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.

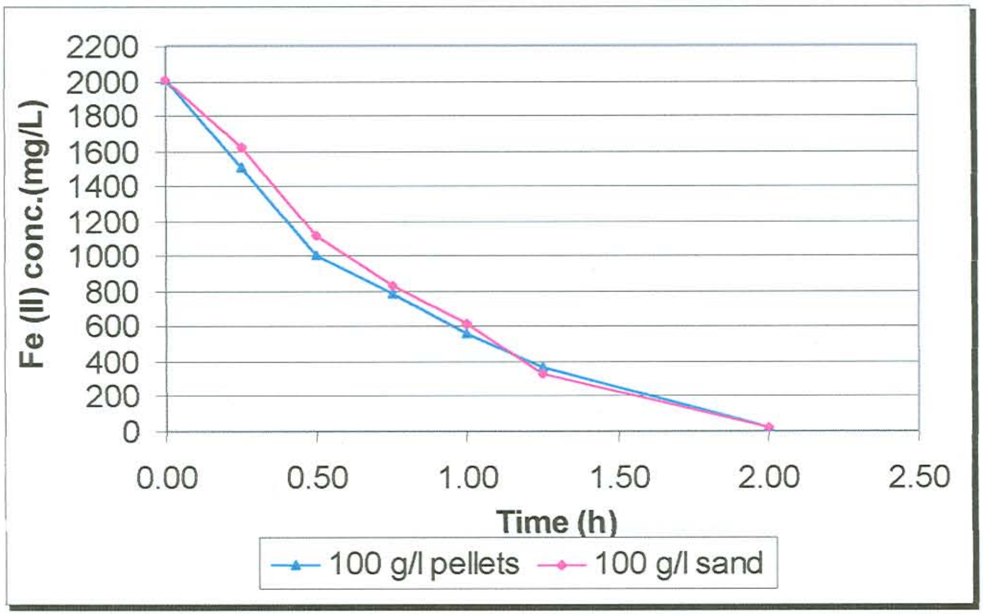

Figure 2: Effect of pellets and sand as support media on the iron (II) oxidation rate

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.

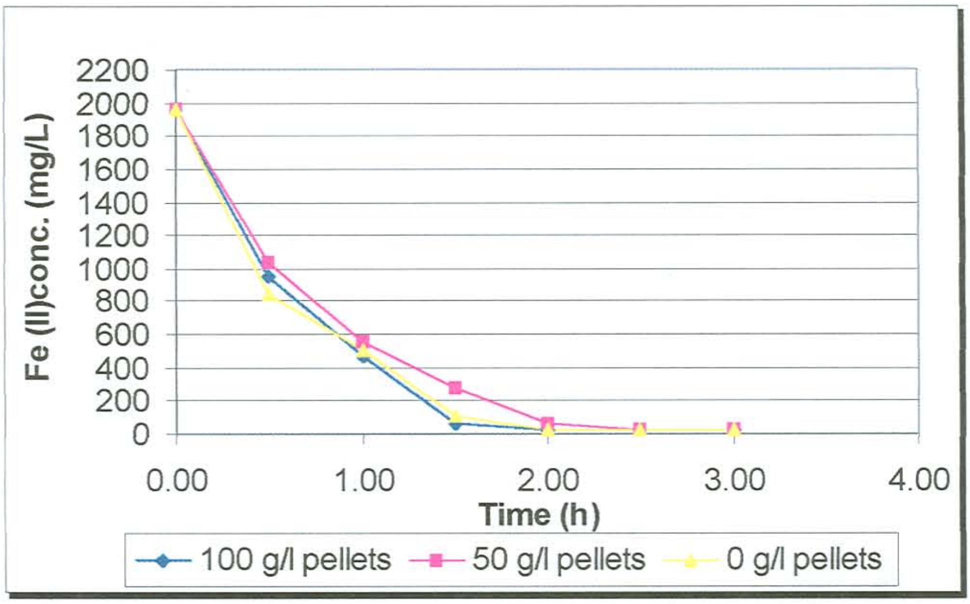

Figure 3: Effect of pellets concentration on the iron (II) oxidation rate

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)

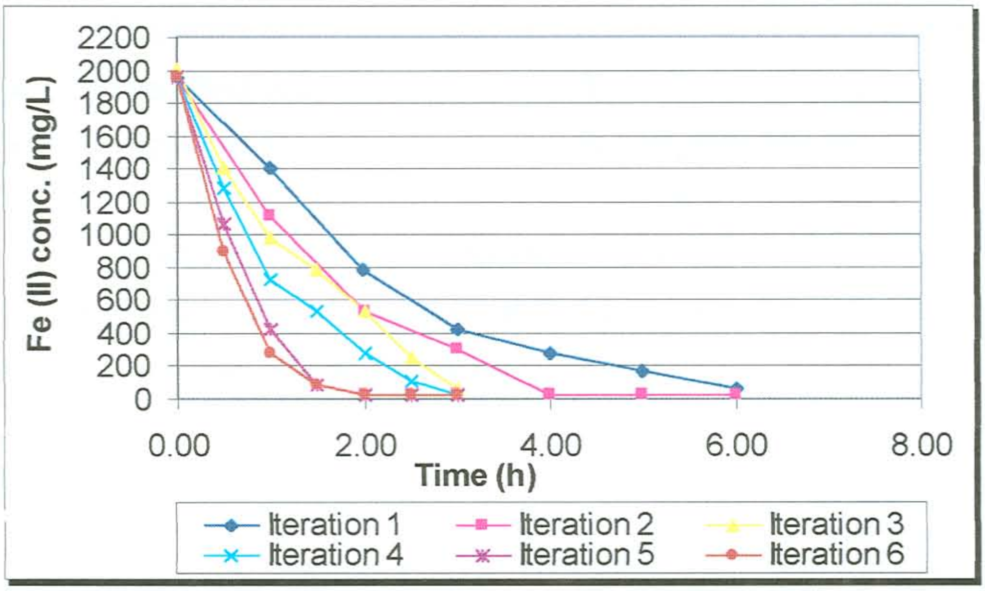

Figure 4: Effect of iterations with sludge (no pellets) on the iron (II) oxidation rate

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 ºC)

Other parameters such as temperature, pH, air flow rate and the initial iron (II) concentration were kept constant at T= 29 ºC, 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.

