Prevention of Acid Rock Drainage (ARD) Formation from Fine Coal and Tailings Fractions by Sulphide Removal: The Role of Bioflotation Reagents

Report to the
Water Research Commission

by

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EXECUTIVE SUMMARY

The South African coal mining industry produces large amounts of sulphide-containing ultrafine waste tailings which pose an Acid Rock Drainage (ARD) generation risk. A two-stage froth flotation process developed at UCT (Harrison, et al., 2013) aims to further process such wastes: the first-stage produces a desulphurised, saleable coal concentrate and the second-stage produces a low-volume, high-sulphide concentrate and a high-volume benign tailings which is suitable for safe disposal without containment. Two key issues which hamper the implementation of this treatment technique are: (1) the high cost of the oleic acid collector required in the first flotation stage and (2) the toxicity of the xanthate collector used in the second-stage which presents issues with the final product stream handling and use. In this study, microorganisms and bioproducts are investigated as potential bioflotation reagents for use in this two-stage flotation desulphurisation of coal ultrafine waste tailings.

Potential microorganisms and bioproducts were identified from the general microbial and specific bioflotation literature, based on evidence that they are attracted to (attach/modify) surfaces of either coal or pyrite, the main sulphide mineral present in the coal wastes. All bioflotation options were required to be non-pathogenic and non-toxic. This led to the selection of *Mycobacterium phlei*, raw algal lipids (RAL) and derived fatty acid methyl esters (FAME) as potential coal bioflotation reagents. The heterotrophs *Bacillus subtilis*, *Bacillus licheniformis*, *Paenibacillus polymyxa*, *Rhodococcus opacus* and *Rhodopseudomonas palustris* as well as the chemolithotrophic *Acidithiobacillus thiooxidans*, *Acidithiobacillus ferrooxidans* and *Leptospirillum ferriphilum* were identified as potential pyrite bioflotation reagents. The performance of these bioflotation reagent options was tested using attachment experiments (for the microorganisms) and by monitoring surface charge changes, followed by batch flotation tests using a 3 ℓ Leeds batch flotation cell.

Both *M. phlei* and algal bioproducts (RAL and FAME) demonstrated excellent technical potential as selective coal (first-stage) bioflotation reagents. In the best case for *M. phlei*, the same overall flotation yield (37-40%) was achieved (with or without an MIBC frother) as the chemical reagent equivalent for a Witbank coal ultrafine waste tailings feed. The *M. phlei* was successful in desulphurising the feed, with a concentrate sulphur recovery of 20-26%, which is comparable to the chemical system. However, the microbial system was only able to upgrade the coal feed from 51% to 57% combustibles, compared to 71% for the chemical system. The RAL and FAME (extracted from *Scenedesmus sp.*) potential was tested using an ultrafine Waterberg coal waste tailings feed. The overall yield was 28 ± 1% for the RAL and 30 ± 2% for the FAME, only marginally below the 35 ± 3% achieved by the chemical system. The FAME system had the best combustible recovery, increasing the combustible content from 52% to 79%, compared to 73% for the RAL system and 77 ± 2% for the chemical system. The FAME system was also best at desulphurising the coal, with only 20% of the sulphur recovered to the concentrate, compared to 28% for the RAL system and 25% for the chemical system. Though the FAME bioflotation reagent performed best, one advantage of the RAL bioproduct was that it created a stable froth in the absence of MIBC, thereby potentially allowing for replacement of both the chemical collector and frother.

For the second-stage flotation, only *P. polymyxa* yielded competitive pyrite flotation results, with all other options achieving overall yields of <1% compared to 95% for the chemical system, despite good initial attachment results. In the most successful bioflotation with *P. polymyxa*, performed at pH 7, an 81% recovery of pyrite was achieved in the first 2 minutes of flotation. Notably, at this pH the *P. polymyxa* had demonstrated near immediate and complete attachment in the attachment experiments and exhibited an essentially neutral surface charge. The pyrite recovery by *P. polymyxa* at pH 4 was lower at only 7%, at which pH a slower rate of attachment was also observed and a marginally positive surface charge. This may indicate that rapid attachment kinetics due to a driving force other than just surface charge is a critical factor determining if bioflotation will be successful.

The economics of producing a bioflotation reagent and implementing the bioflotation was examined by modifying the chemical system process flowsheet and financial analysis (Harrison, et al., 2013). This was done for the *M. phlei* case rather than the algal bioproducts and *P. polymyxa*, because the chemical system profitability analyses found the first-stage collector (oleic acid) to be the most expensive single operating cost and its manufacture uses well-established (and therefore most easily costed) bioprocess engineering technologies. The production of *M. phlei* required the inclusion of a mixing tank for the glycerol-soil growth medium, autoclave, continuous bioreactor and disc-stack centrifuge in the process flowsheet. These additional units accounted for 28% of the total purchase cost of equipment for the plant. The additional units were designed to produce *M. phlei* at a rate of 2×10^{18} cells/h (based on the attachment and bioflotation experiments) which was then sent to a first-stage pre-conditioning tank, after which the process was unchanged from the chemical system. The cost of the growth medium components accounted for 89% of the total raw material costs, with the meat extract (which alone accounted for 69%) having the largest single influence on the net present value (NPV) of the plant. The NPV after 15 years, starting forecasting under current (2016) market conditions and using the same coal pricing basis as Harrison (2013), was found to be –R48 billion. Thus *M. phlei* bioflotation (using current growth data and assuming on site production) was found to be more expensive than the chemical process which was predicted to have a NPV of R50 million after the same period. The NPV remained negative even for the best-case coal price (–R38 billion) and a zero cost for meat extract (–R24 billion). Thus, it is recommended that more research should be done into optimisation of the *M. phlei* production before bioflotation implementation can be considered, including investigation of medium composition, growth rate, maximum possible cell densities, process scale-up and pros/cons of offsite versus onsite production. The RAL or FAME options also require further investigation of large-scale production facilities before this option can be costed.

Thus, technically competitive and non-toxic bioflotation options were successfully identified and demonstrated for both stages of the two-stage froth flotation process for the desulphurisation of ultrafine coal waste tailings, but a preliminary financial analysis based on *M. phlei* usage showed that their production and usage results in a financially less attractive operation. Reduction of growth medium costs in combination with better coal prices, or reduction in the biomass required for bioflotation through process optimisation is needed to make the process feasible. Further research into the production capacity of RAL or FAME options as well as *P. polymyxa* is recommended to be able to cost these systems.

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ACRONYMS & ABBREVIATIONS

ARD	Acid rock drainage
ASTER	Activated sludge tailings effluent remediation
BFA	Bioflotation agent
BSM	Basal salt medium
CCF	Cumulative cash flow
CEPCI	Chemical engineering plant cost indices
DCCF	Discounted cumulative cash flow
EBP	Extracellular bacterial protein
EPS	Extracellular polymeric substance
FAME	Fatty acid methyl ester
IEP	Isoelectric point
IRR	Internal rate of return
MIBC	Methyl isobutyl carbinol
NPV	Net present value
PAX	Potassium amyl xanthate
PBP	Payback period
PCE	Purchase cost of equipment
PGM	Platinum group metals
PZC	Point of zero charge
RAL	Raw algal lipids
ROI	Return on investment
SIBX	Sodium isobutyl xanthate
WCO	Waste cooking oil

GLOSSARY OF TERMS

Acid Rock Drainage (ARD)	The outflow of acidic water from mining sites, caused by the exposure of sulphide minerals to air and water
Activators	A reagent, usually chemical, that enables the flotation of minerals by increasing their surface hydrophobicity
Autotroph	An organism that can synthesize its organic components from inorganic substances, such as CO_2, using chemical energy or sunlight
Biocollector	A biological reagent which increases the hydrophobicity of a mineral causing it to separate and float during the bioflotation process selectively
Bioflotation	The process in which minerals can be separated selectively based on their surface properties, using a biological reagent
Carbon fixation	Conversion process of inorganic carbon (CO_2) to organic compounds by living organisms
Coagulation	Thickening into a coherent mass
Chemoautotroph	An organism that obtains its energy by oxidizing electron donating molecules or inorganic compounds, rather than through photosynthesis, and obtains carbon from CO_2
Chemolithotroph	An organism which obtains its energy through the oxidation of inorganic reduced compounds
Collector	A reagent used to increase the hydrophobicity of a mineral and cause it to separate selectively from other minerals by floating to the surface
Depressant	A reagent used to inhibit the flotation of minerals by increasing their surface hydrophilicity
Depression	The instance where the flotation reagent increases the hydrophilicity of the mineral, causing it to sink rather than float
Extracellular polymeric substances (EPS)	High-molecular weight compounds secreted by microorganisms into their environment
Flocculation	The formation of groups of particles, termed flocs, held together by weak Van der Waal's forces
Flotation	The process in which minerals can be separated selectively based on their surface properties and adherence to gas bubbles
Flotation Efficiency Index	The difference between combustible material recovery to product and non-combustible material recovery to product
Frother	A reagent used to create a stable froth in a flotation cell. This froth is captured as the flotation concentrate.
Gangue	A commercially valueless material associated with the mineral of value
Gram-negative	Bacteria which when using the Gram staining method for characterizing bacteria do not retain the crystal violet stain due to the presence of an outer cell-membrane, external to the peptidoglycan-based cell wall
Gram-positive	Bacteria which when subject to the Gram stain for characterizing bacteria take up the crystal violet stain due to the absence of an outer cell-membrane, allowing direct interaction with the peptidoglycan cell wall
Heterotroph	An organism that assimilates organic carbon into its biomass and cannot assimilate CO_2
Hydrophilic	Strong affinity for water
Hydrophobicity	Water repelling
Isoelectric point	See "Point of zero charge"
Mesophilic	Grows at moderate temperatures between 25ºC and 40ºC
Microbe	Abbreviation for microorganism
Microorganism	A microscopic organism, multi-cellular or single-celled
Point of zero charge (Isoelectric point)	The condition when electrical charge density on the surface of a particle is zero

Sulphur Recovery	The percentage of sulphur being removed from the feed stock that reports to the concentrate
Synergistic	An interaction of multiple elements to produce an effect greater than the sum of their individual effects
Thermophile	An organism that thrives in high temperatures (45-122°C)
Viability	The capacity of a microorganism to reproduce, multiply and form colonies
Vitality	The metabolic activity of a microorganism i.e. ability to grow and metabolise
Zeta potential	The potential difference between the surface of a solid particle in a solution of conducting liquid (e.g. water) and the bulk liquid

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CHAPTER 1: Introduction

1.1 Project background and motivation

South Africa is one of Africa's most important countries in terms of mineral diversity and the quantity of minerals extracted. It is rich in natural resources and is considered to have the largest reserves of gold, coal and platinum group metals (PGM) in the world. However, some of these mining activities produce large volumes of sulphide containing waste materials, such as pyrite, sphalerite and chalcopyrite. Discarded mine waste containing sulphide minerals has the risk of acid rock drainage (ARD) generation. This can be immediate or in the long term, depending on the relative rates of acidification due to sulphide mineral oxidation through the penetration of oxygen and water into the waste (Benzaazoua, et al., 2000) and neutralisation by other mineral components in the ore. As a result, acidic waters, high in dissolved sulphates and trace metals, are released into the soil, affecting water systems, agriculture and all living organisms (Harrison, et al., 2010). This is a growing concern in South Africa as it exacerbates the already existing water scarcity problem and pollutes the environment with associated environmental degradation and impact on human health. The effects of ARD are already experienced in the Witbank and Johannesburg areas through a significant decline in water quality (Geldenhuis & Bell, 1998; Naicker, et al., 2003).

