KINETICS OF THE CHEMICAL AND BIOLOGICAL IRON (II) OXIDATION

by

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SUMMARY

The oxidation of pyrite in the presence of oxygen, water and acidophilic chemolithotrophic iron oxidising bacteria, of the species *Thiobacillus ferrooxidans (T. ferrooxidans)*, leads to the formation of large volumes of Acid Mine Drainage (AMD). AMD is a major environmental pollution problem in coalmines and coalmine dumps in South Africa. Measures to control acid mine drainage include the treatment of acidic effluents by applying limestone neutralization. Prior to limestone neutralization of acid water, iron (II) concentration needs to be oxidized first to iron (III) to prevent downstream oxidation and the formation of acid.

The study presented here concentrated on the different parameters and conditions influencing the iron (II) oxidation rate, both chemically and biologically. The effect of temperature, pH, air concentration, support media, number of iterations, iron (II) concentration, support media concentration, surface area, nutrients, CO_2, HRT and flow rate were investigated.

It was found that, the use of support media had no effect on the chemical iron (II) oxidation rate. When iron (II) was oxidised biologically under batch test, the highest oxidation rate was established when geotextile (GT) was used as the support medium and the initial concentration was 2 g/L. Operating under continuous feeding conditions resulted in the highest oxidation rate when the initial iron (II) concentration varied from 4.5 to 4.8 g/L, GT was used as the support medium and nutrients were added to the reactor. It was established that the removal rates were dependent on the HRT and the optimum HRT was achieved at 8 h.

The conclusion of this study was that the chemical iron (II) oxidation rate depends on the concentration of the sludge ($CaCO_3$). It was also concluded that the biological iron (II) oxidation rate was dependent on the bacterial growth, and that the growth of the micro organisms was influenced by the different parameters.

LIST OF ABBREVIATIONS

AMD	:	Acid Mine Drainage
GT	:	Geotextile
HRT	:	Hydraulic Retention Time
T. ferrooxidans:		*Thiobacillus ferrooxidans*
d	:	day
t	:	time
L	:	litre
mL	:	millilitre
conc.	:	concentration
h	:	hour
min	:	minute
A.F	:	Air flow

CHAPTER 1
INTRODUCTION

South Africa is particularly rich in mineral resources and has been one of the leading raw material exporters in the world for more than a century. The mineral industry has made an important contribution to the national economy, has achieved a high degree of technical expertise and has the ability to mobilize capital for new development.

The main raw minerals are gold, coal, diamonds, platinum, chromium, vanadium, manganese, uranium and iron ore. Coal is second only to gold in the economy of South Africa in total sales value and foreign exchange earnings. Coal continues to play a major role in the production of essential products for development such as electricity, liquid fuels and chemicals (South Africa Department of Minerals, 1998).

The requirements for effective control of water in the mining industry are increasing as mines become deeper and larger and as the environmental controls became more stringent. Mining is implicated as a contributor to water pollution, because most of the geological formations, which are mined, contain pyrites. Mines that contain ore rich in pyrites often produce poor quality water with a low pH and a variety of metal ions. Pyrite is formed from the reaction of sulphur with ferrous sulphide (FeS) to form a highly insoluble crystalline structure, which is very common in bituminous coals and many ore bodies (Brock and Madigan, 1991). Pyrite, when exposed to air and water, produces water high in iron and sulphate concentrations, generally referred to as Acid Mine Drainage (AMD), shown in Plate 1.

Thomas (1970) described AMD as one of South Africa's major pollution problems. It is primarily associated with the mining of coal, although the mining of ores containing other minerals can also lead to the formation of acid drainage (Walsh, 1978). Sulphuric acid is the major pollutant, resulting in mine water with a pH as low as 1.0 in certain instances. The release of acid drainage into water systems increases the acidity, the hardness, the metal ion and suspended solids concentrations.

Lundgren *et al.* (1972) estimated that 3.6 x 10^9 kg of acid is produced each year in South Africa. It was also noted that 90% of the produced pollutants are released into water systems. In addition, concentrations as high as 2.3 x 10^6 kg acid per day were observed in certain mining effluents (Walsh, 1978).

Plate 1: Acid Mine Drainage

AMD is a serious problem since mixing of acidic mine water with natural waters in rivers and lakes can cause severe degradation in the quality of the natural water bodies. This can be ascribed to the fact that both the acid and the dissolved metals are toxic to aquatic life. Such polluted waters are unsuitable for human consumption and industrial use. Collecting and treating mine water to a quality where it can be re-used without restrictions can prevent this source of pollution. Traditionally, iron (II) rich acid mine water was treated with lime using the High Density Sludge (HDS) process. Iron (II) is oxidised at a fast rate through aeration when the pH is raised with lime to pH 7.2 and higher. Recently, lime was replaced with limestone, which has a number of advantages, as:

1. limestone is more readily available,
2. the process control is simplified as no pH-control is required since limestone dissolution essentially occurs at pH-values below 7,
3. limestone is non-hazardous and easy to store.

The raw material can be stockpiled in the open air because $CaCO_3$ is not readily soluble in neutral water. The pH of iron (II)-rich water can only be raised to pH 6 using limestone. During treatment of acid mine water, the iron (II) component needs to be oxidised to iron (III), in order to prevent downstream oxidation of iron (II), which will result in re-acidification of the treated water when exposed to the atmosphere. The following processes were developed to achieve iron (II) oxidation without raising the pH to 7.2.

- **The integrated iron (II) oxidation and limestone neutralization**

In this process, powdered calcium carbonate together with aeration is used to oxidise iron (II) to iron (III), to neutralize acid water and to allow for gypsum crystallization in one reactor (Maree, *et al.*, 1999). In this process the milled limestone (particle size less than 0.1 mm) precipitated to calcium carbonate (e.g. a by product produced by the paper industry).

- **The fluidized-bed limestone neutralization**

In this process crushed limestone (particle size less than 4 mm) is used for neutralization of acid water in a fluidised-bed reactor after iron (II) has been oxidised to iron (III) at low pH. This oxidation process is needed as limestone particles are scaled with a layer of ferric hydroxide and gypsum when iron (II) rich water is fed directly to the limestone neutralization plant. Complete neutralisation of discard leachate containing, 10 g/L $CaCO_3$ and 4 g/L iron (II) can be achieved in a limestone neutralisation fluidised-bed reactor, provided that the iron (II) is oxidised beforehand (Maree, *et al.*, 1998a). This can be achieved through biological iron (II) oxidation at low pH. It was shown that the iron (II) oxidation rate is related to the surface area of

the support medium: when plastic medium (surface area 200 m^2/m^3) was used as support medium a reaction time of 18 h is required to oxidise 4 g/l iron (II) to iron (III) (Maree, *et al.*, 1998b).

1.1 APPLICATIONS FOR IRON (II) OXIDATION

Iron plays an important role in environmental and industrial processes as indicated below:

- Pyrite oxidation in ore. The pyrite oxidation reaction occurs underground during or after mining activities and on the surface of mine dumps that contain pyrite. Pyrite oxidation can occur through direct bacterial attack or through an indirect chemical bacterial mechanism whereby ferric iron oxidizes the pyrite (Silverman, 1967) (Reaction 1):

$$2FeS_2(s) + 2H_2O(\ell) + 7O_2(g) \rightarrow 2FeSO_4(s) + 2H_2SO_4(aq) \quad (1)$$

- Bacterial leaching of gold and other minerals from pyrites rich ore.

- Desulphurization of coal. The microbial oxidation of pyrite present in coal can be represented by the following reaction

$$4FeS_2(s) + 15O_2(g) + 2H_2O(\ell) \rightarrow 2Fe_2(SO_4)_3(s) + 2H_2SO_4(aq) \quad (2)$$

  The ferric ions can oxidise pyrite to ferrous ions and sulphate ions. The ferrous ions formed can be oxidized to ferric ions by the by *T. ferrooxidans* bacteria, and these ferric ions react with more pyrite. Thus there is a progressive, rapidly increasing rate at which pyrite is oxidized, called the *propagation cycle.*

- Metal bioleaching (Nemati *et al.*, 1998). Microbial leaching is generally applicable to both metal sulphides and uranium ores in a slightly acidic

aqueous sulphate solution. The dissolution of metal sulphides is achieved by a series of reactions involving both direct and indirect mechanisms. An important reaction that describes the dissolution of metal sulphide (MeS) ore in an acidic, ferric sulphate medium is:

$$MeS(aq) + Fe_2(SO_4)_3(s) \rightarrow MeSO_4(s) + 2FeSO_4(s) + S(s) \quad (3)$$

- Sulphur production from H_2S. H_2S gas is produced during anaerobic treatment of sulphate-rich water, during oil refinery and by the coal-to-fuel-industry. Sulphur can be produced from H_2S by placing H_2S gas in contact with an iron (III) solution (Reaction 4). The produced iron (II) can be back oxidised to iron (III) at low pH (Reaction 5)

$$H_2S(g) + 2Fe^{3+}(aq) \rightarrow S(s) + 2Fe^{2+}(aq) + 2H^{+}(aq) \quad (4)$$

$$2Fe^{2+}(aq) + 2H^{+}(aq) + O_2(g) \rightarrow 2Fe^{3+}(aq) + H_2O(aq) \quad (5)$$

- Desulphurization of sour gasses and flue gasses (Nemati *et al.*, 1998). Hydrogen sulphide is a highly undesirable component of natural gas and biogas. Removal of H_2S from sour gas is required for reasons of health, safety and corrosion during transmission and distribution, and to prevent sulphur dioxide pollution upon combustion of the gasses. At present, well-established physicochemical techniques for the removal of H_2S dominate the market. These include treatment with alkanolamine and the Clauss processes. The continuing search for more economical processes has led to investigation into microbiological solutions for purifying H_2S and gasses containing SO_2 as well as coal and petroleum. Microbiological processes operate around ambient temperature and atmospheric pressure, thus eliminating the need for heat and pressurization power, cutting the energy cost to a minimum. The relatively high chemical, catalyst and disposal cost of conventional processes are important drawbacks, which may be overcome partly in a biological process as described above.

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CHAPTER 2
THEORETICAL BACKGROUND

2.1 ORIGIN OF AMD

The oxidation of pyrite minerals in ore bodies, by primarily *T. ferrooxidans*, results in the high levels of acidity and the concentration of heavy metal pollutants in the mine drainage. AMD is a common environmental problem in coal mining areas due to the activity of *T. ferrooxidans* (Kleinmann & Crerar, 1979). Coal mined in South Africa contains approximately 3% pyrites (Wagner and Van Niekerk, 1987). It is estimated that about 200 ML/d of acid water is produced in the Gauteng area (Volman, 1984).

2.2 EFFECT OF AMD

Acid water discharged into surface water contributes to the following:

- Salination of surface water: Destruction of river water quality with mine water pollution can render it unsuitable for industrial and potable water supply and unsuitable for irrigation.
- Corrosion: Acidic effluents containing chloride and sulphate ions can lead to corrosion of pipelines.
- Aquatic life: The acidic water from mining industries is harmful to plants and fish life because of its low pH and high concentrations of heavy metals (Hoehn and Sizemore, 1977). Acid mine water can have a pH as low as 2.5 (Barnes and Romberger, 1968).

2.3 TREATMENT OF AMD

Acid water can be treated by the following processes:

- Physical processes, e.g. slurry precipitation, recycle and reverse osmosis (SPARRCO), (Chamber of Mines Research Organization, 1998), gypsum crystallization, ion exchange processes (CHEMEFFCO, undated) and electrodialysis.
- Chemical processes, e.g. precipitation with barium salts, lime (conventional and High Density Sludge (HDS) process) and limestone neutralisation.
- Biological processes, e.g. active and passive biological sulphate removal technology (Bock, *et al.*, 1994, Maree, *et al.*, 1988b and Pulles, 2000).

2.3.1 Chemical treatment of AMD

2.3.1.1 Soluble barium salts treatment

Maree, *et al.,* (1990) described the barium process as an attractive possibility for wide scale application. The chemical treatment technology can be applied directly to acid water. The principle of this technology finds its use in the precipitation of sulphate with barium chloride and results in the removal of ammonia, magnesium, manganese and other heavy metals. The advantage is that by-products like sulphur and NaHS can be derived from H_2S produced during the H_2S stripping stage, and $CaCO_3$ from the softening stage (Adlem, 1991; Bosman *et al.*, 1990; du Preez and Maree, 1994 and Maree *et al.*, 1989).

2.3.1.2 Lime treatment

Traditionally, industries like mining, edible oil, explosive, steel and metal finishing were neutralizing their acidic effluents by treating them with lime. Thompson (1980) reported that the best results were achieved when acid effluent was neutralized with hydrated lime, i.e. calcium hydroxide. Neutralization with lime was applied through the conventional and high density sludge (HDS) processes.

During lime neutralisation in the HDS process, the optimum process configuration is determined by the chemical composition of the feed water, in particular the ratio of iron (II) to iron (III). Should the iron (II) oxidation rate be reduced to less than 1 h, it

will benefit both the limestone and the lime neutralisation technologies. In the case of lime neutralisation, the preferred modified HDS process can be used, which has good lime utilisation efficiency.

Osuchowski, (1992) reported that, when the HDS process was applied, sludge of a density, 10 times higher than that of the conventional process, was produced. The sludge settled faster and was recycled back into the system, using the HDS process.

2.3.1.3 Limestone treatment

Juby, *et al.*, 1985 reported that the gold mining industry in South Africa used 700 000 tons of lime per year for the neutralisation of acidic underground mine water. Therefore, neutralizing AMD with limestone rather than using lime can be considered a cost effective option because limestone is cheap, safe and easy to handle, for example, limestone costs R0.10/kg and lime costs R0.28/kg.

By using the fluidised-bed reactor for limestone neutralisation, the main weaknesses of limestone, such as its low reactivity, scaling with gypsum and hydroxide precipitates that prevented the limestone from being used on a wide basis in the past, can be solved (Maree, *et al.*, 1996). The problem of long reaction time as a result of the low reactivity of limestone is solved in the fluidised reactor because an excessive amount of limestone is in contact with the acid water. Scaling of limestone particles is prevented due to the attrition between the particles under fluidised conditions.

2.4 IRON (II) OXIDATION

Iron is one of the most abundant elements in the earth's crust but is a relatively minor component in aquatic systems because of its relative insolubility in water. It also plays an important role in the mobility and toxicity of other trace elements associated with watersheds affected by abandoned mine lands (Madigan, *et al.*, 1997). Iron exists in two oxidation states, ferrous ion and ferric ion. The form in which iron is found in nature is greatly influenced by pH and oxygen. Oxygen is the only

electron acceptor to oxidize iron (II) (Brock and Madigan, 1991). Iron (II) can be oxidized to iron (III) chemically (reaction 6-9) and biologically (reaction 6-7) underpinned by the following reactions:

$$2Fe^{2+}(aq) + \tfrac{1}{2}O_2(g) + 2H^+(aq) \rightarrow 2Fe^{3+}(aq) + H_2O(l) \quad (6)$$

$$2Fe^{3+}(aq) + 6H_2O(l) \rightarrow 2Fe(OH)_3(s) + 6H^+(aq) \quad (7)$$

$$6H^+(aq) + 3CaCO_3(s) \rightarrow 3Ca^{2+}(aq) + 3CO_2(g) + 3H_2O(l) \quad (8)$$

$$3SO_4^{2-}(aq) + 3Ca^{2+}(aq) \rightarrow 3CaSO_4(s) \quad (9)$$

2.4.1 Chemical Fe (II)-oxidation

Around pH 6.0 – 7.0, ferrous iron is not stable in the presence of oxygen and is rapidly oxidised non-biologically to the iron (III) state. This oxidation is influenced by suspended solids, which act as a catalyst (Maree *et. al.,* 1999).

2.4.2 Biological Fe (II)-oxidation

Biological Fe (II) oxidation is catalysed by *T. ferrooxidans* bacterium at low pH. *T. ferrooxidans* was first isolated by Colmer, *et al.,* (1947) from highly acid water which was produced in an exposed bituminous coal mine. This organism is very common in acid polluted environments, where sulphuric acid is the dominant acid together with a large amount of sulphate. At low pH values, ferric iron does not precipitate as hydroxide but as a complex sulphate mineral called jarosite. Jarosite is a yellowish or brownish precipitate which is responsible for one of the manifestations of acid mine drainage, an unsightly yellow stain called "yellow boy" by U.S. miners (Madigan, *et al.*, 1997). It was noted that *T. ferrooxidans* might accelerate the iron (II) oxidation rate reaction by a factor of 10^6 or more.

2.4.2.1 Characteristics of T. ferrooxidans

T. ferrooxidans bacteria are gram-negative nonsporulating rods, 0.5-0.6µm long, with round ends, occurring singly or in pairs, rarely in short chains. They are motile by

means of a single polar flagellum (Buchanan and Gibbons, 1974). The species is generally characterised by the following properties:

1) Chemolithotrophic (obtaining its energy from the oxidation of inorganic compounds);
2) Autotrophic (utilizing CO_2 as the sole source of carbon);
3) Aerobic (growing in the presence of O_2, may be facultative, obligate or microaerobic);
4) Mesophilic (living in the temperature range near that of warm blooded animals);
5) Acidophilic (growing best at acidic pH values).

