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THE USE OF *ASPERGILLUS NIGER* FOR THE REMOVAL OF POTASSIUM AND PHOSPHOROUS FROM THE IRON ORE OF THE SISHEN IRON ORE MINE, SOUTH AFRICA

by

PETER JOHN WILLIAMS

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"Ek verklaar dat die verhandeling wat ek hiermee aan die Universiteit van Pretoria vir die PhD (Mikrobiologie)-graad voorlê, my eie werk is en nie vantevore deur my aan enige ander tersiêre inrigting vir enige graad voorgelê is nie."

"I certify that the thesis hereby submitted to the University of Pretoria for the degree of PhD (Microbiology) is my own work and has not previously been submitted by me in respect of a degree at any other tertiary institution."

Signature: _____

Date: _____

This thesis is dedicated to my wife, Julie, and daughter, Mackenzie

"We must not forget that when radium was discovered no one knew that it would prove useful in hospitals. The work was one of pure science. And this is proof that scientific work must not be considered from the point of view of the direct usefulness of it. It must be done for itself, for the beauty of science, and then there is always the chance that a scientific discovery may become like the radium, a benefit for humanity."

Marie Curie (1867-1934)

Lecture at Vassar College, May 14, 1921

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LIST OF ABBREVIATIONS

Acetyl-CoA	-	Acetyl-coenzyme A
AGI	-	Acute Gastrointestinal Illness
ARI	-	Acute Respiratory Illness
ATP	-	Adenosine Tri-phosphate
BIF's	-	Banded Iron Formations
bp	-	Base Pair
CI	-	Consistency Index
CO_2	-	Carbon Dioxide
$CuFeS_2$	-	Chalcopyrite
CuS	-	Copper Sulphide (Covellite)
Cu_2S	-	Chalcocite
$CuSO_4$	-	Copper Sulphate
dH_2O	-	Distilled Water
DNA	-	Deoxyribonucleic Acid
Fe	-	Iron
FeS_2	-	Iron Pyrite
$FeSO_4$	-	Ferrous Sulphate
$Fe_2(SO_4)_3$	-	Ferric Sulphate
H_2SO_4	-	Sulphuric Acid
HIV	-	Human Immunodeficiency Virus
K	-	Potassium
KCl	-	Potassium Chloride
K_2O	-	Potassium Oxide
$K_2O.Al_2O_3.4SiO_2$	-	Leucite
$K_2O.Al_2O_3.6SiO_2$	-	Silicide
$K_2O.SiO_2$	-	Potassium Silicate
Mg-ADP	-	Magnesium-Adenosine Diphosphate
Mg-ATP	-	Magnesium-Adenosine Triphosphate
$MgCl_2$	-	Magnesium Chloride
mm	-	Millimetre
mM	-	Millimolar

mmol	-	Millimole
Mt	-	Metric Tonnes
NADH	-	Nicotinamide adenine dinucleotide
ND	-	Not Detected
NH_4^+	-	Ammonium Ion
NiS	-	Nickel Sulphide
$NiSO_4$	-	Nickel Sulphate
OF	-	Oxidation-fermentation
P	-	Phosphorous
PCR	-	Polymerase Chain Reaction
rDNA	-	Ribosomal Deoxyribonucleic Acid
RI	-	Retention Index
RNA	-	Ribonucleic Acid
SO_2	-	Sulphur Dioxide
SX-EW	-	Solvent Extraction-Electrowinning
TAE	-	Tris-Acetate-EDTA
TBR	-	Tree Bisection-Reconnection
TCA	-	Tricarboxylic Acid
Tris-HCl	-	Tris-Hydrogen Chloride
µℓ	-	Microlitre
µmol	-	Micromole
UO_2	-	Uranium Dioxide (Uraninite)
UO_3	-	Uranium Trioxide
USA	-	United States of America
ZnS	-	Zinc Sulphide
$ZnSO_4$	-	Zinc Sulphate

LIST OF PUBLICATIONS, SUBMITTED MANUSCRIPTS AND CONFERENCE CONTRIBUTIONS

YEAR

2006 **WILLIAMS PJ, GEYER H, SURRIDGE AKJ, KATABUA J and CLOETE TE.** Bacterial population study of the industrial wastewater and iron ore of the Sishen Iron Ore Mine. 14th Biennial SASM Conference, CSIR Convention Centre, Pretoria, South Africa, 9-12 April 2006.