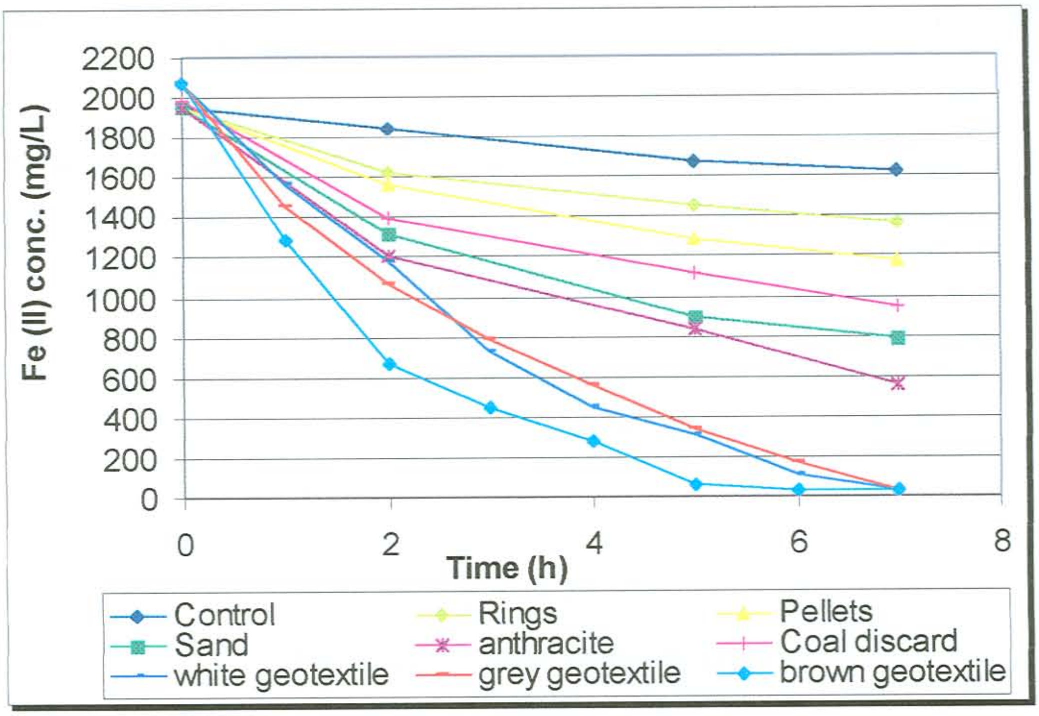

Figure 5: **Effect of the support medium on the iron (II) oxidation rate**

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.

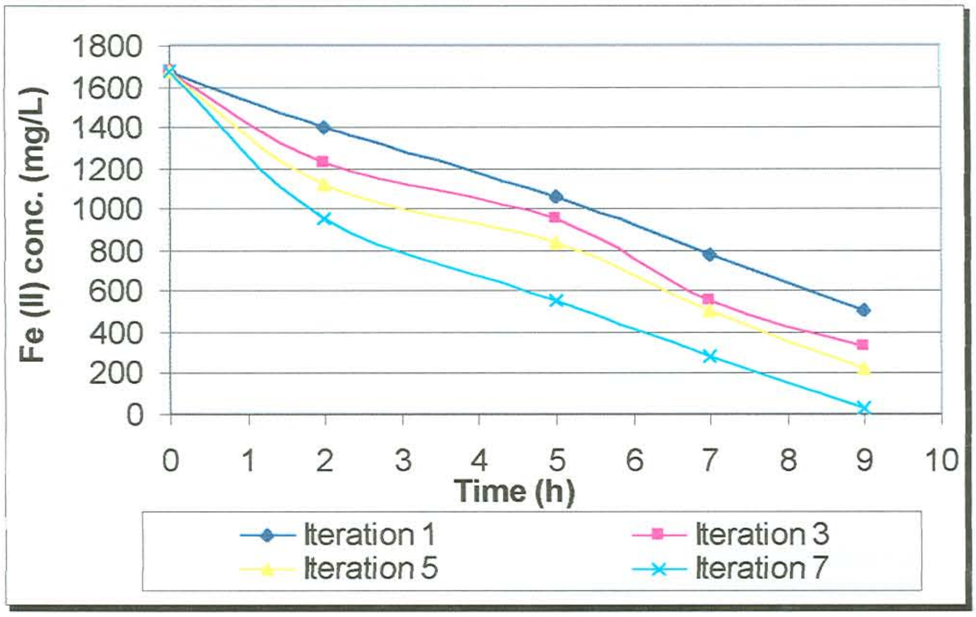

Figure 6: **Effect of iterations when using sand as support media on the iron (II) oxidation rate**

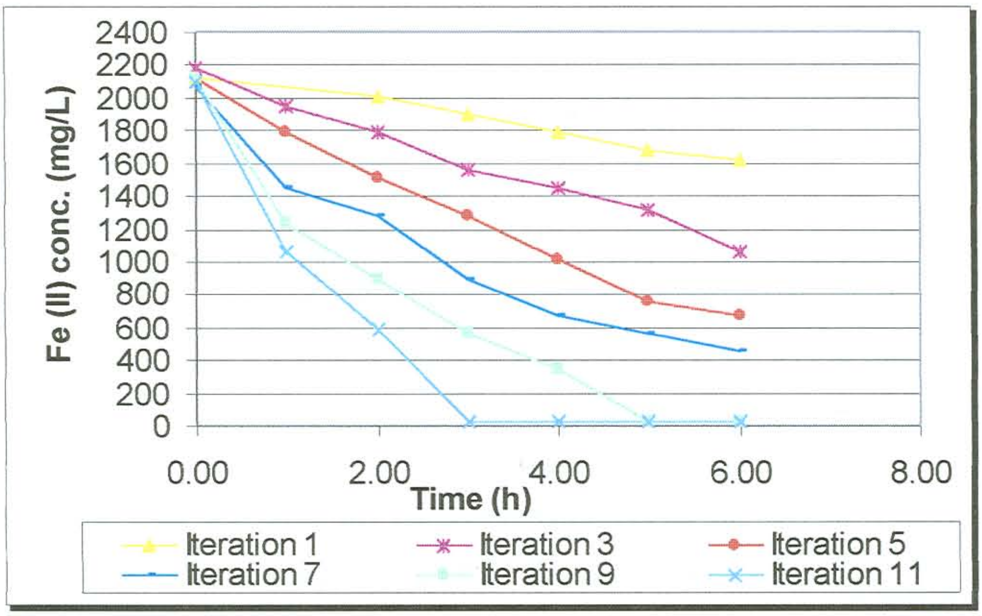

Figure 7: **Effect of iterations when using brown GT on the iron (II) oxidation rate**

