CHAPTER 5

Chemical Leaching of Iron Ore Using a Range of Acids and Oxidative Chemicals

The editorial style of the *Water SA* was followed in this chapter

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5.1 Abstract

It has become important to process lower quality or complex iron ores due to the advancing global technologies and civilization. Phosphorous (P) and particular potassium (K) contained in the iron ore concentrates of the Sishen Iron Ore Mine have a detrimental effect on the steel making process, and therefore, it is becoming increasingly important to remove these alkalis from the charge materials before they enter the blast furnace. The P and K contained within the iron ore concentrate are in non-sulphidic phases, and therefore, these contaminants may be solubilised by a process of complexation using microbially produced inorganic or organic acids (Rawlings, 2005). Therefore, the objective of this study was to determine which acid would be considered to be the best leaching agent for the removal of P and K from the iron ore concentrate of the Sishen Iron Ore Mine. Citric acid proved to be the best leaching candidate for the removal of K from the iron ore, reducing the K content by 21.16%, while sulphuric acid reduced the P content by 27.58%, however, sulphuric acid is the most corrosive of the acids tested, removing 1.14% of the iron from the ore. Although no P was removed from the iron ore when using citric acid, further analyses are needed to determine whether higher leaching temperatures would play a role in the removal of P from the iron ore.

Key words: Phosphorous, potassium, iron ore concentrate, citric acid

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5.2 Introduction

As a result of the advancing global technologies and civilisation, there has been a progressive depletion of high-grade mineral deposits (Jian and Sharma, 2004).

Consequently, it has become increasingly important to process lower-grade or complex ores, as well as industrial and mining wastes (Torma, 1986). Elements such as phosphorous (P) and potassium (K) contained within the lower quality iron ore have a detrimental effect on steel making process, whereby these alkali's cause cracks to form in the refractory lining of blast furnaces (Yusfin *et al*., 1999). As a result the steel making companies charge penalties when purchasing iron ore that contains P and K above certain concentrations. Most steel making companies in the leading industrial nations have established limits on the concentrations of P and K that may be present in the charge materials without changing the smelting practice (Yusfin *et al*., 1999). Therefore, it is becoming increasingly important to remove these alkalis from the charge materials before they enter the blast furnace (Yusfin *et al*., 1999).

Conventional bioleaching involves the oxidation of insoluble metal sulphides to form corresponding soluble metal sulphates, enabling their removal from complex ore bodies (Rohwerder *et al*., 2002; Rawlings, 2005). The P and K contained within the iron ore concentrate are, however, in non-sulphidic phases, and therefore, conventional bioleaching processes are not viable for their removal from the ore. Metals in certain non-sulphide minerals, such as the iron ore concentrate of the Sishen Iron Ore Mine may be solubilised by a process of complexation using microbially produced inorganic or organic acids (Rawlings, 2005). Therefore, the objective of this study was to determine which acid would be considered to be the best leaching agent for the removal of P and K from the iron ore concentrate of the Sishen Iron Ore Mine.

5.3 Materials and Methods

5.3.1 Sample Selection

Iron ore samples (10 kg) of a –5+1 mm particle size were received from Sishen Iron Ore Mine for leaching analysis. The samples were stored in a dry environment for analysis.

5.3.2 Phosphorous and Potassium Phase Determination of the Untreated Iron Ore

The phases in which the P and K are present within the iron ore were determined by X-Ray Diffraction (XRD) analysis conducted at the Department of Geology, University of Pretoria, South Africa, as well as Kumba Resources, Ltd.

5.3.3 Chemical Preparation of Leaching Agents

All leaching chemicals used in this study were acquired from Merck, Darmstadt, Germany, with the exception of propionic acid, which was acquired from British Drug Houses, Ltd, Poole, UK. The leaching chemicals used during this study are listed in Table 5.1. 1 M leaching solutions were prepared by diluting the concentrated form of all chemicals in distilled water. The leaching solutions were stored at 25ºC until use.

TABLE 5.1
Leaching Chemicals for the Removal of P and K from the Iron Ore of the Sishen Iron Ore Mine

Chemical	**Chemical Formula**
Acetic Acid	$C_2H_4O_2$
Citric Acid	$C_6H_8O_7$
Ferric Sulphate	$Fe_2(SO_4)_3$
Hydrochloric Acid	HCl
Lactic Acid	$C_3H_6O_3$
Oxalic Acid	$C_2H_2O_4$
Propionic Acid	$C_3H_6O_2$
Sulphuric Acid	H_2SO_4

5.3.4 Chemical Leaching of Iron Ore

All leaching experiments were conducted in 250 ml Erlenmeyer flasks. Briefly, 100 ml of each of the leaching solutions was added to 100 g of iron ore in separate 250 ml glass Erlenmeyer flasks. A 100 g iron ore sample containing 100 ml of distilled water was used as a negative control. The flasks were incubated at 25ºC for 5 days. After the incubation the leaching solutions were removed and the iron ore washed five times with distilled water. The treated iron ore samples were dried in an oven at 150ºC for 24 h. The iron ore samples were stored in a dry environment for chemical analysis.

