratory-scale studies, alkalinity was generated when the pH of the effluent was raised from 3.6 to 8.0.

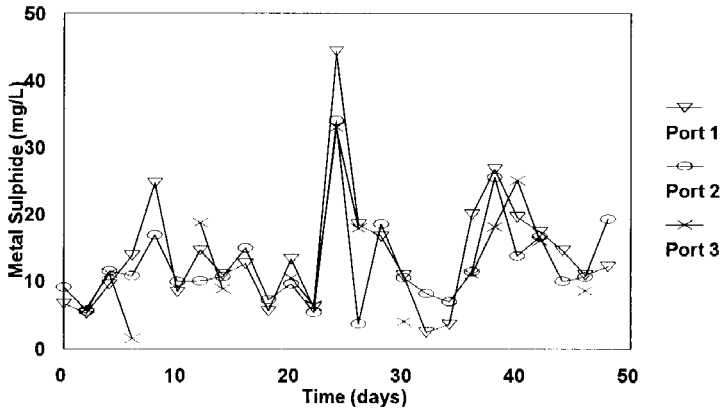

Figure 5.7 Metal sulphide precipitates in a pilot-scale sulphate reducing STR fed sewage sludge as a carbon source

These results indicate increased sulphate reduction efficiency compared to that reported in a laboratory scale sulphate reducing reactor.

5.3.2 The Effect of Solid Recycle on Biological Sulphate Reduction under Optimal Conditions

The results pertaining to sulphate and COD removals when the recycle was introduced in the sulphate reducing reactor are shown in figure 5.8 and 5.9 respectively. The results show improved COD reduction by 5%, when the COD content was reduced from 2 123 to 650mg/L representing the optimum reduction efficiency of 70%.

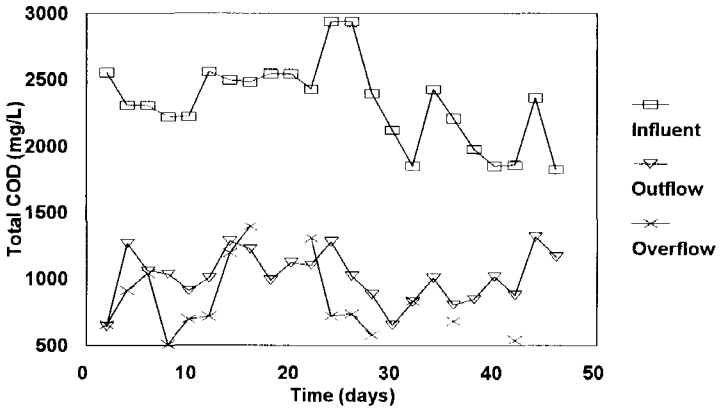

Figure 5.8 Total COD reduction in a pilot-scale sulphate reducing STR fed sewage sludge as a carbon source with solids recycle

The concentration of sulphate was reduced from 1 066 to 100mg/L representing the maximum removal efficiency of 90% in an anaerobic reactor and 70% in a overflow vessel, and remained within the range of 75 and 80%

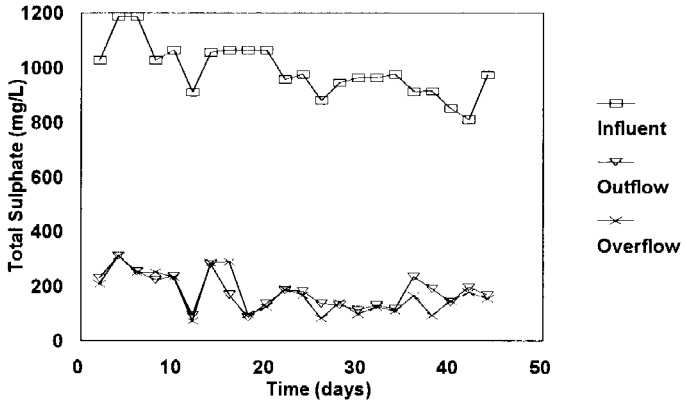

Figure 5.9 Sulphate removal in a pilot-scale sulphate reducing STR fed sewage sludge as a carbon source with solids recycle

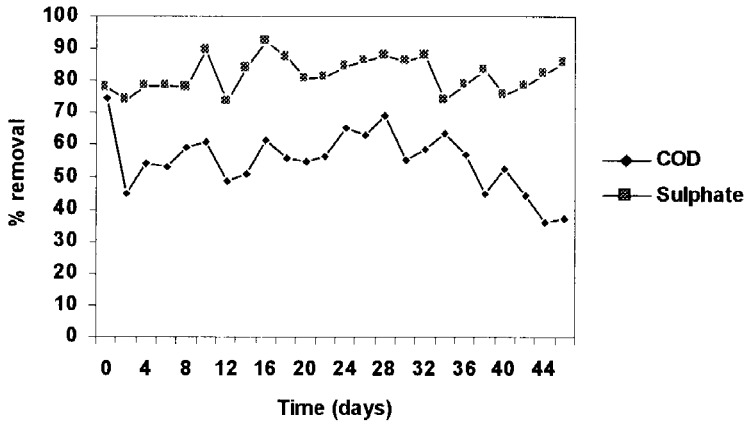

Figure 5.10. Percentage COD and sulphate removals in a pilot-scale sulphate reducing reactor fed sewage sludge as a carbon source with solids recycle

The SO_4 : COD ratio of 1 066 : 2 123mg/L or 1:2 was reduced 100mg/L SO_4 and 650 mg/L COD or 1 : 6.5 representing COD : SO_4 utilization ratio of 1 : 1.5. This increased efficiency could be attributed to the presence of easily biodegradable organic matter from solids to supply an energy source for sulphate reducers.

Figure 5.11 reports the effect of solids recycle on sulphide production and solids accumulation in the effluent reactor. The optimum sulphide production with solids recycle was increased to 90mg/L in the sulphate reducing reactor. Furthermore, sulphide production of 70mg/L was observed in an overflow vessel. The concentration of total solids in the reactor increased from 1 500 to 2 500mg/L with an increase in sulphide production. This could probably be due to utilization of easily biodegradable organic matter from the recycle.

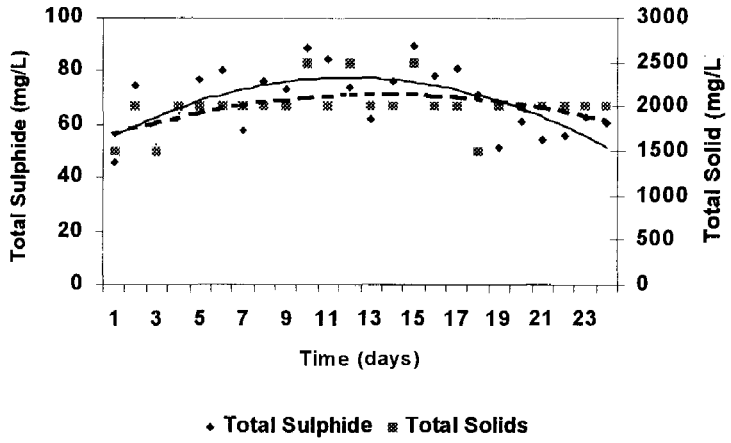

Figure 5.11 Total sulphide production and total solids accumulation in a sulphate reducing STR fed sewage sludge as a carbon source with solids recycle (—Total Sulphide, ----Total Solids)

Measurement of dissolved sulphide and metal sulphide precipitates in the effluent reported in figure 5.12 and 5.13 followed a similar trend to that reported on total sulphide production.