2.4.2.2 Growth kinetics and the effects of physicochemical parameters

Studies (M. Nemati and C. Webb, 1996) showed that the growth of *T. ferrooxidans* and its ability to oxidise ferrous iron are highly dependent on pH, temperature and the concentration of ferrous iron.

❖ pH

With ferrous iron as the energy source, *T. ferrooxidans* bacteria are able to grow in an environmental pH between 1.0 and 6.0 with an optimum between 2.0 and 2.5.

❖ Temperature

Most strains of *T. ferrooxidans* characterized with respect to temperature are mesophilic with temperature optima between 20°C and 40°C. The optimum temperature is said to be pH dependent, showing a lower optimum temperature with decreasing pH (Ahonen, *et al.*, 1989).

❖ Concentration of ferrous iron

The growth of *T. ferrooxidans* and its ability to oxidise ferrous iron is significantly influenced by the ferrous iron concentration of its environment.

❖ Carbon dioxide

T. ferrooxidans is a chemoautotroph with a requirement for carbon dioxide as its cellular carbon source.

❖ Oxygen

T. ferrooxidans is an obligate aerobe, which requires oxygen as an electron acceptor for growth.

Table 1 summarises the biological oxidation rates of ferrous iron achieved in different bioreactor configurations with free or immobilized cells of *T. ferrooxidans*, as well as operational conditions of the different studies. A comparison of the kinetic data achieved in the systems utilizing freely suspended cells with those obtained in immobilized cell bioreactors reveals that the immobilization of *T. ferrooxidans* has led to a significant improvement in biological oxidation of ferrous iron. The improved oxidation rates are mainly due to the higher concentrations of biomass within the immobilized cell bioreactor. Indeed, the immobilized bacteria are usually resistant to the washing out of the bioreactor.

The data presented in Table 1 also indicate the superiority of packed-bed bioreactors among the various bioreactor configurations. Strong shear effects in the fluidised-bed bioreactors and rotating biological contactors is a counterforce which effectively reduces the formation of *T. ferrooxidans* biofilms and results in a lower biomass hold-up within the bioreactor. As a consequence a lower efficiency with respect to oxidation of ferrous iron is usually observed in these bioreactors.

Table 1: Kinetics of bio oxidation of ferrous iron achieved in various bioreactor configurations with free or immobilized cells of *T. ferrooxidans*

Bioreactor	pH	Temp. (°C)	Support media	Oxid. rate g Fe/L d	References
Chemostat-free cells	2.2	28	-	10.1	MacDonald & Clark 1970
Chemostat-free cells	2.3	32	-	12.7	Guay *et al.*, 1977
Chemostat-free cells	1.8	30	-	5.04	Smith *et al.*, 1988
Chemostat-free cells	1.3	30	-	18.48	Halfmeier *et al.*, 1993
Packed-bed	1.5	25	Calcium alginate	29.3	Lancy & Tuovinen, 1984
Packed-bed	1.3	23	Glass beads	79.2	Grishin & Tuovinen, 1988
Packed-bed	1.8	30	Siran-glass ring	86.4	Halfmeier *et al.*, 1993
Trickle-bed	2.3	28-30	Polyurethane foam BSP	105.6	Nemati and Webb, 1996
Inverse-fluidised bed	1.3-2.2	13-38	Expanded polystyrene	37.9	Karamanev and Nikolov, 1988
Fluidised-bed	1.3-1.5	23	Activated carbon	37.9	Grishin & Tuovinen,1988
Circulating-bed	2.3	28	Polyurethane foam BSP	37.4	Armentia and Webb, 1992
Rotating biological contactor	2.6-3.2	9-12	Polyethylene	8.16	Olem and Unz, 1977
Rotating biological contactor	1.0-2.6	10-40	Polyvinyl chloride	18.7	Nakamura et.al., 1986
Rotating biological contactor	2.0-2.5	18	Polyvinyl chloride	33.6	Nikolov *et al.* 1986

(M. Nemati *et al.* Biochemical Engineering Journal I (1998))

2.4.2.3 *Immobilisation of the microorganisms*

During the past years, process engineering of the aspects of iron (II) oxidation have been studied extensively (Loi, *et. al.* 1993). These studies have been aimed mainly at improving the rate of ferrous iron oxidation, the principal factor affecting the cost effectiveness of industrial processes. Several experimental systems with batch and continuous flow modes of operation have been used and various reactor types designed, trying to obtain better results. The natural propensity of *T. ferrooxidans* to grow on surfaces makes it an ideal organism for cell immobilisation. The use of immobilised cells, leading to high and stable cell concentration within the bioreactor has been addressed as a promising method for improving reactor performance (M. Nemati and C. Webb, 1996).

Various immobilisation methods, including the use of glass beads, ion exchange resin, activated carbon, sand and polystyrene particles as carriers for passive immobilisation of *T. ferrooxidans* (Grishin and Tuovinen, 1988, Karamanev and Nikolov, 1988, Halfmeier *et al.*, 1993) have been used. The carrier may exert a catalytic effect on oxidation or have surface properties that accelerate the bacterial adsorption and biofilm formation. The concept of biomass support particles (BSP) introduced and patented by Atkinson, *et al.* (1978, 1980), involves providing a structure within which the organism can grow. The development of a wide variety of applications has been reviewed by Webb and Dervakos (1996). Polyurethane foam BSP has been shown to be a suitable support for the passive immobilisation of *T. ferrooxidans* cells (Armentia and Webb, 1992, Nemati and Webb, 1995). In addition to known immobilisation materials, GT (a firm cloth used in the road construction) can be used as a novel immobilisation agent because it has marked advantages, for example, a high surface area.

2.5 RESPIROMETRY

2.5.1 Description

Respirometry is a method used to measure the metabolic activity of cells. Commercial respirometers fall into three general classifications: manometric, either constant volume or pressure; dissolved oxygen depletion devices; or oxygen replacement systems. The basic principle of a manometric respirometer is the determination of the oxygen weight changes in a closed system by measuring or responding to pressure changes at constant temperature and volume or volume changes at constant temperature and pressure. A dissolved oxygen depletion device uses an oxygen sensitive probe to take measurements of the depletion of oxygen from solution or headspace gases. Oxygen replacement systems measure the oxygen uptake rate by adding very small increments to the reactor in response to small pressure changes due to oxygen uptake. The sum of the incremental addition forms the cumulative oxygen uptake curve.

Respirometers are classified by their use for either batch tests or continuous monitoring. Batch test respirometers are used in laboratory settings in which samples are placed into reactor vessels and allowed to incubate (water bath) for periods ranging from a few minutes to months. Continuous monitoring respirometers are used for on-line instruments, which yield a rapid response to an input waste stream or chemical dose. Laboratory instruments normally are manometric or oxygen depletion measurements. Only batch type respirometers will be covered in this study.

Operational procedures for respirometers vary widely, in general the output of a respirometer is a continuous curve of oxygen uptake. The respirometric measurements require undiluted sample and continuous mixing of the samples to provide uniform contact between microorganisms, substrate and oxygen. The oxygen uptake characteristics are measured in a natural state.

2.5.2 Advantages of using respirometry

The Micro-Oxymax^{TM} Respirometer, is an automated instrument that can run 10 samples simultaneously under different experimental conditions (sample and nutrient concentrations, pH, temperature, volume, etc) using the same oxygen and carbon dioxide gas sensors. The instrument requires a small sample volume and gives rapid results. A real time graphic display of the measured parameters (gas consumption or production rates, total gas consumed or produced and the respiratory exchange rate) allowing continuous monitoring of the experiment is also provided.

2.5.3 Application

Different processes can be studied using the respirometer, for example, sludge and wastewater treatment, BOD measurements, biotoxicity and biosupplement testing, biofilm and soil respiration, bioremediation monitoring and yeast bacteria contamination

2.6 KINETICS OF IRON (II)-OXIDATION

Du Preez and Maree (1994) showed that iron (II) can be oxidised to iron (III) in the presence of acidophilic iron-oxidising bacteria, such as *Ferrobacillus ferrooxidans*, and precipitated as $Fe(OH)_3$ at pH values greater than 3 (equation 10):

$$2Fe^{3+}(aq) + 6H_2O(\ell) \rightarrow 2Fe(OH)_3(s) + 6H^+(aq) \quad (10)$$

Maree, *et al.* (1998a) showed that:

- The relationship between the iron (II) oxidation rate and the specific surface area is given by the equation:

$$-d[Fe^{2+}]/dt = 0.21 \times S^{½}$$

where S = Surface area in m^2g

- Iron (II) (2g/L) was oxidised effectively under continuous conditions in a plastic medium filter at an iron (II) oxidation rate of 2 g Fe/(L.d).

- Ferric hydroxide precipitated slowly on the plastic medium as a result of the solubility of ferric hydroxide that is exceeded during iron (II) oxidation under acidic conditions.

It was showed that, through redox potential measurements, that the biological oxidation of ferrous iron is governed by the following rate equation:

$$d[Fe^{2+}]/dt = 1.62 \times 10^{11}[X][H^+][Fe^{2+}]\ PO_2e^{-58.77/RT} \quad (11)$$

where

[X] = concentration of bacteria
$[H^+]$ = concentration of hydrogen ions
$[Fe^{2+}]$ = concentration of ferrous iron
PO_2 = partial pressure of oxygen
R = universal gas constant
T = absolute temperature

The equation applies to atmospheric conditions at $[Fe^{2+}] < 0.056$ kg/m^3, T < 25°C and at pH values above 2.2. Below pH 2.2, the oxidation rate was shown to be independent of H^+ concentration.

Nemati and Webb (1997) studied the kinetics of ferrous ion bio-oxidation by *T. ferrooxidans*. Effects on the rate of the reaction were determined for ferrous ion concentration in the range 0.25 to 30 kg/m^3, bacterial concentration in the range 3.25 x 10^7 to 4.5 x 10^8 cells/mL and temperature ranging from 20 to 35 °C. Applying the experimental data and an approach based on Michaelis-Menten kinetics they proposed the following model for bacterial oxidation of ferrous ion:

$$\frac{d[Fe2+]}{dt} = \frac{K_0 e^{-Ea/RT}[X][[Fe^{2+}]}{K_m\{1 + [X]/K_i\} + [Fe^{2+}] + \{1 - [X]/\beta\}[Fe^{2+}]^2/\alpha}$$

where,

$[Fe^{2+}]$ = ferrous ion concentration, kg/m^3

X = bacterial concentration, cells/ml

T = temperature, °K

The model, that excluded the effects of temperature, ferrous ion and bacterial concentrations, also incorporated terms for substrate and cell inhibitions. Values for the terms in the models were as follows:

$K_0 = 6\ 438\ kg\ m^{-3}\ h^{-1}/cells\ ml^{-1}$

$K_m = 0.0672\ kg/m^3$

$K_i = 2.68 \times 10^7$ cells/mL

$E_a = 68.4$ kJ/mol

$\alpha = 26.1\ kg/m^3$

$\beta = 7.8 \times 10^8$ cells/mL

Stumm and Lee (1961) determined the following relationship between the iron (II) oxidation rate and pH in the absence of micro organisms (equation 12).

$$-d[Fe(II)]/dt = k[Fe(II)][OH^-]^2PO_2 \qquad (12)$$

where

$-d[Fe(II)]/dt$	-	rate of iron (II) oxidation;
k		reaction rate constant;
[Fe(II)]	-	iron (II) concentration (moles/L);
$[OH^-]$	-	hydroxide concentration (moles/L);
PO_2	-	partial pressure of oxygen (mm Hg).

It appears (Maree *et al*, 1994) that iron (II) oxidation at pH levels less than 4 is catalysed by bacterial activity.

The relative importance of various factors in terms of their influence on the rate of iron (II) oxidation was determined by a series of controlled tests in which the dependence on the rate of one variable at a time was determined. The iron (II) oxidation rate was assumed to have the following functional form:

$$-d[Fe^{2+}]/dt = k[Fe^{2+}]^{n1}.[O_2]^{n2}.[SM]n3 \quad (13)$$

where .

$-d[Fe^{2+}]/dt$ or R	=	rate of iron (II) oxidation
k	=	reaction rate constant
$[Fe^{2+}]$	=	iron (II) concentration (moles/L)
$[O_2]$	=	oxygen concentration (moles/L)
SM	=	reactor surface area (m^2/m^3)

By varying the value of only one parameter in a series of experiments, say $[Fe^{2+}]$, equation 14 can be written as:

$$-d[Fe^{2+}]/dt = K[Fe^{2+}]^{n1} \quad \text{or} \quad \log(-d[Fe^{2+}]/dt) = \log K + n_1 \log [Fe^{2+}] \quad (14)$$

where $K = k[O_2]^{n2}.[SM]^{n3}$

The contribution, n_1, of iron (II), to the overall reaction rate was determined from the slope of the graph obtained by plotting log R versus log $[Fe^{2+}]$. It was pointed out that this behaviour is in line with the behaviour of iron (II) oxidation in the pH range 2 to 3 where the rate is directly proportional to the square root of the medium specific surface area (Maree *et al.*, 1998a).

2.7 PRECIPITATION OF FERRIC COMPOUNDS

Precipitation of ferric compounds results when *T. ferrooxidans* is grown on ferrous sulphate. The pH value initially increases due to the removal of acid when iron (II) is oxidized (reaction 15) and increase again due to hydrolysis of the ferric ion (reactions 16 to18).

$$4Fe^{2+}(aq) + O_2(g) + 4H^+(aq) \rightarrow 4Fe^{3+}(aq) + 2H_2O(\ell) \quad (15)$$

$$Fe^{3+}(aq) + H_2O(\ell) \rightarrow FeOH^{2+}(aq) + H^+(aq) \quad (16)$$

$$Fe^{3+}(aq) + 2H_2O(\ell) \rightarrow Fe(OH)_2^+(aq) + 2H^+(aq) \quad (17)$$

$$Fe^{3+}(aq) + 3H_2O(\ell) \rightarrow Fe(OH)_3(s) + 3H^+(aq) \quad (18)$$

A competing reaction for the hydrolysis is the formation of basic ferric hydroxysulphates (jarosites) with the general formula $MFe_3(SO_4)_2(OH)_6$, where M = K^+, Na^+, NH_4^+ or H_3O^+. Jarosite precipitation is also an acid producing reaction (reaction 19).

$$3Fe^{3+}(aq) + M^+(aq) + 2HSO_4^-(aq) + 6H_2O(\ell) \rightarrow MFe_3(SO_4)_2(OH)_6(aq) + 8H^+(aq) \quad (19)$$

The formation of jarosite is highly dependent on pH. Ferric ion precipitation has the following detrimental effects on biological iron (II) oxidation (Nemati and Webb, 1998):

- Iron (III) concentration in solution that serves as the leaching medium or adsorbent for H_2S is reduced.
- Precipitates in immobilization matrices may limit the amount of biomass retention by occupying the bulk of the available space.
- Precipitates create kinetic barriers because of the slow diffusion of reactants and products through the precipitation zone.
- Precipitates tend to block pumps, valves, piping and other equipment.
- The tendency for precipitates to cover mineral sites on ore particles adversely affects microbial leaching.

2.8 AIM OF THE STUDY

The aim of this study was to obtain improved iron (II) oxidation rates so that limestone neutralization can be applied to AMD.

The objective of the study was to determine the effect of the following parameters on the rate of iron (II) oxidation, chemically and biologically

1) Chemical oxidation (the pH is raised to 6.5 with the addition of $CaCO_3$)

- Type of support media (plastic pellets and sand). In this study sand and pellets were used as support media because these media do not easily scale up with $CaCO_3$ as compared to the other media, for example, GT.
- Support media concentration: 0, 50 and 100 g/L pellets were tested.
- Number of iterations: The aim was to observe the effect of an increase in concentration of $CaCO_3$ on the iron (II) oxidation rate.

2) Biological oxidation

The influence of the following parameters on the bacterial growth was determined:

- Type of support media: The following media (pellets, sand, plastic rings, coal discard, anthracite and geotextile) were tested because they provide a surface area on which the bacteria can adhere.
- Number of iterations (iteration is a repeat of the experiment with the same support medium): Iterations were increased to determine the formation of a biofilm on the support medium.
- Media concentration: Brown geotextile plates were used.

- Iron (II) concentrations: The iron (II) concentration was varied form 2 to 20 g/L.
- Surface area: The BET surface area analyser (Micromeritics Flowsorb II 2300) was used to measure the surface area of the media at different iterations.
- Nutrients: 2mL/L of the hydroponic nutrient powder which contains macro and micro elements was used.
- Hydraulic retention time: The range of HRT evaluated from 24 to 6h,

$$HRT = \frac{\text{volume of the reactor (L)}}{\text{flow rate (L/d)}}$$

- CO_2 concentrations: 3% of CO_2 was bubbled into a reactor vessel through a diffuser.
- Air flow: The air flow rate of 3, 5.6 and 8.9 mL/L was tested.
- pH: The pH 1.7, 2.0 and 2.3 was tested.
- Temperature: The temperature was varied from 25 to 30^{0}C.