One proposed approach to ARD mitigation is to prevent its formation from waste rock and tailings through the desulphurisation of waste materials prior to their disposal. This strategy removes the potential risk of ARD formation, avoiding long term liability. This desulphurisation may occur by reaction of the sulphide or by its physical separation, provided it is sufficiently liberated. One route by which to achieve this is desulphurisation flotation. This ARD prevention strategy for wastes is attractive as it can produce a large sulphide-lean tailings fraction as well as a low volume concentrated sulphide-rich fraction (Benzaazoua, et al., 2000; Hesketh, et al., 2010; Kazadi Mbamba, et al., 2012). The benefit of desulphurisation flotation is that the low volume sulphide-rich concentrate is much easier to manage and control than a large volume of untreated waste stream (Kazadi Mbamba, 2011) which means the need for lined tailings facilities can be minimised with associated cost savings. Furthermore, potential uses for both the benign and concentrated fractions have been proposed by Harrison et al. (2013), allowing both waste minimisation and the maximising of resource productivity.

Desulphurisation flotation utilises the difference between the surface properties of the sulphide minerals and the other components in the waste sample. A suite of chemical reagents is used in the process: collectors and depressants typically enhance the inherent hydrophobicity and hydrophilicity respectively of targeted components of the feed stream and frothers produce a stable froth during flotation, permitting recovery of the floated material. There is a growing interest in the development of bioflotation reagents, owing to concern regarding the environmental effects of the chemical reagents used during flotation, especially with respect to soil contamination (EPA, 1994; Govender & Gericke, 2011; Jiang, et al., 1998). This is particularly pronounced in the use of two-stage flotation for desulphurisation of tailings and fine coal wastes, owing to the large reagent usage of these processes (Kazadi Mbamba, 2011; Kazadi Mbamba, et al., 2012; Hesketh, et al., 2010). Furthermore, the chemical reagents are very costly, comprising the largest fraction of the total desulphurisation flotation operating costs for the treatment of fine coal wastes (Jera, 2013; Harrison, et al., 2013).

Bioflotation, using microbial cultures or their products instead of the chemical reagents, has the potential of offering a lower cost and more environmentally benign approach to the desulphurisation flotation process. It has been found that select microorganisms may enhance the beneficiation of minerals through bioflotation or bioflocculation. Numerous studies have been performed on the selective flotation or depression of minerals using microorganisms or their products and a variety of microorganisms have been found to show potential. These include *Leptospirillum ferrooxidans*, *Acidithiobacillus ferrooxidans*,

Acidithiobacillus thiooxidans, *Bacillus subtilis*, *Mycobacterium phlei*, *Rhodococcus opacus*, and *Paenibacillus polymyxa* (Govender & Gericke, 2011; Dwyer, et al., 2012; Sharma, 2001; Nagaoka, et al., 1999; Vilinska & Hanumantha Rao, 2008; Hanumantha Rao & Subramanian, 2007; Chandraprabha, et al., 2004; Deo & Natarajan, 1997; 1998) (Patra & Natarajan, 2008a; 2008b; 2003).

In this study, the potential of bioflotation, using either microorganisms or biologically produced biochemicals, is considered with the aim of reducing the environmental burden and enhancing the cost-effectiveness of the two-stage flotation process for desulphurization of fine coal waste and tailings from mineral sulphide processes, aimed at ensuring long-term prevention of ARD formation by risk removal.

1.2 Project aims

The project aims set out in the proposal for this study are as follows:

- Identification of potential bioflotation reagents for each of fine coal and pyrite
- Assessment of the technical feasibility of the potential bioflotation reagents for the sequential flotation separation of coal and pyrite relative to the chemical two-stage flotation system performance
- Compilation of an integrated process flowsheet
- Assessment of the environmental burden associated with the bioflotation process
- Assessment of the profitability of the bioflotation system compared to the chemical two-stage flotation system

1.3 Project scope and limitations

The project assesses the bioflotation potential of a large number of microorganisms and makes use of a few different mineral samples, focussed on coal fines and fine discards. The scope of the experiments therefore needed to be limited to manage the number of potential experiments. These limitations include:

- Microorganisms were grown using the recommended media only, with no modifications made
- The effect of the microorganisms' age was not considered as an optimisation factor
- Flotation was performed at the conditions recommended by the existing chemical two-stage flotation literature (volume, pulp density, temperature, aeration rate, impeller speed), without testing for optimisation of the biological system
- Flotation tests were performed at two pH's for each microorganism only: neutral pH (chemical two-stage process conditions) and pH 2 or 4, circa the microorganism's point of zero charge
- Bioflotation was only conducted at a single microbial concentration, either correlated to the attachment experiments for the microorganism or limited by the maximum growth kinetics of the microorganism
- Potential coal bioflotation microbial options were only tested on either the Witbank or Waterberg South African ultrafine coal waste tailing samples as the samples were limited in size
- Economic analyses were performed based on 2016 costs and using the results of only the most successful small scale bioflotation screening tests for which only limited optimisation was possible

CHAPTER 2: Background: Mine Waste Desulphurization through Two-Stage (Bio)Flotation

2.1 Introduction

Large amounts of solid mine waste are produced annually in South Africa, foremost in the gold sector, followed by the PGM and coal sectors. This is illustrated in Figure 1 which shows the approximate solid waste generated by each sector of the South African minerals industry in 1999. The appropriate disposal, treatment or containment of these solid wastes needs to be identified properly in order to minimise potential negative environmental impacts.

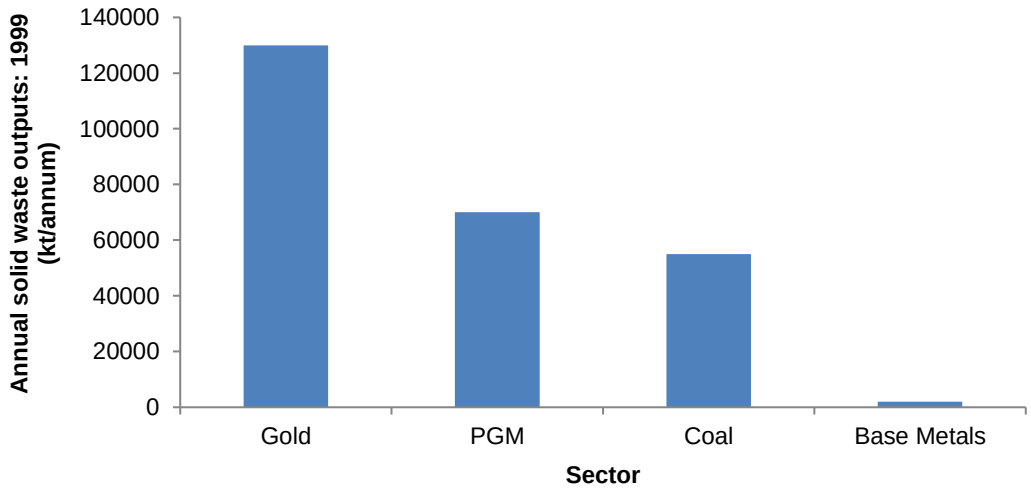

Figure 1: Solid waste generated by mining industry sectors in South Africa (adapted from Stewart (1999))

One such detrimental impact associated with metal sulphide containing wastes (prevalent in the gold and coal mining sectors) is acid rock drainage (ARD) generation which has already led to environmental problems, most notably a decline in water quality (Geldenhuis & Bell, 1998; Naicker, et al., 2003). This project aims to examine the potential of bioflotation as a treatment option for the mitigation of this risk.

In this literature review, we examine the background to the motivation for the bioflotation project, considering ARD formation, the chemical two-stage flotation process and the theorised mechanisms of bioflotation. Attention is then given to those microorganisms or microbial products that have been evidenced in the literature to be attracted to (attach/modify) surfaces of either coal or sulphide minerals, especially pyrite, and whether they have shown bioflotation success. This latter section is then used in Chapter 3 to inform the selection of potential bioflotation reagents for the two-stage flotation process.

2.2 Acid Rock Drainage (ARD)

2.2.1 ARD formation

Acid rock drainage (ARD) is a naturally occurring process involving the chemical reaction of sulphide minerals through oxidation or solubilisation reactions, or both. Sulphide minerals which undergo oxidation reactions only are termed “acid insoluble” minerals, whereas those which may undergo reaction with protons in addition to oxidation reactions are termed “acid soluble” (Schippers & Sand, 1999). Pyrite, an acid insoluble mineral, may undergo oxidation in the presence of oxygen and water according to the following generalised equation:

$$2FeS_2 + 7O_2 + 2H_2O \rightarrow 2Fe^{2+} + 4SO_4^{2-} + 4H^+ \quad (1)$$

Although mineral oxidation by oxygen is often the initial step in ARD generation in a disposal scenario, oxygen quickly becomes a secondary reactant in the presence of soluble ferric iron under acidic conditions.

$$FeS_2 + 14Fe^{3+} + 8H_2O \rightarrow 15Fe^{2+} + 2SO_4^{2-} + 16H^+ \quad (2)$$

Here, the rate of pyrite oxidation is increased, with ARD generation occurring over a shortened time frame. The generation of protons following the oxidation of the sulphide minerals by either oxygen or ferric iron or both may result in leaching reactions of the acid soluble mineral phases present in the mining wastes. For example, the dissolution of galena (PbS) through reaction with protons results in the solubilisation of the lead metal species, adding to the environmental burden related to heavy metal toxicity associated with ARD pollution.

$$PbS + 2H^+ \rightarrow Pb^{2+} + H_2S \quad (3)$$

Following this reaction, the hydrogen sulphide product may undergo further reactions with ferric iron and protons, resulting in an elemental sulphur product in the absence of sulphur-oxidising microorganisms (Schippers & Sand, 1999).

The regeneration of the leaching agents occurs through the oxidation of ferrous iron product to ferric iron by oxygen. Under acidic conditions, however, the presence of iron and sulphur oxidising microorganisms additionally contributes to the regeneration of the ferric iron and protons (Watling, 2006; Rohwerder, et al., 2003). The rate of microbial regeneration of the leaching agents is often orders of magnitude greater than that obtained under abiotic conditions (Nordstrom & Alpers, 1999). Ferric iron regeneration through microbial ferrous iron oxidation is presented in the following equation:

$$4Fe^{2+} + O_2 + 4H^+ \xrightarrow{iron-oxidising\ microorganisms} 4Fe^{3+} + 2H_2O \quad (4)$$

Similarly, the presence of sulphur oxidising microorganisms may regenerate the protons necessary for solubilisation of the acid soluble mineral phases. In well-established ARD generating environments, the extent of this oxidation is such that the majority of the sulphur in the aqueous phase is present as sulphate ions (Johnson, 2003).

$$2S + 3O_2 + 2H_2O \xrightarrow{sulphur-oxidising\ microorganisms} 2H_2SO_4 \quad (5)$$

2.2.2 Current ARD prevention and treatment techniques

Current ARD prevention measures focus on limiting the contact of the sulphide minerals with oxygen and water. The most commonly implemented ARD prevention technique is the use of seals and covers to prevent the penetration of oxygen and water into the mine waste. However, preventing the contact of oxygen and water with large volumes of mine waste is very difficult and does not address the long-term problems of ARD generation (Jera, 2013).

In terms of treatment techniques, common practice is the blending of mine waste with neutralising agents such as limestone after which the waste is disposed in the open pits formed during mining through a process known as backfilling (Jera, 2013). However, limestone is poorly soluble and thus the

neutralisation ability of limestone is restricted. The oxidation of mine waste in backfills is a significant ARD concern if the mine waste has not been adequately isolated from oxidising conditions and the neutralisation ability of limestone is restricted (Kazadi Mbamba, 2011; Benzaazoua, et al., 2008).

Furthermore, existing ARD prevention and treatment techniques are both costly and fail to offer a long-term solution to ARD generation (Kazadi Mbamba, 2011), particularly owing to neglect of the need to balance the kinetics of the rate of release of neutralising capacity and acid generation.