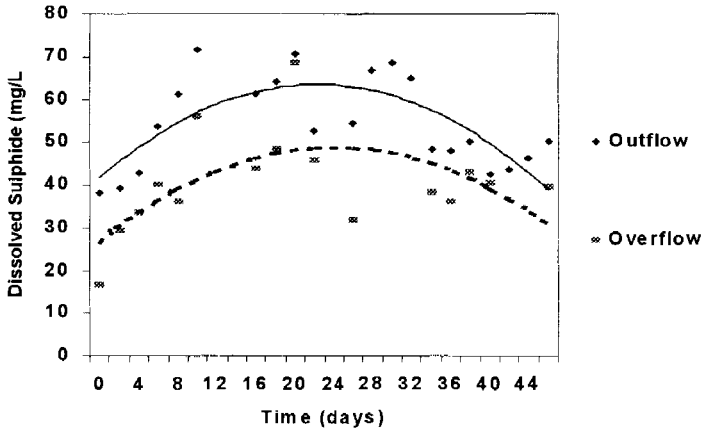

Figure 5.12 Dissolved sulphide production in a pilot-scale sulphate reducing STR fed sewage sludge as a carbon source with recycle (—Outflow, ---- Overflow)

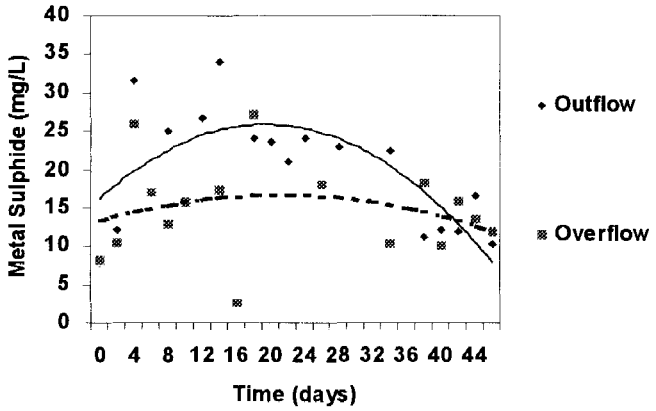

Figure 5.13 Metal sulphide precipitates in a pilot-scale sulphate reducing STR fed sewage sludge as a carbon source with recycle (— Outflow, ---- Overflow)

5.4 CONCLUSION

The study reported here demonstrated the scale-up evaluation of sewage sludge as a carbon source for sulphate reduction in synthetic drainage wastewaters. The increased efficiency of sulphate reduction at pilot-scale compared to that reported in a laboratory-scale suggest that reactor size of a pilot-scale system enhanced substrate diffusion required by SRB to carry out their metabolic activities. Furthermore, the efficiency of pilot-scale sulphate reducing reactor was increased by introduction of solid recycle into the anaerobic reactor. This increased efficiency could be attributed by the presence of readily biodegradable organic particulates from the solid recycle to provide carbon source for sulphate reduction. In large-scale anaerobic treatment processes, introduction of effluent recycle into the anaerobic reactor has been reported to increase process efficiency and to minimize the operational cost (Romli *et al.*, 1994).

The feasibility of sulphate reduction utilizing hydrolysate of sewage sludge as a carbon source, coupled with the reduced operational costs involved, suggest that the system described in this study has a considerable potential application for treatment of large AMD volume flows. Generation of alkalinity and sulphide associated with the growth of SRB fed sewage sludge as a carbon source could provide an efficient method for the treatment of AMD containing heavy metals.

CHAPTER SIX

HEAVY METAL REMOVAL BY SULPHIDE PRECIPITATION

6.1 INTRODUCTION

Acid mine drainage (AMD) is a widespread problem at abandoned mine sites, and results from the oxidation of pyrite in exposed surfaces in underground mines, overburden spoils and mine wastes (Singh, 1992). AMD may be extremely acidic (pH as low as 2) and enriched with iron, aluminium, manganese, sulphate and heavy metals such as lead (Pb), mercury (Hg), cadmium (Cd), and in some cases thorium (Th), and uranium (U) nuclides (Eckenfelder, 1989).

The conventional processes for the treatment of AMD metal laden effluents from industries involve, neutralization by addition of alkaline reagents such as limestone, lime, sodium hydroxide, and sodium carbonate. The construction and chemical costs make these systems unattractive (Robinson & Robb, 1995; Gazea *et al.*, 1996). The high density sludge (HDS) lime process is the most often used method to remove metals form AMD. However, the process cannot be relied upon, because the minimum solubilities of the various metals do not occur at the same pH values and often the presence of complexing agents such as ammonia and EDTA prevents effective precipitation. In addition the hydroxide sludge quantities may be substantial and are generally difficult to dewater due to the amorphous particle structure (Tunay & Kabdasli, 1994).

The potential method for the removal of heavy metals from industrial effluents involves the precipitation of metals as insoluble sulphide. The sulphide is generated biologically via dissimilatory sulphate reduction where sulphate reducing bacteria reduce inorganic sulphate or other oxidized forms of sulphur to sulphide under anaerobic conditions. The sulphide combines with metal cations to form insoluble or sparingly soluble metal sulphides. If the sulphide is generated in excess of metal precipitation requirement, it contributes to alkalinity and raises the pH of acidic effluents (Singh, 1992). The major advantages of sulphide precipitation are the removal of chromium and dichromates without requiring the reduction of chromium to its trivalent state, a reduced tendency to resolubilize, and a lower sludge volume production (Elsenberg *et al.*, 1985; McFadden *et al.*, 1985; McAnnally *et al.*, 1984).

The objective of this study was to evaluate the practical application of sulphide and alkalinity generated through activities of SRB fed sewage sludge as a carbon source to the precipitation and removal and heavy metals such as copper (Cu), cadmium (Cd), iron (Fe), nickel (Ni), lead (Pb) and zinc (Zn) in solution.

6.2 MATERIALS AND METHODS

6.2.1 Effluent

The sulphide-rich effluent obtained from pilot-scale anaerobic digester fed sewage sludge for sulphate reduction was used to study the precipitation of heavy metals (Cd, Cu, Fe, Ni, Pb and Zn) in solution.

6.2.2 Metal Solutions

The metal solutions were prepared individually by dissolving sulphate salts in deionised water to give stock solutions at 1000mg/L, which were later diluted as required. The controls were prepared by adjusting the pH of the metal solution to 2.0 by addition of HCl.