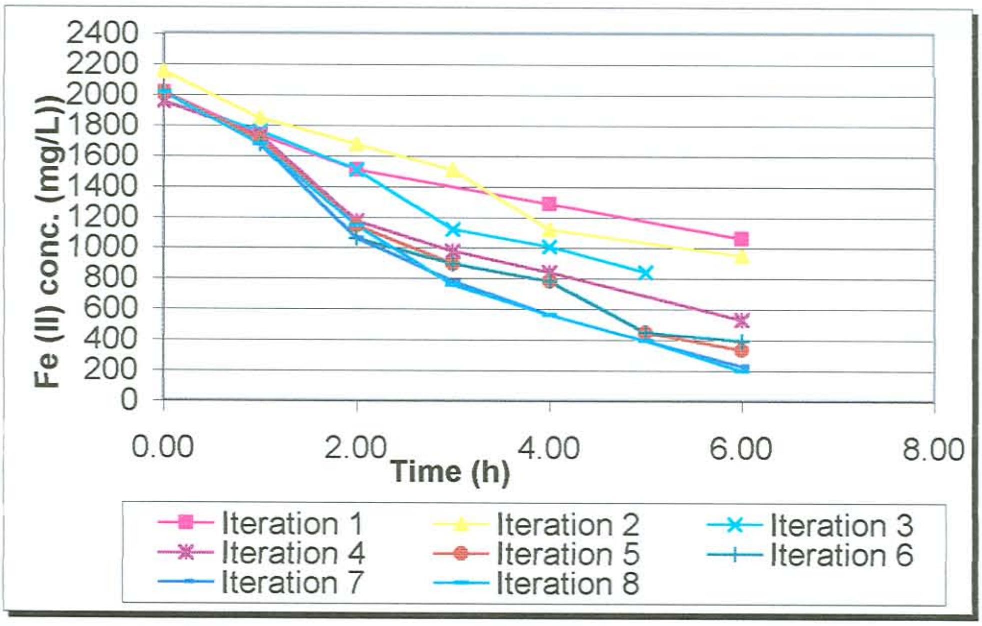

Figure 8: **Effect of iterations when using grey GT on the iron (II) oxidation rate**

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.

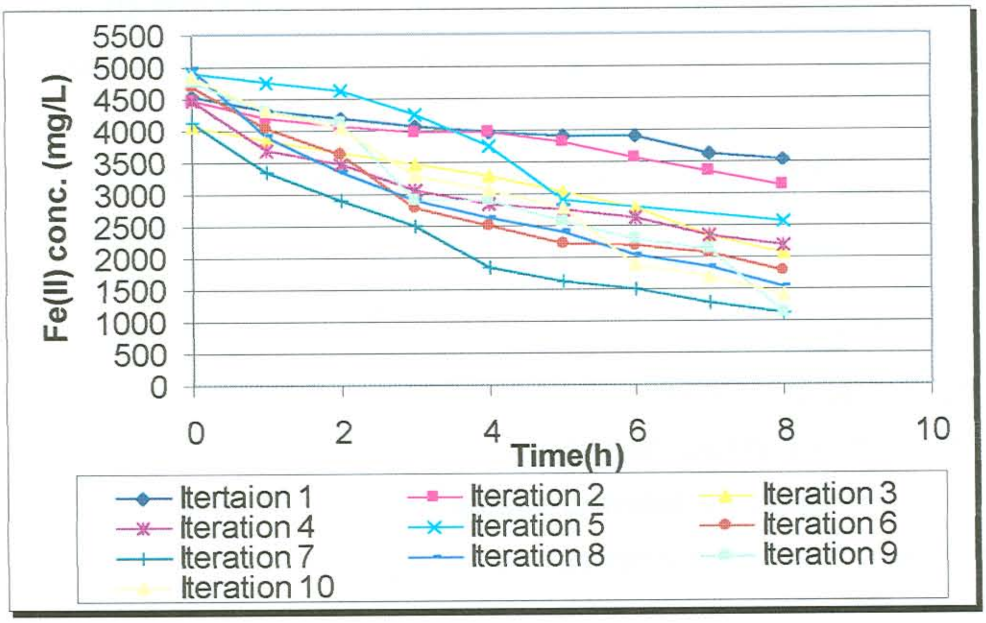

Figure 9: Effect of iterations and surface area on the iron(II) oxidation rate

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.