Desulphurisation of mine waste has been proposed as a new ARD mitigation strategy, providing an alternative solution to the ARD prevention and treatment techniques (Benzaazoua, et al., 2000; Harrison, et al., 2010; Harrison, et al., 2013). This technique operates in conjunction with cleaner production strategies by reducing mine waste volumes through the beneficiation of the waste to yield additional products (Reddick, 2006). In a study completed by Lottermoser (2003), desulphurisation during mineral processing is considered the best technique for addressing ARD generation. This approach may reduce the volume of acid-producing mine waste significantly, leading to more effective management strategies (Benzaazoua, et al., 2000; Kazadi Mbamba, et al., 2012).

2.3 Froth Flotation Separation

Froth flotation is a physico-chemical separation process that utilises the different surface properties of particles to induce separation within a liquid medium (Wills, 1997). More specifically it exploits the varying degrees of hydrophobicity and hydrophilicity of different mineral and solid types and hence their varying floatability. It is a versatile and important mineral processing technique that has a wide application across a number of industries. It is generally a cost-effective process and has been used in the minerals industry since early in the 19th century (Dube, 2012).

2.3.1 Process of flotation

The principle of froth flotation is illustrated in Figure 2. The fine solids to be separated are suspended in a solution to form a pulp and fed to the flotation vessel. When air is passed through the pulp, the hydrophobic particles attach to air bubbles and float to the surface. The air bubbles form a froth at the top of the flotation vessel which is then removed; this allows for the recovery of the floated solid particles as well as any particles that have become entrapped or entrained in the froth. The remaining hydrophilic fraction stays wetted in the liquid and is removed in the tailings. An impeller ensures that the pulp remains well mixed and reduces bubble size (Harris, 2001).

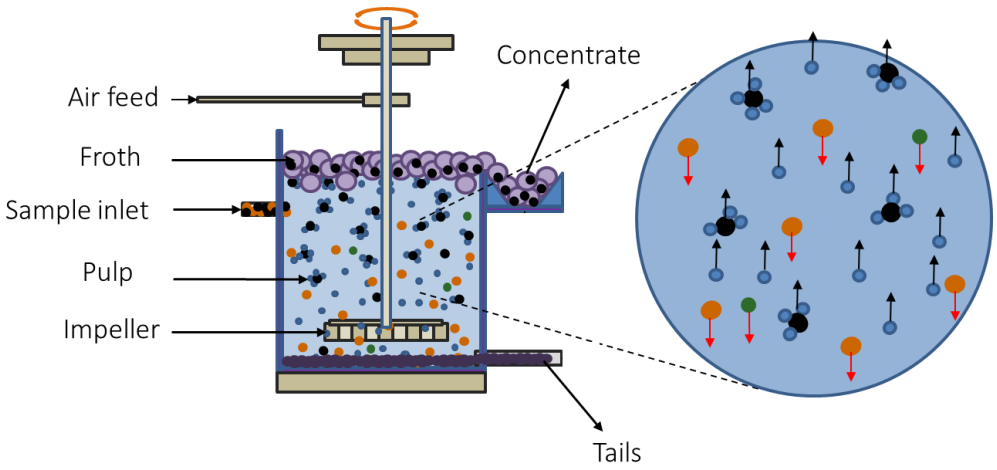

Figure 2: Schematic of a typical flotation setup

The froth flotation process is governed by the surface properties of solids and these surface properties are typically enhanced or controlled by chemical flotation agents. The reagents used in flotation can be classified as collectors, frothers, promoters, modifiers and depressants (Dube, 2012). Collectors can be further classified based on the electrical charge associated with the polar group into ionic (anionic and cationic) and non-ionic surfactants (Leja, 1982). Frothers are also grouped based on their solubility in water: soluble and partially soluble frothers (Booth & Freyberger, 1962).

2.3.2 Surface properties of particles

The hydrophobicity of a particle is most directly affected by its polarity (Wills, 1997). Non-polar minerals are characterised by relatively weak molecular bonds (Van der Waals forces) and therefore do not readily attach to water molecules. Hence, they are hydrophobic. Polar minerals, consisting of strong covalent or ionic surface bonds, react easily with water molecules and are therefore hydrophilic. Surface charge (zeta potential) also affects the separation of minerals and sulphides in water solutions because of the attraction and repulsion between unlike and like charged surfaces respectively (Patra & Natarajan, 2004a). The more charged a surface is (positive or negative), the less hydrophobic it becomes. Differences in the surface charge of the various minerals are therefore crucial for the effective separation of sulphide minerals using froth flotation (Wills, 1997).

The attachment of the minerals to air bubbles is the most important aspect of froth flotation separation and is highly dependent on the surface properties of the solids, the solvent and the gas. Attachment occurs through adhesion between the air bubble and the particle (Wills, 1997). The strength of adhesion is dependent on the physical forces of attraction and repulsion between the surfaces and the liquid. This is defined by the contact angle between the liquid and the particle. It is observed that the greater the contact angle between the liquid and the surface, the higher the degree of hydrophobicity (floatability) of the particle. Figure 3 shows how the contact angle is representative of the degree of hydrophobicity. Particles exhibiting high levels of hydrophobicity are more inclined to attach to air bubbles and rise to the surface of the liquid whereas particles that are more hydrophilic are likely to remain in solution and settle (Wills, 1997).

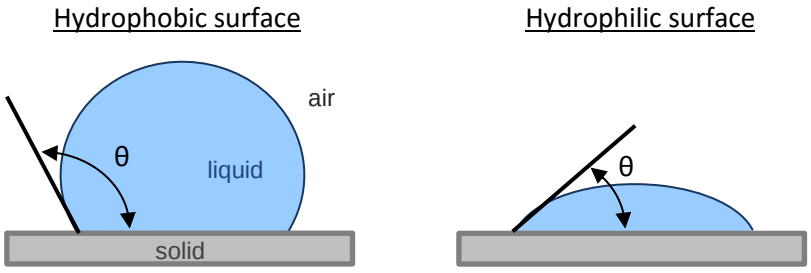

Figure 3: Hydrophobic vs. hydrophilic contact angles

It has been observed that coal has a larger contact angle than other related minerals such as quartz and pyrite as a result of a higher hydrophobicity (Kazadi Mbamba, 2011; Raichur, et al., 1996). Furthermore, ash components like pyrite and quartz have been shown to be hydrophilic. This inherent floatability of coal makes it easy to separate through froth flotation.

2.4 Desulphurisation of Mine Waste by Flotation

The desulphurisation of mine waste for ARD prevention may be achieved using froth flotation. The process utilises the difference in surface properties of the coal, sulphide minerals and gangue in the waste sample. The process results in the formation of a sulphide-rich concentrate fraction as well as a higher volume sulphide-lean benign tailings. The benefit of desulphurisation by flotation is that the

sulphide-rich concentrate has a low volume and is thus much easier to manage and control than large volumes of mine waste (Kazadi Mbamba, et al., 2012).

2.4.1 Two-stage flotation process for desulphurisation of coal tailings

The technical feasibility of a two-stage desulphurisation flotation process for copper and coal mine waste tailings has been shown by Hesketh et al. (2010) for hard rock tailings and by Kazadi Mbamba et al. (2011, 2012) for fine coal discards. Figure 4 illustrates the two-stage flotation process using an ultrafine coal waste stream. The proposed desulphurisation process is separated into two stages: coal flotation separation and sulphide flotation separation. In the first-stage, ultrafine coal waste is floated with a saleable coal product stream recovered as a concentrate. The tails remaining after the first-stage flotation undergo desulphurisation flotation to remove the acid producing pyrite (Kazadi Mbamba, et al., 2012). The separation results achieved when the process was applied to the coal tailings waste sample are presented in Table 1.

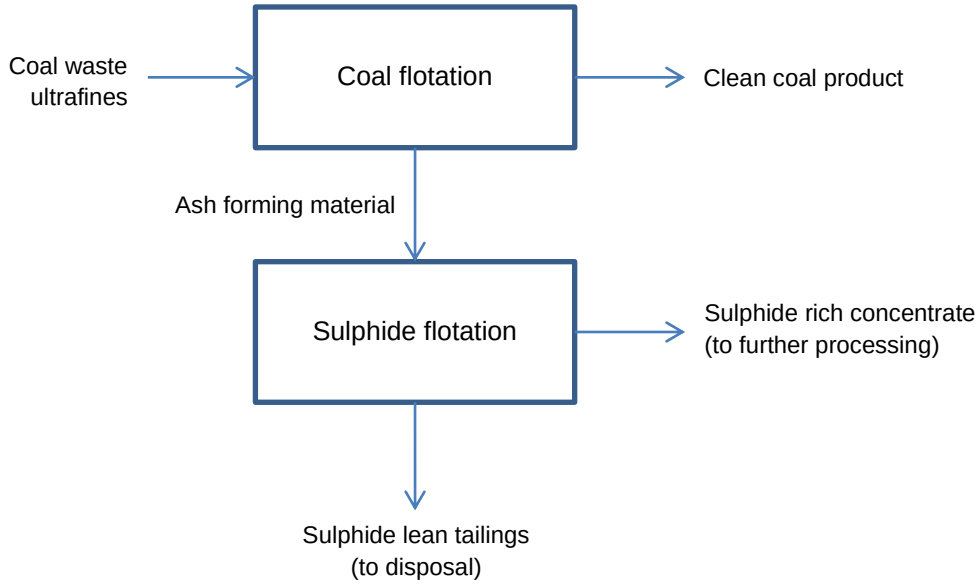

Figure 4: Two stage ultrafine coal waste desulphurization process (adapted from Kazadi Mbamba et al. (2012))

Table 1: Two stage ultrafine coal waste flotation results (adapted from Kazadi Mbamba et al. (2012))

Product	Amount	Weight percent	
		Ash	Total sulphur
First-stage Reagent addition:	1.86 kg/t Dodecane 0.11 kg/t MIBC		
Feed	100	34.4	1.08*
Clean coal concentrate	19.7	13.5	0.48
Ash forming tailings	80.3	38.8	0.91
Second-stage Reagent addition:	2.33 kg/t PAX 0.11 kg/t MIBC 0.93 kg/t Dextrin		
Feed (tailings from stage 1)	80.3	38.8	0.91
Sulphide rich concentrate	13.1	28.9	2.68
Sulphide lean (benign) tailings	67.2	40.8	0.38

* some of the S in feed to stage 1 is in the form of sulphate, reporting to the solution

One of the benefits of desulphurisation by flotation lies in the potential uses for the sulphide-rich concentrate and the (benign) sulphide-lean tailings. Although further research is required and ongoing

in this field, Harrison et al. (2013) have provided potential uses for both the sulphide concentrate and benign tailings process streams. These uses have the potential to enhance the economic feasibility of desulphurisation flotation significantly and thus provide an advantage over other desulphurisation techniques. Potential downstream processes are being further assessed in the associated project WRC K5/2231. Possible uses for the sulphide-rich concentrate fraction identified in these reports include sulphuric acid production, ferric sulphate production, enhancement of high temperature base metal heap bioleaching, and use in replenishing depleted nutrients to the soil. The large volume benign tailings fraction, on the other hand, has the possibility of being used in the construction of roads, in paste backfill, in disposal of waste rock as covers or neutralising material or both, and potentially in the development of matrixes for soils and as a cement additive.

2.4.2 Two-stage desulphurisation flotation reagents

The chemical reagents used in the two-stage desulphurisation flotation process are given in Table 1. Different combinations of reagents are used in each stage in order to target the selective flotation separation of coal in stage 1 and pyrite in stage 2.

Methyl isobutyl carbinol (MIBC), a short chain alcohol, was found to be the best frother for both stages of the two-stage desulphurisation process. It is used to facilitate air dispersion into fine bubbles, and to stabilize the froth (Kazadi Mbamba, 2011). According to Aplan (1976) and Gupta et al. (2007), advantages of this frother include that it has no collecting properties for gangue, it is most effective at maximising the contact surface area of the bubbles, and only small volumes of MIBC are required relative to other frothing agents.