6.2.3 Experiments

The concentration of sulphide and pH from sulphide rich-effluent were measured prior to experimentation. The metal solutions within the concentrations of 100 and 500mg/L were precipitated with 5ml sulphide-rich effluent at sulphide concentrations of 50 and 85mg/L to determine the removal of different concentrations of heavy metals by sulphide precipitation. Deionized water adjusted to pH 2 by addition HCl was used as the blank for every concentration tested, because heavy metals tend not to precipitate at acidic pH. Instead of sulphide-rich effluent,

deionized water at pH adjusted with NaOH to that of the effluent was used to determine the effect of pH on precipitation of heavy metals. The samples were allowed to stand at room temperature for complete precipitation (1-2hours). Analyses were done in triplicate, and the mean was presented. The concentrations of metals remaining in solution after precipitation were determined on a GBC 909AA Atomic Absorption Spectrophotometer linked to a GBC integrator. All samples were accompanied by five commercial standards for calibration purposes.

6.3 RESULTS AND DISCUSSION

6.3.1 Precipitation of Heavy Metals as Metal-Sulphides

Varying concentrations of metal ions were precipitated with sulphide-rich liquor obtained from the outflow of the pilot plant sulphate reducing digester. Percentage removal of metals was measured. Figure 6.1(a) and (b) illustrates the removal efficiencies of metal ions precipitated with 50mg/L sulphide in effluent at pH 7. The percentage removals of Cu, Pb, Zn shown in figure 6.1(a), indicate that the concentration of copper was reduced from 100 to 4mg/L representing the maximum removal efficiency of 96%. Lead (Pb) exhibited high removal efficiencies ranging between 90 and 98% at metal concentrations tested. About 90% zinc was removed from 100mg/L metal solution. However, increasing the concentration of copper, zinc and cadmium in solution to 500mg/L decreased the removal efficiencies to 60, and 55% respectively.

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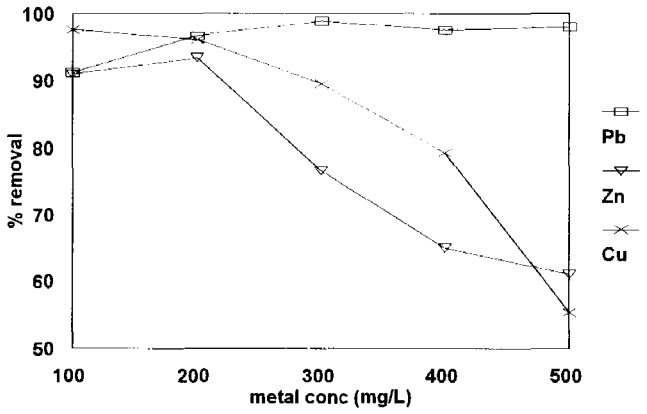

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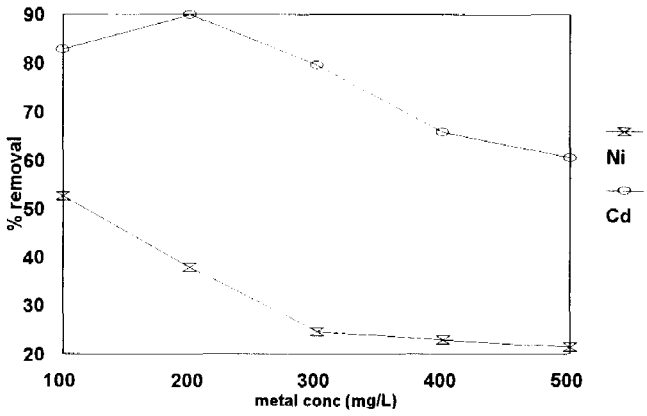

Figure 6.1A and B : Removal of heavy metals from aqueous solution with the addition of 50mg/L sulphide from sulphate reducing reactor outflow liquor.

As shown in figure 6.1b, about 20% of nickel (Ni) was removed at metal concentration between 500 and 300mg/L, and increased to 50% when the concentration was decreased to 100mg/L. The concentration of cadmium was reduced from 100 to 10mg/L representing the maximum removal efficiency of 90%. In general the removal efficiencies of metals increased with a decrease in metal concentration, indicating that the higher the concentration of metal in solution, the lower the removal efficiency.

The profiles of heavy metals precipitation with 85mg/L sulphide at pH 8.0 are shown in figure 6.2. Increased sulphide concentration increased removal of nickel and cadmium to 60 and 90% at 300 and 100mg/L respectively. However, the removal efficiencies of copper, lead and zinc remained higher. These metals are highly insoluble over a broad pH range and their sulphide precipitation was therefore a rapid and efficient process (Christensen *et al.*, 1986). The maximum removal of 70% was obtained for iron precipitation at 200mg/L.

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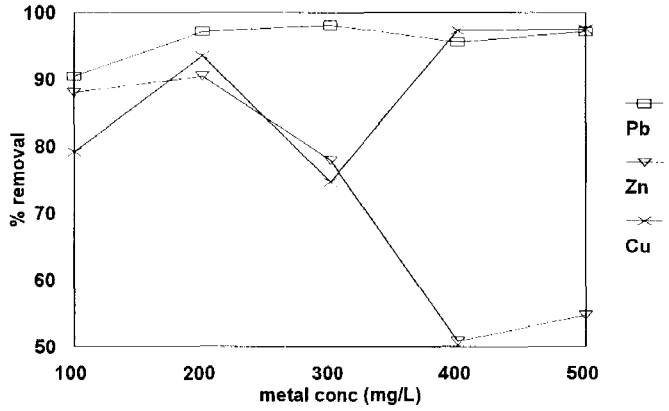

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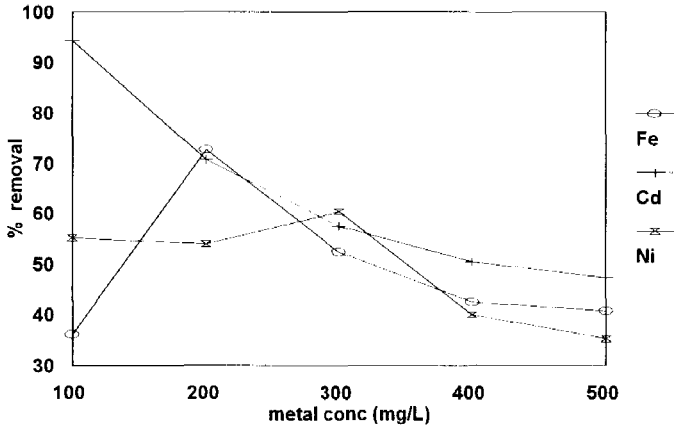


Figure 6.2A and B : Removal of heavy metals from aqueous solution with the addition of 85mg/L sulphide from sulphate reducing reactor outflow liquor

Theoretically, the quantity of sulphide required is approximately 1mg/L for each mg/L (1 : 1) of toxic heavy metal present (Machemer & Wildeman, 1992). According to this prediction, the ratio of sulphide to heavy metals precipitated by 50mg/L sulphide, 7.796*u*M sulphide should be approximately 7.796*u*M. As shown in table 6.1 below, the ratio of sulphide to Pb (7.769 : < 0.750*u*M) exceeded the theoretical prediction at all concentrations tested. The ratio of sulphide to Zn and Cu removal at concentrations less than 300mg/L representing 7.796 : < 7.646 : < 3.5*u*M respectively. The concentration of Ni and Cd were higher than sulphide required, i.e >11.0*u*M between 200 and 400mg/L, and 10*u*M at 500mg/L metal solutions respectively.