CHAPTER 3
MATERIALS AND METHODS

3.1 FEEDSTOCK

During batch studies a synthetic iron (II) solution was used as feed water. The feed water contained: 10 g/L $FeSO_4.7H_2O$, 1.4 g/L H_2SO_4, 0.5 g/L $MgSO_4$, 0.1 g/L $(NH_4)_2SO_4$ and 0.01g/L H_3PO_4. Ferrous sulphate was technical grade (97.5%) whereas the remaining reagents were of analytical grade. The temperature was always maintained at 29 ^{0}C.

Hydroponic nutrients (Kompel Chemicult products) were used as macro and micro nutrients to synthetic iron (II) solution and the coal discard leachate. Table 2 shows the chemical composition of the various elements added to the iron (II) rich solutions.

Table 2: Chemical composition of nutrient solution

Element	Concentration			
	Hydroponic powder		Stock solution (2 g/L)	Feed stock (2 ml/L)
	%	mg/g	mg/L	mg/L
N	6.5	65	130	0.26000
P	2.7	27	54	0.10800
K	13.0	130	260	0.52000
Ca	7.0	70	140	0.28000
Mg	2.2	22	44	0.08800
S	7.5	75	150	0.30000
Fe	0.15	1.5	3	0.00600
Mn	0.024	0.24	0.4	0.00080
B	0.024	0.24	0.48	0.00096
Zn	0.005	0.05	0.1	0.00020
Cu	0.002	0.02	0.04	0.00008
Mo	0.001	0.01	0.02	0.00004

During the continuous studies acid coal discard leachate from the Toe Seep Dam at Navigation (Witbank) was used as feed water. The iron (II) concentration was 4.5 to 5 g/L and the pH was 2.0 (as described in 3.7).

3.2 LABORATORY EQUIPMENT

3.2.1 Batch studies

Plate 2 shows the 1L beakers and stirring mechanisms that were used for executing the batch studies. Plate 3 shows the 3L vertical and horizontal box reactors. The solutions in the beaker reactors were stirred continuously and aerated at a flow rate of 3L/min with compressed air through diffusers (porosity no. 2, 210 x 8mm (OD)). The air to the container reactors and box reactors was distributed through small holes punched into a perspex pipe situated at the bottom of the reactor.

3.2.2 Continuous studies

Plate 4 shows the reactor, which was used to conduct the continuous studies. It had a volume of 15L and a rectangular shape with 19 GT sheets as support medium. The GT sheets were supported in vertical positions by plastic frames. Compressed air was supplied through aerators to the reactors at a flow rate of 3L/min for each aerator.

3.2.3 Respirometry studies

Plate 5 shows the Micro-Oxymax^{TM} Respirometer (Columbus Instruments, Ohio, USA), used to monitor the bacterial respiration. Respirometry is a technique to measure the metabolic activity of cells. The biological oxidation, which is a function of the biomass respiration, can be shown by the O_2 uptake and CO_2 production. Advances in gas sensing and computer technology have resulted in a flexible, accurate and powerful tool to detect and interpret activity signals previously outside the range of classical methods. In the context of waste management, the monitoring

of biological activity can be applied to monitor microbial contamination and biodegradation. In the case of the Fe oxidising bacteria, oxygen is needed in order to carry out the oxidation process. The faster the oxidation process, the more oxygen is needed for respiration. The advantage of using the respirometer is that, 10 samples can be analysed simultaneously (Plate 6).

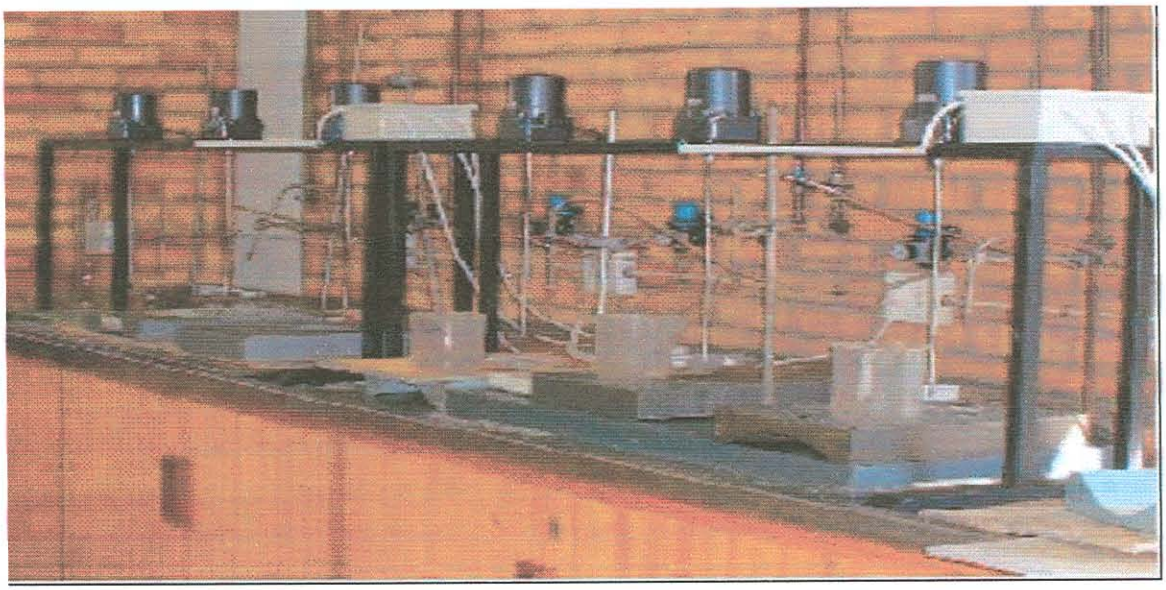

Plate 2: **Batch test conducted in 1L beaker reactors**

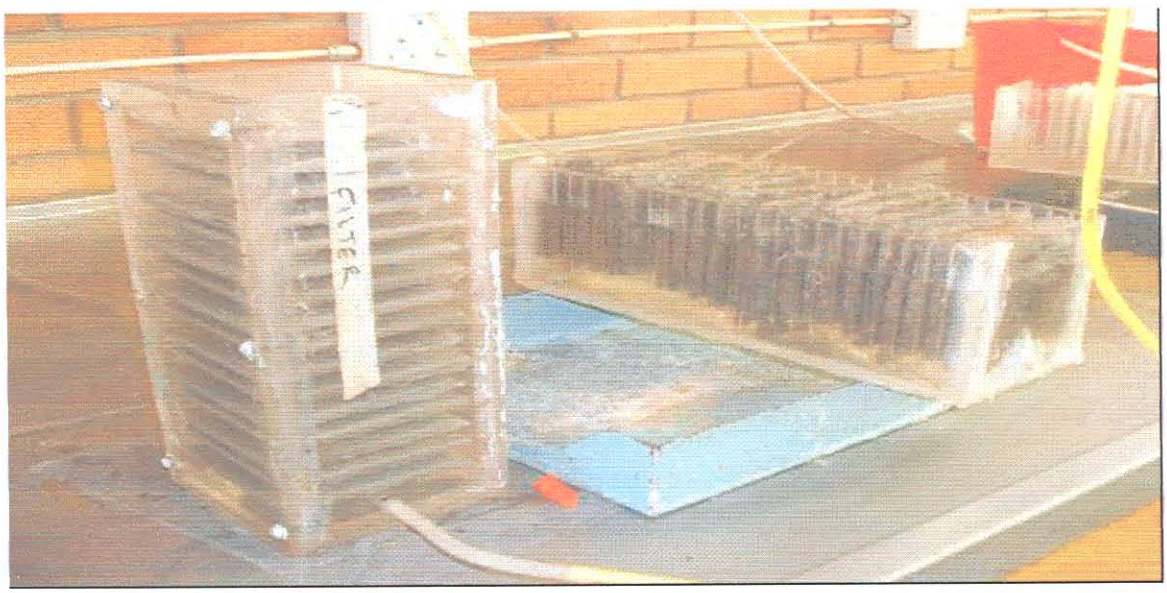

Plate 3: **Batch test conducted in 3L horizontal and vertical plate reactors**

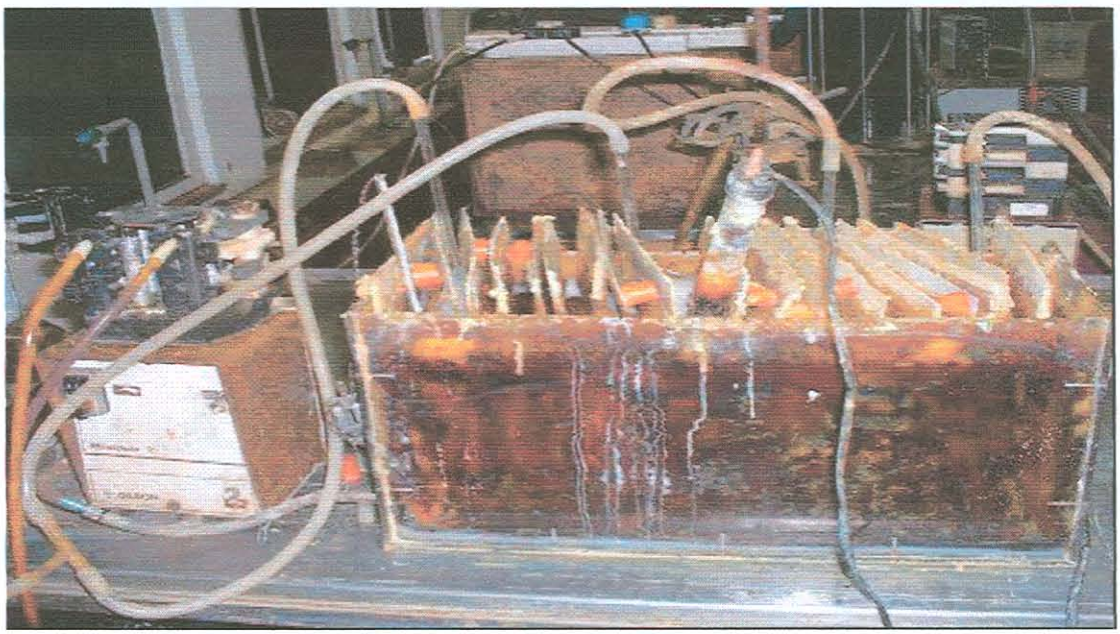

Plate 4: **Laboratory reactor that was used for continuous studies**

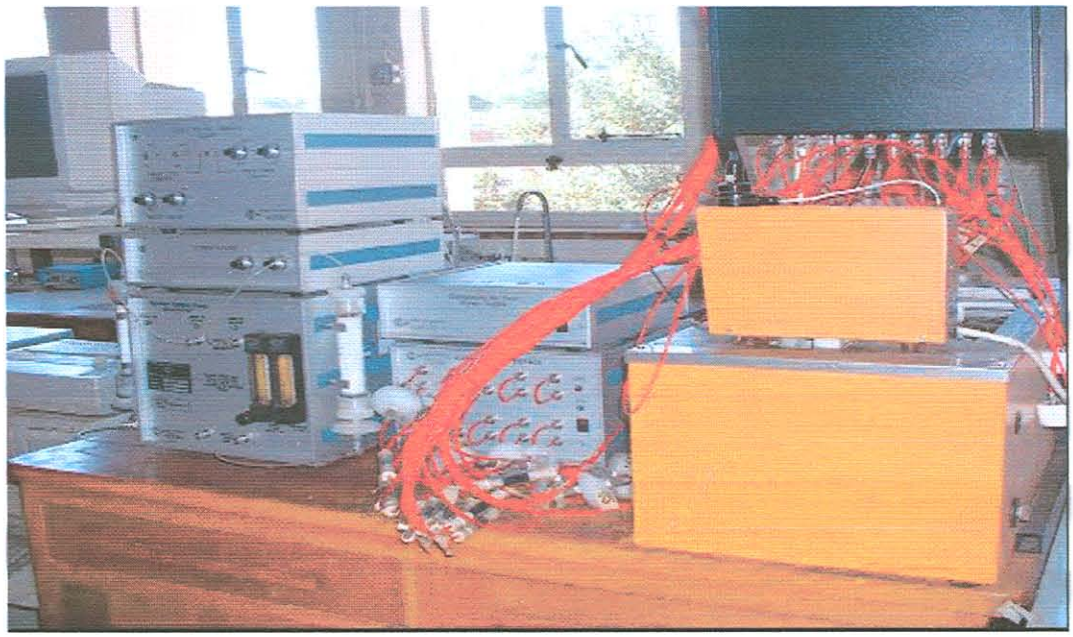

Plate 5: **Respirometer**

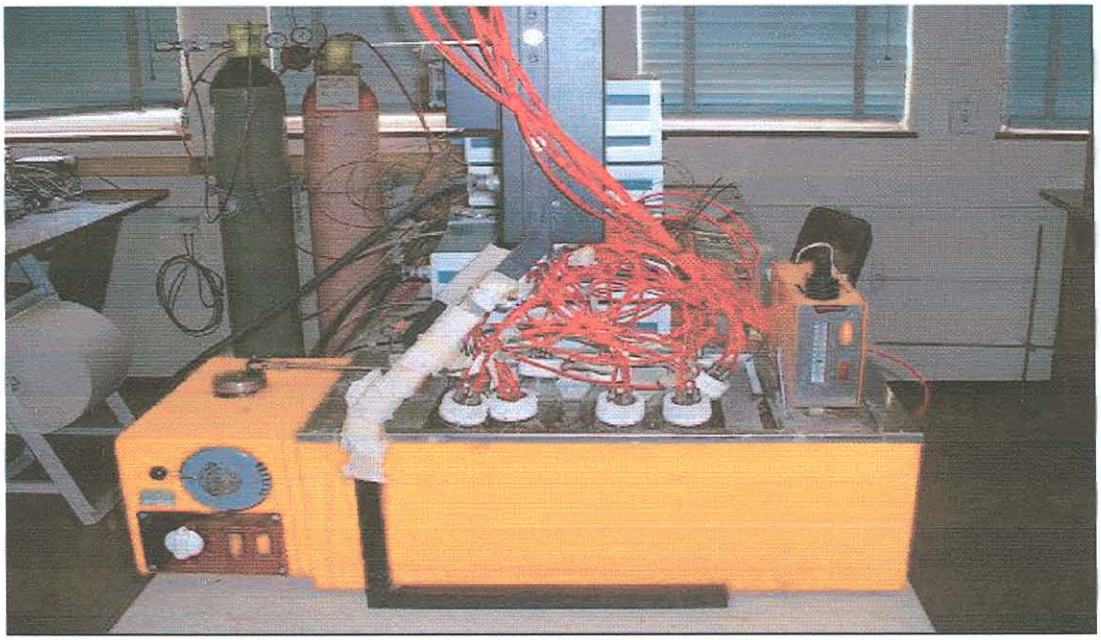

Plate 6: Reactor vessels

3.3 BIOMASS SUPPORT MEDIA

The following media were used to study the possible increase of the iron (II) oxidation rate:

- Sand (Plate 7). Normal building sand.
- Plastic rings (Plate 8). The plastic rings were cut from plastic piping of 5 mm diameter.
- Plastic pellets (Plate 9). The poly ethylene plastic pellets were used.
- Anthracite (Plate 10). Anthracite is a hard coal used for space heating and generating electricity.
- Coal discard (Plate 11). The "discard" is a coal of inferior quality due to its general high pyrites content. It was obtained from Navigation mine, Witbank.

- Geotextile (Plate 12). GT is a fabric made of synthetic material used in road construction and maintenance, where it is placed between the soil and a water pipe. It can also be used between a gabion or a retaining wall. GT is used for the separation or stabilization of two distinct layers of soil, drainage to filter water, erosion control and for reinforcement in situations where the foundation soils are too weak to support a road or other building structure. The fabric is divided into two types: woven (very strong not stretchable) and non-woven (stronger as thickness increases and stretch). The GT was mounted on a perspex plate using silicone.