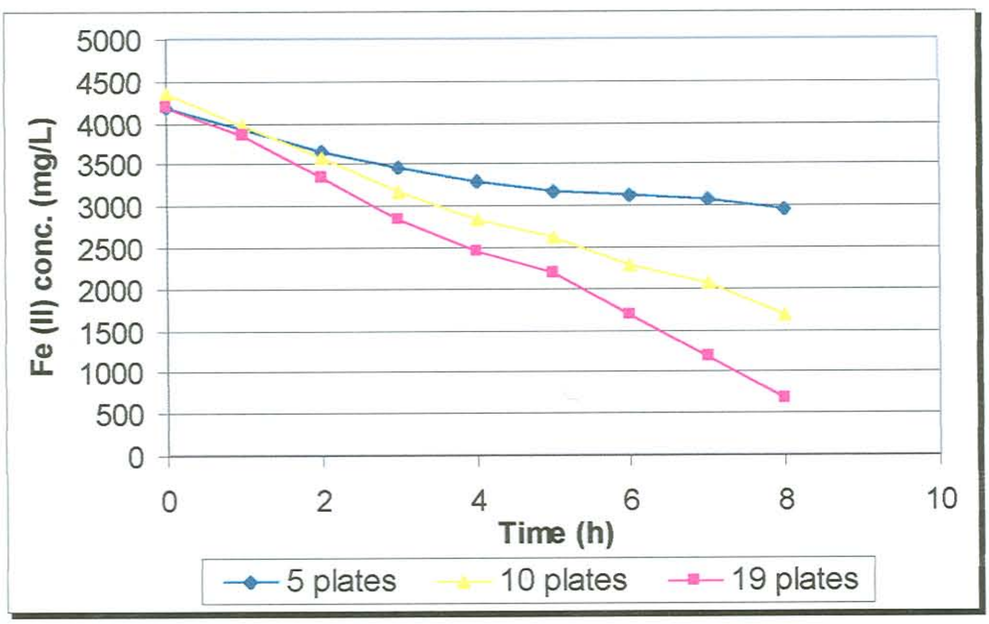

Figure 10: Effect of brown GT plates on the iron (II) oxidation rate

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

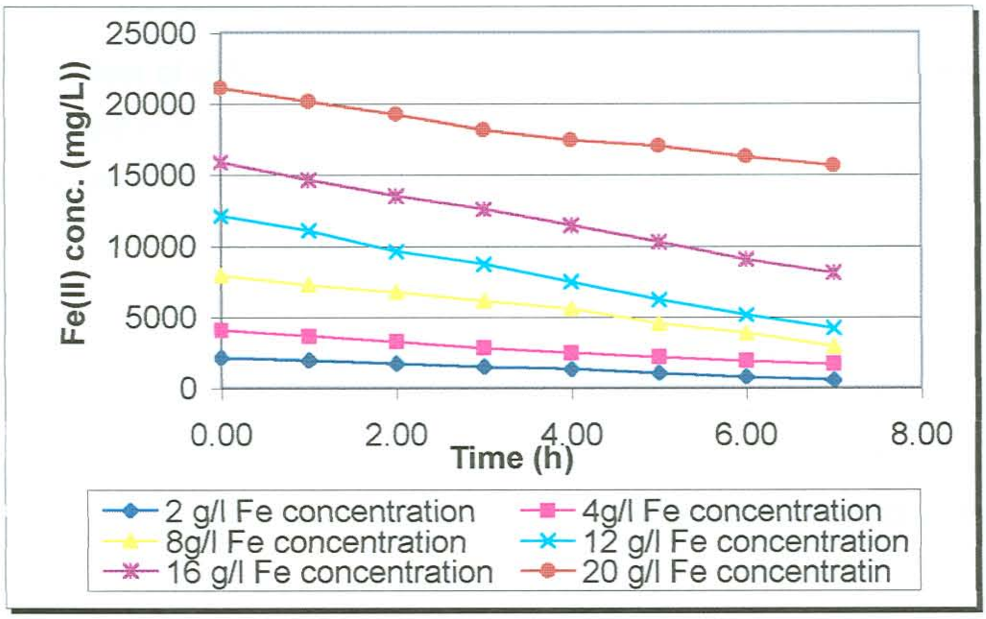

Figure 11: Effect of Fe (II) concentration using GT as support media on the iron (II) oxidation rate

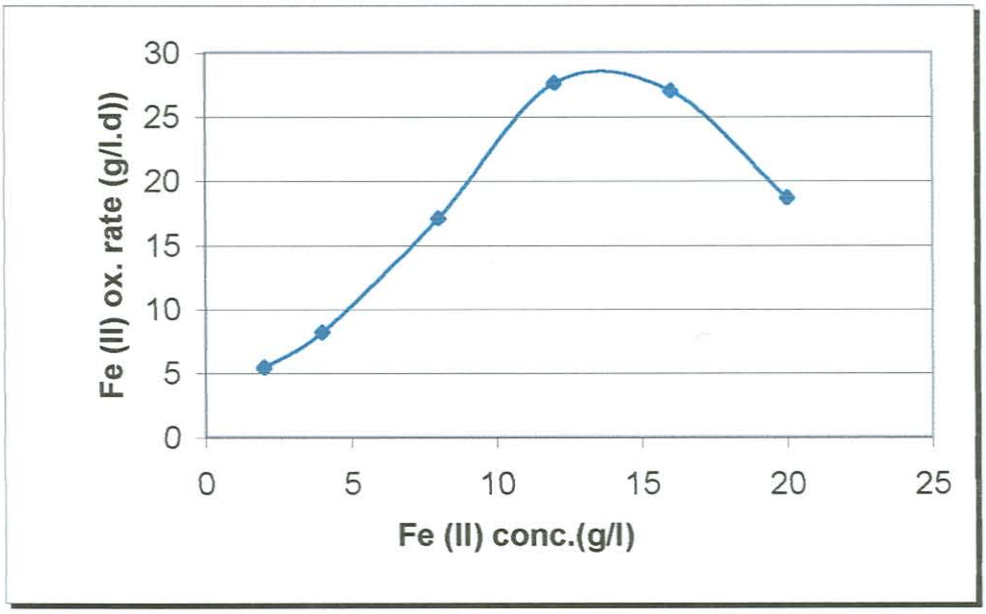

Figure 12: Effect of initial iron (II) concentration on the iron (II) oxidation rate.

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.