Table 6.1 Metal ion concentrations (*u*M) removed by 7.796*u*M sulphide

Metal conc (mg/L)	Pb	Zn	Cu	Cd	Ni
100	0.490	0.702	0.343	52.131	4.379
200	0.493	0.969	0.738	2.019	11.467
300	0.195	7.646	3.494	5.444	30.295
400	0.619	15.430	10.786	1.130	43.857
500	0.747	26.762	34.891	1.007	66.979

For precipitation of heavy metals by 85mg/L sulphide, 13.25*u*M sulphide was required to remove 13.25*u*M of each heavy metal in solution. The concentrations of metal ions removed by 13.25*u*M sulphide are illustrated in table 6.2. The ratio of sulphide to Pb and Cu concentrations, that is 13.25 : <10 : < 8*u*M respectively, indicating that sulphide concentration was in excess for the removal of these metals. However, sulphide concentration appeared to be lower for the removal of Ni and Fe from 300mg/L, Cd and Zn at concentrations higher than 400mg/L metal solutions.

Table 6.2 Metal ion concentrations (μM) removed by 13.25μM sulphide

Metal Conc (mg/L)	Pb	Zn	Cu	Fe	Cd	Ni
100	0.419	0.856	2.360	6.392	18.221	2.828
200	9.494	3.884	14.729	6.374	15.343	8.604
300	1.510	7.616	8.214	18.984	12.054	14.329
400	1.101	22.542	1.339	32.463	3.641	25.873
500	1.191	31.732	2.014	35.667	1.325	62.481

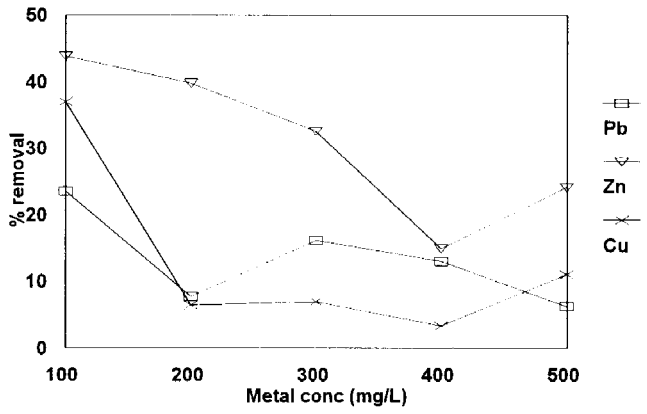
The ratio of lower sulphide to metal concentration obtained in this study suggest that excess heavy metals could have been removed by the presence of carbonates and hydroxides from sulphide-rich effluent which formed complexes with metals and thereby increasing their removal efficiencies (Dvorak *et al.*, 1992). In addition, the presence of traces of iron in effluent could also have enhanced metal removal efficiencies through co-precipitation and adsorption of the ion sulphide (Watson *et al.*, 1995). Sulphide in excess could be used to precipitate high concentrations of heavy metals.

6.3.2 Precipitation of Heavy Metals as Metal Hydroxides

Since pH has been identified as an important factor governing the removal of heavy metals in solution. In order to compare sulphide precipitation with hydroxide precipitation, instead of effluent, deionized water at pH 7.0 and 8.0 was used to study the removal of metals as hydroxide precipitates.

The effect of no sulphide addition on the removal of metals at neutral pH is shown in figure 6.3a and b. The precipitation of heavy metals at pH 7 showed the maximum removal efficiencies of 50, 40% for Cd, Zn, and 20% Cu, Ni, Pb respectively. The lower removal efficiencies of metals could be attributed to the fact that the hydroxide precipitation of these metals require an optimum pH of 11.0 (Peters & Ku, 1985).

A



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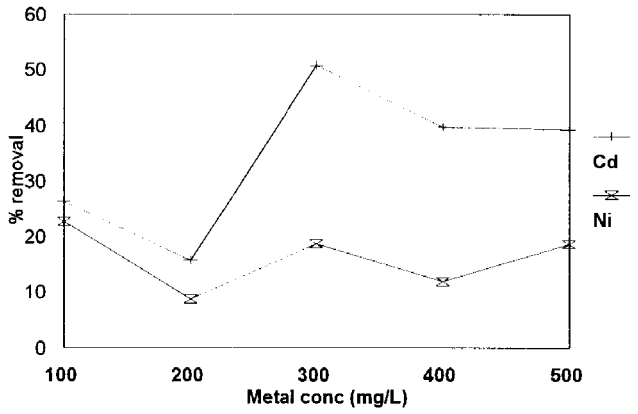

Figure 6.3 A and B : Removal of heavy metals form solution by hydroxide precipitation at pH 7.0

When the pH was increased to 8.0, the removal of metals with the exception of Cd increased representing the maximum removal efficiencies of 50% for Pb and Zn, 35, 30, 20 and 25% Cu, Ni, and Fe respectively. This result indicates that at pH 8.0 the affinity of metal ions to negatively charged hydroxyl group was enhanced. Comparing these results with the results obtained for sulphide precipitation, it is clear that lower residual metal concentrations could be achieved using sulphide precipitation for comparable pH conditions as compared to metal hydroxide precipitation. Furthermore, low solubility of metal sulphides result in higher removal efficiency over the hydroxide process (Whang, 1982).

These results indicate that for effective precipitation of the majority of metals as metal hydroxides, the pH of the effluent has to be maintained at or above pH 8, suggesting that should the pH decrease below 7, the removal efficiency of metals would be affected. It has also been reported that Pd, Cu, Cd, Zn and Ni precipitate at pH values higher than 5.0, while Fe precipitate at pH higher than 2.5 (Sag & Kutsal, 1995).