5.4 Results and Discussion

The chemical composition of the iron ore of Sishen Iron Ore Mine is given in Table 5.2. The average weight % of P and K in the iron ore was found to be 0.14 for both elements as detected by XRF spectrometry. These weight percentages, however, were too low to be detected by XRD analysis at the Department of Geology, University of Pretoria. Through personal communication with a mineralogist at HQP Research and Development, Kumba Resources, Ltd, it was discovered that the only K bearing phase detected thus far in the iron ore of Sishen Iron Ore Mine, has been muscovite, while P has been detected in various phases in the iron ore, including apatite, goyazite and woodhouseite.

TABLE 5.2
Major Elemental Composition of the Iron Ore of the Sishen Iron Ore Mine

Major Elements	Mass %
Silica (SiO_2)	0.89
Titanium (TiO_2)	0.08
Aluminium (Al_2O_3)	1.18
Iron (Fe_2O_3)	96.90
Manganese (MnO)	0.02
Magnesium (MgO)	0.12
Calcium (CaO)	0.07
Sodium (Na_2O)	0.08
Potassium (K_2O)	0.14
Phosphorous (P_2O_5)	0.14
Chromium (Cr_2O_3)	0.01

The average chemical composition of the iron ore after treatment with the various leaching solutions is given in Table 5.3. Citric acid proved to be the best leaching candidate for the removal of K from the iron ore, reducing the K content by 21.42%, while sulphuric acid reduced the P content by 24.43%. Due to data scatter as a result of sample and assay variation however, the actual decrease in K using citric acid may be as low as 7.69%, while the decrease in P using sulphuric acid may be as low as 9.40%. Acetic acid and sulphuric acid proved to have the highest Fe-corroding properties, reducing the Fe content by 1.14% and 1.00% respectively.

TABLE 5.3
Average Chemical Composition of the Iron Ore after Treatment with the Various 1M Leaching Solutions for 5 Days

Major Elements	$C_2H_4O_2$	$C_6H_8O_7$	$Fe_2(SO_4)_3$	HCl	$C_3H_6O_3$	$C_2H_2O_4$	$C_3H_6O_2$	H_2SO_4
SiO_2	1.52	1.14	0.59	0.94	1.36	1.12	0.46	1.19
TiO_2	0.07	0.07	0.08	0.07	0.08	0.07	0.07	0.06
Al_2O_3	1.02	1.05	1.38	1.32	1.09	1.10	1.21	1.11
Fe_2O_3	95.93	96.45	97.21	97.15	96.29	96.39	97.70	96.14
MnO	0.02	0.02	<0.01	<0.01	0.02	0.02	<0.01	<0.01
MgO	0.10	0.10	0.08	0.13	0.12	0.12	0.10	0.07
CaO	0.06	0.05	0.04	<0.03	0.05	0.08	0.05	<0.03
Na_2O	0.07	0.07	<0.05	<0.05	0.06	0.05	<0.05	<0.05
K_2O	0.13	0.11	0.14	0.15	0.14	0.13	0.14	0.13
P_2O_5	0.13	0.14	0.14	0.12	0.13	0.16	0.14	0.10
Cr_2O_3	<0.01	<0.01	<0.01	<0.01	0.01	0.01	<0.01	<0.01

5.5 Conclusions

Citric acid seemed to be the best leaching candidate for the removal of P and K from the iron ore of the Sishen Iron Ore Mine. Although no P was removed by citric acid during this investigation, further analyses are needed to determine whether higher leaching temperatures would play a role in the removal of P from the iron ore. Organic acids, such as citric acid produced by *Aspergillus niger*, possess several carboxyl groups, and therefore can act as chelating agents (Sayer and Gadd, 2001). These carboxyl groups tend to donate protons (H^+), resulting in negatively charged carboxyl groups that are capable of forming stable complexes with several metal cations, such as copper and zinc (Sayer and Gadd, 2001). Therefore, it is possible that these negatively charged carboxyl groups are able to form stable complexes with the K^+ cations present, while the H^+ cations are able to react with the negatively charged phosphorous, resulting in their removal from the iron ore concentrate.

5.6 References

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CHAPTER 6

The Production and Use of Citric Acid for the Removal of Phosphorous and Potassium from the Iron Ore Concentrate of the Sishen Iron Ore Mine, South Africa

