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).

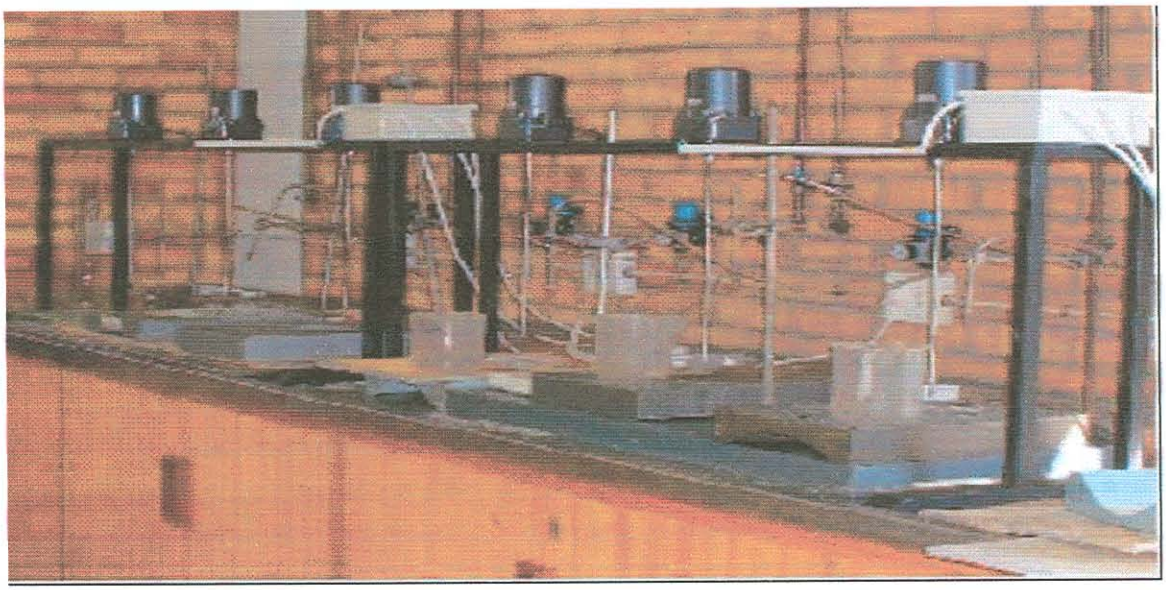

Plate 2: **Batch test conducted in 1L beaker reactors**

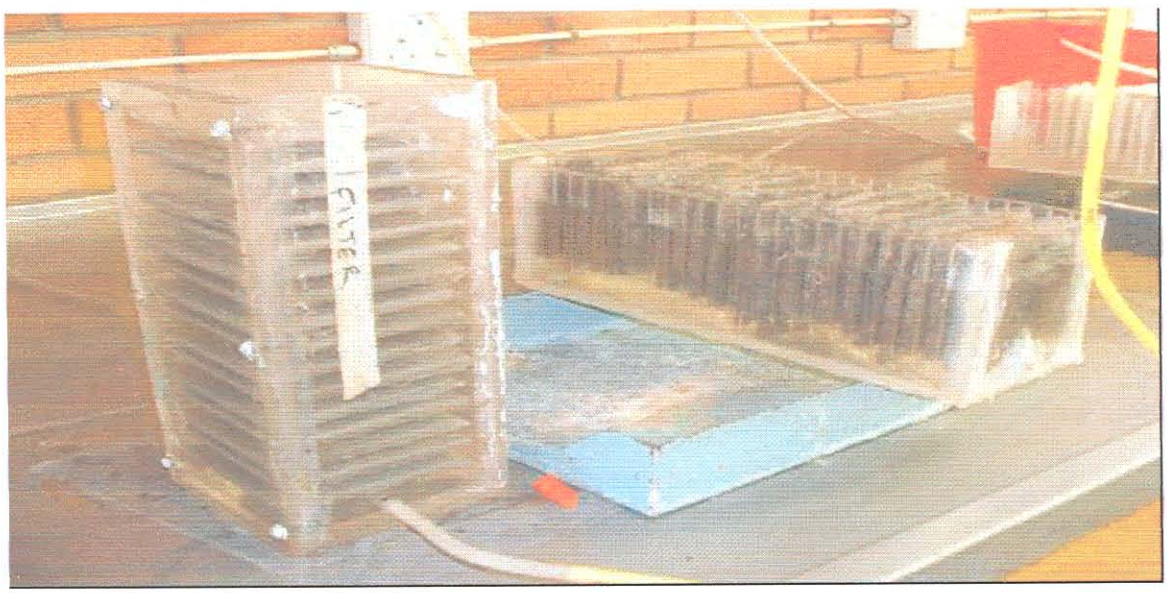

Plate 3: **Batch test conducted in 3L horizontal and vertical plate reactors**