Plate 7: Sand

Plate 8: Plastic rings

Plate 9: **Plastic pellets**

Plate 10: **Anthracite**

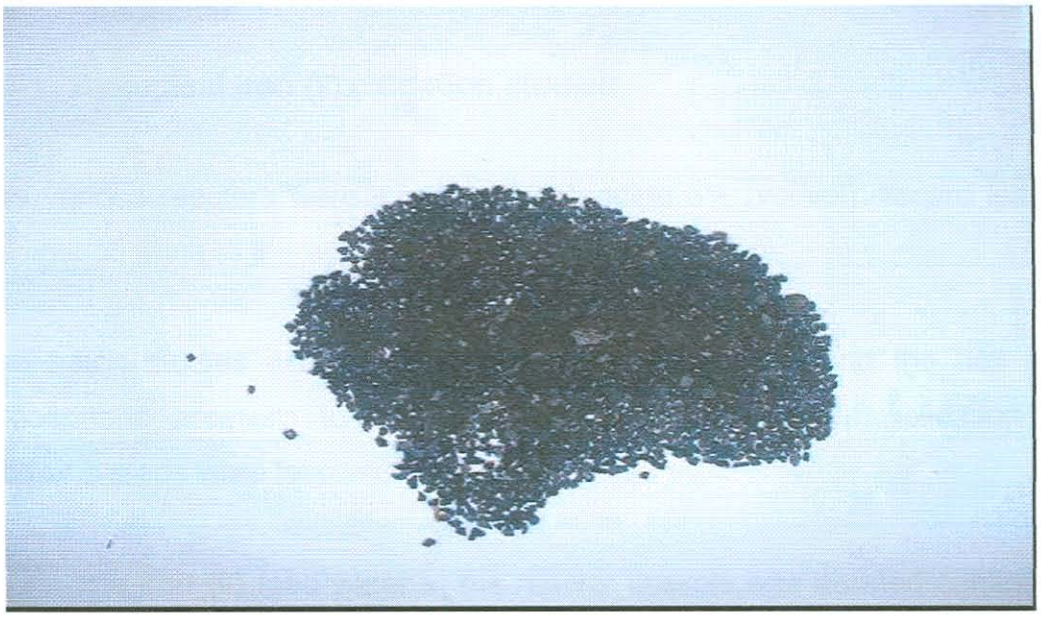

Plate 11: Coal discard

Plate 12: Geotextile mounted on a perspex plate

3.4 PROCEDURE

3.4.1 Biological iron (II) oxidation studies

The various reactors were inoculated by adding 5% (vol/vol) discard leachate from Navigation Mine to the water in the reactor vessels.

The batch studies were carried out using the beakers and the box reactors at atmospheric pressure to determine the biological iron (II) oxidation rate. The following steps were followed:

- The iron (II) feed water in the reactor vessels was aerated continuously until the iron (II) was completely oxidised to iron (III).
- Samples were taken at different intervals, filtered and analysed for the iron (II) concentration as well as the pH as described in 4.7.
- The iron (III) rich water in the reactor was discarded and replaced with fresh iron (II) rich feed water. The same support medium was used repeatedly during consecutive batch runs.
- The procedure was repeated for several iterations (iteration is the repeat of the experiment) until the reaction reached the optimum rate.

3.4.2 Chemical iron (II) oxidation studies

During the chemical studies, the same procedure was followed as in the biological studies. However, 7.8 g/L $CaCO_3$ was added at the start of each experiment in order to raise the pH to 6.5.

3.4.3 Respirometry studies

Ten temperature controlled test chambers were operated simultaneously. The gas concentration changes were monitored in the headspace of the enclosed test chamber (200 mℓ Schott bottle). The frequency at which the test chambers are refreshed by outside air or bottled gas is pre-programmed. For each measurement

the date and time, sample number, gas exchange rates, cumulative gas measurements at that time, incubation temperature and the respiratory exchange ratio are calculated. The data are stored on a disk, which can be processed with a spreadsheet programme after the experiments are completed. In order to maintain the dissolved gas concentration in equilibrium with the air above the sample, the reaction mixture was shaken with a linear shaking water bath for the duration of the experiment.

3.5 EXPERIMENTAL PROGRAMME

3.5.1 Chemical iron (II) oxidation studies

The effects of the following parameters were investigated:

- Support medium
- Media concentration
- Number of iterations

3.5.2 Biological iron (II) oxidation studies

The effects of the following parameters were investigated during batch and continuous studies:

- Support medium
- Number of iterations
- Support media concentration
- Iron (II) concentration
- Reactor type
- Nutrients
- CO_2
- Air flow
- pH

- Temperature

3.5.3 Respirometry studies

The effects of the following parameters were investigated:

- Support media
- Nutrients and microorganisms
- Iron (II) concentration

3.6 ANALYTICAL TECHNIQUES

Samples (20 mL) were taken at different intervals and filtered through a Whatman No 1 filter paper. The iron (II) determinations were carried as follows:
The 10 mL of 1N H_2SO_4 (dispenser), 10 mL Zimmerman Reinhardt (ZR) reagent (dispenser) and 10 mL sample (pipette) were added into the flask. The solution was titrated with a 0.1 N $KMnO_4$ until the colour appeared light pink and the titration value noted (Vogel, 1989). The pH determinations using a 691 Metrohm pH meter were carried out manually according to procedures described in Standard Methods (APHA, 1985).

The surface areas were measured using a BET surface area analyser (Micromeritics FlowSorb II 2300). This area analyser permits the measurement of

(1) surface area by a single determination,
(2) surface area by a multipoint procedure,
(3) total pore volume in one step,
(4) the distribution of pore wall area and pore volume as a function of pore size.

In this study the surface area was performed by a single determination.

The calibration equation of the instrument is given in reaction 20.

$$S = v\left[\frac{T(K)}{Rm.Temp.}\right]\left[\frac{Atm.Press.}{760}\right]\left[\frac{A*N}{M}\right]\left[1-\frac{P}{P_0}\right] \quad (20)$$

$$= v\left[\frac{273.2K}{295.2K}\right]\left[\frac{760mmHg}{760mmHg}\right]\left[\frac{6.023*10^{23}\,mol/g\,X16.2*10^{-20}\,m^2}{22414\,cm^3/g}\right]\left[1-\frac{0.3*760mmHg}{775mmHg}\right]$$

$$= 2.84\ v$$

where:

S = surface area in m^2

T/K = thermodynamic temperature

Rm. Temp = room temperature (22 ^{0}C)

Atm. Press = atmospheric pressure

A = avogadro's number

N = area of each adsorbed gas molecule

M = molar volume of the gas

P = pressure of gas adsorbed (30% N_2 x atmospheric pressure)

P_0 = saturation pressure

The equation means that a syringe injection of v = 1.00 cm^3 of nitrogen at 22 ^{0}C and 760 mmHg should produce an indicated surface area of 2.84 m^2

The following procedure was carried out:

Nitrogen gas adsorption at the boiling point of nitrogen was measured for specific masses of the samples. The volume of nitrogen liquid adsorbed is related to the surface area of the samples. A schematic diagram of the instrument is given in Figure 1.

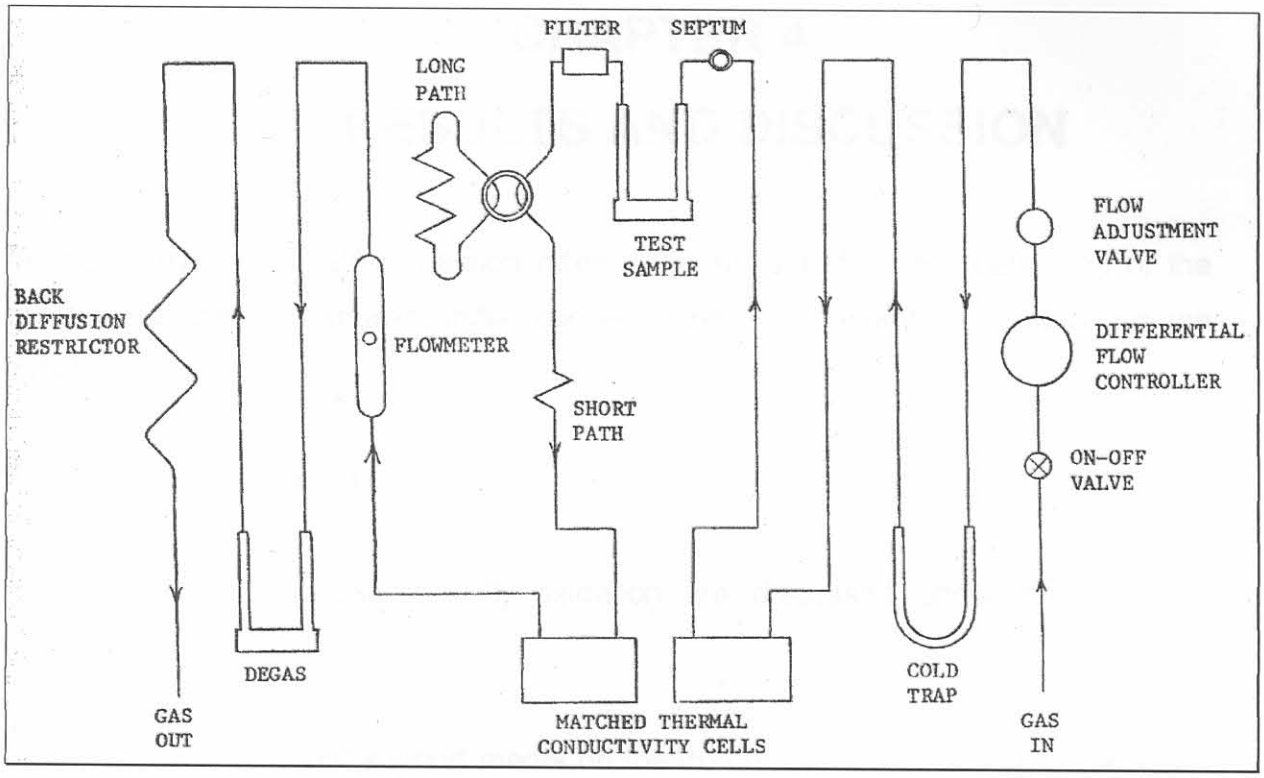

Figure 1: Schematic Diagram of a BET surface area analyser (Micromeritics FlowSorb II 2300

CHAPTER 4
RESULTS AND DISCUSSION

In this study the oxidation reaction rates were measured for the beginning of the reaction for several different initial concentrations of reactants by calculating the initial slope.

4.1 CHEMICAL IRON (II) OXIDATION

The results of chemical iron (II) oxidation are discussed under the following headings:

- Effect of different support media on the iron (II) oxidation rate (100g/L pellets and sand)
- Effect of support media concentration on the iron (II) oxidation rate (0, 50 and 100 g/L pellets)
- Effect of increasing iterations on the iron (II) oxidation rate (1-6)

Other parameters such as temperature, pH, air flow rate, the initial iron (II) concentration and the limestone concentration were kept constant at T = 29 °C, pH = 6.5, A.F = 3L/min, Fe(II) = 2g/L iron (II) and [$CaCO_3$] = 7.8 g/L, respectively.

4.1.1 Support media

In this study sand and pellets were used as support media because these media do not easily scale up with $CaCO_3$ as compared to the other media, for example, GT. When the initial rates of the graphs in figure 2 were measured, the results showed that pellets as support media gave oxidation rates of 48.3 as compared to the rate of 42.9 g Fe/(L.d) when sand was used as a support media, while the reaction time in both cases was 2.00 hours (Fig.2). In both cases the number of iterations was 6. The higher oxidation rate when using pellets as support media may be due to a better air distribution in the case of the pellets as opposed to the sand as support media.

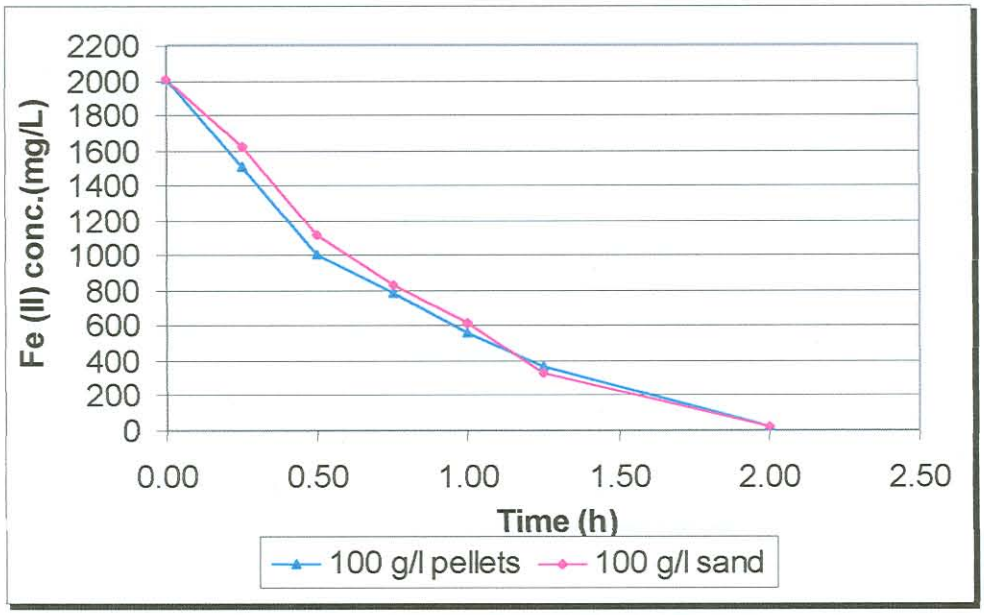

Figure 2: Effect of pellets and sand as support media on the iron (II) oxidation rate

4.1.2 Support media concentration (Pellets)

The experimental results as shown in Fig. 3 were obtained after 6 iterations. When the initial slope was calculated to the graphs in Fig. 3, the oxidation rates were 48.9, 44.0 and 50.2 g Fe/(L.d) using 100, 50 and 0 g/L pellets, respectively. These results showed that the pellets had no significant effect on the Fe (II) oxidation rates. It can be concluded that when applying chemical iron (II) oxidation, the use of support media does not influence the reaction rate and the support media also inhibit the reaction.

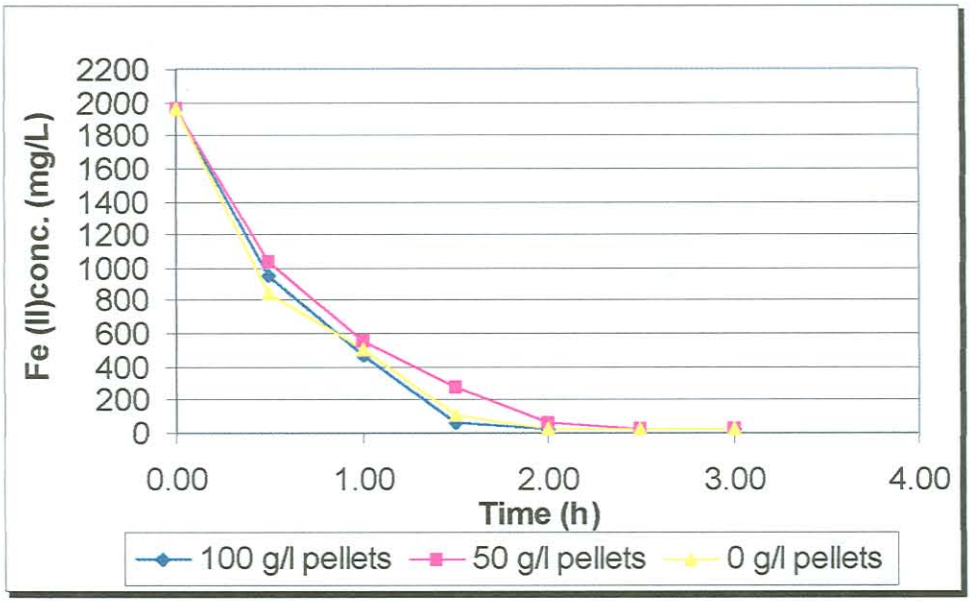

Figure 3: Effect of pellets concentration on the iron (II) oxidation rate

4.1.3 Iterations

Figure 4 shows the effect of number of iterations when no support medium was used. The graphs in Fig. 4 showed that, when one iteration was applied, the reaction time was 6h, while when the iterations increased (2, 3, 4, 5 and 6 iterations), the reaction time decreased (from 4, to 3, to 2.5, to 1.5 and 1.5 h, respectively). The initial slope was measured from graphs as shown in Fig. 4. The results showed that increasing iterations from 1, 2, 3, 4, 5 and 6, the reaction rates increased from 14.1, 17.1, 24.6, 29.5, 36.9 and 40.2 g Fe/(L.d), respectively. The graphs furthermore showed that at iteration number 5, the reaction occurred at optimum rate, as can be seen from the graphs representing iterations 5 and 6. The increase in oxidation rate with the number of iterations can be ascribed to the increase in sludge concentration in the reactor and to the suspended solids formed ($Fe(OH)_3$) and $CaSO_4$, as described in 2.4), which both act as a catalyst to the reaction (Maree *et. al.,* 1999)

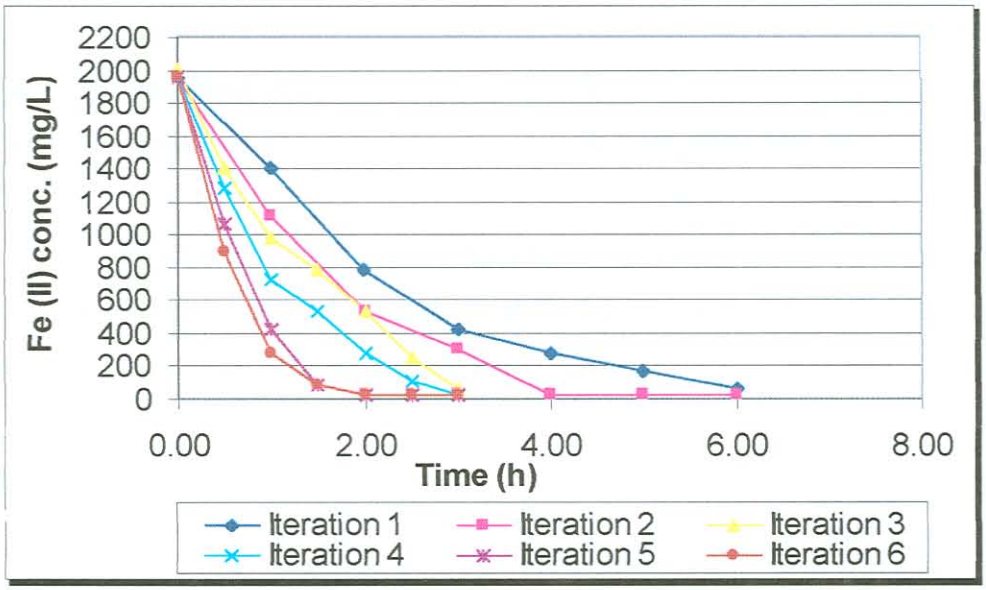

Figure 4: Effect of iterations with sludge (no pellets) on the iron (II) oxidation rate

4.2 BIOLOGICAL IRON (II) OXIDATION

4.2.1 Batch studies

The results of biological iron (II) oxidation are discussed under the following headings:

- The effect of support media on the iron (II) oxidation rate (sand, plastic rings, plastic pellets, coal discard, anthracite and geotextile)
- The effect of iterations on the iron (II) oxidation rate (1 to 14)
- The effect of the support media concentration on the iron (II) oxidation rate (GT plates)
- The effect of the initial iron (II) concentration on the iron (II) oxidation rate (2 to 20 g/L)
- The effect of reactor type on the iron (II) oxidation rate (horizontal and vertical reactor)
- The effect of nutrients on the iron (II) oxidation rate (2mL/L hydroponic nutrient)

- The effect of CO_2 on the iron (II) oxidation rate (3%)
- The effect of pH on the iron (II) oxidation rate (1.7, 2.0 and 2.3)
- The effect of air flow on the iron (II) oxidation rate (3.0 , 5.6 and 8.9mL/min)
- The effect of temperature on the iron (II) oxidation rate(25 to 30 °C)

Other parameters such as temperature, pH, air flow rate and the initial iron (II) concentration were kept constant at T= 29 °C, pH = 2.0, A.F = 3L/min, Fe (II) conc. = 2g/L respectively, unless otherwise stated.