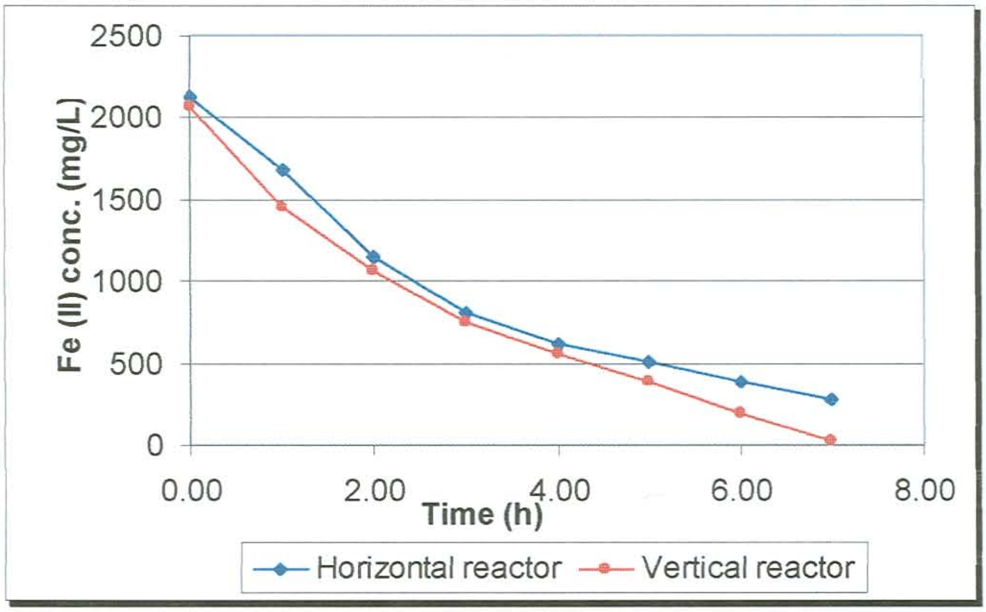

Figure 13: Effect of reactor system (type) on the iron (II) oxidation rate

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).

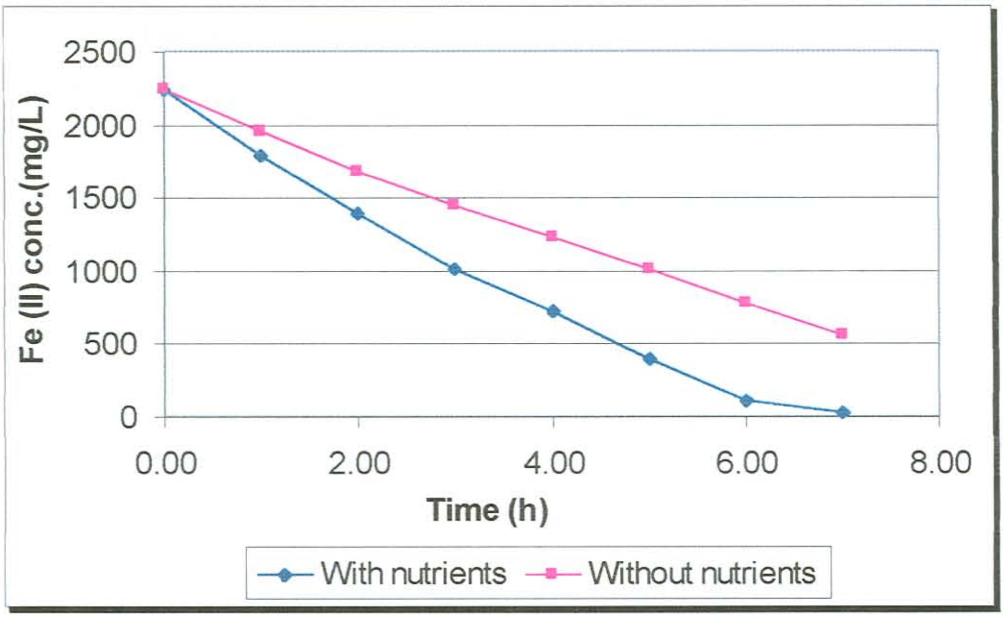

Figure 14: Effect of Nutrients on the iron (II) oxidation rate

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,

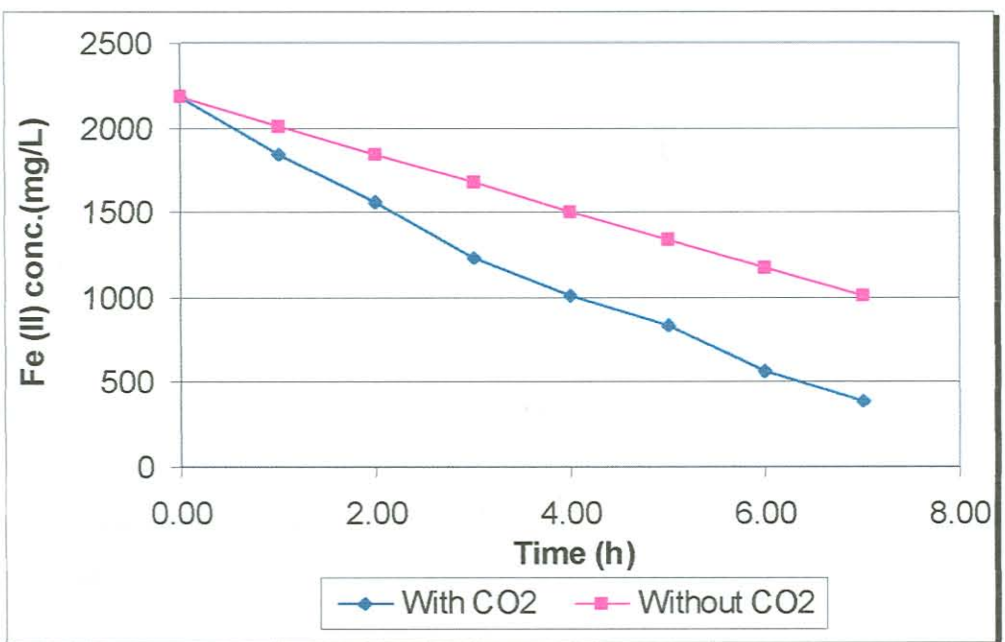

Figure 15: Effect of CO_2 on the iron (II) oxidation rate

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.