The recovery of the valuable coal to the concentrate in the first flotation stage was targeted using dodecane as a collector to enhance the coal's hydrophobicity. The collector works by forming a monolayer on the surface of the particle, thereby creating a film of non-polar hydrophobic hydrocarbons and increasing the particle's floatability. Other collectors tested by Kazadi Mbamba (2011) were oleic acid and kerosene. Oleic acid was reported to result in the best recovery of the combustibles. They attributed this to a stronger interaction between the oleic acid molecule and the aromatic sites on the coal surface than between an aliphatic hydrocarbon chain and the coal surface because of the strong π-bonding between the hydrophobic component of the coal surface and the oleic acid double-bond. However, selectivity of the aliphatic oils (kerosene and dodecane) for the hydrophobic carbonaceous material was greater than that of oleic acid and as a result they produced a cleaner coal with a lower sulphur content.

The desulphurisation flotation process which occurs in the second-stage relies on the modification of the surface properties (charge and hydrophobicity) of pyrite, the key sulphide mineral associated with ARD generation, to make it amenable for separation (Murphy & Strongin, 2009). Conventionally inorganic materials such as xanthates, cyanides, sulphides and ferro-cyanides are used as flotation reagents for the removal of pyrite (Konstantine, et al., 1992; Pearse, 2005). The most effective collector reagents for desulphurisation flotation using an ultrafine coal waste stream have been demonstrated to be xanthates, namely potassium amyl xanthate (PAX), sodium isobutyl xanthate (SIBX) and sodium ethyl xanthate (SEX). Xanthates interact with most sulphide minerals, forming dixanthogen or metal xanthate on the mineral surface (Misra & Chen, 1995; Shen, et al., 1997) with dixanthogen having been identified as the main xanthate species responsible for the surface changes induced on pyrite (Jiang, et al., 1998). Jiang et al. (1998) found that when floating pyrite with a xanthate collector, the solution pH and particle size strongly affect the reaction. Kazadi Mbamba (2011) achieved the best sulphide recoveries in the second-stage of the two-stage desulphurisation of coal using a PAX collector, as presented in Table 1. This is due to its high sulphide mineral selectivity as well as the fact that xanthates have no affinity for the hydrophilic gangue particles (Kazadi Mbamba, 2011).

The third reagent used in the second-stage process is dextrin, a starch, which acts as a coal depressant to ensure that coal is not recovered in the sulphide rich product stream. The dextrin oxidizes the surface of coal, thus destroying its natural hydrophobicity and inhibiting its floatability.

2.4.3 Environmental effect and cost of reagents

In assessing the economic feasibility of desulphurisation of coal waste ultrafines by flotation and the associated sensitivity analysis, the cost of chemical reagents has been identified as a major contributor with potential improvements affecting the process economics significantly (Harrison, et al., 2013). These chemical reagents have been estimated to comprise approximately 59% of the total operating costs of the desulphurisation of a Witbank coal using a flotation process (Jera, 2013). Expanding this study to consider desulphurisation of five different ultrafine coal waste samples, the reagent cost contributions estimated vary between 35 and 64% (Harrison, et al., 2015). Xanthate, in particular, is required in high concentrations, hence large quantities, with economic and potential environmental consequences (Jiang, et al., 1998). In some instances, such as the flotation of iron sulphide minerals, the selectivity of xanthate is low and thus lime is needed to suppress the floatability of iron, increasing the plant costs further (Shen, et al., 1997). Therefore, the financial viability of desulphurisation flotation is largely dependent on the amounts of chemical reagents used and the cost of these reagents.

An additional concern are the environmental effects of the chemical reagents owing to the nature of the chemicals and the high concentrations and amounts required for the handling of large volumes of tailings or fine coal wastes (Govender & Gericke, 2011; Harrison, et al., 2013). Several chemical reagents used in desulphurisation flotation are moderately unstable and have the tendency to decompose once discharged into the environment. Furthermore, the chemical reagents have been associated with soil contamination (EPA, 1994). The xanthate collectors display toxicity, which may lead to downstream water pollution if degradation does not occur rapidly.

These two factors strongly motivate for the investigation of alternative flotation reagents for desulphurisation. Bioflotation reagents are one such possibility and are the focus of this study.

CHAPTER 3: The Potential of Bioreagents for Coal and Sulphide Flotation

Bioflotation falls under the broader topic of bio-beneficiation in which microbial cultures act either as collectors, surface modifiers or depressants to enhance the separation of sulphide minerals either by flocculation or flotation (Farahat, et al., 2008). Advantages of using microbial cultures over chemical reagents include lower toxicity, being environmentally benign, biodegradability and effectiveness over a wide range of temperature and pH (Subramanian, et al., 2003; Hamid & Abbas, 2011).

As in chemical flotation, bioflotation can separate minerals selectively based on their surface properties. It was first reported by Solojenleen in 1976 who described the possibility of ore flotation in the presence of sulphate reducing bacteria (Khoshdast & Sam, 2011). The floatability of hydrophobic minerals may be enhanced through exposure to microorganisms; conversely, microbial cultures may enhance the hydrophilicity of minerals. Thus, microbial cultures may be used as either collectors or depressors, depending on the minerals and microorganisms used.

3.1 Bioflotation Mechanisms

Alteration of the mineral surface by the microbial culture is essential to facilitate bioflotation. This alteration to the mineral surface occurs in one of three ways (Dwyer, et al., 2012; Vilinska & Hanumantha Rao, 2008; Hosseini, et al., 2005):

1. Microbial attachment of the cultures to the surface of the minerals such that the particle surface characteristics are largely determined by those of the microorganism
2. Microbially catalysed oxidation whereby the culture oxidises the iron and sulphur present in the minerals (as described in Equations (4) and (5))
3. Microbial production of Extracellular Polymeric Substances (EPS) which coat the mineral and microbe surface

Microbial attachment to the minerals is postulated as the desired mechanism for surface chemistry alteration, since it can occur rapidly within a period of 5 to 15 minutes (Africa, et al., 2010; 2013a; 2013b; Harneit, et al., 2006), consistent with typical flotation contacting times of around 1 hour or less (Oner, 2013; Olson, 1991) whereas microbial oxidation and EPS formation typically occur over a comparatively longer time frame.

The primary reversible attachment of the microbial culture onto the mineral surface is dependent on their coming into contact with one another as well as physico-chemical attraction. The initial attachment is governed by interactions such as (Dwyer, et al., 2012; Sharma, et al., 2001):

- electrostatic interactions (surface charge)
- hydrophobic interactions
- acid-base interactions
- Van der Waals forces

These factors are dependent on the structure of the microbial cell envelope. There are two broad categories of bacterial cell envelope: Gram-positive and Gram-negative, with the main difference being that the Gram-positive bacteria have a thicker peptidoglycan cell wall, making up some 90% of the cell envelope, giving increased structural strength (Hancock, 1991). An additional outer cell membrane is present external to this cell wall in Gram-negative bacteria (Dwyer, et al., 2012; Harrison, 2011). Gram-negative bacteria may also have an outer layer of lipopolysaccharides. While bacteria are the microorganisms most commonly discussed in the bioflotation literature, it must be noted that the cell envelopes of yeasts, fungi, archaea and algae, all of which are also reported associated with biomining

applications, have very different cell envelopes. For example, algae are most commonly characterised by cellulosic walls, while the primary components of the yeast and fungal cell walls are β-1,3-glucan, β-1,6-glucan, mannoprotein and chitin.

As described in Section 2.3.2, the surface charge of a mineral affects the likelihood of polar interactions with water molecules and is thus a key factor in flotation. Many traditional chemical flotation reagents act by altering the surface charge of select minerals, thereby influencing their floatability.

Bacterial cell surfaces are charged due to the presence of functional groups such as carboxyl (-COOH), amino ($-NH_2$) and hydroxyl (-OH) groups (Sharma, 2001). These functional groups originate from cell wall components such as lipopolysaccharides, lipoproteins and bacterial cell surface proteins. Typically, at neutral pH, bacteria have an overall negative surface charge owing primarily to the presence of peptidoglycan found in the cell walls (Chandraprabha & Natarajan, 2009). This component, rich in carboxyl and amino groups, forms the backbone of the cell wall (Chandraprabha & Natarajan, 2009). Because most mineral substrates also have negative surface charges at neutral pH, this results in repulsion between bacteria and these surfaces. However, attachment of the microorganisms to the mineral surface can have a pronounced effect on the surface charge of the mineral, thereby influencing its floatability. The surface charge of bacteria is highly pH dependent (Smith, et al., 1995). This is because the pH strongly affects the ionization of the surface groups (amino, carboxylate, phosphate), and hence the charge on the cell envelope. An electrical double layer is formed at the interface between counter-ions and charged groups. The thickness of this double layer is directly affected by the electrolyte and its ionic strength (Chandraprabha & Natarajan, 2009). As the pH rises, the surface charge of both minerals and bacteria decrease (becomes more negative) (Raichur, et al., 1996). It is usually best to operate at a pH that reduces the surface charge of the mineral surface and bacteria to as close to zero as possible or to the region of opposite charge, to minimise the repulsion forces and maximise attraction. Zeta potential, also known as the isoelectric point (IEP), is most commonly used to characterise the surface charge properties of the microorganisms and mineral surfaces, much like solid-solutions interfaces (Chandraprabha & Natarajan, 2009).

Bacterial cell walls contain varying amounts of lipids, which inherently exhibit hydrophobic behaviour (Chandraprabha & Natarajan, 2009). The lipids therefore render cells naturally hydrophobic. This is complemented by the effect of a combination of other hydrophobic structures present on the cell wall, including proteins (Hanumantha Rao & Subramanian, 2007). Thus, the degree of the hydrophobicity of the cell walls varies depending on the chemical composition and physical arrangement of the cell wall outer layers (Chandraprabha & Natarajan, 2009). Due to the inherent hydrophobicity of microbial cells, they have the tendency to absorb to solid surfaces as they repel from the polar molecules that comprise water (Chandraprabha & Natarajan, 2009). The strength of the hydrophobic repulsion of a microbial cell away from water often overpowers the electrostatic repulsion between the solid and the bacteria, resulting in an overall attraction as both repel from water (Chandraprabha & Natarajan, 2009). However, because the surface charge increases the likelihood of polar interactions with polar molecules (water), the more charged the cell surface is, the less hydrophobic it is. The overall bacterial adhesion is therefore dependent on the balance of bacterial and solid surface charges and hydrophobicity (Chandraprabha & Natarajan, 2009).

Secondary irreversible attachment of microorganisms to the mineral surface occurs as a result of the formation of a biofilm due to the release of EPS which hinders microbial detachment (Dwyer, et al., 2012). The formation of the biofilm may also trap surface ions onto the minerals, resulting in a change in surface charge (Hanumantha Rao & Subramanian, 2007).

3.2 Bioproducts as Bioflotation Reagents

3.2.1 Biofrothers

Biofrothers are metabolic products of microorganisms and include both organic and inorganic reagents such as mineral acids, fatty acids, polysaccharides, proteins and chelating agents (Subramanian, et al.,

2003). The advantages of biofrothers over chemical frothers include lower toxicity (being typically environmentally benign), biodegradability and effectiveness over a wide range of temperature and pH (Subramanian, et al., 2003; Hamid & Abbas, 2011). They are considered promising potential substitutes for synthetic chemicals from petrochemical origin (Khoshdast & Sam, 2011).