4.2.1.1 Support media

Figure 5 compares the biological iron (II) oxidation rate for various support media, under the experimental conditions as described in Table 3. Table 3 also shows the reaction rates for the various support media, when initial slopes were calculated. It was noted that:

- The reaction rate without any media is 1.3 g Fe/(L.d)

- The reaction rate with media varied between 4.1 and 18.1 g Fe/(L.d), depending on the type of medium.

The reaction rate increased in the following sequence: plastic rings, plastic pellets, coal discard, sand, anthracite, white GT, grey GT and brown GT. When comparing the oxidation rates, it can be noted that the brown GT as a support media provided the best results as opposed to the other tested media (e.g. sand). These improved results can be ascribed to the nature of the textile that accelerated the bacterial adsorption and biofilm formation (a complex, multicellular structure formed when microorganisms attach or colonize to a surface, Nemati and Webb, 1999). Due to the porosity of the GT, air can penetrate easily into the fibres and due to its texture it provides a large surface area on which the bacteria can adhere.

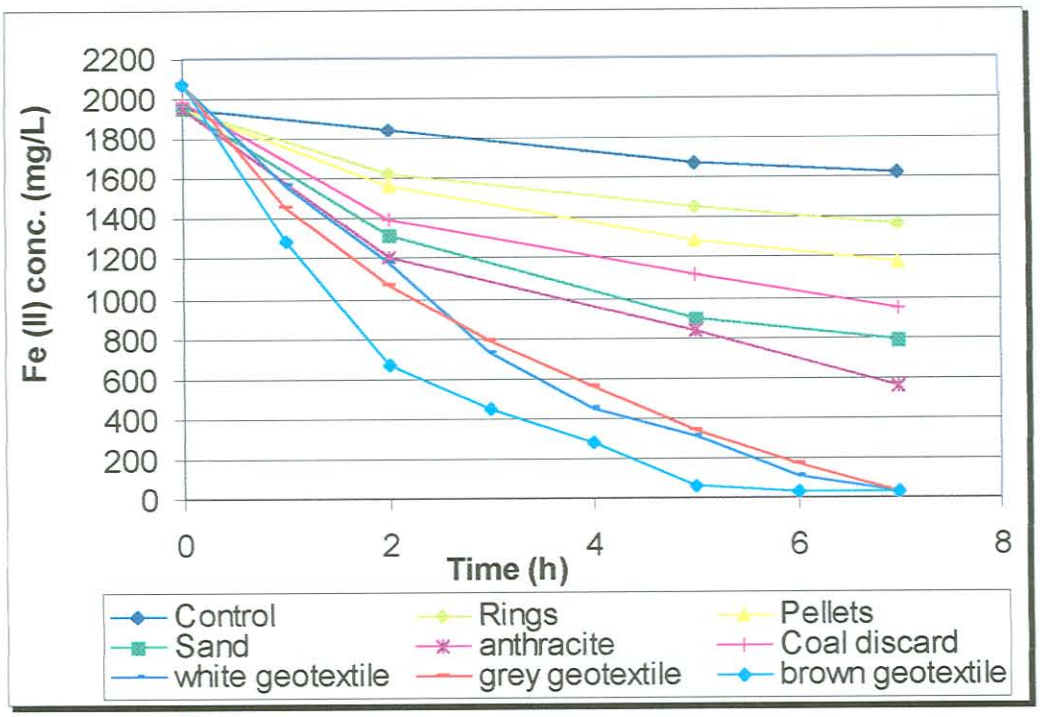

Figure 5: **Effect of the support medium on the iron (II) oxidation rate**

Table 3 **Effect of the support medium on the rate of iron (II) oxidation**

Media type	Iron (II) conc. (mg/L)	Media conc. g/L	Iron (II) oxidation rate (g Fe/(L.d))
Control	1955	100	1.3
Plastic rings	1955	100	4.1
Plastic pellets	1955	100	4.7
Coal discard	1955	100	7.0
Sand	1955	100	7.7
Anthracite	1955	100	9.0
White GT	2066	100	10.7
Grey GT	2066	100	12.1
Brown GT	2066	100	18.1

Experimental conditions: A.F = 3L/min, pH = 2.0, temperature = 29 ^{0}C and number of iterations = 8.

4.2.1.2 Iteration

Figures 6, 7, 8 and Table 4 show the effect of the number of iterations on the rate of iron (II)-oxidation, using artificial iron (II) rich water and various types of support media (sand, brown GT and grey GT, respectively). In the case of brown and grey GT the empty volume of the sheets amounted to 40% of the volume filled with water. By measuring the initial slope of the graphs, the following rates were obtained for the various media:

- Sand. The oxidation rates were calculated to be 3.4, 5.4, 6.7 and 8.7 g Fe/(L.d) for iterations 1, 3, 5 and 7 respectively (Figure 6).
- Brown GT. The oxidation rates were calculated to be 1.3, 5.4, 8.0, 14.7, 21.4 and 24.8 g Fe/(L.d) for iterations 1, 3, 5, 7, 9 and 11 respectively. (Figure 7).
- Grey GT. At iteration 8 the reaction rate was 10.7 g Fe/(L.d) while at iteration 1 the reaction rate was 2.0 g Fe/(L.d) (Figure 8).

It is noted that the reaction rates increased with the increase in iterations. This increase in reaction rate can be assigned to the biomass growth on the support media (Nemati and Webb, 1996). Every time the iteration was carried out, the biomass attached and grew on the support media, forming a biofilm. This form of microbial growth can greatly affect the rate of microbial metabolism. On surfaces microbial numbers and activity are usually much greater than in free water, because of the adsorption effects (Madigan, *et al.*, 1997). According to Brock and Madigan (1991) surface areas are considered microbial habitats and the bacterial cells can attach to a surface by a way of adhesive polysaccharides, which are excreted by the cells.

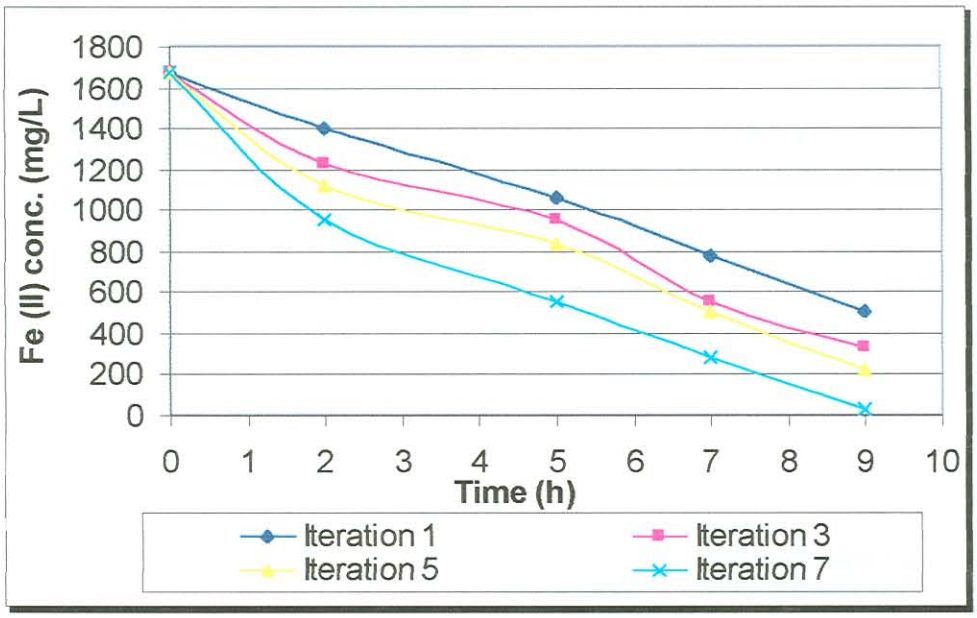

Figure 6: Effect of iterations when using sand as support media on the iron (II) oxidation rate

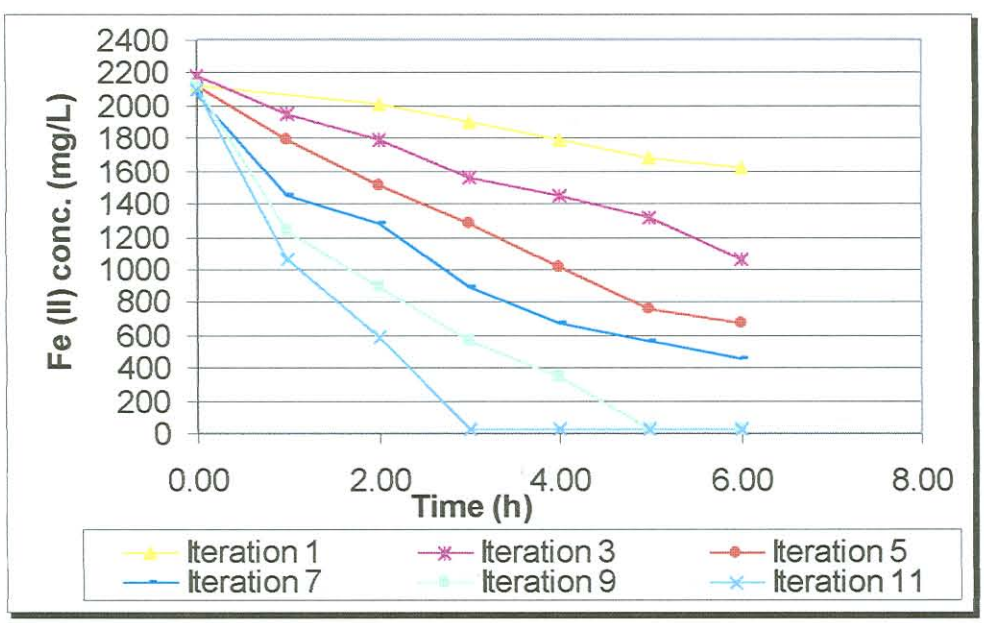

Figure 7: Effect of iterations when using brown GT on the iron (II) oxidation rate

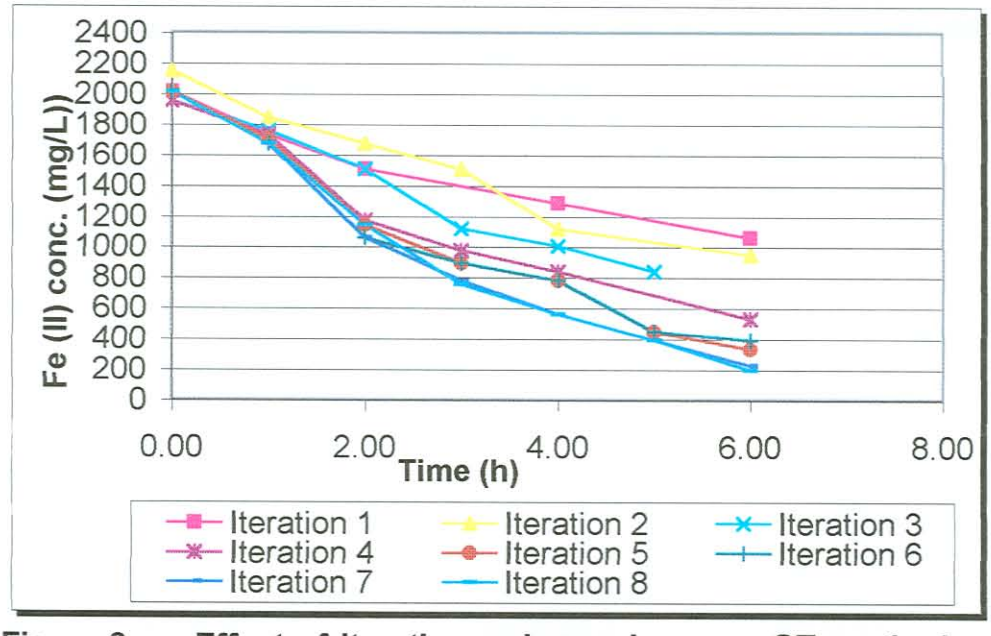

Figure 8: Effect of iterations when using grey GT on the iron (II) oxidation rate

Table 4 Effect of iterations on the rate of iron (II) oxidation for various support media

Iterations	Fe(II) oxidation rate (g Fe/(L.d))		
	Sand	Grey GT	Brown GT
1	3.4	2.0	1.3
3	5.4	5.4	5.4
5	6.7	7.4	8.0
7	8.7	8.0	14.7

Table 5 and figure 9 shows the effect of surface area on the rate of iron (II) oxidation with respect to number of iterations (using the BET surface analyser) when discard leachate was used as the feed water and brown GT was used as a support media. It is noted that the iron (II) oxidation rate increased with the increased number of iterations, while the surface area of the geotextile decreased. The results indicated that the iron (II) oxidizing bacteria formed a biofilm on the geotextile. Therefore, it was concluded that geotextile has a high surface area that accelerated the bacterial

adsorption and biofilm formation. Due to the porosity of the geotextile structure, the air could penetrate easily to make contact with the oxidizing biomass.

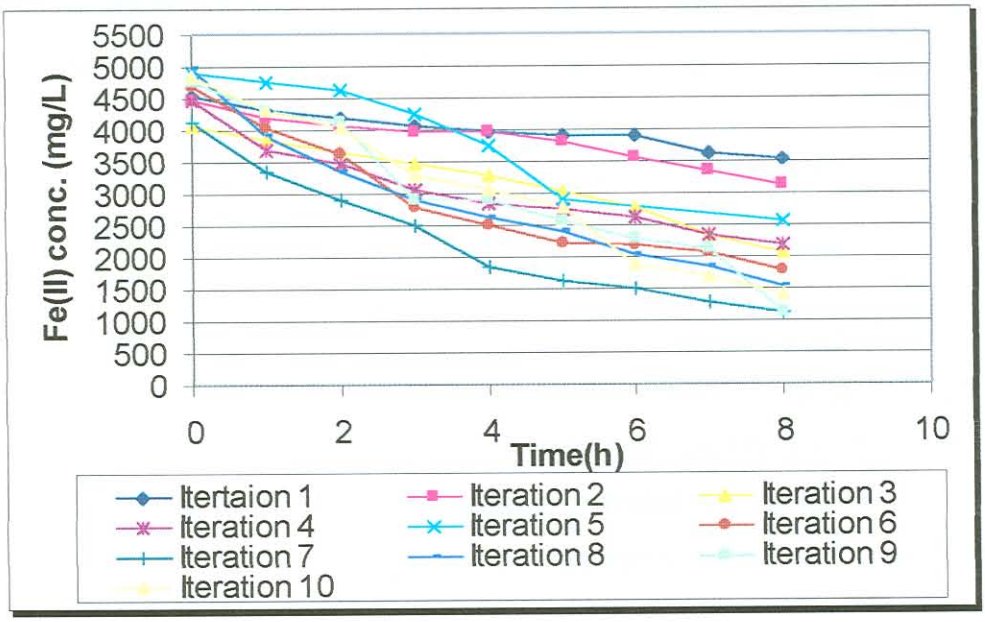

Figure 9: Effect of iterations and surface area on the iron(II) oxidation rate

Table 5 Change in surface area of the brown GT with respect to number of iterations

Iteration	Fe (II) oxidation Rate (g Fe/(L.d))	Surface area (m^2/g)
1	2.7	2.5
2	3.62	2.3
3	5.89	1.7
4	6.08	1.2
5	7.96	0.9
6	8.38	0.8
7	8.78	0.7
8	9.23	0.6
9	10.03	0.4
10	10.62	0.3

4.2.1.3 Media concentration

Figure 10 shows the effect of support media concentration on the rate of iron (II) oxidation for consecutive batch runs under the following experimental conditions:

- Brown GT plates submerged in discard leachate and aerated with compressed air.