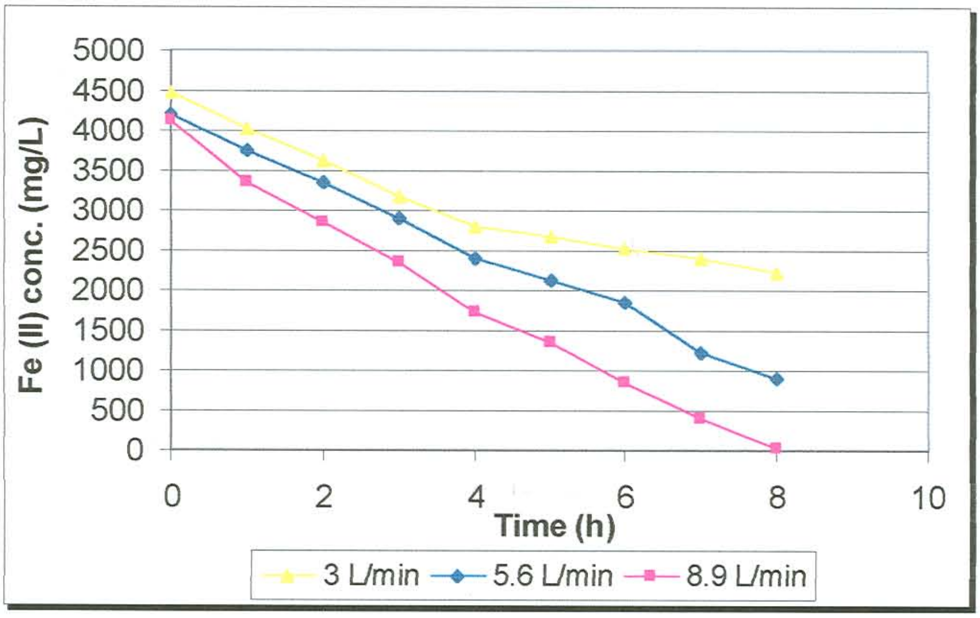

Figure 16: Effect of air flow on the iron (II) oxidation rate

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.

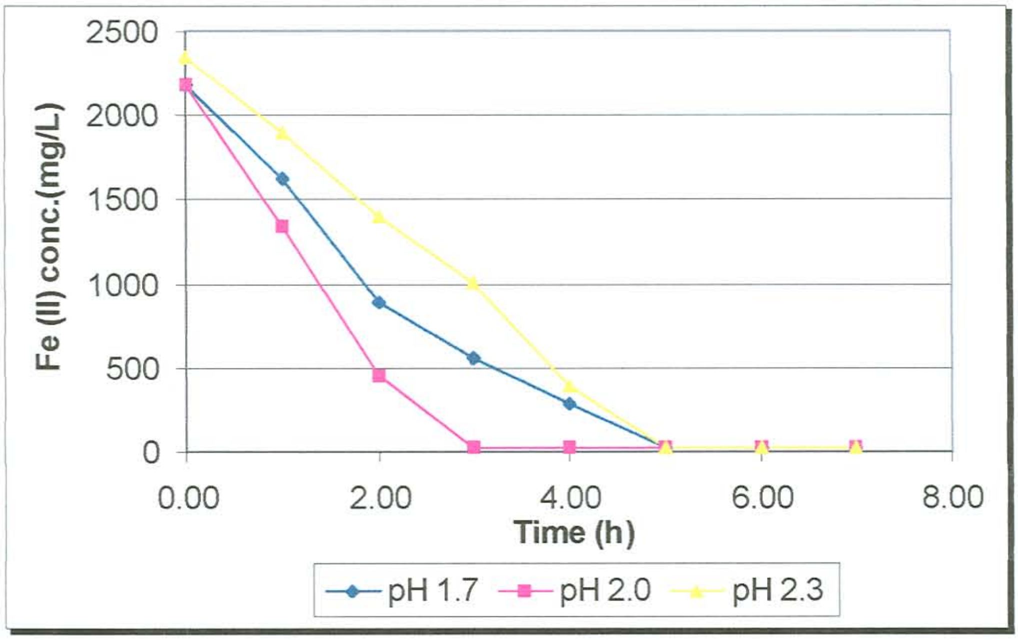

Figure 17: Effect of pH on the iron (II) oxidation rate

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.

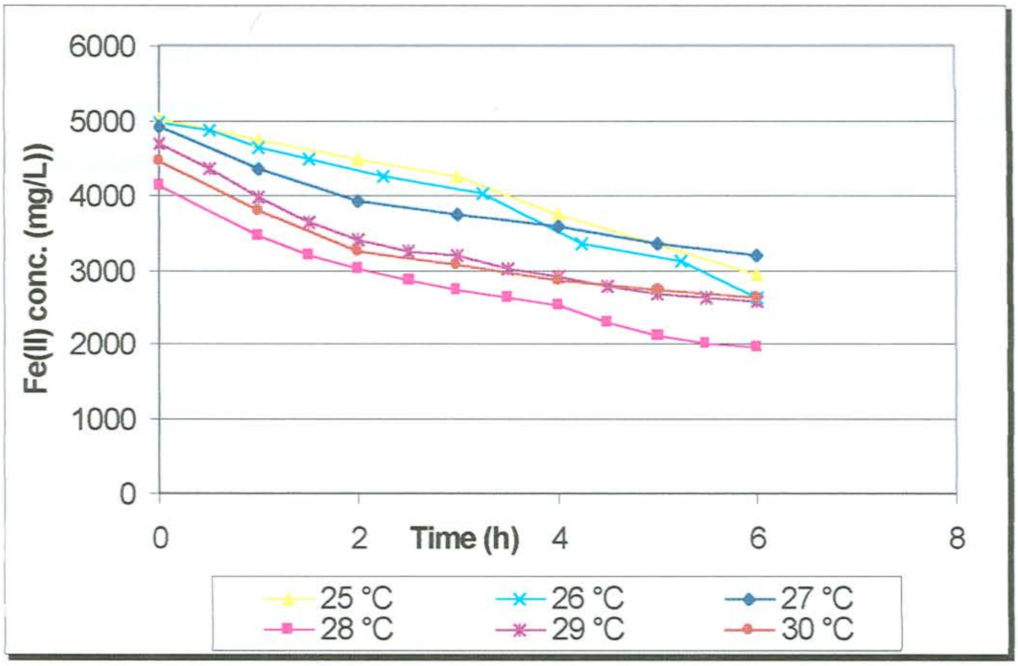

Figure 18: Effect of Temperature on the iron (II) oxidation rate

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.