In relation to the body of work done on the use of microorganisms as bioflotation agents, little work has been done on the identification of potential microbial products for use as biofrothers. Khoshdast and Sam (2011) report that much work has been done on rhamnolipids, produced by *Pseudomonas aeruginosa* strains. Fazaelipoor et al. (2009) studied the frothing characteristics and flotation applicability of rhamnolipid-type biological surfactants used as a biofrother. They found that they showed better surface-activity and static frothability (frother height and half-life) in comparison with MIBC. Khoshdast and Sam (2011) concluded that the decrease in surface tension and the resultant increase in froth stability resulted for two reasons. Firstly, the biofrothers tested have higher molecular weights than MIBC. High molecular weight increases the viscosity and hence the stability of the froth within the flotation cell. Secondly, MIBC is a short chain alcohol and thus only possesses one –OH group while biofrothers have several oxygenate units. Interactions between these oxygenate units and the water molecules in the float cell occurs through hydrogen bonding, allowing the molecules to lie flat on the surface of the bubble (Khoshdast & Sam, 2011). These hydrogen bonds decrease the surface tension of the water film through increased film stability, leading to subsequent increase in froth stability.

3.2.2 Biocollectors

Fatty acids, which may be biologically derived, are considered universal collectors as they can be used to float many materials. They are generally non-selective; hence an appropriate modifier may be required for enhanced selectivity. Some common examples of fatty acids are oleic acid and linoleic acids (cis-9, cis-12 and cis-15) (Leja, 1982). They are typically synthesized by hydrolysis, hydrogenation of petroleum products or from animal fats, vegetable oils and microbial lipids. They are considered environmentally friendly as well as economical for flotation applications (Dube, 2012). Hikmet et al. (2004) reported that a mixture of fatty acids performed better than a single fatty acid as a mixture facilitated a variety of adsorption capabilities. Thus, a mixture of fatty acids can adsorb onto different oxygen groups on the coal surface, thereby permitting higher coal recovery from flotation processes. Oleic acid, the current chemical coal collector in the two-stage flotation desulphurisation process, is a fatty acid flotation reagent option which tends to form an acid soap dimer complex that is highly surface active. The presence of such dimer complexes minimises the surface tension of solution which could further enhance the flotation process (Dube, 2012).

Vegetable oils, comprised mainly of a wide variety of fatty acids, have been found to be an effective collector for coal flotation. Olive oil and soybean oil provided a substantial increase in the recovery of combustibles for low rank coals (Alonso, et al., 2000). Additionally, these oils were found to be effective for agglomerating coal fines at very low oil concentrations; the coal fines were postulated to interact with the fatty acids through hydrogen bonding (Alonso, et al., 2002). In a study conducted by Vasumathi et al. (2013), a vegetable oil based, eco-friendly reagent was developed as a replacement for the diesel-frother system. After encouraging results were obtained at laboratory scale, trials were conducted of a 30 t/h process. The single reagent was found to be superior to the conventional diesel-frother system in terms of yield and ash content. This implied that better separation and selectivity could be achieved in the system, improving the flotation performance of fines in the flotation circuit. After performing a cost analysis, it was found that the single reagent system was more cost effective compared to the diesel-frother system. Hence vegetable oils, namely crude soybean and olive oils, have been reported as promising alternatives for the recovery of coal fines (Vasumathi, et al., 2013).

Tall oil, a by-product of the pulp and paper industry, is a complex fatty acid mixture which has been shown to float the majority of minerals including phosphate and hematite (Kou, et al., 2010). It is mainly comprised of oleic (C18:1 cis-9), linoleic (C18:2 cis,cis-9,12), palmitic (C16:0), and rosin (C20, aromatic) acids. The use of tall oil fatty acids as collectors for coal flotation provided an improved combustible

recovery (Hines, et al., 2011). Furthermore, tall oil lowers the surface tension of slurry which further enhances the bubble surface area in the flotation cell.

Algae are photosynthetic organisms that range from unicellular microscopic microalgae to multicellular macrophytic forms distributed ubiquitously in the environment. Algae accumulate varied forms of lipids and lipid content (Kumari, et al., 2013). Research has found that microalgae produce lipids that are similar to those produced in most vegetable oils (Stanley, et al., 2010). The lipids produced are of two main classes: polar lipids (including phospholipids and glycolipids) and neutral lipids (mono-, di-, and triglycerides, isoprenoids, and waxes). The latter are accumulated under stress conditions like nitrogen limitation, and exhibit the more desirable properties for conversion to biodiesel (Schlagermann, et al., 2012; Griffiths, et al., 2012). The neutral lipids which are extracted by *n*-hexane are converted to fatty acids by esterification and transesterification, the product specifically being fatty acid methyl esters (FAMEs) with a general chemical formula $CH_3(CH_2)_nCOOCH_3$ (Laskowski, 2013; Kumari, et al., 2013; European Biofuels Technology Platform, 2011). A study by Griffiths et al. (2012) on 11 microalgal species from different environments showed that *Scenedesmus sp.* and *Chlorella vulgaris* were the best in terms of lipid content and lipid productivity (649 mg/ℓ and 106 mg/ℓ·day, respectively, for *Scenedesmus*, and 597 mg/ℓ and 67 mg/ℓ·day, respectively, for *C. vulgaris*). These microalgae produced lipids which met about 99% of the requirements for biodiesel quality according to the European standard EN 14214 (Griffiths, et al., 2012; Knothe, 2006). The FAME profile from Griffiths et al (2012) also showed that the carbon chain length of the fatty acids ranged from 14 to 20 carbon atoms per molecule, with a higher proportion being unsaturated fatty acids, which correspond to the carbon length in popular reagents used as collectors for coal.

Since biodiesel is a mixture of FAMEs, the suitability of microalgal lipids as coal collectors can be supported by the work done by Yi et al (2015) which used a mixture of FAMEs, coined bioflotation agent (BFA), from transesterification of glycerides in waste cooking oil (WCO). In comparison to petrol-diesel, BFA proved to be an efficient, economic and environmentally friendly reagent as it reduced the production cost of clean coal by US$ 0.836 for each ton of fine coal slurry processed from the WCO-to-BFA-to-Coal flotation process, the energy consumption by 13% and the carbon dioxide emission by 76%. Similar studies were done using biodiesel on oxidised coal and the outcome of the research was that biodiesel was better than diesel in terms of combustible matter recovery (≈75% vs 58% with 12% and 11% ash content, respectively) and flotation efficiency index (34.44% at biodiesel dosage of 7 kg / ton vs 29.31% at diesel dosage of 10 kg / ton) (Xia, et al., 2013). The researchers concluded that biodiesel was better than diesel because of the abundant unsaturated fatty acids which interact with the oxygen groups on the oxidised coal. The success of biodiesel in coal collection was attributed to the similarity in chemical structure of the FAMEs to conventional collectors such as oleic acid, diesel, paraffin and dodecane (Kazadi Mbamba, 2011; Han, 1983; Curl & O'Donnell, 1977; Sisa, et al., 2003).

3.2.3 Extracellular polymeric substances (EPS) and extracellular bacterial proteins (EBP)

Govender and Gericke (2011) investigated the possibility of using mixed bioleaching consortia and their EPS as bioflotation reagents. The results obtained demonstrated the potential of the use of EPS derived from pure cultures as a bioflotation reagent. It was found that free EPS obtained from microbes growing on chalcopyrite has a higher affinity for chalcopyrite during flotation compared to the EPS extracted from other systems (pyrite and sphalerite). The use of free EPS resulted in higher chalcopyrite recoveries as opposed to when bacterial cells containing bound EPS were used. Additionally, higher recoveries were reported in cases where the free EPS used was extracted from systems at higher temperatures (45°C and 70°C). It was further found that EPS isolated from these systems consisted mainly of carbohydrates. However, high amounts of proteins, uronic acids and carbohydrate:protein (C:N) ratios were found (Govender & Gericke, 2011) at these higher temperatures when compared to the free EPS extracted from other systems.

The metabolically secreted proteins in EPS have been known to induce hydrophobicity on various minerals whilst polysaccharides confer hydrophilicity on others (Vilinska and Rao, 2008). Extracellular proteins essentially serve as a hydrophobic agent with higher surface hydrophobicity and lower surface charge and are related to higher dispersion and flotation tendencies. Therefore, in a flotation process, proteins can aid in the selective separation of sulphide minerals such as pyrite and chalcopyrite. Patra and Natarajan (2004a) compared the performance of whole cells of *P. polymyxa*, against extracted EPS and EBP for the bioflotation separation of pyrite and chalcopyrite from oxide gangue minerals. The EBP was reported to outperform the EPS and whole cells due to it imparting a greater degree of hydrophobicity to the gangue minerals. The EPS experiments in this study did not significantly outperform chemical free controls.

3.3 Microorganisms as Bioflotation Reagents

3.3.1 Heterotrophic microorganisms

Certain heterotrophic bacteria contain the properties necessary to float pyrite. Heterotrophs are organisms that are not capable of fixing carbon and need a source of organic carbon for growth. Heterotrophs that have been investigated and found as potential bioflotation reagents for the removal of pyrite from a number of mineral deposits include *Bacillus subtilis*, *Mycobacterium phlei*, *Rhodococcus opacus*, *Sulfolobus acidocaldarius* and *Paenibacillus polymyxa*, all of which are dealt with in turn in the following subsections.

3.3.1.1 Bacillus subtilis

Bacillus subtilis is a rod-shaped bacterium which is Gram-positive and neutrophilic and is often naturally associated with various mineral deposits. Sarvamangala et al. (2013) reported the use of *B. subtilis* for the flotation of pyrite from galena. Mineral interactions with these bacterial cells rendered pyrite more hydrophilic and galena more hydrophobic at a neutral pH. The pH and presence of ions significantly influences the charge on the microorganism. *B. subtilis* moves through its point of zero charge, on moving from pH 5.0 to pH 7.5 in the presence of 0.05 mM Cr^{3+}, while in the presence of Cu^{2+} this occurs in the pH range 6 to 9 (Smith, et al., 1995).

3.3.1.2 Mycobacterium phlei

Mycobacterium phlei is a Gram-positive bacterium which is fast growing and rod shaped with a mycolic covered surface. Its cell envelop consists of an inner membrane (cell wall) with a thick peptidoglycan layer (Raichur, et al., 1996). The cell wall contains varied amounts of sugars, alcohols, teichoic acids and phosphates and can contain between 30% and 60% lipids (Raichur, et al., 1996). According to Raichur *et al*. (1996), *M. phlei* has a negative surface charge and has a surface contact angle of 112°, therefore being highly hydrophobic. The bacterium renders the mineral hydrophobic after its adhesion to the mineral surface, thereby allowing flotation. However, its surface chemistry has been reported to be dependent upon the culture medium and the growth rate (Raichur, et al., 1996) and different effects on floatability have been described. The bacterium's negative surface charge means that electrostatic and hydrophobic interactions govern its attachment to minerals which are less negatively charged (Smith & Misra, 1991; Hanumantha Rao & Subramanian, 2007). The cell concentration and mineral particle size have been shown to influence the adhesion and can result in mineral flocculation with fine particles (Dwyer, et al., 2012).

The bacterium has been used for the desulfurization of coal through flocculation and flotation (Raichur, et al., 1996; Chandraprabha & Natarajan, 2009; Mishra, et al., 1993). The bacterium was used by Raichur et al. (1996) to separate mineral matter from coal by flocculation and flotation. The coal particles flocculated due to hydrophobic interaction and *M. phlei* bridged between the particles. The inorganic matter in coal flocs was then separated by column flotation techniques. *M. phlei* has also been used as a flotation depressant in the anionic flotation of apatite ($Ca_{10}(PO_4)_6(OH,F,Cl)_2$) and dolomite ($CaMg(CO_3)_2$) (Zheng & Smith, 1997).

M. phlei has been used in the flotation of non-sulphide minerals such as haematite (Fe_2O_3), due to the microorganism's strong hydrophobicity (Smith, et al., 1995). The use of *M. phlei* for the selective separation of pyrite has given varying results, with Atkins et al. (1987) and Hanumantha Rao and Subramanian (2007) reporting that it acts as a depressant due to increasing the hydrophilicity of the minerals, while Nagaoka et al. (1999) and Smith et al. (1995) report that it acts as a biocollector by increasing the hydrophobicity of the minerals.