When calculating the initial slope of the graphs, the results showed that, increasing the number of plates from 5, 10 and 19, the oxidation rates increased from 4.1, 7.2 and 10.2 g Fe/(L.d) respectively. This can be ascribed to the fact that when more plates are used, more surface area is provided for bacterial growth. It can thus be assumed that the number of micro organisms on the plates is proportional to the surface area of the plates.

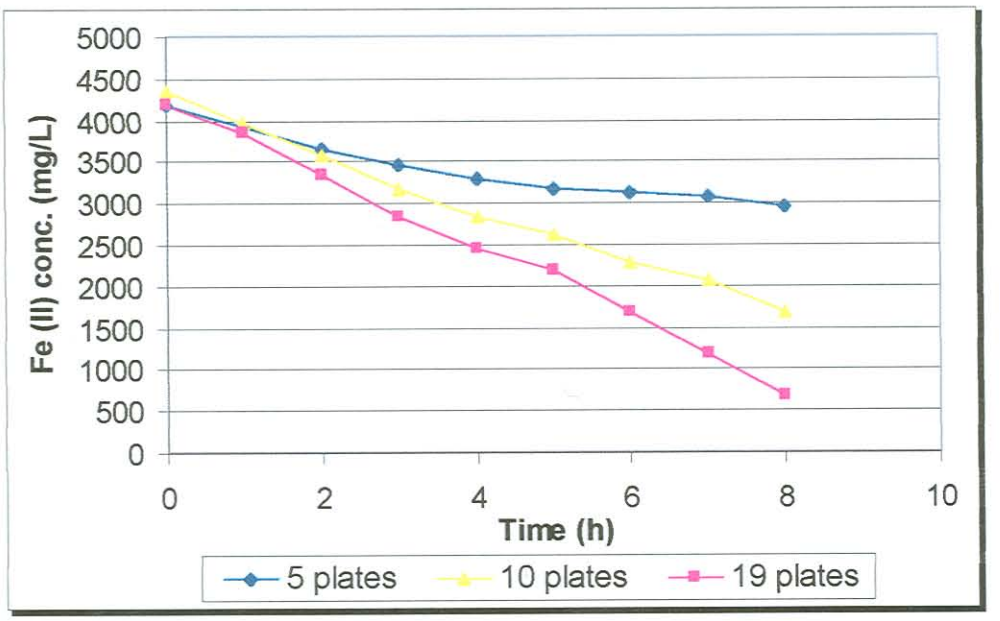

Figure 10: Effect of brown GT plates on the iron (II) oxidation rate

4.2.1.4 *Iron (II) concentration*

The brown GT was found to be the most suitable medium for iron (II) oxidation and used as medium in this experiment to determine the optimum iron (II) concentration.

Figures 11 and 12 and Table 6 show the effect of the initial iron (II) concentration on the rate of iron (II) oxidation, using artificial iron (II)-rich water as the feed water and brown GT as support medium. The initial slopes of the graphs in Figure 11 were determined and the results in Table 6 show that, when 2g/L Fe (II) was used, the oxidation rate was 5.4 g Fe/(L.d). Further increases in Fe (II) concentration from 4 to 12 g/L resulted in an increase of the oxidation rates from 9.5 to 27.6 g Fe/(L.d) respectively. Silvermann and Lundgren (1959) reported that the growth of *T. ferrooxidans* and its ability to oxidise ferrous iron is significantly influenced by the concentration of ferrous iron. Similar observations were also reported by Kelly and Jones (1978).

The results also illustrated that the initial Fe (II) concentrations of 12 and 16g/L gave almost similar results, 27.6 g Fe/(L.d) and 26.1 g Fe/(L.d), respectively. However, employing higher initial concentrations (20 g/L) of ferrous iron inhibited the growth of *T. ferrooxidans*. Increasing the initial iron (II) concentration to 20 g/L resulted in a decrease in oxidation rates (22.5 g Fe/(L.d)).

Table 6 Effect of iron (II) concentration on the iron (II) oxidation rate.

Initial iron (II) concentration (g/L)	Fe (II) oxidation rate (g Fe/(L.d))
2	5.4
4	9.5
8	17.2
12	27.6
16	26.1
20	22.5

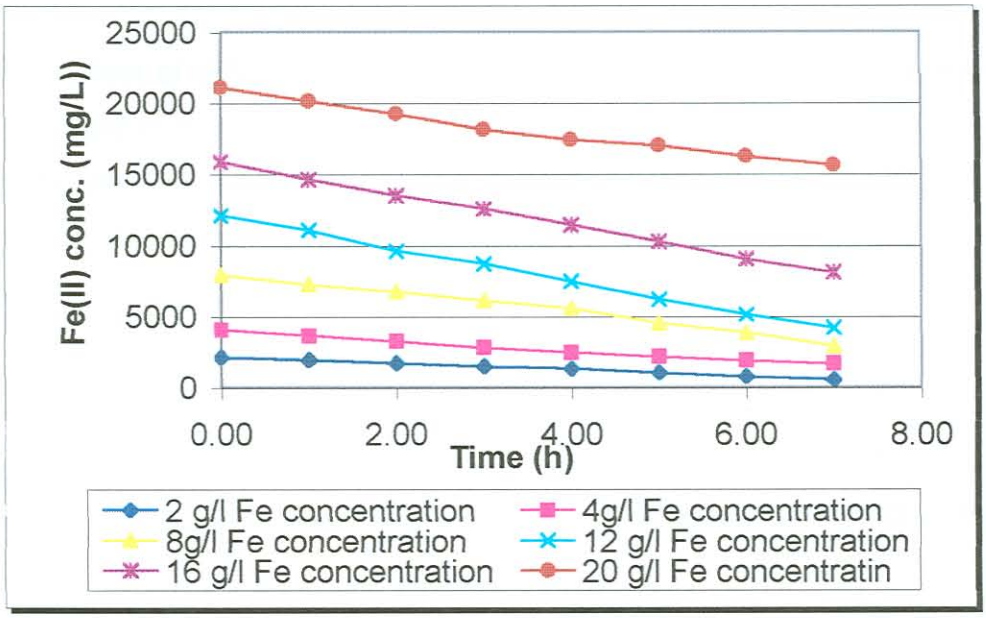

Figure 11: Effect of Fe (II) concentration using GT as support media on the iron (II) oxidation rate

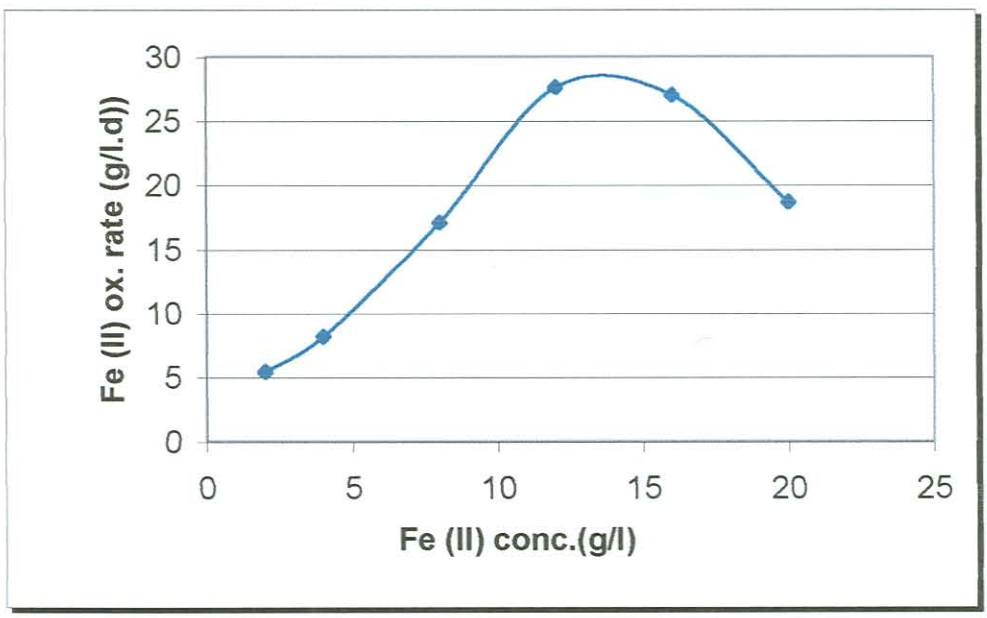

Figure 12: Effect of initial iron (II) concentration on the iron (II) oxidation rate.

The graph in figure 12 shows that the highest oxidation rate was achieved when the initial iron concentration was 14.0 g/L.

4.2.1.5 *Reactor type*

The effect of different types of reactor systems (vertical and horizontal reactors, as indicated in plate 2) was investigated. The initial rates were calculated by the graphs in figure 13 and the results were 12.1 and 11.7 g Fe/(L.d) for the vertical and horizontal reactor, respectively. These results showed that the oxidation rate using the vertical reactor was slightly higher than when using the horizontal reactor under the same experimental conditions. This finding can possibly be credited to the better distribution of air in the vertical reactor. In this reactor type, the air forced its way from the bottom of the reactor to the top. The results obtained seem to indicate that, this manner of air flow improved the contact time between the micro organisms and the air.

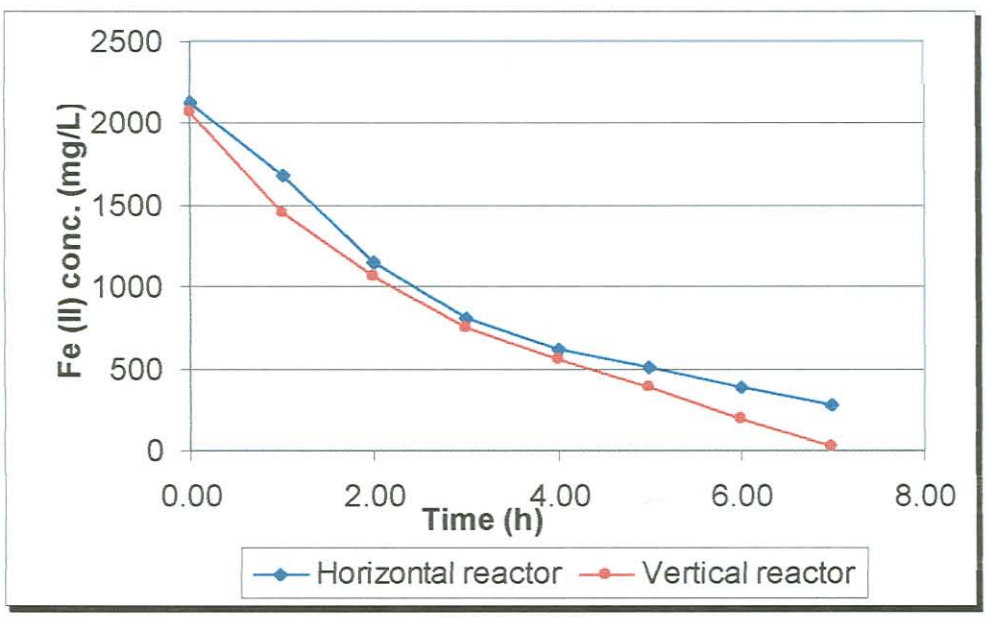

Figure 13: Effect of reactor system (type) on the iron (II) oxidation rate

4.2.1.6 *Nutrients*

Figure 14 shows the effect of nutrient addition. In the case where 2 ml/L of the hydroponic nutrients were added, the reaction time was 7h, while with no nutrients added to the reactor, the reaction time was longer than 7h. When the initial slope of the graphs was calculated, the results indeed demonstrated that the use of nutrients resulted in a higher iron (II) oxidation rate (8.4 g Fe/(L.d)) as compared with no

addition of nutrients (5.7 g Fe/(L.d)). These results confirm that nutrients are the building blocks for the growth of bacterial cells (Brock and Madigan, 1991).

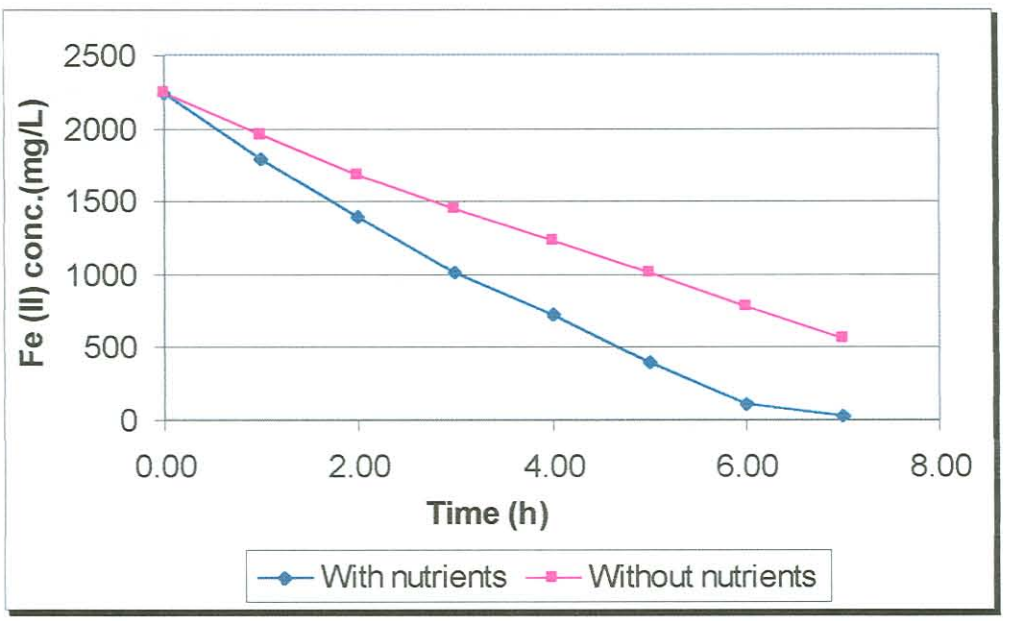

Figure 14: Effect of Nutrients on the iron (II) oxidation rate

4.2.1.7 CO_2

Figure 15 shows the effect of carbon dioxide on the iron (II) oxidation rate. When linear regression was applied to the graphs in figure 15,

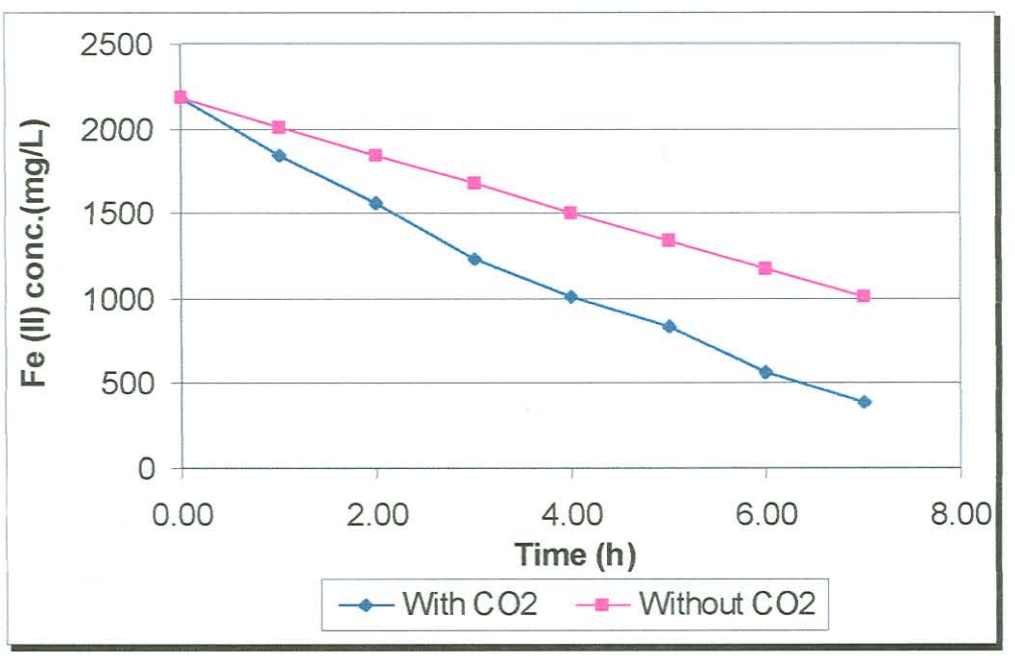

Figure 15: Effect of CO_2 on the iron (II) oxidation rate

it is noted that the reaction rate was faster when CO_2 was bubbled into the reactor vessel (6.1 g Fe/(L.d) as compared to when no carbon dioxide was added (4.0 g Fe/(L.d). The results supported the findings of Nemati and Webb (1998). They indicated that *T. ferrooxidans* needs CO_2 as its carbon source for growth. Holuigue *et al* (1987) and Barron (1990) demonstrated that the availability of CO_2 is important for achieving optimal growth rates and maximum cell yields.