The editorial style of *Hydrometallurgy* was followed in this chapter

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6.1 Abstract

The depletion of the richer iron ore worldwide has made it necessary to process lower quality iron ore. Certain substances, such as phosphorous (P) and potassium (K), contained within the iron ore have a detrimental effect on the smelting process during steel manufacturing. Therefore, international steel making companies charge penalties when purchasing iron ore concentrates containing high concentrations of P and K. To date, lower quality iron ore has been blended with high quality iron ore in an attempt to alleviate the P and K concentrations in the export iron ore product, however, the ratio of low quality iron ore to high quality iron ore is increasing, and thus becoming an escalating problem within the economic functioning of the Sishen Iron Ore Mine. It has, therefore, become necessary to develop an economically viable and environmentally friendly process to reduce the high P and K concentrations contained in the iron ore concentrate of the Sishen Iron Ore Mine, resulting in the minimizing of the penalties charged by the steel making companies. During this study solid substrate- and submerged fermentation using *Aspergillus niger* were compared for the production of citric acid, which is to be used for the chemical leaching of P and K from the iron ore concentrate. It was found that submerged fermentation proved to be more economical and efficient, producing a maximum citric acid concentration of 102.3 g.l^{-1} in 96 h of fermentation. "Heap leaching" simulation experiments was found to be uneconomical due to the addition of fungal growth medium every 5 days as a result of growth factor depletion within this time, however, this process removed 17.65% K and no P from the iron ore concentrate. In contrast, chemical leaching of P and K from the iron ore concentrate proved to be most efficient when using a 1M citric acid leaching solution at 60ºC, removing 35.29% P and 23.53% K contained within the iron ore concentrate. Therefore, the most economical and efficient process for the removal of P and K from the iron ore concentrate of the Sishen Iron Ore Mine, involves a two-stage process whereby citric acid is produced by *A. niger*, followed by the chemical leaching of the P and K from the iron ore concentrate using a 1M citric acid leaching solution at 60ºC.

Key words: Iron ore concentrate, phosphorous, potassium, solid substrate citric acid fermentation, submerged citric acid fermentation, heap leaching, chemical leaching

6.2 Introduction

The depletion of the richer iron ore deposits worldwide, as a result of the advancing global technologies and civilisation, necessitated the processing of lower quality iron ore (Jian and Sharma, 2004). The phosphorous (P) and potassium (K) contained within the lower quality iron ore have a detrimental effect on the smelting process during steel making in blast furnaces (Yusfin *et al*., 1999). These compounds are deposited on the surface of the coke, where they act as a catalyst in the gasification of carbon in the presence of carbon dioxide (CO_2) (Yusfin *et al*., 1999). The presence of especially K in the coke leads to the formation of $K_2O.SiO_2$ and $K_2O.Al_2O_3.2SiO_2$, which leads to an increase in the coke volume and its subsequent fracture (Yusfin *et al*., 1999). In addition, K penetrates the monolithic alumosilicate lining of the furnace, resulting in the formation of silicide or leucite, and the subsequent rearrangement of the crystalline lattice of the refractories (Yusfin *et al*., 1999). This results in the creation of stresses that cause cracks to form in the refractory lining, leading to its subsequent destruction (Yusfin *et al*., 1999). Therefore, as a result of the negative financial impact due to the destruction of the refractory lining, the steel making companies charge penalties when purchasing iron ore concentrates containing high concentrations of P and K. The limits on alkali concentrations range from 0.25% mass in Japan to 0.55% mass in Switzerland (Yusfin *et al*., 1999).

In the past, lower quality iron ore was blended with high quality ore to "dilute" the P and K concentrations in the final iron ore product, which is exported to the international steel making plants. Similar practices have been reported from other parts of the world, such as the Hamersley Province in Australia where low-phosphorous ore (0.05% P) is blended with high-phosphorous ore (0.10% P), the former being the major component of the blend (Dukino *et al*., 2000). To date, the blending of different quality iron ores has minimised the penalties charged by the steel making companies, however, the ratio of low quality iron ore to high quality iron ore is increasing, and thus becoming an escalating problem within the economic functioning of the Sishen Iron Ore Mine. It has, therefore, become important to develop an economically viable and environmentally friendly process to reduce the high P and K concentrations contained in the iron ore concentrate to improve the quality of ore that is being exported to the international steel making companies.

During a previous study whereby a range of inorganic- and organic acids were tested for the removal of P and K from the iron ore concentrate of the Sishen Iron Ore Mine, it was discovered that citric acid (2-hydroxypropane-1,2,3-tricarboxylic acid) proved to be the best leaching agent for the removal of P and K without a major reduction in the iron content (0.46%) of the ore (Chapter 5). Citric acid is an intermediate in the tricarboxylic acid (TCA) cycle, which is widely used in the food, beverage, pharmaceutical and cosmetic industries, but also has other applications in the textile, electroplating and bioremediation industries (Wang and Liu, 1996; Tran *et al*., 1998; Ates *et al*., 2002). The most popular microorganism for the large-scale production of citric acid is the white-rot fungus *Aspergillus niger*, due to its high citric acid productivity at low pH without the secretion of toxic metabolites (Kim, 2004). Citric acid production by *A. niger* involves two main metabolic pathways, namely: 1) the catabolic pathway of hexoses to pyruvate and Acetyl-coenzyme A by glycolysis, and 2) citric acid formation by the TCA cycle (Alvares-Vasquez *et al*., 2000). During cell propagation and maintenance, complete oxidation of glucose leads to the production of adenosine triphosphate (ATP), CO_2 and H_2O (Jianlong, 2000). Depending on the growth conditions and concentrations of end products, *A. niger* is able to control when to stop the full respiration process during the TCA cycle, resulting in the production of citric acid (Jianlong, 2000).