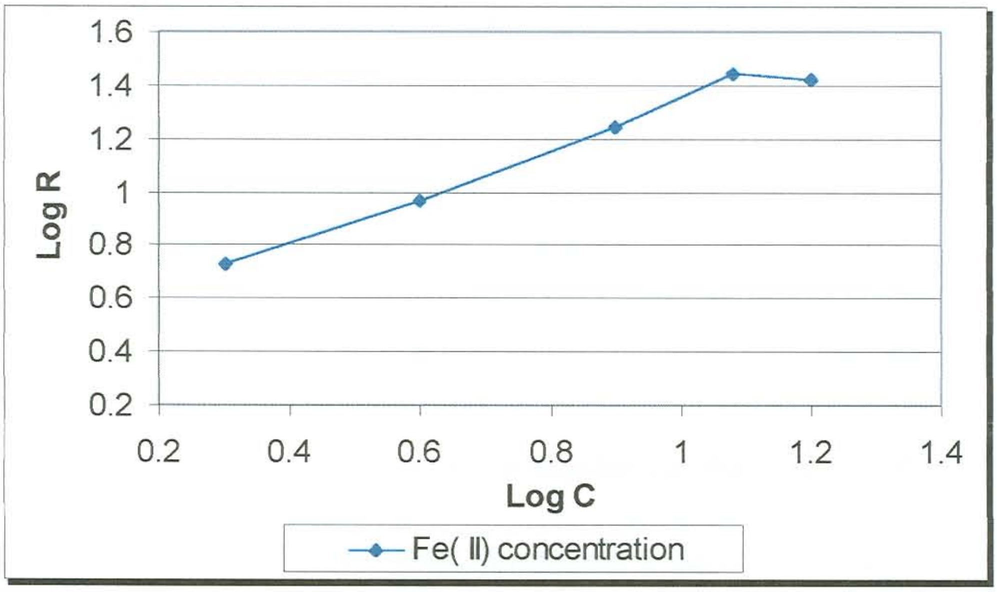

Figure 19: Effect of Fe (II) concentration on the kinetics of iron (II) oxidation

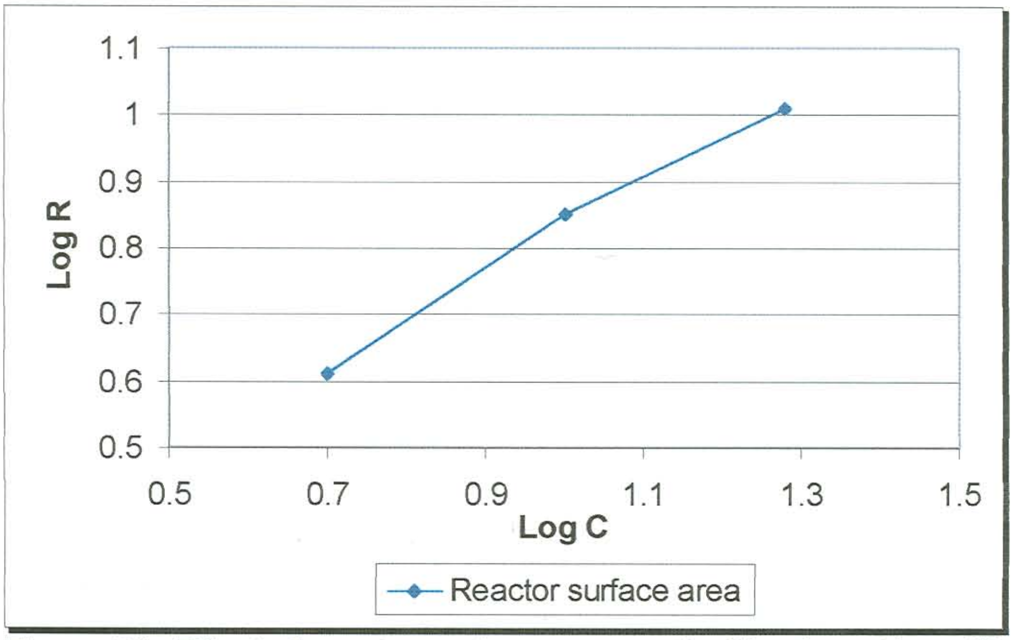

Figure 20: Effect of reactor surface area on the kinetics of iron (II) oxidation

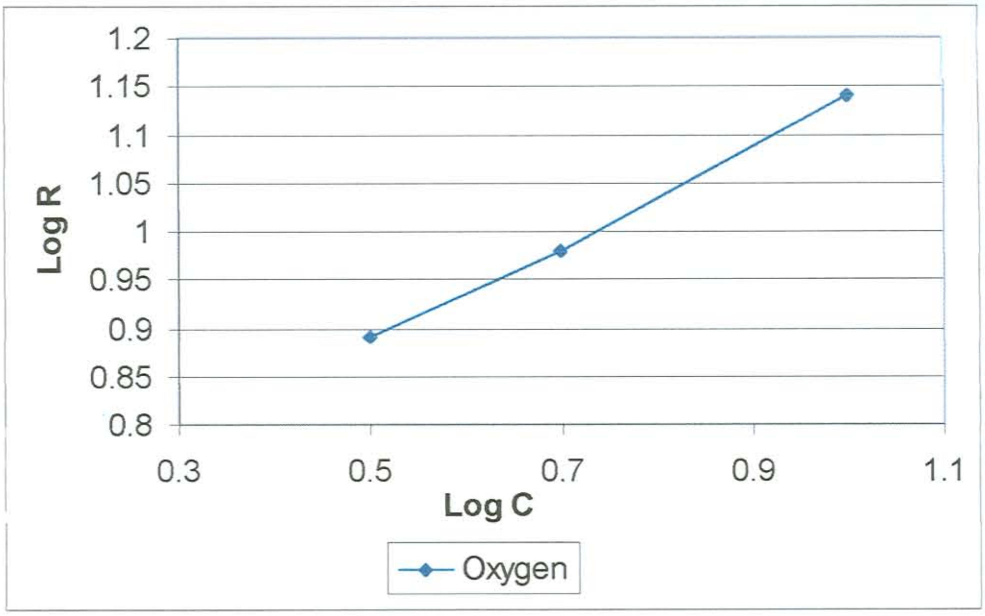

Figure 21: Effect of oxygen concentration on the kinetics of iron (II) oxidation

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).