4.2.1.8 Air flow

Figure 16 shows the effect of the air flow. The effect of the amount of air on the iron (II) oxidation rate was tested using GT as the support material at various air flow rates (one unit of air = 3L/min, two units of air = 5.6 L/min and three units of air = 8.9 L/min). The reaction rates using the initial slopes were calculated to be 7.8 g Fe/(L.d), 9.5 g Fe/(L.d) and 13.9 g Fe/(L.d) for one, two and three units of air, respectively. These results can be assigned to the increased respiration rate of *T. ferrooxidans.* When more air was supplied, the respiration rate of the biomass increased, resulting in faster iron degradation rates.

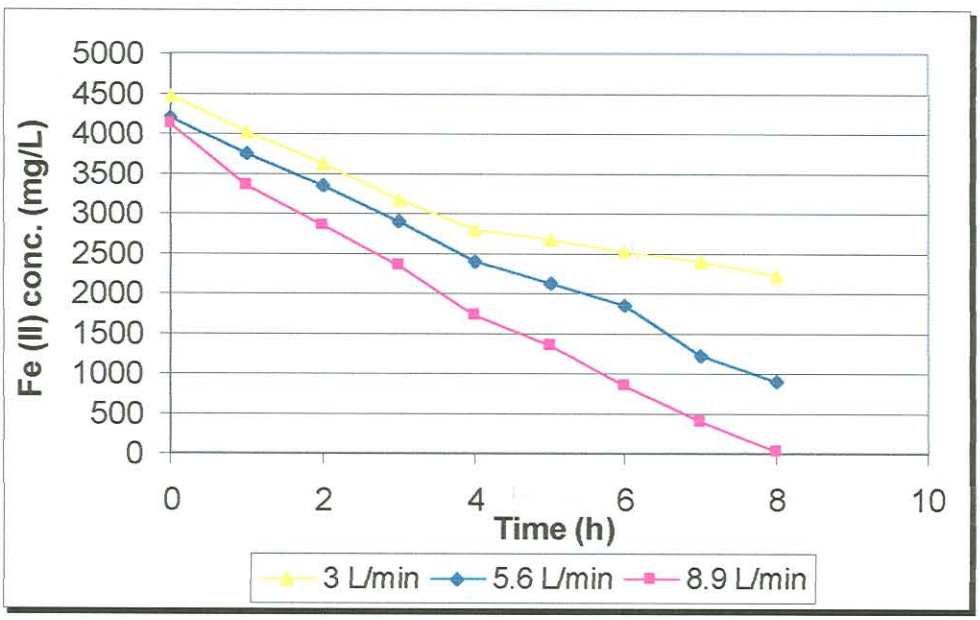

Figure 16: Effect of air flow on the iron (II) oxidation rate

4.2.1.9 pH

Figure 17 shows the effect of pH on the rate of iron (II)-oxidation, using the brown GT as support medium. The initial slope was determined from the graphs and the results show that, at pH 1.7 the oxidation rate achieved was 15.4 g Fe/(L.d) and at pH 2.3 the oxidation rate was 11.4 g Fe/(L/d). The highest oxidation rate achieved was 20.8 g Fe/(L.d) at pH 2.0.

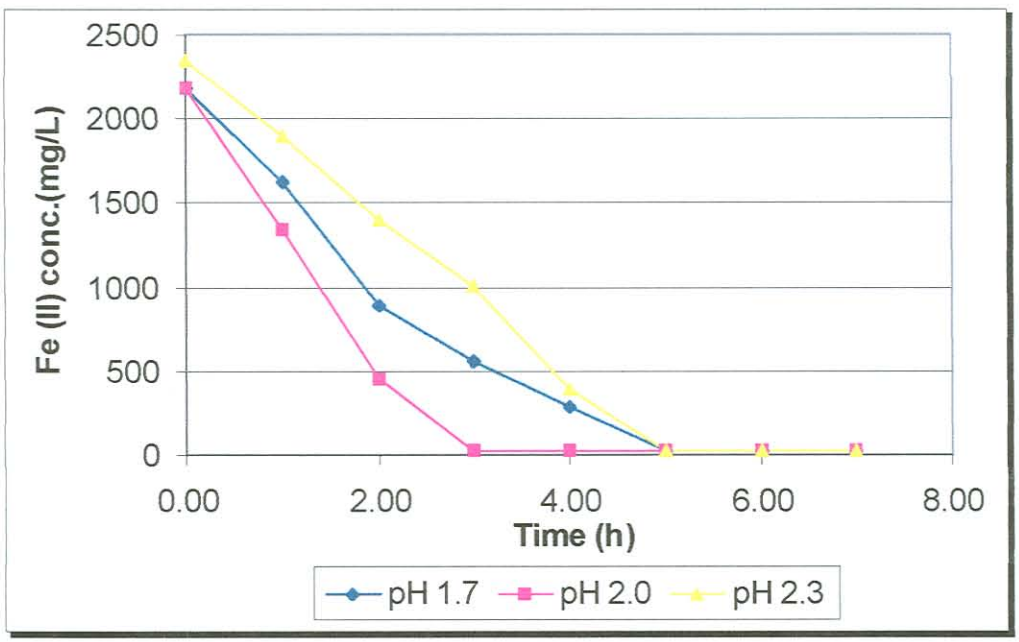

Figure 17: Effect of pH on the iron (II) oxidation rate

4.2.1.10 Temperature

Figure 18 shows the effect of different temperatures on the rate of iron (II) oxidation. When the reaction rates were calculated, the results were found to be 6.7, 8.3, 12.1, 13.7, 15.8 and 14.7 g Fe/(L.d) for 25, 26, 27, 28, 29 and 30 °C, respectively.

These results indicate that the optimum oxidation rate was achieved when the temperature was 29 °C.

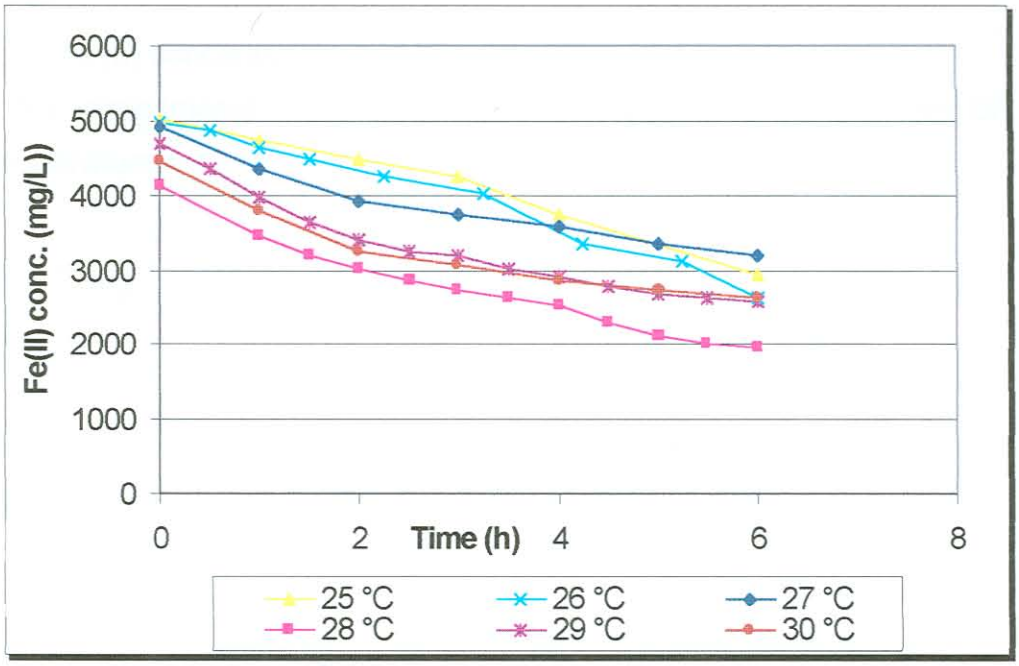

Figure 18: Effect of Temperature on the iron (II) oxidation rate

4.2.2 Kinetic studies

The data in Table 7 and graphs in figures 19, 20 and 21 show the effect of various factors on the kinetics of iron (II) oxidation.

Table 7 Effect of various factors on the kinetics of iron (II) oxidation

Variable	Value Concentration	Rate (g Fe/(L.d))	Log C*	Log R*
Fe (II) (g/L)	2	5.4	0.30	0.73
	4	9.5	0.60	0.98
	8	17.2	0.90	1.24
	12	27.6	1.08	1.44
	16	26.1	1.20	1.42
SM (m^2/m^3)	5	4.1	0.70	0.61
	10	7.2	1.00	0.86
	19	10.2	1.28	1.01
O_2 (air) (L/min)	3	7.8	0.50	0.89
	5	9.5	0.70	0.98
	8.9	13.5	1.00	1.14

*C = Fe (II) concentration and *R = Iron (II) oxidation rate

Other experimental conditions: Type of support media = brown GT, pH = 2.0, temperature = 29 ^{0}C.

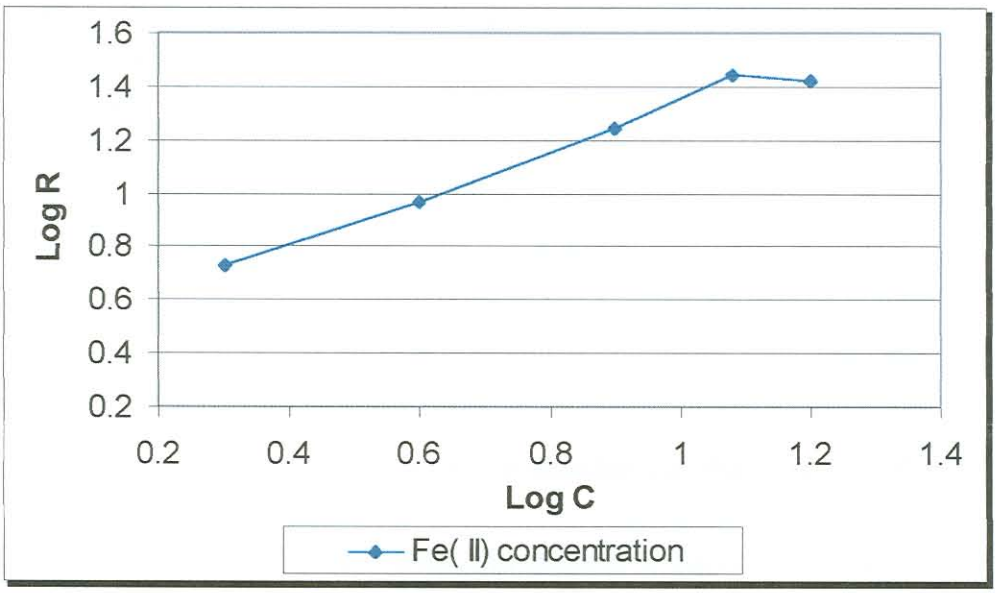

Figure 19: Effect of Fe (II) concentration on the kinetics of iron (II) oxidation

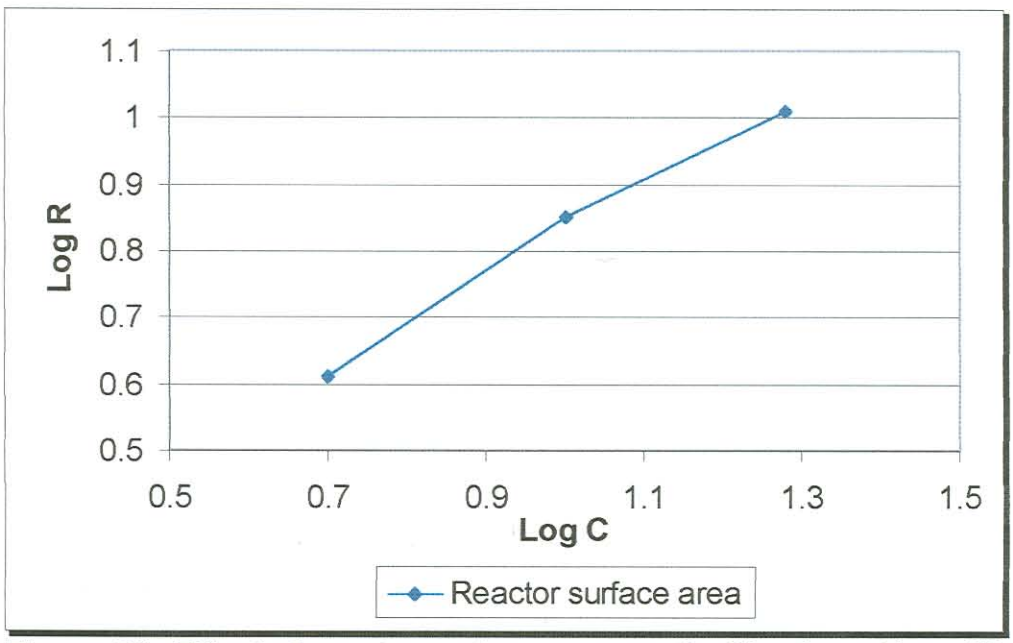

Figure 20: Effect of reactor surface area on the kinetics of iron (II) oxidation

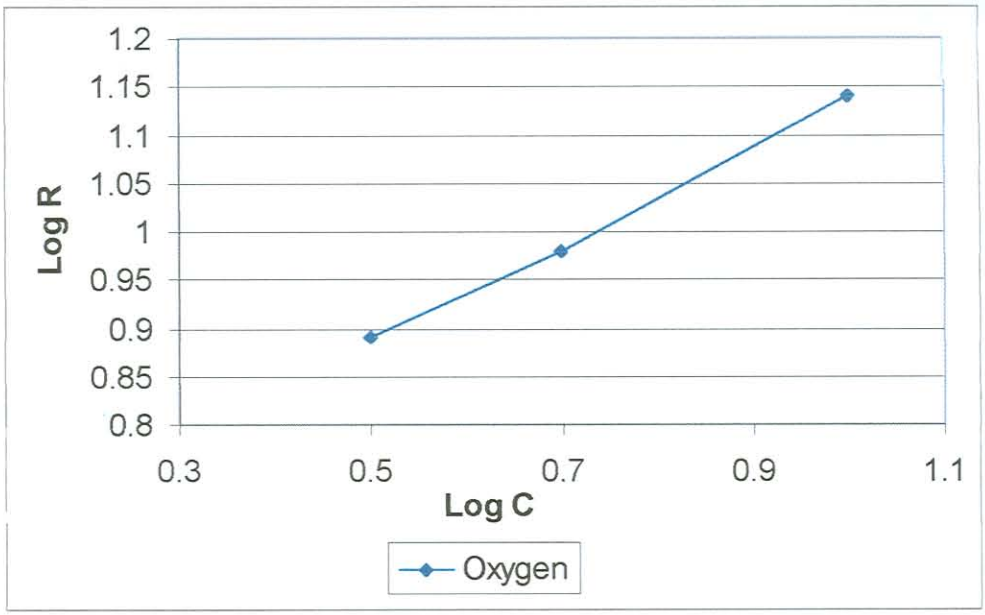

Figure 21: Effect of oxygen concentration on the kinetics of iron (II) oxidation

When calculating the slopes from the graphs in figures 19 to 21, the results showed that the rate of iron (II) oxidation is of order 1, 1 and 0.5 in respect to Fe^{2+}, SM and O_2 concentrations, respectively. These findings suggest that the rate equation for the biological iron (II) oxidation should be modified for suspensions to:

$$-d[Fe^{2+}]/dt = k[Fe^{2+}]^1.[SM]^1.[O_2]^{0.5} \quad (21)$$

where,

$-d[Fe^{2+}]/dt$	=	rate of iron (II) oxidation
k	=	reaction rate constant
$[Fe^{2+}]$	=	iron (II) concentration
SM	=	reactor surface area
O_2	=	oxygen concentration

4.2.3 Continuous Studies

Kinetic studies showed that brown GT is the most suitable support medium to support biological iron (II) oxidation. Further studies were carried out to determine process performance under continuous conditions using coal discard leachate as feed water. The results are shown in Table 8.