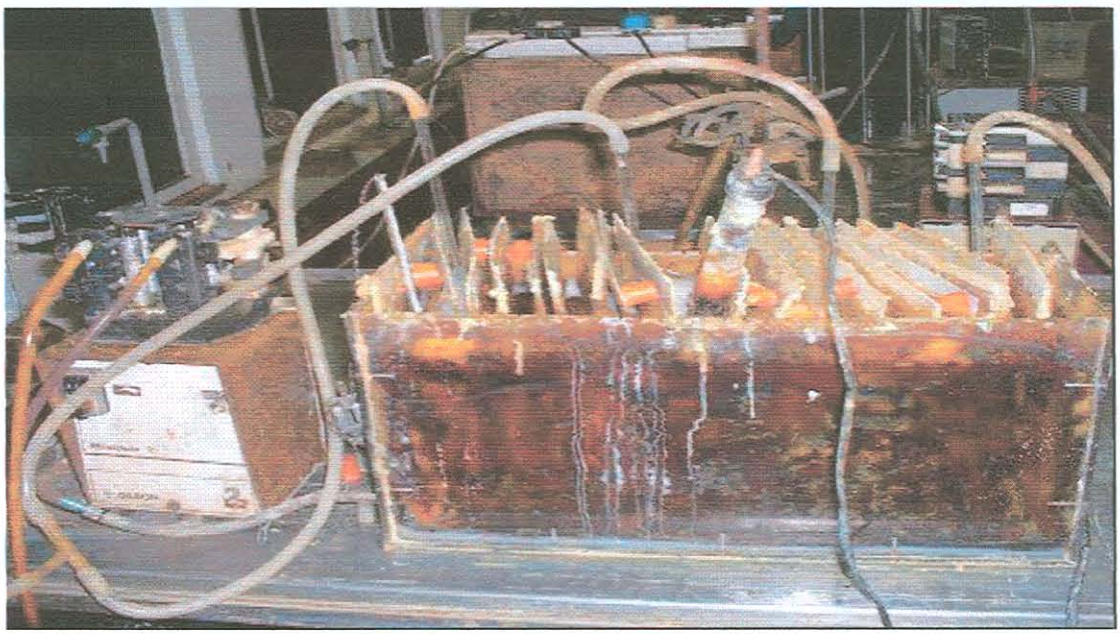

Plate 4: **Laboratory reactor that was used for continuous studies**

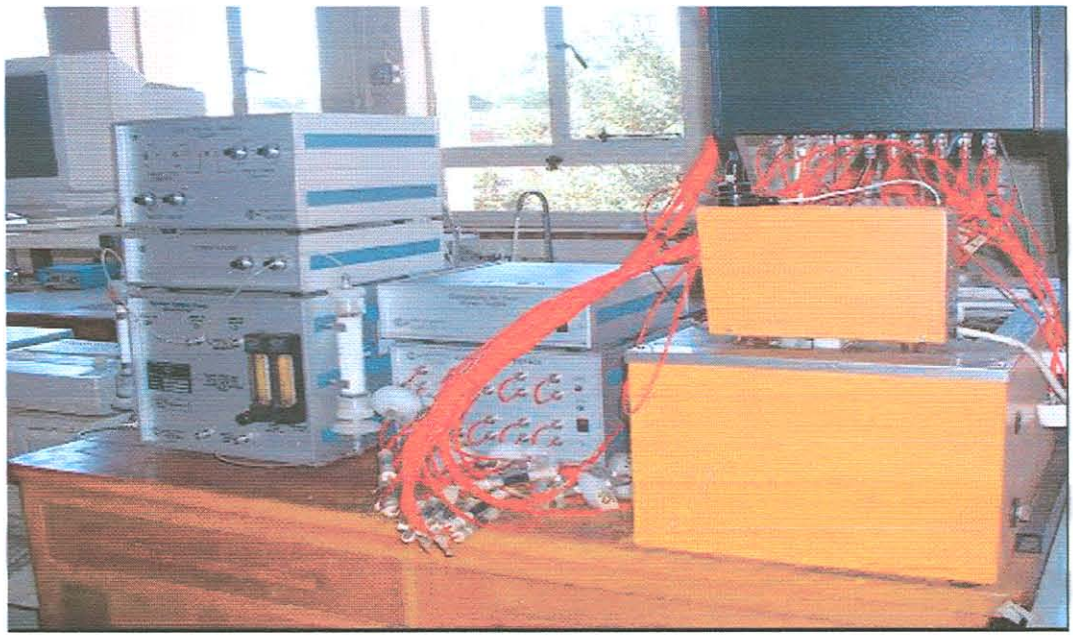

Plate 5: **Respirometer**

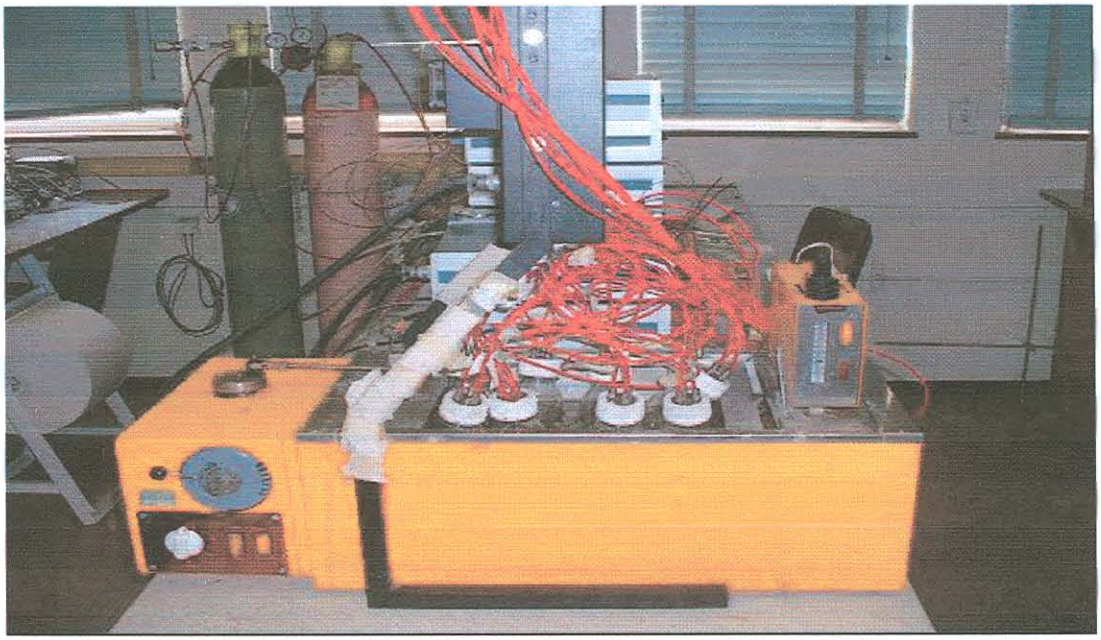