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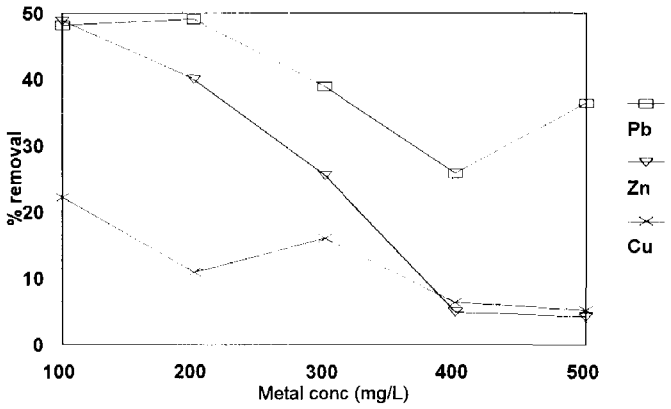

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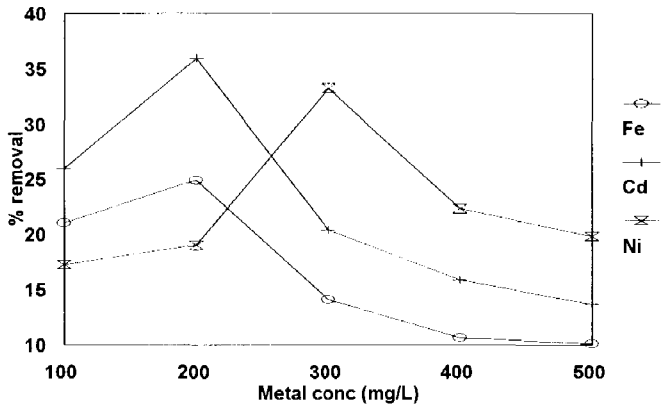

Figure 6.4A and B : Removal of heavy metals from solution by hydroxide precipitation at pH 8.0

6.4 CONCLUSION

The sulphide rich-liquor outflow effluent from the sulphate reducing digester has proved to be more successful for the removal of heavy metals in solution as metal sulphides. Extremely low residual metal concentrations were achieved using sulphide precipitation compared to hydroxide precipitation. The total removal efficiencies of heavy metals varied with concentration of metals in solution, sulphide concentration and pH. Precipitation of heavy metals with sulphide concentration of 85mg/L at pH 8.0 resulted in the removal efficiency greater than 90% for Cu, Pb, and Cd, and greater than 60% for Ni and Fe. However, 50mg/L at pH 7.0 sulphide decreased the removal efficiency of Ni by 10%, while the removal of other metals were not affected by lower sulphide concentration.

Treatment of AMD containing high concentrations of heavy metals by hydrogen sulphide and alkalinity generated through sulphate reduction could prevent and remediate metal pollution of the receiving waters. However, the practical application of this technology for treatment of AMD metal laden effluents ultimately depend on its economics, especially the cost of carbon source. The use of less expensive industrial organic waste products such as molasses and anaerobically digested cattle wastes, tannery effluent and algal biomass have been previously reported to be useful organic carbon sources for sulphate reduction for removal of heavy metals from AMD (Maree & Strydom, 1985; Ueki *et al.*, 1988; Boshoff *et al.*, 1996). The use of sewage sludge for generation of sulphide and alkalinity through activities of sulphate reducing bacteria could provide a low-cost treatment technology for the removal of heavy metals in AMD.

CHAPTER SEVEN

GENERAL DISCUSSION AND CONCLUSION

7.1 DISCUSSION

The industrial development in South Africa has increased the volume of wastewater to be disposed of, whereas the capacity of the receiving water to accept acid mine drainage (AMD) pollution is limited (Kiestra & Eggers, 1986). Forced by the legislation acting through the National Water Bill set out by the Department of Water Affairs and Forestry (Perkins, 1998; Viljoen *et al.*, 1998), industries are looking for the least-cost solution for the required reduction of AMD pollution to prevent the resultant deterioration of the environmental quality.

The "active" biological AMD treatment systems based on the activities of sulphate reducing bacteria (SRB), has been identified as a potentially valuable processes for treating AMD. The scale-up application of this process is constrained by the availability and the cost of carbon source. The feasibility of sewage sludge to provide a low-cost, readily available carbon source and electron donor for sulphate reduction was evaluated at laboratory-scale using a biological sulphate reducing stirred tank reactor (STR). The objective was to utilize the readily biodegradable components of the sewage sludge for the production of sulphide and removal of sulphate. The process successfully demonstrated the feasibility of sewage sludge to provide a carbon source for

sulphate reduction. During the steady state operation of biological sulphate reduction, 87mg/L total sulphide was produced. This resulted in generation of alkalinity by raising the pH of acidic water from 6.0 to 7.8, and heavy metals contained in the effluent precipitated as insoluble metal sulphides. In addition, sulphate and COD reduction efficiencies of 80 and 50% were obtained respectively. The COD : SO_4 utilization ratio of 1 : 4 assumes no utilization of electron flow to methanogenesis. However, it is also known that in nutritionally rich environments, such as anaerobic digesters fed sewage sludge, and animal waste, sulphate reduction does not compete with methanogenesis for energy sources (Butlin,1956; Ueki *et al.*, 1988). The low utilization of organic carbon (as COD reduction) obtained during this study was probably due to low hydrolytic rate of organic particulates from sewage sludge and/or slow conversion rate of biodegradable compounds. These results suggest that the presence of readily biodegradable or soluble organic matter to provide the carbon source for SRB, while the process was developed, was limited and thus low efficiencies of sulphate reduction was obtained during the start-up of operation.

Sewage sludge is a complex carbon source, consisting mainly of carbohydrates, proteins and lipids, (Gujer & Zehnder 1983), which are energy sources for SRB. During anaerobic digestion for sulphate reduction, SRB do not degrade these organic substrates, they usually depend on the activities of fermentative bacteria for the supply of energy through the process of hydrolysis (Hansen, 1988). Studies on carbohydrate degradation has never been shown to occur directly via sulphate reducers, with one exception for fructose (Cord-Ruwisch *et al.*, 1986). Instead, they use the fermentation products arising by Entner-Doudoroff pathway fermentation such as alcohols,

pyruvate, lactate, and hydrogen. However, some strains use amino acids directly (Stams, 1985), or by interspecies hydrogen transfer (Nonninga & Gottschalk,1987). The presence of enzymes for conventional TCA cycle in SRB, perform the anaplerotic function for carbohydrate degradation, while degradation of the higher fatty acids forms exclusively acetate which occurs by the classical β–oxidation of the aliphatic carbon chains (Postgate, 1984).

To investigate the complexity of sewage sludge as a carbon source, hydrolytic studies were carried out to investigate the formation of easily biodegradable carbon. Because hydrolysis is greatly influenced by a range of operational parameters including pH, the objective of this study was to examine the effect of pH on substrate degradation and the production of soluble particulate matter during anaerobic digestion of sewage sludge in a three stage laboratory reactor system. During the studies without pH control, the pH in the three anaerobic reactors progressively decreased to from 7.2 to 6.7 over time. The acid generation from fermentation products of carbohydrate metabolism such as organic acids, alcohols, aldehydes and ketones and glycerol from lipid hydrolysis was an indication of induced hydrolysis (Gottschalk, 1986).

The pH changes during anaerobic digestion affected the degradation pattern of organic substrate solubilization. Lipids were the most degraded (66%) at pH 7.3, carbohydrates (43%) at pH 6.8, and proteins (30%) at pH 7.1. Interestingly, the high hydrolytic efficiencies of these substrates was observed in R1 and R2 rather than R3.