Citric acid contains three carboxyl groups, which tend to donate protons (H^+), resulting in negatively charged carboxyl groups that are capable of forming stable complexes with several cations (Sayer and Gadd, 2001). Therefore, it would be possible that these negatively charged carboxyl groups might form stable complexes with the positively charged K cations present, resulting in its removal from the iron ore concentrate. Therefore, the objective of this study was to develop a "bioleaching" process, whereby citric acid is produced and used to remove the P and K from the iron ore concentrate of the Sishen Iron Mine. In addition, a heap leaching process was investigated whereby *A. niger* was directly applied to the iron ore concentrate of the Sishen Iron Ore Mine.

6.3 Materials and Methods

6.3.1 Microorganism and Preparation of Inoculum

A freeze-dried sample of *Aspergillus niger* NRRL 567 was obtained from the American Type Culture Collection (ATCC, Rockville, MD, USA) and stored at 4ºC. *A. niger* spores were produced on Potato Dextrose Agar (PDA) (Merck Laboratories, Darmstadt, Germany) (Appendix A) at 30ºC and were sub-cultured at bi-weekly intervals. Spores were harvested after 7 days of incubation by adding 10 ml of 0.1% Tween 80 (Merck) solution to each plate. Spore inoculates of 1.0×10^7 spores.ml^{-1} were prepared using a haemocytometer.

6.3.2 Solid Substrate

Sphagnum Peat Moss (PM) (Schultz Company, Mississauga, Ontario, Canada) supplemented with glucose was used to simulate a sugar-rich by-product. The PM was sterilised in an autoclave at 121ºC for 15 min. The PM was wetted to moisture content (MC) of 80% wet weight with deionised water supplemented with various salts and glucose (Considine *et al.*, 1987).

6.3.3 Basal Salt Solution for Solid Substrate Fermentation

The basal salt solution used to wet the dried Peat Moss (DPM) and to provide basic nutrients to the fungal culture contained the following (Appendix A): 3.84 g.l^{-1} $(NH_4)_2SO_4$, 10.98 g.l^{-1} KH_2PO_4, 1.01 g.l^{-1} NaCl, 1.01 g.l^{-1} $MgSO_4.7H_2O$ and 1.01 g.l^{-1} $FeSO_4.7H_2O$ (Kim, 2004). After the addition to PM, the solution gave the following salt concentration in terms of kg DPM: 15.36 g $(NH_4)_2SO_4$, 43.92 g KH_2PO_4, 4.04 g NaCl, 4.04 g $MgSO_4.7H_2O$ and 4.04 g $FeSO_4.7H_2O$.

6.3.4 Fermentation Medium for Submerged Fermentation

The fermentation medium for the submerged fermentation was prepared using distilled water (dH_2O) and contained the following (Appendix A): 150 g.l^{-1} D-glucose, 2.5 g.l^{-1} $(NH_4)_2SO_4$, 0.5 g.l^{-1} $MgSO_4.7H_2O$, 2.0 KH_2PO_4, 0.1×10^{-3} g.l^{-1} $Fe_2(SO)_4.24H_2O$, 0.1×10^{-3} g.l^{-1} $ZnSO_4.7H_2O$ and 0.06×10^{-3} g.l^{-1} $CuSO_4.5H_2O$.

6.3.5 Fermentation Conditions for Solid Substrate Fermentation

Solid substrate fermentation was conducted in 500 ml Erlenmeyer flasks holding 25 g of DPM wetted with 100ml of the basal salt solution and 967.88 g.kg^{-1} DPM glucose (Merck) (Kim, 2004). The solid substrate was inoculated with 1ml of *A. niger* NRRL 567 inoculum (1.0×10^7 spores.ml^{-1}). The Erlenmeyer flasks and their contents were incubated at 30ºC for 6 days. The fermentation procedures were conducted in duplicate.

6.3.6 Fermentation Conditions for Submerged Fermentation

Submerged fermentation was conducted in 500 ml Erlenmeyer flasks. Briefly, 250 ml of fermentation medium was inoculated with 1 ml of *A. niger* NRRL 567 inoculum (1.0×10^7 spores.ml^{-1}). The Erlenmeyer flasks and their contents were incubated at 30ºC for 6 days. The fermentation procedures were conducted in duplicate.

6.3.7 Analytical Procedure

For each sampling procedure of the solid substrate fermentation, 5 g of wet sample of PM was harvested from each flask and placed in 50ml of dH_2O, followed by incubation in a shake incubator for 60 min at 150 revolutions per min (rpm) and 25ºC. The supernatant was filtered through a 0.45µm syringe filter (Millipore), followed by the analyses for pH and citric acid quantification. The sampling of the submerged fermentation entailed the aseptic extraction of 5 ml of the fermentation medium from each flask to be used for analyses. The extracted medium was filtered through a 0.45µm syringe filter (Millipore), followed by the analyses for pH and citric acid quantification. The abovementioned analyses were performed daily for 6 days.