3.3.1.3 Paenibacillus polymyxa

Paenibacillus polymyxa is a chemo-organo heterotroph which is found associated with many mineral deposits, being responsible for the fixation of nitrogen in soils. It is Gram-positive and neutrophilic (Patra & Natarajan, 2003; 2008a; 2008b) and produces extracellular polysaccharides (Hanumantha Rao & Subramanian, 2007).

Strains of *P. polymyxa* have been demonstrated for the beneficiation of bauxite and in studies performed on calcite ($CaCO_3$), haematite (Fe_2O_3), corundum (Al_2O_3), kaolinite ($Al_2Si_2O_5(OH)_4$) and quartz (SiO_2), surface changes were induced by the bacteria-mineral interactions (Patra & Natarajan, 2003; Deo & Natarajan, 1997; 1998). Calcite, haematite and corundum became more hydrophilic due to the adhesion of bacterial excreted polysaccharides, while kaolinite and quartz became more hydrophobic as a result of the adhesion of bacterial excreted proteins (Deo & Natarajan, 1997; 1998; Hanumantha Rao & Subramanian, 2007). During the majority of these adhesion processes, it was found that electrostatic forces governed the adhesion. For quartz, on the other hand, chemical forces played an additional role (Hanumantha Rao & Subramanian, 2007).

Subramanian et al. (2003) investigated using *P. polymyxa* in the selective separation of the sulphide minerals galena (PbS) and sphalerite (ZnS). The bacteria caused selective depression of galena from the sphalerite. *P. polymyxa* strains were also adapted to sulphide minerals such as pyrite (FeS_2) and chalcopyrite ($CuFeS_2$), thereby influencing their relative tolerance of and so attraction to these minerals.

Patra and Natarajan (2003) looked at the possibility of separating pyrite from quartz and calcite by flotation with the *P. polymyxa* cells. They found that the cells had the highest affinity towards pyrite, followed by calcite and lastly quartz and thus effective separation of these minerals is possible. Preconditioning with *P. polymyxa*, when using a xanthate collector, was found to enhance mineral recoveries (Patra & Natarajan, 2003; 2008a).

Patra and Natarajan (2008a) stated that together chalcopyrite and pyrite could be depressed from mineral mixtures when preconditioned with *P. polymyxa* while galena, sphalerite and quartz were selectively floated.

3.3.1.4 Rhodococcus opacus

Rhodococcus opacus is another highly hydrophobic bacterium with a net negative charge and is able to adhere to inert mineral surfaces. It has been used as a biocollector in the separation of haematite (Fe_2O_3) from quartz (SiO_2), due to its high affinity towards the haematite particles. It has more recently been used as a flotation collector for magnesium or calcite ($CaCO_3$), as well as for the removal of the heavy metals cadmium and zinc from wastewaters (Dwyer, et al., 2012).

3.3.1.5 Sulfolobus acidocaldarius

Hanumantha Rao and Subramanian (2007) suggest that *Sulfolobus acidocaldarius*, a thermophilic archaeon, can be used to remove pyrite from coal. The optimum growth temperature for this thermophile is 70-75°C. It has a depressive effect due to the microorganism's hydrophilicity. Schippers (2007) observed that several species of the order *Sulfolobales* have metal sulphide, iron and sulphur-compound oxidizing abilities, but metal sulphide oxidation has not been demonstrated with *Sulfolobus acidocaldarius*.

3.3.2 Chemolithotrophic bacteria

Some chemolithotrophic bacteria occur naturally in the presence of minerals in mine water and selectively attach to sulphides, making them possible reagents for sulphide flotation. Studies on microbial species have focused on chemoautotrophic bacteria such as *Leptospirillum ferrooxidans*, *Leptospirillum ferriphilum* and *Acidithiobacillus ferrooxidans* for their iron oxidizing abilities, and *Acidithiobacillus thiooxidans* and *Acidithiobacillus caldus* for their sulphur oxidizing ability.

The main advantages of using of chemolithotrophs for bioflotation are:

1. Chemolithotrophs are native to the ore and occur naturally in an acid rock drainage environment (Schippers, 2007). This means that, for industrial implementation, contamination of the microbial cultures with other cultures during bio-flotation will be inconsequential (Emerson, et al., 2012).
2. The microbial cultures are accustomed to growing on pyrite which results in the selective adherence of the microbial cultures to pyrite during bioflotation (Misra, et al., 1996). This is important considering that pyrite is the most significant sulphide mineral contributor to ARD.
3. The microbial cultures have an affinity for iron and sulphur as they act as oxidising catalysts (Natarajan, 2008; Suzuki, 2001).
4. The mixed mesophilic cultures exhibit synergistic attachment properties when in a mixed culture as compared to pure cultures (Florian, 2012).
5. Certain species show selective attachment to mineral sulphides over gangue minerals and may show preferential attachment to specific mineral sulphides (Africa, et al., 2013a).

A further motivation for the utilisation of chemolithotrophs over heterotrophs is the fact that heterotrophs require an organic carbon source while chemolithotrophs do not. However, heterotrophs have a faster growth rate and hence higher secretion of EPS (Sharma, et al., 2001). Due to the slower growth rate of chemolithotrophs, the alteration in surface chemistry of the minerals is likely to be primarily due to microbial attachment over the time period of contacting for flotation as the autotrophic chemolithotrophs require several days to produce significant EPS biofilm on the mineral surface (Africa, et al., 2010; Africa, et al., 2013b).

3.3.2.1 Chemolithotrophic bacterial attachment

Numerous studies have been conducted on the attachment of *A. ferrooxidans* and *Leptospirillum* spp. to pyrite and chalcopyrite due to the importance of this for bioleaching operations. This affinity renders them potentially useful for bioflotation of sulphide minerals (Vilinska, 2007). The adsorption of *A. ferrooxidans* to pyrite has been reported to be more efficient than adsorption onto chalcopyrite and arsenopyrite (Chandraprabha, et al., 2004), with the adsorption onto pyrite having been found to occur within 10-15 minutes (Das, et al., 1999; Natarajan & Das, 2003). Furthermore, under acidic conditions the microbial attachment occurred at an even faster rate (Natarajan & Das, 2003). Africa et al. (2013a) reported enhanced attachment degree and efficiency for *A. ferrooxidans* and *Leptospirillum* spp. grown on sulphide mineral compared to only on ferrous ion in solution.

Through the use of spectroscopy, Diao et al. (2013) established that at a pH of 2.05 *A. thiooxidans*, with a water contact angle of 12°, was slightly more hydrophobic than *L. ferrooxidans* which had a water contact angle of 9°. Pyrite has a water contact angle of 67° and is considerably more hydrophobic than silica which has a water contact angle reported as 4° (Diao, et al., 2013). This is an indicator of the natural floatability of pyrite, with a high natural selective separation of pyrite from a silica – pyrite sample. According to Razatos et al. (1998), the microbial adhesion is enhanced by the surface hydrophobicity of the mineral, which induces stronger microbial adhesion onto pyrite. Surface roughness may also impact microbial adhesion, with an increased surface roughness resulting in an increased microbial attachment (Eginton, et al., 1995).

Diao et al. (2013) investigated the adhesion force between the microbes and the mineral surface. These forces aid microbial attachment and subsequently lead to surface chemistry alteration. They observed the maximum adhesion at the lowest pH while the silica surface demonstrated some resistance to *A. thiooxidans* adhesion. The resistance of *A. thiooxidans* adhesion increases the ability of the microbes to bring about selective separation due to the change in surface chemistry of pyrite differing to the change induced on the silica. The adhesion force of *L. ferrooxidans* to silica was similar to adhesion force of *A. thiooxidans* to pyrite whereas the adhesion force of *L. ferrooxidans* to pyrite was higher that its adhesion force to silica. Thus, in a *L. ferrooxidans* dominated culture the adhesion to pyrite is the most significant factor to selective separation.

In a study of microbial attachment to silica and pyrite minerals by Harneit et al. (2006), cell attachment to silica was determined to only be 18% compared to the 80-90% cell attachment to pyrite. This is confirmed by Africa et al. (2013a) who showed 74 to 79% attachment of *L. ferriphilum* and *A. ferrooxidans* to pyrite, 58 to 64% attachment to chalcopyrite and 50% or less attachment to low grade ore and quartz, typically around 25%. With low grade ores, preferential attachment was observed in the regions of the sulphide mineral grains (Africa, et al., 2010). The difference in adhesion forces to these different minerals could not account fully for this difference in cell attachment. Hence, the difference in cell attachment between silica and pyrite may also be attributed to the difference in surface roughness (Eginton, et al., 1995). According to Liu et al. (2006) microbes attach selectively to sites with high surface energy, where the surface energy is related to the zeta potential. The zeta potential of pyrite at a pH of 2 was determined to be around 16 mV while the silica's zeta potential was determined to be around 3 mV (Liu, et al., 2006; Xu, et al., 2003). Similarly, Africa et al. (2010) demonstrated preferential attachment at surface defects on the pyrite and chalcopyrite surfaces.

3.3.2.2 Leptospirillum spp.

L. ferrooxidans pure cultures have been reported to oxidise pyrite, chalcopyrite and sphalerite successfully (Lens & Pol, 2000). However, *L. ferrooxidans* oxidises only the ferrous iron present in pyrite and not the sulphur (Suzuki, 2001). Due to the non-sulphur oxidising properties of *L. ferrooxidans*, their mechanism of attachment to pyrite and their influence on the surface properties of pyrite differ to those of *A. ferrooxidans* (Vilinska & Hanumantha Rao, 2008).

Vilinska and Hanumantha Rao (2008) have shown that *L. ferrooxidans* has a greater affinity towards chalcopyrite on pyrite-chalcopyrite flotation systems than *A. ferrooxidans*, with more cells absorbing onto the chalcopyrite surface. Vilinska and Hanumantha Rao (2008) concluded that as the microbial concentration of *L. ferrooxidans* within the flotation cell increased so the recovery of pyrite in the combined chemical and bioflotation decreased even in the presence of a xanthate collector. This indicates that *L. ferrooxidans* has a substantial ability to depress pyrite.

It must be noted that the differentiation between *L. ferrooxidans* and *L. ferriphilum* was recognised in the earlier 2000s for mineral bioleaching (Coram & Rawlings, 2002). It is expected that the authors above may be referring to cultures that were more dominantly *L. ferriphilum*.

3.3.2.3 Acidithiobacillus ferrooxidans

A. ferrooxidans has an affinity for pyrite due to its ability to oxidise the pyrite to form ferric iron and sulphate (Lens & Pol, 2000). The bacterium adheres to pyrite preferentially compared with chalcocite (Cu_2S), molybdenite (MoS_2), millerite (NiS), and galena (Nagaoka, et al., 1999; Misra & Chen, 1995). This allows for selective removal of pyrite to the tailings in bioflotation with *A. ferrooxidans* acting as a depressant (Nagaoka, et al., 1999; Vilinska & Hanumantha Rao, 2008; Mehrabani, et al., 2011).

Studies have shown that the prior application of *A. ferrooxidans* can reduce the use of xanthate in the flotation of pyrite significantly, while having minimal effects on chalcopyrite. The bacterial cells adsorb to the pyrite surface and act as a depressant, reducing its floatability, when floating chalcopyrite from pyrite. It is thus possible to float chalcopyrite from pyrite selectively using *A. ferrooxidans* (Vilinska & Hanumantha Rao, 2008; Govender & Gericke, 2011; Hosseini, et al., 2005; Chandraprabha, et al.,

2004). Depression of 20% of pyrite was reported, with chalcopyrite still floating at 60-80% recovery and arsenopyrite largely unaffected (Vilinska, 2007; Chandraprabha, et al., 2004; Sharma, et al., 2001; Nagaoka, et al., 1999). Hosseini et al. (2005) reported that pyrite depression could be increased to around 50% recovery when using double the bacterial cell concentration. The use of *A. ferrooxidans* enhanced flocculation of the sulphide minerals while negligibly affecting non-sulphide minerals. Chandraprabha et al. (2004) found that individually conditioning pyrite and chalcopyrite with *A. ferrooxidans* cells prior to the use of a xanthate collector resulted in pyrite depression and chalcopyrite flotation. However, conditioning the minerals together in this sequence resulted in poor selectivity, due to the activation of pyrite by the dissolved copper from chalcopyrite. It was then found that by reversing the order of conditioning, such that the xanthate collector was used prior to interaction with the bacterial cells, better selectivity could be achieved. Hosseini et al. (2005) looked at the interactions of sulphide copper mineral deposits containing pyrite and found that selective flotation was enhanced by sulphur grown *A. ferrooxidans* as opposed to ferrous grown cells. Africa et al. (2013a) have described the change in surface properties of *A. ferrooxidans* as a function of growth conditions. Maximum attachment of cells to pyrite was achieved after only 5 to 10 minutes, while attachment to other sulphide minerals (chalcopyrite, sphalerite and galena) was much slower (Harneit, et al., 2006).