These results suggested the accumulation of unsolubilized particulate matter in R3. However, the production of soluble organic carbon was not enhanced, probably due to pH alterations in the anaerobic reactors. It was therefore clear that optimization of pH for sewage sludge hydrolysis was necessary in order to stabilize COD solubilization and degradation of organic particulates.

Analysis of the degradation behaviour of the organic classes studied at pH 6.5, 7.5 and 8.5 revealed major differences in the extent of their hydrolysis. Carbohydrates and proteins were extensively degraded at pH 6.5, while lipids degradation was observed at pH 7.5. A similar trend of carbohydrate degradation has been observed by Eastman & Ferguson (1981), in a continuous flow reactors treating primary sludge at pH values between 5.0 and 6.0. Breure *et al.*, (1986) also reported that the activity of enzymes involved in carbohydrate degradation is optimal at pH 6.5. This suggests that the activities of the enzymes involved for degradation of these substrates were induced at these pH values. Although many proteolytic organisms prefer neutral pH environments, proteolytic enzymes may exhibit their maximum activity at different pH values ranging from 2 to 10 (Elefsiniotis & Oldham, 1994). The reduction of COD during anaerobic digestion indicated the utilization of readily biodegradable organic constituents of the sewage sludge. Furthermore, pH of 7.5 and 8.5 did not induce solubilization of organic matter, while pH 6.5 resulted in remarkably high solubilization, which was attributed to the high efficiencies of carbohydrate (65%), and protein (53%) hydrolysis occurring at this pH.

Since anaerobic digestion of complex organic compounds is accomplished by the presence of hydrolytic enzymes, it would therefore be expected that the hydrolysed sewage sludge would have a characteristic enzyme profiles. The enzymes present in the sewage sludge were characterised using API-ZYM^{TN} system and their activities were determined as a function of pH in the same system used for hydrolytic studies. Among a wide range of enzymes characterized, glucosyl hydrolases, phosphatases and aminopeptidases were dominant in R1 and R2, indicating the availability of these enzymes to organic substrates from sewage sludge in these reactors. In general, the enzyme activities correlated with the degradation pattern of organic substrates obtained as a function of pH.

The success of laboratory-scale studies led to the scale-up evaluation of biological sulphate reduction in synthetic mine drainage wastewaters utilizing sewage sludge as a carbon source. The facilities for the operation being readily available at Grahamstown Works. The investigations were carried out to compare the efficiency of the pilot-scale STR with the laboratory-scale sulphate reducing reactor for optimization of biological sulphate reduction. The pilot-scale sulphate reducing reactor showed an improved performance over time similar to that reported for laboratory-scale. At steady state operation, sulphate and COD removal efficiencies increased by 5 and 25% (i.e 85% and 75%) respectively compared to those obtained in the laboratory- scale reactor, while no change in sulphide production was observed. Furthermore, the effect of solids recycle on the performance of pilot-scale sulphate reducing reactor was evaluated at steady state operation. The introduction of the solids recycle increased sulphate removal efficiency further to 90%, and no increments in COD removal was obtained. The sulphide production was also

increased to 90mg/L with an increase in total solids concentration. These results suggest utilization of readily biodegradable organic matter from the solids recycle.

The practical application of biological sulphate reduction to remove heavy metals was examined by precipitating a range of metals viz. Cu, Cd, Fe, Zn, Pb, and Ni at concentrations ranging from 100to500mg/L with sulphide-rich solution of 50 and 80mg/L from the outflow liquor of pilot-scale sulphate reducing reactor fed sewage sludge as a carbon source. The precipitation of metals with both sulphide concentrations resulted in the removal efficiencies greater than 90% for Cu, Pb, and Cd and greater than 60% for Fe with the exception of Ni removal which decreased by 10% with 50mg/L sulphide precipitation.

7.2 CONCLUSION

Sewage sludge is an easily available waste substrate to provide a low-cost carbon source and electron donor compared to high-cost feeds studied in the past, for biological sulphate reduction in acid mine drainage wastewaters. The anaerobic digestion, specifically hydrolysis of sewage sludge through the release of extracellular enzymes by fermentative bacteria under optimal conditions prior to its use for sulphate reduction could improve the degradation of organic particulate matter to supply energy source for SRB. This would provide easily biodegradable and soluble organic carbon source for sulphate reduction. The application of anaerobic sulphide production in this medium, to the generation of alkalinity, precipitation and removal of metals sulphides from mine wasterwaters would eliminate any possible release of toxic hydrogen sulphide

gas, while maintaining the high levels of heavy metal removal. The scale-up application of biological sulphate reduction utilizing sewage sludge as a carbon source is possible for the treatment of sulphate and heavy metal containing industrial effluents.

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APPENDICES

APPENDIX I : PREPARATION OF ANALYTICAL REAGENTS

1.0 SYNTHETIC MINE WATER

Reagent	Quantity for 750L	Quantity for 1000L
K_2SO_4	37.32g	49.76g
$FeSO_4.7H_2O$	75g	100g
$CaSO_4.2H_2O$	300g	400g
$NaSO_4$	267.15g	356.2g
$(NH4)_2SO_4$	148.5g	198g
ddH_2O	750L	1000L

2.0 COD DETERMINATION

COD Solution A and B

3.0 SULPHATE DETERMINATION

3.1 Buffer Solution A

Reagent	Quantity
$MgCl_2.6H_2O$	30g
$CH_3COONa.3H_2O$	5g
KNO_3	1g
CH_3COOH	20ml
ddH_2O	880ml

3.2 Barium Chloride

Reagent	Quantity
$BaCl_2$	50g
ddH_2O	1000ml

4.0 SULPHIDE DETERMINATION

4.1 Amine-Sulphuric Acid Stock Solution

Reagent	Quantity
NN-Dimethyl-*p*-phenyldiamine dihydrochloride	2g
HCl	500ml

4.2 Ferric Chloride

Reagent	Quantity
$FeCl_3.6H_2O$	8g
HCl	500ml

5.0 PROTEIN DETERMINATION

5.1 Solution A : stored at 4°C

Reagent	Quantity
$CuSO_4.5H_2O$	0.5g
$NaC_6H_5O_7.2H_2O$	1g
ddH_2O	100ml

5.2 Solution B: Stored at 4°C

Reagent	Quantity
Na_2CO_3	20g
NaOH	4g
ddH_2O	1000ml

5.3 Solution C : This reagent is prepared fresh on the day of the experiment.

Solution A + Solution B

5.4 Solution D: Store in a dark brown bottle (to prevent photo-oxidation)

Reagent	Quantity (ml)
Folin-Ciocalteu Phenol	500ml
ddH_2O	500ml

6.0 CARBOHYDRATE DETERMINATION

6.1 Phenol Reagent

Reagent	Quantity(g/L)
Phenol	50g
ddH_2O	1000ml

6.2 Sulphuric Acid

100% Sulphuric Acid

7.0 LIPID DETERMINATION

7.1 Extraction Solvent per gram of sample

Reagent	Quantity
Chloroform	10ml
Methanol	20ml

7.2 Washing Solvent

Reagent	Quantity
KCl	8.8g
ddH_2O	1000ml

8.0 METAL SOLUTIONS

Metal solutions prepared individually in a volume of 1000ml ddH_2O with concentration of 1000mg/L

Reagent	Quantity
$CuSO_4.5H_2O$	3.487g
Cd $SO_4.8/3H_2O$	2.282g
$FeSO_4.7H_2O$	4.970g
$NiCl_2.H_2O$	4.05g
$PbSO_4.H_2O$	1.598g
$ZnSO_4.7H_2O$	4.397g

9.0 REAGENTS FOR ENZYME ASSAYS (API-EZM)

ZYM A and B Reagents : as described by the manufacturer (Separation Scientific C.C. Co.)