6.3.8 Citric Acid Concentration Analysis

Citric acid concentrations were determined by spectrophotometry at 420nm after adding pyridine and acetic anhydride as described by Marier and Boulet (1958). Briefly, 1 ml of the sample was added to a test tube containing 1.3 ml of pyridine (C_5H_5N) (Merck), followed by the addition of 5.7 ml of acetic anhydride ($C_4H_6O_3$) (Merck). The contents of the test tube was mixed by swirling the tube and immediately placed in a constant-temperature (22ºC) water bath. Colour development was allowed for 30 min, followed by reading the colour intensity at 420nm with the blank set on 100% transmission. The citric acid concentration was determined by referring to a standard curve for citric acid concentration. Citric acid concentrations were expressed per kg of DPM for solid substrate fermentation and $g.l^{-1}$ for submerged fermentation.

6.3.9 Chemical Leaching of Iron Ore Concentrate with Citric Acid

The produced citric acid was used for the chemical leaching of the iron ore concentrate. A 1M concentration of citric acid was added to a 40g sample of iron ore concentrate (–5+1mm particle size) in a 250ml Erlenmeyer flask. 40g of iron ore concentrate containing 40 ml of distilled water was used as a negative control. The flasks were incubated at 25ºC for 5 days. In addition, the leaching procedure was conducted using different concentrations (0.25M, 0.5M, 0.75M and 1M) of citric acid at a leaching temperature of 60ºC for 5 days. After the incubation the leaching solutions were removed and the iron ore concentrate washed five times with distilled water. The treated iron ore concentrate samples were dried in an oven at 150ºC for 24 h. The iron ore concentrate samples were stored in a dry environment for chemical analysis.

6.3.10 "Heap Leaching" of Iron Ore Concentrate Using Aspergillus niger

A "heap leaching" simulation experiment was conducted in 500 m*l* Erlenmeyer flasks. Briefly, spore suspensions were prepared as in 6.3.1. The fermentation medium used during these experiments was the same as used for submerged citric acid fermentation (6.3.4). Iron ore concentrate (500g) was placed in each Erlenmeyer flask and mixed with 50 m*l* of fermentation medium inoculated with 1 m*l* of the spore inoculum (1.0×10^7 spores.ml^{-1}). The flasks and their contents were incubated at 30ºC for a period of 40 days.

6.3.11 Chemical Analysis of the Treated Iron Ore Concentrate

The chemical content of the treated iron ore concentrate was analysed by the Department of Geology, University of Pretoria, Pretoria, South Africa, using XRF spectrometry of the major elements. Briefly, the samples were ground to <75µm in a Tungsten Carbide milling vessel, roasted at 1000ºC, followed by the fusing of 1g of sample and 9g $Li_2B_4O_7$ into a glass bead. Major element analysis was executed on the fused bead using a ARL9400XP+ spectrometer. Another aliquot of the sample was pressed into a powder briquette for trace element analysis.

6.4 Results and Discussion

Previous research has suggested that citric acid is excreted from *A. niger* cells in response to unfavourable intracellular conditions that lead to increased levels of tricarboxylic acids through anaplerotic pathways during growth in a high glucose concentration environment (Legiša and Mattey, 2007). It is suggested that polyols, in particular glycerol, may play an important role as osmoregulators in *A. niger* cells, which explains the ability to grow in environments where high glucose concentrations prevail (Legiša and Kidrič, 1989). Therefore, a high initial glucose concentration of 967.88 g.kg^{-1} DPM and 150 g.*l*$^{-1}$ was used for solid substrate- and submerged fermentation respectively, to ensure that citric acid is excreted from the *A. niger* mycelia.

Solid substrate- and submerged citric acid production by *A. niger* NRRL 567 at 30ºC is illustrated in Figure 6.1 and 6.2 respectively. Citric acid excretion commenced after approximately 24 h during the solid substrate fermentation, compared to 48 h during submerged fermentation. This may be attributed to the abnormal spore germination in the form of bulbous cells during the early stages of growth, followed by a sudden change in morphology to highly branched filamentous hyphae, which are responsible for the citric acid overflow (Legiša *et al*., 1981). During the germination of the fungal spores the pentose phosphate pathway is predominant, followed by a switch to glycolysis before the onset of citric acid excretion (Legiša and Mattey, 1986a; Röhr *et al*., 1987).