Plate 6: Reactor vessels

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**

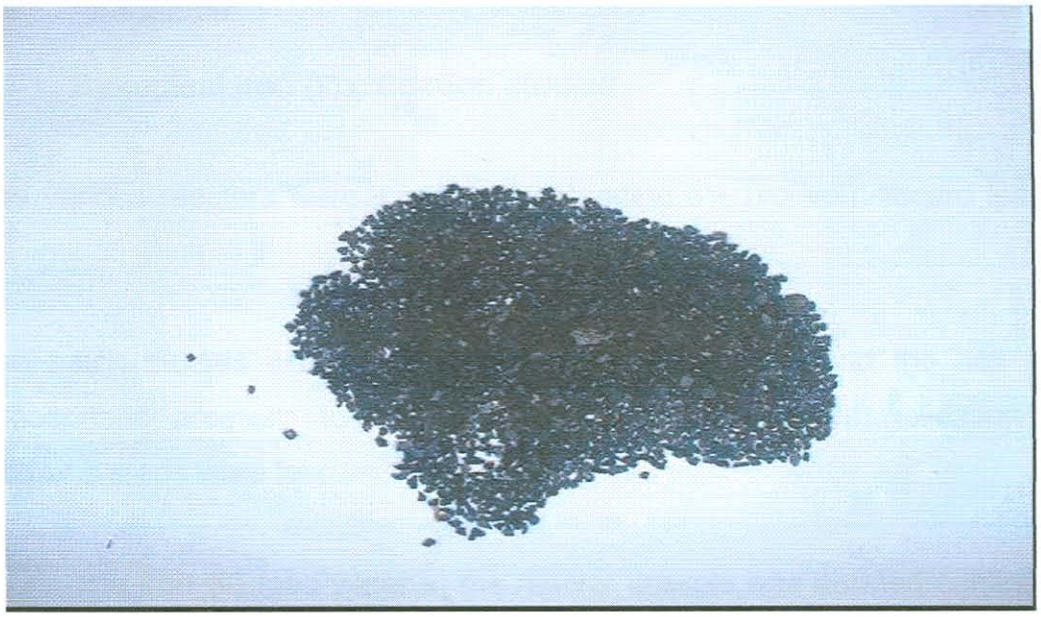

Plate 11: Coal discard

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.

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