APPENDIX II : PROTOCOLS FOR ASSAYS

1. COD DETERMINATION : SQ 118 Method

Principle : The Solutions A and B are filled into a reaction cell together with the sample solution. After digestion with the thermoreactor TR 205, the solution is analysed with photometer SQ 118 (Merck Test Kit).

1.1 Analysis

1. Measure out 3ml sample into a COD reaction cell;
2. Add 0.3ml of Solution A and 2.3m Solution B and mix;
3. Insert the reaction cell into the TR 205 and heat up for 2hours at 148°C;
4. Remove the reaction cell from the TR 205 and cool for 5minutes;
5. Blank with water and measure the concentration of COD (mg/L) with photometer SQ 118.

2. SULPHATE ASSAY : Turbidimetric Method

Principle : Sulphate ion is precipitated in acetic acid by addition of barium chloride to form crystals of uniform size. Light absorption of barium sulphate suspension is measured by spectrophotometer and sulphate concentration is determined by components of the reading standard curve (APHA, 1985).

2.1 Preparation of Sulphate Standard

Prepare 0.1485g Na_2SO_4 in 1000ml ddH_2O to get a final concentration of 100mg/L solution;

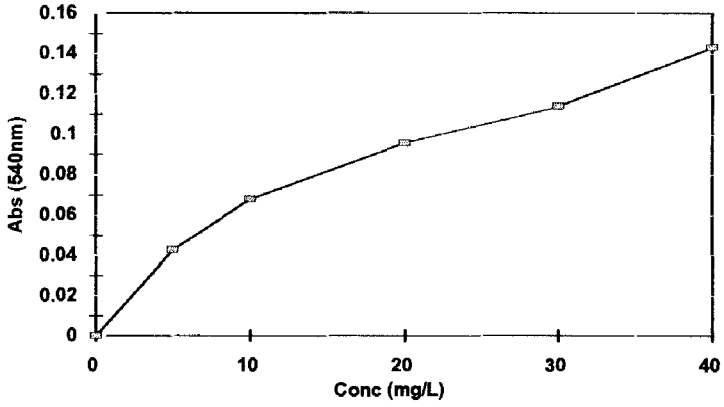
Space standards at 5mg/L intervals between 0-40mg/L as shown in table 1A

Table 1A Preparation of Sulphate Standard

Conc (mg/L)	Stock Solution (ml)	dd H2O (ml)
0	0	100
5	5	95
10	10	90
20	20	80
30	30	70
40	40	60

2.2 Analysis

1. Measure out 5ml sample;
2. Add 1ml buffer solution;
3. Add 500*u*l $BaCl_2$ solution and vortex mix;
4. Read absorbance at 420nm.



Regression Output	
Constant	0
Std Err of Y Est	0.021323
R Squared	0.698139
No. of Observations	5
Degrees of Freedom	4
X Coefficient (s)	0.003914
Std Err of Coef.	0.000388

Figure 1A Sulphate standard working curve

3. SULPHIDE ASSAY

Principle : This method involves the bluish colour formation by *NN*-diethyl-*p*-phenylenediamine in the presence of iron (III) ions. The intensity of the colour is proportional to the concentration of sulphide (Rees *et al.*, 1971).

3.1 Preparation of Sulphide Standard

Make up a stock solution of $Na_2S.9H_2O$ by dissolving 0.789g in 500ml ddH_2O;

Add the following volumes of stock solution to 100ml volumetric flasks and make up with ddH_2O as shown in table 2A.

Table 2A Preparation of Sulphide Standard

Conc (mg/L)	Stock Solution (ml)	ddH_2O (ml)
0	0	100
0.2	2	98
0.4	4	96
0.6	6	94
0.8	8	92
1.0	10	90

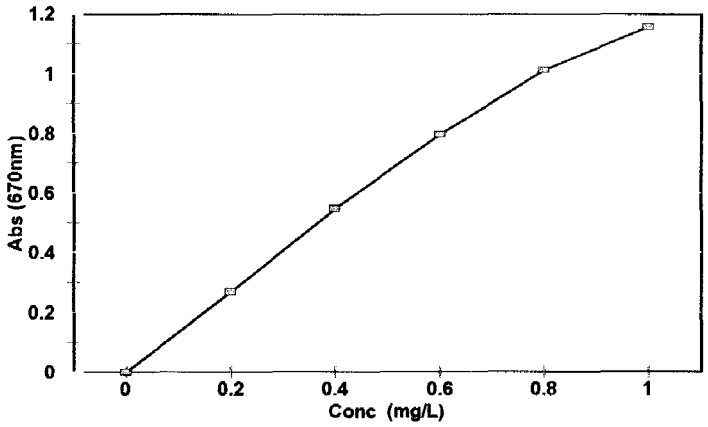
3.2 Analysis

1. Measure out 5ml sample;

2. Add 500*ul* of each amine-sulphuric acid and ferric chloride reagents;

3.Allow the reaction to develop for an hour;

4.Read the absorbance on spectrophotometer at 670nm.



Regression Output

Constant	0
Std Err of Y Est	0.047882
R squared	0.982627
No. of Observations	5
Degrees of Freedom	4
X coefficient(s)	1.237727
Std Err of Coef.	0.032282

Figure 3A Sulphide standard working curve

4. PROTEIN ASSAY : Folin-Lowry Protein Method

Principle: This assay employs two colour-forming reactions to assay protein concentrations photometrically. Firstly, copper ions, present in the alkaline copper reagent, react with peptide bonds to form a deep bluish colour. Secondly, the complex inorganic salt mixture of the Folin-Ciocalteu reagent is responsible for further colour development. This reagent is a solution of sodium tungstate and sodium molybdate in phosphoric and hydrochloric acid. Phosphomolybdate generated in this solution imparts the yellow colour to the reagent and is reduced by the copper-treated free or peptide-bound tyrosine and tryptophan residues present in the solution. The intensity of a blue-green colour produced by this reaction is proportional to the protein concentration (Lowry *et al.*, 1951) .