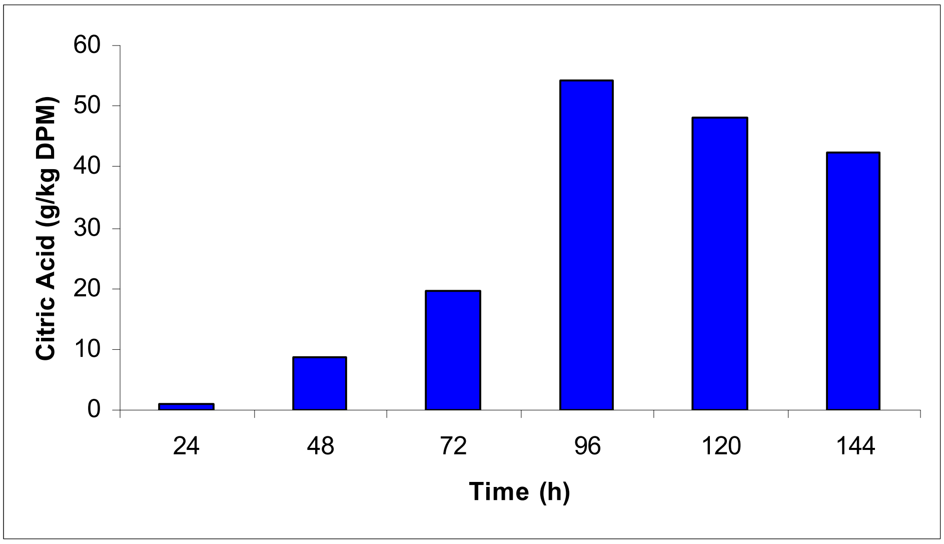

FIGURE 6.1

Diagrammatic Representation of the Solid Substrate Citric Acid Production from PM by *Aspergillus niger* NRRL 567 grown at 30ºC

An increase in citric acid productivity was observed until maximisation at approximately 96 h (54.2 g.kg^{-1} DPM and 102.3 g.l^{-1} respectively for solid substrate- and submerged fermentation) (Figures 6.1 and 6.2). Similar observations have been recorded, where little or no citric acid could be detected in the medium during the first 24 h of fermentation, while a relatively slow excretion rate was observed during the second day of fermentation, followed by a sudden increase in citric acid productivity thereafter (Röhr and Kubicek, 1981; Legiša and Mattey, 1986b; Ruijter *et al.*, 1997; Papagianni *et al.*, 2005). During the productive phase of citric acid accumulation, the direct conversion of hexoses to pyruvate via glycolysis becomes predominant, starting after approximately 24 h and accelerating after 40-50 h of growth in a batch system (Legiša and Mattey, 1986a). It is, however, not clearly understood what the mechanism causing the shift of glucose degradation from the pentose phosphate pathway to glycolysis is (Legiša and Mattey, 2007).

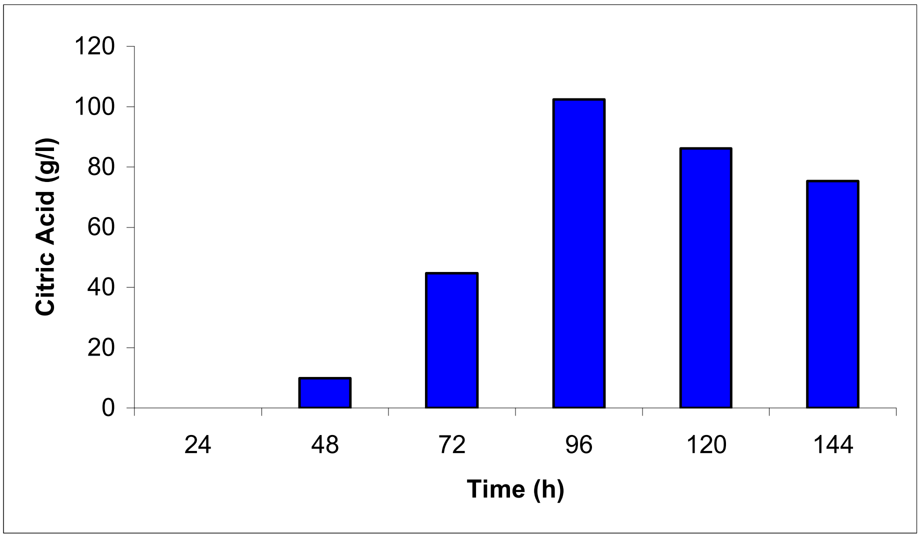

FIGURE 6.2

Diagrammatic Representation of the Submerged Citric Acid Production by *Aspergillus niger* NRRL 567 grown at 30°C

To attain increased citric acid yields, nitrogen in the medium must be limiting (Kristianen and Sinclair, 1978). Citric acid production is only established once the nitrogen in the medium is depleted (Papagianni *et al.*, 2005). The bulk of the ammonium is removed from the medium between 20 and 25 h, followed by the release of protons into the fermentation medium (Papagianni *et al.*, 2005). In the early stage of citric acid production (<24 h), it seems that a chain of events is established where fungal growth leads to ammonium uptake, which leads to proton release (Papagianni *et al*., 2005). Papagianni and colleagues (2005) suggested that a nitrogen compound must be produced and excreted by the mycelium, as the increase in biomass cannot be due to an accumulation of ammonia in the biomass of the fungus. It was subsequently reported that the nitrogen compound in question was in fact glucosamine, as identified by HPLC analysis (Papagianni *et al*., 2005). Following the detection of glucosamine, Papagianni and colleagues (2005) also investigated the formation, release and fate of the aminated compound throughout the fermentation process. It was found that the highest detected concentration of glucosamine in the medium was attained when using optimal concentrations of glucose and ammonium for citric acid production. Glucosamine excretion started at approximately 15 h of fermentation, followed by the start of citric acid production at about 24 h. It appears that the sudden increase in citric acid production is preceded by the sudden increase in glucosamine excretion into the medium by *A. niger*. Glucosamine appeared to be stable in the medium until approximately 85 h, where after it was reduced and subsequently depleted at 126 h of fermentation. This correlates with the citric acid production peak of 54.2 $g.kg^{-1}$ DPM at 96 h for solid substrate fermentation and 102.3 $g.l^{-1}$ for submerged fermentation, followed by the decline of citric acid production thereafter (Figures 6.1 and 6.2). Therefore, when comparing the fate of glucosamine and citric acid production during the fermentation process, it appears that glucosamine plays a direct role in citric acid production. Although the direct role of glucosamine during fermentation is unclear, it is suggested that it acts as a storage compound and is utilised by the fungus during the course of fermentation (Papagianni *et al*., 2005).