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

$$= 2.84\,v$$

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.

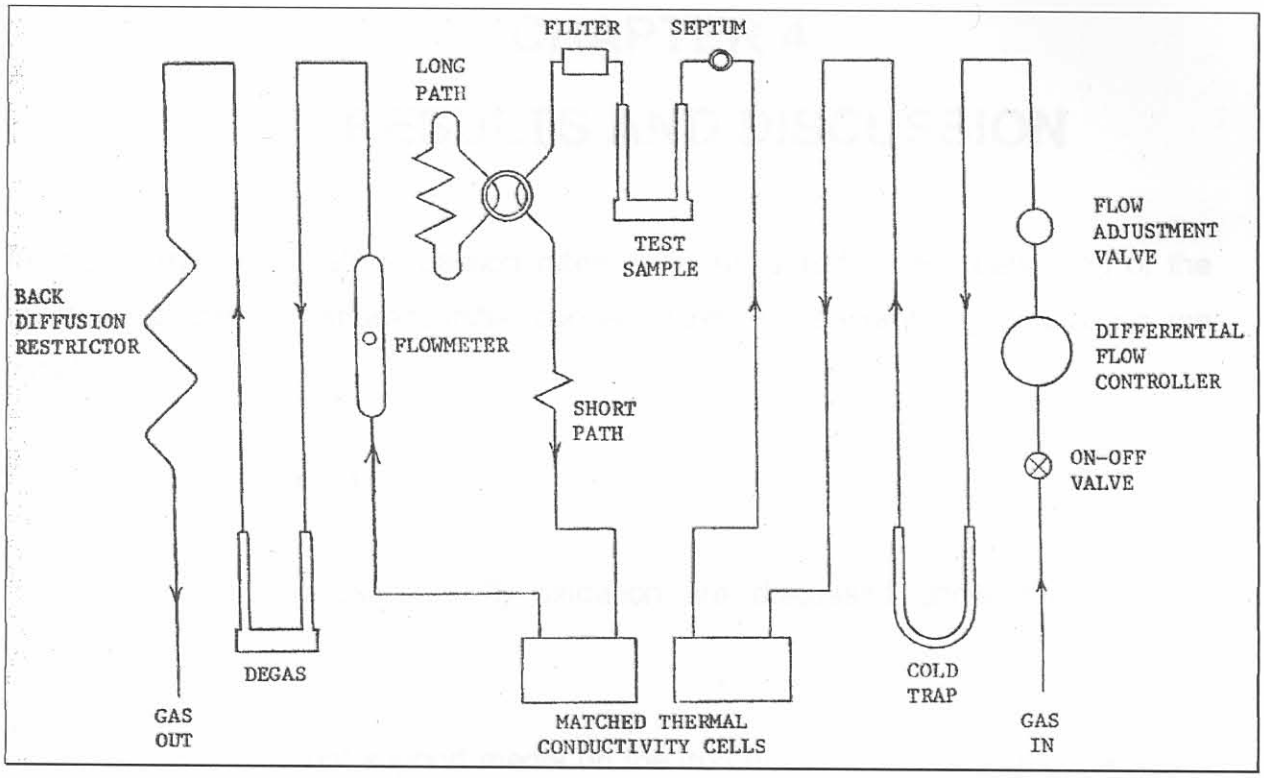

Figure 1: Schematic Diagram of a BET surface area analyser (Micromeritics FlowSorb II 2300