A. ferrooxidans has been used for the desulphurisation of coal due to the attachment of the microbial cells to the pyrite, rendering the mineral surface hydrophilic (Smith, et al., 1995; Dwyer, et al., 2012; Pesic & Kim, 1993). Pyrite can be depressed selectively from coal-pyrite mixtures using *A. ferrooxidans* as a biological pre-treatment, followed by a conditioning with a xanthate collector (Rao, et al., 1991; Misra & Chen, 1995; Hanumantha Rao & Subramanian, 2007). According to Misra and Chen (1995) even upon the addition of a PAX collector, the sulphur remained depressed. Thus, as with *L. ferrooxidans*, the recovery decreased even in the presence of the PAX collector. It was therefore concluded that the biological depression was caused by the surface oxidation of sulphur-containing compounds on pyrite, generating hydrophilic jarosite (Misra & Chen, 1995). Jarosite is formed in the reaction:

$$3Fe^{3+} + X^{+} + 2HSO_4{}^{-} + 6H_2O \leftrightarrow XFe_3(SO_4)_2(OH)_6 + 8H^{+} \quad (6)$$

where $X^{+} = K^{+}, Na^{+}, NH_4{}^{+}, H_3O$. The fact that depression of pyrite occurs even in the presence of PAX means that *A. ferrooxidans* has a substantial ability to act as a depressant. However, microbial catalysed oxidation occurs over a longer time period than traditional flotation.

Amini et al. (2009) compared the kinetics of biological and conventional flotation of coal. Conventional flotation tests were conducted using sodium cyanide as a depressant whereas *A. ferrooxidans* was used as the bio-depressant in the biological experiment. It was found that bioflotation could be more effective for coal deposits with higher percentage of sulphur as pyrite. The kinetic parameters derived indicated that bacteria could be used as a suitable alternative depressant for pyrite in coal flotation. In addition, the total selectivity index for the biological tests was reported to be higher than in the conventional tests. Thus, the bacterial system was more effective in separating gangue from coal than conventional flotation methods.

3.3.2.4 Acidithiobacillus thiooxidans

Efficient separation of pyrite from quartz has been achieved through bioflocculation with both *A. thiooxidans* and *A. ferrooxidans* (Natarajan & Das, 2003).

The selective flotation of chalcopyrite from pyrite has also been achieved using a combination of bioflotation with *A. thiooxidans* and chemical flotation with potassium isopropyl xanthate (Chandraprabha & Natarajan, 2006). The maximum recovery of pyrite was between 30 and 40%, meanwhile the chalcopyrite recovery by flotation remained at 70 to 80% (Chandraprabha & Natarajan, 2006; Vilinska, 2007). Stronger adhesion forces were generally seen between *A. ferrooxidans* and chalcopyrite than *A. thiooxidans* and chalcopyrite, with increased adhesion forces for both bacteria at a low pH of roughly 2 (Diao, et al., 2014). Flotability of pyrite with *A. thiooxidans* was found to be highest

under acidic conditions, due to the change in solubility of the iron hydroxides at lower pH (Chandraprabha & Natarajan, 2006). This suggests that at a low pH *A. thiooxidans* acts as a collector. It is well recognised that the point of zero charge for these acidophilic autotrophs occurs at a pH in the vicinity of pH 2 (Africa, et al., 2010).

3.3.3 The potential of microorganisms for coal and pyrite flotation

Table 2 provides a summary of the microorganisms discussed in the literature in terms of bioflotation. Both the growth characteristics of the microbes and their performance in terms of flotation behaviour is given. It is seen that microbes can act as either depressants or collectors, depending on the type of microorganism, mineral and physico-chemical conditions. While the literature on microbial adhesion provides substantial insight into factors affecting the hydrophobicity and surface charge, these considerations have yet to be properly linked to their role in bioflotation. It is essential that the influence of culture conditions on surface properties (Bromfield, et al., 2011; Africa, et al., 2013a) be considered and that the effect of pH on surface charge and associated flotation performance be reported.

Table 2: Summary of microorganisms reported in the bioflotation literature and the findings into the possibility of pyrite flotation with each, as referenced in Section 3.3.
(H = heterotrophic, A = autotrophic. X represents positive flotation, ? indicates non-definitive results)

Microorganism	Growth Type	Hydrophobic	Hydrophilic	Pyrite collector	Pyrite depressant	Pyrite separation from			
						Coal	Gold	Base Metals	Other
Bacillus subtilis	H		X		X			Galena	
Mycobacterium phlei	H	X		?	?	X		Iron ore	
Rhodococcus opacus	H	X		?	?			Iron ore	
Sulfolobus acidocaldarius	H		X		X	X			
Paenibacillus polymyxa	H		X		X			Chalcopyrite Galena Sphalerite	Quartz Calcite
Leptospirillum ferrooxidans	A		X		X			Chalcopyrite Sphalerite	
Acidithiobacillus ferrooxidans	A		X		X	X		Arsenopyrite Chalcopyrite Chalcocite Millerite Galena	Quartz Molybdenite
Acidithiobacillus thiooxidans	A	?	?		X			Chalcopyrite	Quartz

3.4 Selection of Bioflotation Reagents for Investigation

Following consideration of the information collated, a selection of microorganisms and bioproducts were identified as key bioflotation reagents to investigate for the flotation separation of coal and, subsequently, pyrite (the sulphidic mineral of concern) in the two-stage desulphurisation flotation process. To be considered, the microorganisms or bioproducts were required to be non-pathogenic.

The microorganisms selected fall into two subcategories:

- Microorganisms that have already been applied successfully in reported coal or pyrite bioflotation studies

- Microorganisms that have not been reported in previous bioflotation studies, but are known to exhibit selective coal or pyrite affinity, as this has been demonstrated to be a requirement for biobeneficiation

3.4.1 Selection of potential bioflotation reagents for separation of coal

A single potential bioflotation reagent was identified from the literature (Section 3.3.1.2) for the selective flotation of coal from sulphide mineral and gangue in the first-stage of the desulphurisation flotation process, namely:

- *Mycobacterium phlei*

Numerous studies have demonstrated its selective affinity for coal and confirmed that it exhibits the hydrophobic properties required for a coal collector, making it an ideal candidate. However, its applicability to coals in South African remained untested prior to this study.

Flotation of the coal using biologically synthesised fatty acids, specifically algal lipids from *Scenedesmus sp.*, was investigated in this study. This is motivated by Kazadi Mbamba's (2011) finding that flotation recovery of South African coal in the two-stage process was optimised using an oleic acid collector, another fatty acid.

3.4.2 Selection of potential bioflotation reagents for separation of pyrite

The potential bioflotation reagents identified from the literature for the flotation separation of pyrite from gangue mineral in the second-stage of the desulphurisation flotation process are listed below.

Heterotrophs

- *Bacillus subtilis*
- *Paenibacillus polymyxa*
- *Rhodococcus opacus*

Chemolithoautotrophs

- *Acidithiobacillus thiooxidans*
- *Acidithiobacillus ferrooxidans*
- *Leptospirillum ferrooxidans*

All these microorganisms have been reported in the literature to show selective affinity to the pyrite (or other sulphide minerals) or gangue/coal, thereby facilitating the bioflotation separation of these substances. In the current 'chemical' second-stage of the desulphurisation process, the pyrite is floated and the gangue is depressed. This may potentially be reversed in the bioflotation process, depending on whether the microorganism renders a select mineral more or less hydrophobic or hydrophilic. *R. opacus* is hydrophobic and consequently is expected to facilitate the flotation of the mineral to which it shows preferential attachment (Sections 3.3.1.4). By contrast *P. polymyxa* has the effect of depressing the mineral to which it has selectively attached as it renders it more hydrophilic (Section 3.3.1.3). *B. subtilis* has shown differing effects, depending on the mineral to which it has attached (Section 3.3.1.1). The acidophilic chemolithotrophs *L. ferrooxidans* (or *L. ferriphilum*), *A. ferrooxidans* and *A. thiooxidans* have been reported to selectively attach to and depress pyrite in preference to other minerals (Section 3.3.2).

Two microorganisms that have not been tested before for their bioflotation potential will be considered in this project. These are:

- *Bacillus licheniformis*
- *Rhodopseudomonas palustris*

Bacillus licheniformis is a gram-positive endospore-forming bacterium which is closely related to *B. subtilis* (Rey, et al., 2004), a microorganism that has been identified from the existing bioflotation literature to be a potential bioflotation reagent. This similarity therefore motivates investigation of *B. licheniformis*. *R. palustris* is a rod-shaped gram-negative purple bacterium which can grow by any one of the four modes of metabolism that support life (Larimer, et al., 2004):

- photoautotrophic / photosynthetic (energy from light and carbon from carbon dioxide),
- photoheterotrophic (energy from light and carbon from organic compounds),
- chemoheterotrophic (carbon and energy from organic compounds) and
- chemoautotrophic (energy from inorganic compounds and carbon from carbon dioxide)

This versatility in growth mechanism suggests that it may have potentially interesting interactions and affinities to the different mineral species present in the flotation process and motivates its investigation. It is also reported to have quorum sensing abilities (Platt & Fuqua, 2010), where a population is able to regulate gene expression in response to changes in the population density through the production and release of chemical signal molecules by the bacteria (Miller & Bassler, 2001). This too may have interesting implications for its use as a bioflotation agent.

Prospecting for additional potential microbial flotation reagents was done using consortia of microorganisms from different mining environments, namely:

- a thiocyanate (SCN-) degrading mixed culture containing autotrophic and heterotrophic species from an Activated Sludge Tailings Effluent Remediation (ASTER TM) reactor
- a mixed mesophilic sulphur oxidizing culture

3.5 Additional Bioflotation Implementation Considerations

Bioflotation is influenced by mineral particle size, pulp density, pH and inoculum concentration (Hanumantha Rao & Subramanian, 2007). These are therefore important factors to consider in the assessment of bioflotation potential of a microorganism. Microbial health (vitality and viability) and the stability of the flotation system must also be considered.

The mineral size and pulp density are predetermined for the two-stage flotation process and are therefore not considered as optimisation parameters in this study. The inoculum concentration must be high enough to ensure that it is not limiting.

3.5.1.1 pH

The pH of the flotation liquor is critical in both chemical flotation and bioflotation as both the charge of the minerals and microbial cultures are dependent on the pH (Smith & Misra, 1991). As a result, microbe-mineral interactions are strongly affected by the pH of the solution. Bacterial cells have macromolecules that are polyelectrolytes because they carry charged groups such as carboxyl, phosphate and amino groups (Mesquita, et al., 2003). The pH of the solution strongly affects the ionization of these surface groups which is how cells gain charge. This implies that depending on the pH, the net charge in the cell wall can be positive, negative or zero (Kim, et al., 2015). It is thus favourable to work at a pH that reduces the surface charge to as close to zero as possible to minimize repulsion forces between bacterial cells and mineral particles. For example, Patra & Natarajan (2004a) showed that *P. polymyxa* cells had a high affinity for pyrite and chalcopyrite from pH 2-6 with a slight decrease in adsorption in the alkaline pH range. Using zeta potentials, the authors showed that as the pH increased, a net surface negative charge resulted for both bacterial cells and minerals. This led to electrostatic repulsion forces which were responsible for the decrease in adsorption density.