The results of continuous studies are discussed under the following headings:

- The effect of support medium (GT)
- The effect of nutrients (2mL/L hydroponic nutrients)
- The effect of HRT (24 to 9h)

Table 8 Effect of different parameters on the iron (II) oxidation rate

Parameters				
Feed rate (L/d)	***HRT (h)***	***No support media***	***Brown Geotextile***	***Nutrients (2 mL/L) & Brown G.T***
		Iron (II) oxidation rate (g Fe/(L.d)		
15	24.0	4.20	4.20	4.08
20	18.0	6.01	6.37	6.48
25	14.4	7.12	7.33	7.45
30	12.0	7.60	8.66	8.60
35	10.3	8.27	9.71	10.10
40	9.0	8.34	10.98	12.02
45	8.0	8.30	11.02	12.10
50	7.2	5.02	8.46	11.03
55	6.5	4.56	7.37	9.40
60	6.0	4.01	7.17	7.88
65	5.5	3.25	6.35	7.82

Other experimental parameters: Iron (II) concentration of discard leachate varied from 4.5 to 4.8 g/L, pH = 2.0, temperature = 29 ^{0}C and A.F = 3L/min

Table 8 shows the effect of the use of support media and addition of the nutrients. The results show that that when the support media was used, the highest oxidation rate was 8.30 g Fe/(L.d) and when no media was used the highest oxidation rate was 11.02 g Fe/(L.d). It can be concluded that the use of support media is important for the bacteria on which to adhere. Furthermore, the results in table 8 indicated that the addition of nutrients had a positive effect on the iron (II) oxidation rate. The results showed that when nutrients were added the highest oxidation rate was 12.10 g

Fe/(L.d) while when no nutrients were added the oxidation rate was slightly lower at 11.02 g Fe/(L.d). Brock and Madigan (1991) indicated that micro organisms need nutrients for building new cell material (growth) and for generating energy. It can also be seen from the table that the optimum HRT for the continuous study was obtained at 8 h when GT was used as a support media and nutrients were added.

4.3 RESPIROMETER RESULTS

Respirometer studies were carried out to confirm the results as obtained from the batch and the continuous studies, as shown in Table 8 and sections 4.2.1.4 to 4.2.1.10. The results showed the significant effect of adding the support media, micro organisms and nutrients. All experiments were conducted at pH 2.0 and at a temperature of 29 ^{0}C. The graphs in the figure 22 to 24 show the oxygen uptake rate and the cumulative oxygen rate during the reaction.

The results of the respirometer are discussed under the following headings:

- The effect of support medium on the oxygen uptake rate (GT)
- The effect of micro organisms and nutrients on the oxygen uptake rate
- The effect of the initial Fe (II) concentration on the oxygen cumulative rate (2 to 20 g/L)

4.3.1 Support media

The results of the effect of support media on the oxygen uptake rate are shown in fig. 22. Microorganisms (1mL), nutrients (2mL) and CO_2 (3%) were added to the reactor vessels (200mL).

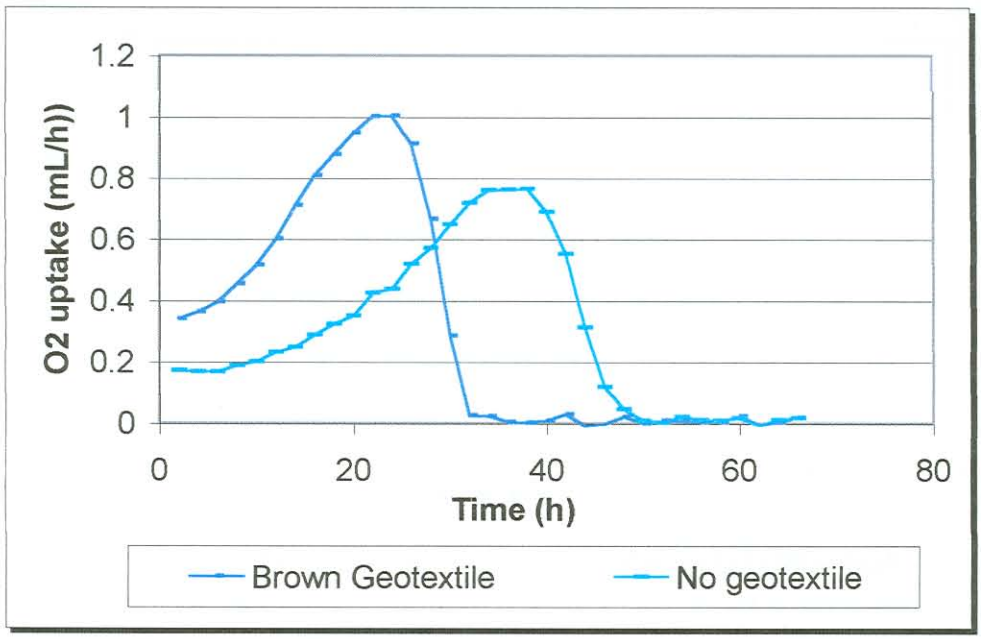

Figure 22: Effect of support media on the oxygen uptake rate

The graphs in fig. 22 show that, when no support media was used, the maximum oxygen uptake rate was almost 0.75mL/h and when the GT was used as a support media, the maximum oxygen uptake rate was 1.0mL/h. The graphs furthermore show that when more oxygen was utilised the iron (II) oxidation reaction was faster (24h) as compared to 38h, when less oxygen was utilised. The higher oxygen uptake rate can be ascribed to the surface area of the GT on which the bacteria adhere. When the microorganisms attach to a surface they form a multicellular structure called a biofilm (Characklis and Marshall, 1990).

4.3.2 Microorganisms and nutrients

The experimental results of different parameters on the oxygen uptake rate are shown in Figure 23.

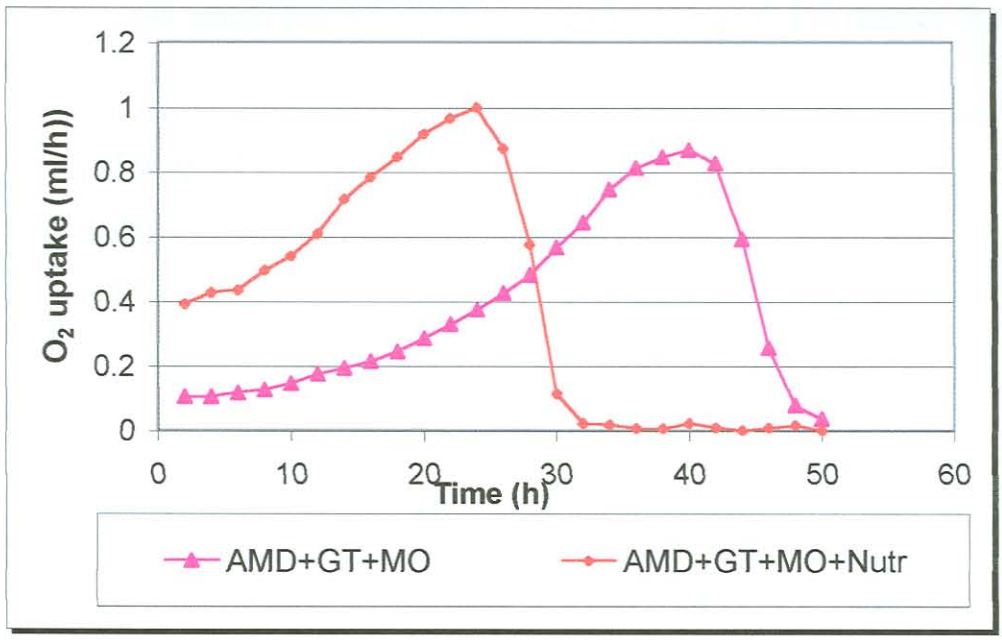

Figure 23: Effect of micro organisms and nutrients on the oxygen uptake rate

The graphs in Figure 23 show that when no nutrients were added, the maximum oxygen uptake rate was 0.85 mL/h at 40h. When nutrients were added the maximum oxygen uptake rate increased to 0.98 mL/h at 25h. It was concluded that the more oxygen used by the bacteria the faster the oxidation reaction and the less oxygen used the longer the reaction time. Brock and Madigan (1991) showed that microorganisms need nutrients for growth to assimilate various organic compounds for the use of new cell material.

4.3.3 Iron (II) concentration

The results of the effect of initial iron (II) concentration are given in figure 24. The graphs in fig 24 showed that increasing the concentration of Fe (II) from 2 g/L to 15 g/L resulted in the increase in oxygen cumulative rate (10 to 89.1 ml O_2) at the reaction time starting from 44h to 96h. The results further illustrated that increasing the Fe (II) concentration to 20 g/L decreased the oxygen cumulative rate to 81.6 ml while the reaction time increased to 110h. Silvermann and Lundgren (1959) showed that the growth of *T. ferrooxidans* is influenced by the concentration of ferrous iron. However, the inferior results achieved in the presence of high concentrations of iron (II) can partly be contributed to the inhibition effect of ferrous iron on the growth of *T.*

ferrooxidans which leads to lower biomass hold-up in the reactor (Kelly and Jones, 1978)

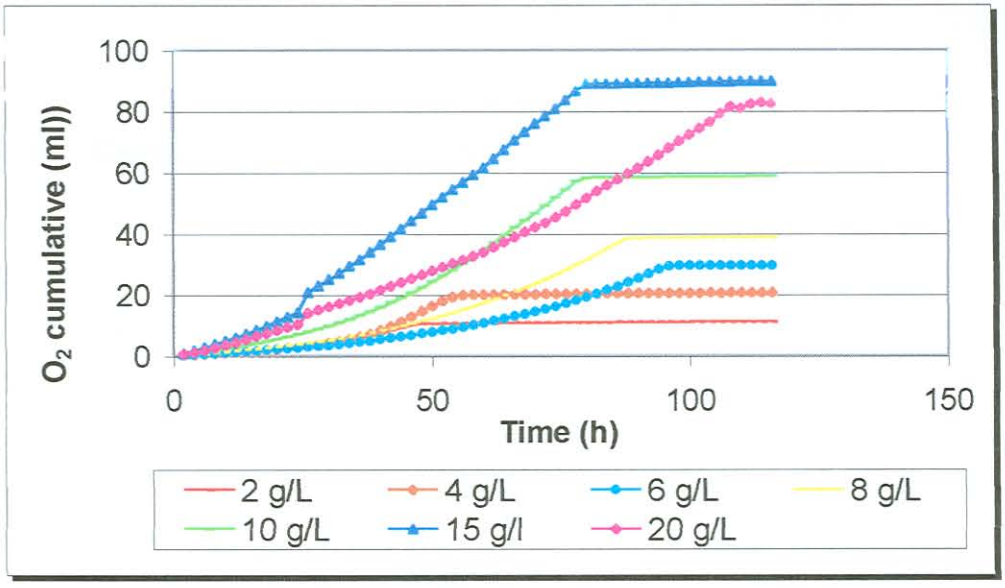

Figure 24: Effect of Fe (II) concentration on cumulative O_2 rate

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CHAPTER 5
CONCLUSIONS

5.1 CHEMICAL FE (II) OXIDATION

When iron (II) was oxidised chemically, the highest iron (II) oxidation rate achieved was 50.2 g Fe/(L.d) when no media was added. The results showed that the addition of $CaCO_3$ had a positive effect on the oxidation rate, however the addition of support media did not have the same effect. This finding can be ascribed to the fact that when $CaCO_3$ is added to the aerated iron (II) solution, the pH increases from 2.0 to 6.5. At pH higher than 6 the iron (II) becomes unstable in the presence of oxygen and thus the iron (II) oxidised to iron (III). The sludge formed during this reaction is composed of ferric hydroxide ($Fe(OH)_3$) and gypsum ($CaSO_4$), (as described in 2.4). It can be assumed that the two suspended solids act as catalysts to the oxidation reaction because the oxidation rate improved from 44.0 to 50.2. The investigation further showed that, the increase in number of iterations from 1 to 6 resulted in the increase in oxidation rates from 14.1 to 40.2 g Fe/(L.d). It was noticed that the suspended solids (catalysts) concentration in the reactor vessel increased.

5.2 BIOLOGICAL FE (II) OXIDATION

5.2.1 Batch studies

Iron (II) can be oxidised to iron (III) at low pH due to the presence of iron oxidising bacteria. The obtained results showed that the oxidation rate was influenced by bacterial growth, which can be increased by providing the following support parameters:

1) Temperature

The results as obtained from this study showed that optimum temperature for the biological iron (II) oxidation was 29^{0}C. At this temperature, the oxidation rate was 15.8 g Fe/(L.d).

2) pH

The optimum pH for the growth and catalytic activity of the *T. ferrooxidans* was found to be 2.0 as it provided the highest oxidation rate of 20.8 g Fe/(L.d).The oxidation rates at pH 1.7 and 2.3 were 15.4 and 11.4 g Fe/(L.d), respectively.

3) Air flow

It was found that the increase in the air flow (3, 5.6 and 8.9 L/min) resulted in an increased Fe (II) oxidation rate (7.8, 9.5 and 13.9 g Fe/(L.d), respectively). It can be concluded that when more air is available, the respiration rate of the *T. ferrooxidans* increase resulting in a faster iron degradation rates. The results show that sufficient air should be available when conducting the biological iron (II) oxidation.

4) Iron (II) concentration

The highest oxidation rate (27.0 g Fe/(L.d)), was achieved when the initial iron (II) concentration was 14.0 g/L. Increasing the Fe (II) concentration to 20 g/L resulted in a decrease in the oxidation rate. It can be assumed that the poorer results achieved in the presence of the higher Fe^{2+} concentrations can partly be attributed to the inhibition effect of ferrous and ferric iron on the growth of *T. ferrooxidans*.

5) Support media

Of the different support media tested (sand, rings, pellets, discard, anthracite, white GT, grey GT and brown GT), brown GT gave the highest oxidation rate (24.8 g Fe/(L.d)). It was concluded that GT has a high surface area, which accelerated the bacterial adsorption and biofilm formation. Due to the porosity of the GT structure,

the air could penetrate easily to contact the oxidizing biomass. This result showed the importance of supplying sufficient air to the microorganisms in the formed biofilm.

6) Media concentration

The results of this study showed that increasing the number of GT plates from 5, 10 to 19, gave an increase in the iron (II) oxidation rate, 4.1, 7.2 and 10.2 g Fe/(L.d), respectively. It can be concluded that when the concentration of support media increase the micro organisms have enough surface area on which to adhere and as a result their growth multiplies.

7) Iterations

It was found that the iron (II) oxidation rate increased with the increased number of iterations, while the surface area of the support media decreased. When the number of iterations increased, the iron oxidising bacteria concentration in the reactor vessel increased as well, which resulted in an increased biofilm formation on the GT as support medium.

8) Nutrients

The oxidation rate of 8.4 g Fe/(L.d) was achieved when nutrients were added to the reactor vessel and when no nutrients were added the oxidation rate was 5.7 g Fe/(L.d). The finding shows that the *T. ferrooxidans* require the nutrients to build new cell material for growth.

9) CO_2

This investigation showed that the addition of CO_2 resulted in an increase in the oxidation rate of 6.1 g Fe/(L.d) as compared to the oxidation rate of 4.0 g Fe/(L.d) when no CO_2 was added to the reactor vessel. It can be concluded that the *T. ferrooxidans* uses CO_2 as its source of carbon for growth.

5.2.2 Continuous Studies

The results of the continuous reaction study confirmed the results as obtained from the batch studies. It was found that the highest iron (II) oxidation rate was 12.20 g Fe/(L.d) when GT was used as the support media and when the nutrients were added. It was further concluded that the optimum HRT for the continuous study was obtained at 8 h. Thus the faster the feed flow rate, the better the oxidation results. These results are beneficial for the mining industry as it indicates that optimal iron oxidation rates can be achieved at low HRT when treating higher volumes of mine water.

5.2.3 Kinetic studies

Kinetic studies showed under acidic conditions, the rate equation for the biological oxidation is determined by the surface area of the support medium.

5.3 RESPIROMETER STUDIES

The results as obtained from the respirometry studies showed when more oxygen is utilised the iron (II) oxidation reaction becomes much faster. The highest oxygen cumulative rate obtained was 89.1mL under the conditions that, the iron (II) concentration was 15 g/L, GT was used as a support media, and the addition of 1mL biomass and 2mL nutrients.

5.4 GENERAL DISCUSSION

The results of this study showed that the highest oxidation rate of 24.8 g Fe/(L.d) was obtained when the temperature was 29 °C, pH 2.0, when GT was used as support media when the nutrients and CO_2 were added to sustain the bacteria. The results do not compare favourably with the results of Nemati and Webb (1996) (as described in Table1). Nemati and Webb conducted their study under the following conditions: temperature was 30 °C, pH 2.0 and polyurethane foam BSP was used a support media. The highest oxidation rate achieved by Nemati and Webb can be ascribed to the fact that in their study pure cultures of *T. ferrooxidans* were used while in this

study microorganisms which are naturally present in the AMD were used. Nemati and Webb furthermore utilized polyurethane foam BSP as support media as opposed to GT in this study. The advantage of polyurethane foam BSP is the higher surface area, however, due to the lightness of the polyurethane foam BSP, the particles float in the reactor which in a full scale plant result in disturbances and thus to malfunction of the plant. GT as a support media offers the benefit that it does not float in water and it is cheaper.

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