4.1 Preparation of Protein Standard

Dissolve100mg bovine serum albumin stock solution in 100ml distilled water. Prepare standards ranging from 20 to100*u*g/ml as shown in table 3A

Table 3A Preparation of Protein Standard

Conc (*u*g/ml)	Sock solution (*u*g/ml)	ddH_2O
0	0	100
20	20	80
40	40	60
60	60	40
80	80	20
100	100	0

4.3 Analysis

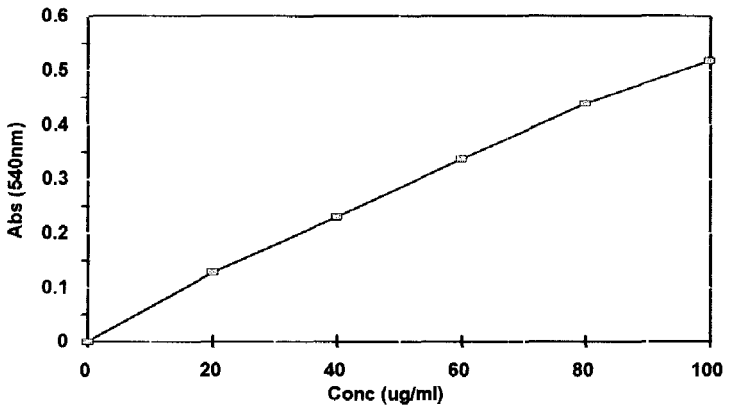
1. Bring a sample solution to 0.5ml with distilled water;

2. Add 2.5 ml Solution C;

3. Vortex and let stand at room temperature for 5-10minutes;

4. Add 0.25ml Solution D and vortex;

5. After 20-30minutes read the absorbance at 750nm.



Regression Output	
Constant	0
Std Err of Y Est	0.014194
R Squared	0.995737
No. of Observations	6
Degrees of Freedom	4
X Coefficient(s)	0.005186
Std Err of Coef.	0.00017

Figure 2A Protein standard working curve

5. CARBOHYDRATE ASSAY : Phenol-Sulphuric Acid Method

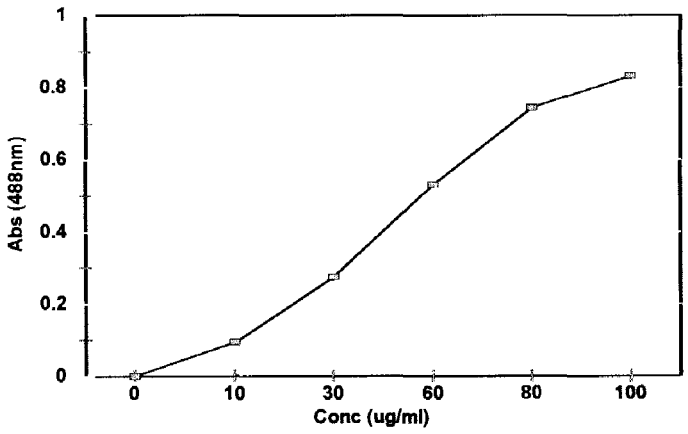
Principle : This method is based on the colour reaction between carbohydrate and phenol reagent in a strong sulphuric acid. The mechanism of the colour development is that polysaccharides are hydrolysed during heating by acid to monosaccharides. Formation of an orange colour from this reaction is measured spectrophotometrically (Dubois *et al.*, 1956).

5.1 Preparation of Carbohydrate Standard.

Dissolve 100mg of glucose powder into 100ml of 0.15 % (wt/vol) benzoic acid, store at 5°C. Prepare standards ranging from 10 to 100*u*g/ml.

Table 4A Preparation of Carbohydrate Standard

Conc (*u*g/ml)	Stock solution (*u*g/ml)	ddH_2O(ml)
0	0	100
10	1	99
40	4	96
60	6	94
80	8	92
100	10	90



Regression Output

Constant	0
Std Err of Y Est	0.030546
R Squared	0.993683
No. of Observations	6
Degrees of Freedom	4
X Coefficient (s)	0.008603
Std Err of Coef.	0.000343

Figure 4A Carbohydrate standard working curve

5.2 Analysis

1. To 1ml sample add 1ml of phenol reagent;
2. Shake on a whirl mixer;
3. Add 5ml Sulphuric acid and allow to cool for 15minutes;
4. Measure the absorbance at 488nm.

6. LIPID ASSAY

1. Measure out 10ml sample to a clean porcelain evaporating dish;
2. Heat at 100°C for an hour in an oven;
3. Homogenise the solids contained in evaporating dish by adding 1ml methanol;
4. Add 2ml of chloroform and continue homogenisation for 2min;
5. Centrifuge at 5000rpm for 3-5 minutes the mixture, when solids remaining is resuspended in chloroform-methanol (2:1 by volume), and homogenise for 3min;
6. Centrifuge again and rewash with fresh solvent;
7. The combined supernatants are then transferred to a test tube;
8. Add one fourth of the total volume of 0.88% KCl;
9. Draw off the aqueous (upper) layer, the solvent contained in the bottom layer containing the lipid is removed on a rotary evaporator;
10. Lipid content is then determined by weight.

7.0 SOLIDS DETERMINATION

Total Solids

This is the measurement of all solid material (both soluble and suspended) in the effluent.

Soluble Solids

This is the measurement of solids dissolved in the aqueous phase of the effluent and water. The concentration of soluble is determined by centrifugation at 5000rpm for 15minutes to remove large solid material and filtration through a GF/C microfibre glass filters.

Total Suspended Solids

Total Suspended solids are the solids suspended and floating in the effluent.

7.1 Preparation of Evaporating Dish

1. Heat clean porcelain evaporating dish at 105°C for an hour in an oven;
2. Place the dish in a desiccator and allow to cool to room temperature;
3. Store in desiccator until needed, and weigh immediately before use.

7.2 Analysis

1. Switch on a steam bath and weigh evaporating dish immediately before use;
2. Transfer 10ml of the filtrate or supernatant solution to weighed evaporating dish;
3. Dry at 105°C in an oven for an hour;

4. Place the dish in a desiccator and allow to cool to room temperature and re-weigh.

API-ZYM

Principle : The API-ZYM^{TN} gallery is composed of microtubes, the bottom of which forms a support especially designed to contain the enzymatic substrate. This support allows the contact of between the enzyme and insoluble substrate.

EQUATIONS

1. Dilution Rate

$$D \text{ (per day)} = F/V$$

2. Hydraulic Retention Time (HRT)

$$HRT \text{ (per day)} = V/F$$

V = Volume of Anaerobic Reactor (Litre), **F** = Feeding rate(Litre per day)

3. Total and/or Dissolved Solids

$$(mg/L) = \frac{(B - A) \times 100 \times 10\,000}{a}$$

Total Suspended Solids = Total Solids - Total Dissolved Solids

A = weight of dried sample + dish (g), **B** = weight of dish (g) , **a** = aliquot sample (ml)

APPENDIX III : PUBLICATIONS

Molipane NP, Hart O, Boshoff G, Duncan J & Rose PD (1998) Biological removal of sulphate and heavy metals from synthetic mine drainage wastewaters utilizing sewage sludge as a carbon source. Water Institute of Southern Africa Biennial Conference, Cape Town.