The initial pH decreased proportionally with the increase in citric acid concentration during both solid substrate- and submerged fermentation. In both fermentation processes the pH decreased during the first 96 h of fermentation as a result of citric acid production during this period. The maximum decrease of the pH of 1.34 and 2.17 respectively during solid substrate- and submerged fermentation was reached after 96 h, which correlates with the maximum citric acid concentrations achieved at this point during fermentation. In both solid substrate- and submerged fermentation the pH increased after

96 h due to the decreased citric acid concentrations in the fermentation media. During both fermentation processes it is evident that the evolution of the pH of the PM occurred in parallel with the citric acid concentration during the fermentation process. Therefore, the pH evolution may be used as an indirect indicator of citric acid production during the fermentation process.

The chemical composition of the untreated iron ore concentrate of the Sishen Iron Ore Mine is given in Table 6.1. XRF spectrometry of the iron ore concentrate revealed a P concentration of 0.17 % mass, a K concentration of 0.17 % mass and a Fe concentration of 96.90 % mass.

TABLE 6.1
Chemical Composition of the Untreated Iron Ore Concentrate of the Sishen Iron Ore Mine

Major Elements (Oxides)	% Mass
Silica (SiO_2)	1.89
Titanium (TiO_2)	0.08
Aluminium (Al_2O_3)	1.18
Iron (Fe_2O_3)	96.90
Potassium (K_2O)	0.17
Phosphorous (P_2O_5)	0.17

The average percentage removal of the major elements from the iron ore concentrate of the Sishen Iron Ore Mine by chemical leaching at for 5 days using different concentrations of citric acid is given in Table 6.2. Based on the results listed in Table 6.2 it is evident that the leaching temperature and the citric acid concentration plays an important role during the chemical leaching of the P and K from the iron ore concentrate. Leaching at 30ºC using a 1M citric acid leaching solution resulted in a 17.65% removal of both phosphorous and potassium, while at 60ºC a 35.29% and 23.53% removal of phosphorous and potassium, respectively, occurred (Table 6.2). Using a 1M citric acid leaching solution at 60ºC also resulted in the increased removal of silica (15.87%), aluminium (18.64%) and iron (0.92%) as compared to leaching at 30ºC, whereby only 11.02% of aluminium, 0.46% of iron and no silica was removed from the iron ore concentrate (Table 6.2). Therefore, it is clear that chemical leaching of P and K from the iron ore concentrate occurred more efficiently at a higher temperature (60ºC), which is most likely due to the fact that chemical reactions occur more efficiently at higher temperatures.

TABLE 6.2
The Average Percentage Removal of the Major Elements from the Iron Ore Concentrate by Chemical Leaching for 5 Days Using Different Concentrations of Citric Acid

Major Elements	1M Citric Acid; 30ºC	0.25M Citric Acid; 60ºC	0.5M Citric Acid; 60ºC	0.75M Citric Acid; 60ºC	1M Citric Acid; 60ºC
Silica (SiO_2)	0.00	0.00	0.00	7.41	15.87
Titanium (TiO_2)	12.50	0.00	0.00	12.50	12.50
Aluminium (Al_2O_3)	11.02	0.00	3.39	13.56	18.64
Iron (Fe_2O_3)	0.46	0.00	0.42	0.89	0.92
Potassium (K_2O)	17.65	5.88	5.88	17.65	23.53
Phosphorous (P_2O_5)	17.65	0.00	23.53	35.29	35.29

Citric acid concentrations of 0.25M and 0.5M resulted in the removal of only 5.88±5.88% of K, while at citric acid concentrations of 0.75M and 1M the K removal increased to 17.65±5.88% and 23.53±5.88%, respectively. The decrease of K using citric acid concentrations of 0.25M and 0.5M, however, could be as a result of data scatter due to sampling and assay variation, and thus may be regarded as insignificant. The actual percentage decrease of K using citric acid concentrations of 0.75M and 1M may also be lower than recorded in Table 6.2 as a result of data scatter. When taking the data scatter into account, the actual decrease in K may be as low as 6.88% and 12.5% using a citric acid concentration of 0.75M and 1M respectively.