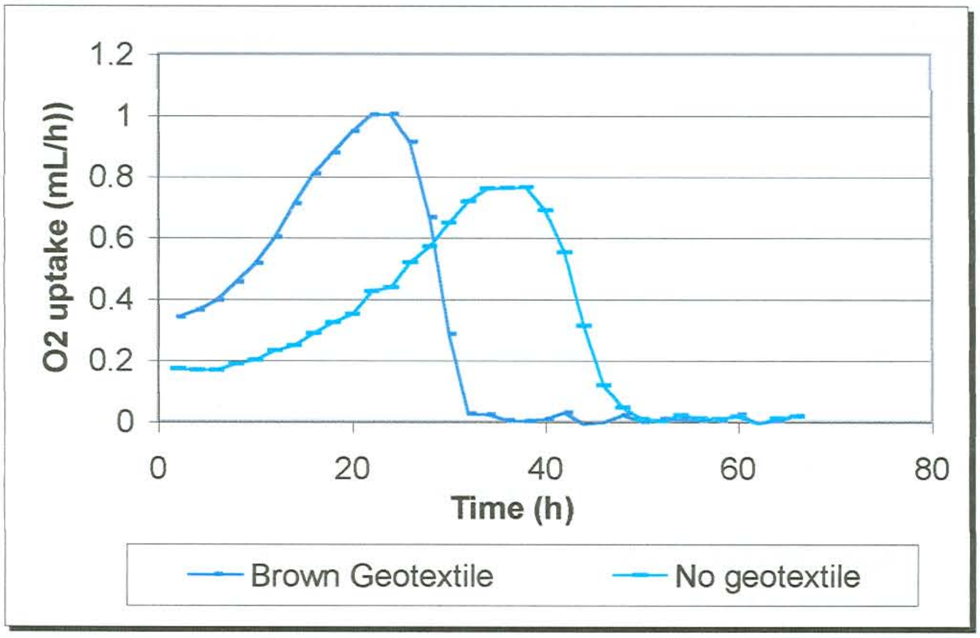

Figure 22: Effect of support media on the oxygen uptake rate

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.

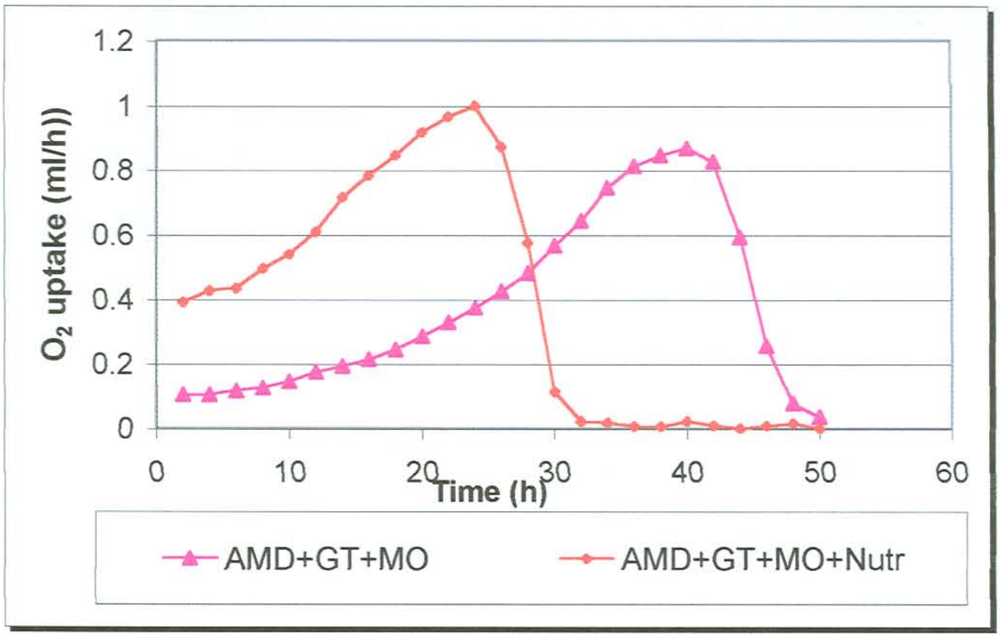

Figure 23: Effect of micro organisms and nutrients on the oxygen uptake rate

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)

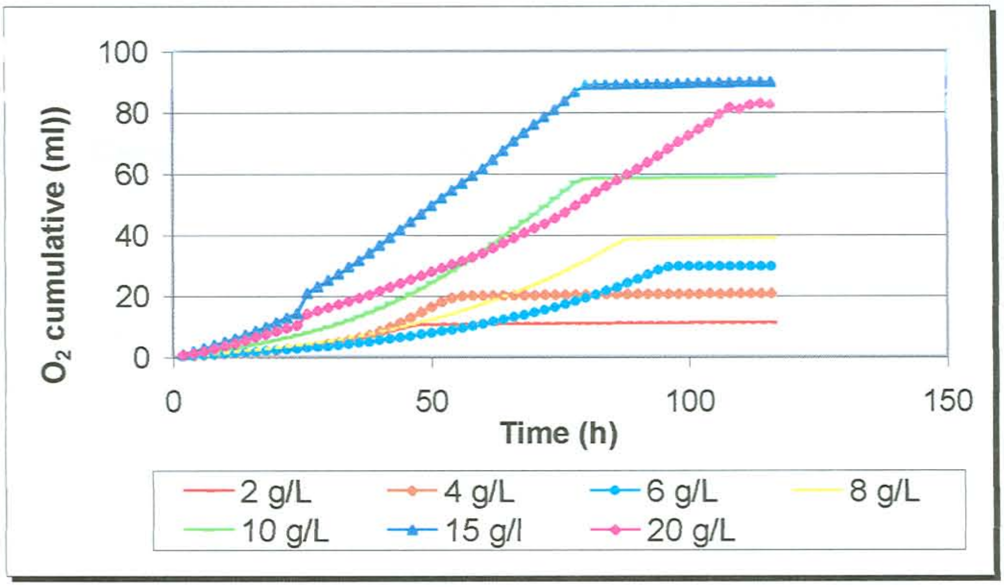

Figure 24: Effect of Fe (II) concentration on cumulative O_2 rate

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