2008 **WILLIAMS PJ and CLOETE TE.** The use of *Aspergillus niger* for the removal of phosphorous and potassium from the iron ore of the Sishen Iron Ore Mine, South Africa. Bio-08 SASM-Biotech SA-SASBMB Conference, Grahamstown, South Africa, 21-25 January 2008.

2008 **WILLIAMS PJ, SURRIDGE AKJ and CLOETE TE.** Microbial Community Study of the Process- and Groundwater of the Sishen Iron- Ore Mine, South Africa. *Water SA* **34(5)**.

2008 **WILLIAMS PJ and CLOETE TE.** Microbial Community Study of the Iron Ore Concentrate of the Sishen Iron Ore Mine, South Africa. *World Journal of Microbiology and Biotechnology* **24(11):** 2531-2538.

2008 **WILLIAMS PJ and CLOETE TE.** 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. Submitted for publication in *World Journal of Microbiology and Biotechnology*.

2008 **WILLIAMS PJ and CLOETE TE.** The Use of *Aspergillus niger* for the Removal of Phosphorous and Potassium from the Iron Ore Concentrate of the Sishen Iron Ore Mine, South Africa. Submitted for publication in *Hydrometallurgy*.

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PETER JOHN WILLIAMS

PROMOTOR: Prof TE Cloete (University of Pretoria)
DEPARTMENT: Microbiology and Plant Pathology
Faculty of Natural and Agricultural Sciences
University of Pretoria
DEGREE: PhD (Microbiology)

SUMMARY

As global technologies and civilisation have advanced, the utilization of diverse mineral products has increased, resulting in the progressive depletion of high-grade mineral deposits. Consequently, metal production has to rely more on the use of lower-grade or complex ores, as well as metal extraction from mining and industrial wastes. 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, resulting in the international steel making companies charging penalties when purchasing iron ore concentrates containing high concentrations of P and K. 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 no microbial bioleaching candidates could be isolated from the aquatic environment in order to develop an economical process to remove the P and K from the iron ore concentrate.

The most likely candidate for the removal of P and K from the iron ore concentrate would seem to be *Acidithiobacillus ferrooxidans* that was isolated from the iron ore concentrate, however, this bacterium produces sulphuric acid, which is undesired in the iron ore industry due its corrosive properties. Furthermore, the P and K contained in the iron ore is in a non-sulphidic phase, 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. Chemical leaching of the iron ore concentrate using citric acid 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. The results of the chemical leaching suggested that a 1M citric acid leaching solution be used at 60ºC for 5 days for the chemical leaching process, as the most P and K is removed from the iron ore concentrate using these leaching conditions. The possibility to use *A. niger* as a bioleaching microorganism was also investigated, due to its ability to produce organic acids such as citric acid, which has the ability to remove P and K from the iron ore concentrate by chemical leaching. Compared to chemical leaching, which requires high concentrations of citric acid and/or high leaching temperatures, bioleaching using *A. niger* offers a more economical method with similar efficiency for the removal of P and K from the iron ore concentrate of the Sishen Iron Ore Mine. The most economically viable process for the removal of P and K from the iron ore concentrate, although not as efficient as chemical leaching using citric acid at high temperature, proved to be the bioleaching process using *A. niger* with a bioleaching pulp density of 33% at 30ºC for 10 days. This is the first report of the use of *A. niger* for the use in any bioleaching process.