Similar results were observed for P removal, whereby 23.53±3.40% P was removed when using a citric acid concentration of 0.5M, compared to 35.29±5.88% P removal when 0.75M and 1M citric acid concentrations were used. The actual P removal, however, may be as low as 11.33% when using a citric acid concentration of 0.5M and 20.0% when using citric acid concentrations of 0.75M and 1M. When comparing the chemical leaching process at 60ºC on the iron ore concentrate, it was evident that the process occurs more efficiently at higher citric acid concentrations. The presence of a higher negatively charged carboxyl group concentration at higher citric acid concentrations leads to the formation of more stable complexes with the K cations present, resulting in a higher removal of K from the iron ore concentrate. Phosphorous is removed as a result of hydrolysis whereby the H^+ cations, which are released from the carboxyl groups of citric acid, form stable complexes with the negatively charged P, resulting in its removal from the iron ore concentrate. Higher citric acid concentrations result in higher H^+ cation concentrations, and therefore, more P can be removed from the iron ore concentrate.

Other parameters that must be taken into account during chemical leaching of P and K from the iron ore concentrate are contact time and particle size of the iron ore concentrate. During this investigation it was found that the maximum P and K removal from the iron ore concentrate occurred after 5 days of chemical leaching, with no increase in P and K removal thereafter. The particle size of the iron ore concentrate is a limiting factor during chemical leaching, and determines how much of the P and K, contained within the iron ore particles, are actually exposed to acid attack, which takes place during the chemical leaching process. As the particle size of the iron ore concentrate decreases, the surface area of the particles are increased, therefore, exposing a larger amount of the P and K contained within the iron ore concentrate to acid attack. Therefore, if treating iron ore with a smaller particle size than –5+1mm, more P and K may be removed from the iron ore concentrate, however, the iron ore concentrate with the particle size of –5+1mm is the final iron ore concentrate product exported from the Sishen Iron Ore Mine, and therefore, it was used during this study to investigate the removal of P and K from the iron ore concentrate using the smallest marketable particle size from the mine.

The heap leaching simulation experiments using *A. niger* for the removal of P and K from the iron ore concentrate of the Sishen Iron Ore Mine yielded various problems. *Aspergillus niger* was only able to proliferate on the surface of the iron ore concentrate layer in the flask, possibly due to the anaerobic conditions which may prevail within the iron ore concentrate layer. Therefore, oxygen would have to be introduced throughout the iron ore heap when using this type of technology. In addition, after a period of 5 days the fungus started to sporulate, indicating the depletion of growth factors needed for fungal growth to occur. After the addition of more growth medium to the inoculated iron ore concentrate, fungal growth recommenced, followed by sporulation 5 days thereafter. Therefore, heap leaching technology would require the addition of fungal growth medium every 5 days, making this type of technology uneconomical due to the high costs of growth factors such as glucose.

During the 40 day heap leaching simulation, the pH of the leachate solution decreased from 4.00 to 3.42, indicating that a low concentration of citric acid was produced due to the limiting of the carbon source (glucose) in the system. XRF analysis revealed that 17.65% K and no P was removed from the iron ore concentrate during the 40 day period. Thus, compared to chemical leaching of the iron ore concentrate using a 1M concentration of citric acid produced by *A. niger*, heap leaching technology is less efficient as less P (0% vs 35.29%) and K (17.65% vs 23.53%) is removed over a much longer contact time.

6.5 Conclusions

During solid substrate- and submerged fermentation using *A. niger* a high concentration of glucose was required to ensure that citric was excreted from the fungal mycelia. The maximum citric acid yield of 54.2 $g.kg^{-1}$ DPM by solid substrate fermentation and 102.3 $g.l^{-1}$ by submerged fermentation was attained after a period of 96 h in both processes. The fermentation media of both solid substrate- and submerged fermentation contained similar constituents, with the difference that the submerged fermentation medium contained less of each constituent. In addition, solid substrate fermentation required peat moss as a solid substrate, which increases the costs incurred by using this fermentation process. Therefore, taking all the variable factors into account it is suggested that submerged fermentation be used for citric acid production by *A. niger*, as this fermentation process was found to be more economical and produced more citric acid in the same time.

Chemical leaching of the iron ore concentrate proved to be more efficient than "heap leaching", as more P and K was removed from the iron ore concentrate, as well as in a shorter time frame. In addition, using "heap leaching" technology proved to be uneconomical in this case, as growth medium will have to be added every 5 days to enhance the fungal growth. It is suggested that a 1M citric acid leaching solution be used at 60ºC for the chemical leaching process, as the most P and K is removed from the iron ore concentrate using these leaching conditions. Therefore, the most economical and efficient process for the removal of P and K from the iron ore concentrate of the Sishen Iron Ore Mine, involves a two-stage process whereby citric acid is produced by *A. niger*, followed by the chemical leaching of the P and K from the iron ore concentrate using a 1M citric acid leaching solution at 60ºC.

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