Furthermore, the pH affects the viability of the microbial cultures within the flotation cell. At higher pH the microbial cultures tend to coagulate, thus inhibiting their effectiveness during bioflotation (Vilinska & Hanumantha Rao, 2008).

3.5.1.2 Froth stability

When considering microbial replacements to chemical collectors and depressants, a frothing agent such as MIBC (widely reported to have low toxicity and therefore suitable for inclusion in bioflotation processes) or a biological frothing agent is still required to ensure froth stability. Froth stability is essential during flotation to prevent mineral fall back which happens when mineral that has been brought to the top of the float cell falls back down into the float cell before it can be removed because the bubbles burst. Microbial cultures have been found to have potential to decrease the surface tension of the bubble surface and hence froth stability within the flotation cell (Khoshdast & Sam, 2011).

Bioflotation has been conducted under acidic conditions in a number of studies in order to prevent the coagulation of the microbial cultures (Vilinska & Hanumantha Rao, 2008; Schippers, 2007). However, the stability of the MIBC frothing reagent used during bioflotation decreases significantly with decreasing pH and can therefore result in decreased recovery and grade or grade of concentrate (Gupta, et al., 2007).

3.5.1.3 Microbial health

Two aspects of microbial health may affect the performance of bioflotation reagents, namely the microbial vitality and viability.

The microbial vitality refers to the metabolic activity of the microorganism and is affected by the physicochemical environment and culture history. It can be quantified by calculating the rate of resource utilisation of a microbial substrate, the growth rate or the rate of product formation. For example, the vitality of chemolithotrophs may be determined in terms of the capacity of microorganisms to oxidise inorganic materials such as iron and sulphur. To date, limited research has been done on the effect of flotation reagents on the vitality of microbial cultures.

Microbial viability is a measure of the ability of the microbial cell to reproduce. Viability can also be negatively affected in the presence of flotation reagents. The rate of decrease in microbial viability can be affected by the concentration of the flotation reagent and time of exposure to the flotation reagent (Talaro & Talaro, 1999). As with microbial vitality, limited research is reported on the effect of flotation reagents on the viability of microbial cultures.

CHAPTER 4: Methodology for Testing of Bioflotation Reagent Potential

4.1 Approach for Testing Flotation Potential of Bioreagents

A systematic approach for testing the potential of the identified microorganisms and bioproducts as bioflotation reagents was developed based on the methods presented in the literature for similar studies. The methods are consistent across the bioflotation literature and are intrinsically linked to standard microbiology and flotation chemistry experimental approaches.

Following the culturing of the microorganism or recovery of the desired biologically produced substance (methodology detailed in Section 4.3), assessment of a microorganism's or bioproduct's bioflotation potential was assessed in two steps: (1) initial screening experiments followed by (2) flotation tests, provided the former was successful. These experiments were done using coal and other mineral samples that had been selected as being representative of typical ultrafine waste tailing feeds to the different stages of the two-stage flotation process. The selection and characterisation of these samples is presented in Section 4.2.

Initial bioflotation potential screening experiments were used to assess a microorganism's affinity to the individual components of South African coal waste tailings targeted in the two stages of the flotation process (namely coal, sulphide mineral and gangue components). This included attachment experiments, the background motivation and details for which are presented in Section 4.4. In the most promising cases surface property measurements were used to further interrogate the observed affinities, as explained in Section 4.4.2.

Flotation experiments, the methodology for which is given in Section 4.5, were performed using either real samples or in some cases pure or 'constructed' samples, comprising of the individual mineral components combined in representative ratios. The latter tests were performed if complete liberation of the mineral of interest was desired to ensure clear results.

4.2 Coal and Mineral Selection and Preparation

Initial bioflotation potential screening experiments assessed a microorganism's affinity to the individual components of South African coal waste tailings targeted in the two stages of the flotation process (namely coal, sulphide mineral and gangue components). Following this pre-selection process, only the most promising microorganisms were tested on representative and real samples.

Table 3 summarises the results of mineralogical characterization tests previously done on a number of ultrafine coal waste samples from different coal mining regions in South Africa. The analysis revealed that pyrite (FeS_2) was the only acid generating sulphide mineral present in the coal samples (Kazadi Mbamba, et al., 2012; Iroala, et al., 2014). The most abundant gangue minerals included kaolinite ($Al_2Si_2O_5(OH)_4$), quartz (SiO_2), gypsum ($CaSO_4 \cdot 2H_2O$) and calcite ($CaCO_3$). Quartz and kaolinite together represented, at the minimum, about 80% of the coal samples. Based on these data, the key 'pure' non-coal components whose microbial-mineral interactions should be tested were pyrite (sulphide mineral) and quartz and kaolinite (gangue). It was found not to be possible to test with kaolinite as an isolated mineral because its particle size distribution in pure form was too fine for flotation; therefore, only pyrite and quartz were considered. A 95.3 wt% pyrite mineral concentrate (courtesy of BHP Billiton) was used for this.

The representative samples of fine coal waste tailings used in the experiments originated from both the Witbank and Waterberg regions. The sample of Witbank coal fines used had an ash content determined as 48.6 ± 0.02 % and LECO analysis indicated that the sulphur content of the coal fines was

4.02 ± 0.26 %. The sample of the Waterberg coal had an ash content of 47.8 ± 0.08 % and sulphur content of 4.57 ± 0.31 %.

The optimal particle size for flotation is generally in the range of 10-100 µm (Rahman, et al., 2012). The raw samples were therefore milled and screened to ensure that their particle size distribution fell into this range. The minerals were additionally washed with an acidified water solution to remove any soluble secondary precipitates prior to use. The resulting particle size distributions for selected samples are given in Appendix A.1.

Table 3: Mineralogical characterization of samples of South African coal ultrafine waste (adapted from Kazadi Mbamba et al. (2011) and Iroala et al. (2014))

Mineral	Mineral composition	Concentration in coal (wt%)		
		Middleburg	Witbank	Waterberg
Quartz	SiO_2	29.4	41	41
Kaolinite	$Al_2Si_2O_5(OH)_4$	59.16	46	38
Epsomite	$MgSO_4 \cdot 7(H_2O)$	1.65	<2	<2
Gypsum	$CaSO_4 \cdot 2H_2O$	4.03	5	5
Jarosite	$KFe_3^{3+}(OH)_6(SO_4)_2$	0.76	<2	<2
Pyrite	FeS_2	1.13	5	<2
Siderite	$FeCO_3$	0.55	Not-detectable	<2
Calcite	$CaCO_3$	2.99	<2	4
Dolomite	$CaMg(CO_3)_2$	0.33	Not-detectable	8

4.3 Culture and Bioproduct Preparation

The microorganisms identified as potential bioflotation reagents were cultured by inoculating a volume of the pure or mixed strain in growth medium (appropriately chosen for each microorganism) and grown under conditions that allow optimum growth for each strain, based on standard operating procedure or literature.

4.3.1 Microbial growth conditions

4.3.1.1 Growth conditions for Mycobacterium phlei

M. phlei was cultured using a glycerol-soil liquid medium (see Appendix A.2.5) in Erlenmeyer flasks on a shaking platform. From the growth curves generated by conducting cell counts, the doubling time was calculated to be 3.5 hours with a maximum specific growth rate, μ_{max}, of 0.202 hr^{-1}. The maximum concentration achieved fell in the range of $2.8\text{-}4.6\times10^8$ cells/mℓ.

4.3.1.2 Growth conditions for chemolithotrophs

The feasibility of using the acidophilic chemolithotrophs *A. thiooxidans, A. ferrooxidans* and *L. ferriphilum* for pyrite flotation separation from gangue was assessed using a mixed mesophilic culture grown on acidified 0K basal salt medium (BSM) (pH 2.0) (see Appendix A.2.1) supplemented with 2 wt% pyrite as a ferrous substrate for the iron oxidising microorganisms. This was done in Erlenmeyer flasks and incubated at 30ºC on a shaking platform.

4.3.1.3 Growth conditions for Bacillus licheniformis

Bacillus licheniformis cultures were grown using Tryptic-Soy liquid medium (see Appendix A.2.3) in Erlenmeyer flasks and incubated at 30ºC on a shaking platform for 48 hours. From a growth curve constructed using absorbance readings, the maximum specific growth rate, μ_{max}, was calculated to be 0.2 h^{-1} and the doubling time was 3.4 h. These corroborate values commonly reported in literature. The

μ_{max} (and thereby doubling time) varies with the type of medium used to culture the microorganism (Verster, et al., 2013). Stationary phase cultures grown for 48 hours were used for all experiments.

4.3.1.4 Growth conditions for Bacillus subtilis

Bacillus subtilis cultures were grown aerobically using Tryptic-Soy liquid medium (see Appendix A.2.3) in Erlenmeyer flasks and incubated at 30ºC on a shaking platform for 48 hours. Stationary phase cultures grown for 48 h were used for all experiments.

4.3.1.5 Growth conditions for Rhodococcus opacus

Rhodococcus opacus cultures were grown aerobically using GYM Streptomyces medium (see Appendix A.2.2) in Erlenmeyer flasks and incubated at 30°C on a shaking platform. Stationary phase cultures grown for 48 h were used for all experiments.

4.3.1.6 Growth conditions for Rhodopseudomonas palustris

Rhodopseudomonas palustris cells were grown anaerobically using a nitrogen gas sparged medium, modified *Rhodospirillaceae* medium (see Appendix A.2.4). The culture was grown at pH 7.4 and 32°C under illumination with tungsten filament lamps. From a growth curve constructed using absorbance readings, the maximum specific growth rate, μ_{max}, was calculated to be 0.028 h^{-1} and the doubling time was 24.8 h^{-1}.

4.3.1.7 Growth conditions of Paenibacillus polymyxa

Paenibacillus polymyxa cultures were grown aerobically using modified Bromfield medium (see Appendix A.2.5) in a New Brunswick bioreactor. This was required due to challenges with poor growth kinetics in shake flask experiments and hence biomass quantity limitations. The culture was grown at 30ºC, 0.5 vvm and agitated at 300 rpm. A 2 M potassium hydroxide (KOH) solution was used to control the pH and keep it constant at pH 7. Stationary phase cultures grown for 12 h were used for all experiments.

4.3.2 Microalgal growth and lipids extraction

The microalgae grown for lipid production were cultured under limited nitrogen (150 mg/ℓ) using Bold's basal medium (see Appendix A.2.7). Previous studies (Griffiths, et al., 2012) have shown that lipid productivity is enhanced at limited nitrogen concentrations with a compromise, however, on biomass productivity. The algae were grown in batch cultures in airlift photobioreactors with a working volume of 3.2 ℓ. Carbon dioxide-enriched air (1% CO_2) was sparged at a rate of 2 L/min, with light being provided by 18 W fluorescent tubes (250 µmol photons m^{-2} s^{-1} at the reactor surface). Starter cultures were grown in 500 mℓ glass bottles, under normal air, for 10 to 15 days before inoculation into the airlift reactors. The reactors were operated for at least 25 days to maximise lipid production (Mandal & Mallick, 2009).

Lipid extraction was performed on the dewatered biomass using the Axelsson-Gentili single-step method for total lipids (Axelsson & Gentili, 2014) and the *in-situ* transesterification method for FAMEs (Griffiths, et al., 2010). A rotary evaporator was used to recover the lipids from the solvents used in the extraction process. The raw algal lipids and FAMEs extracted were stored in absolute ethanol for later use in the first-stage of the bioflotation of coal.

4.4 Characterisation of Bioflotation Potential

4.4.1 Attachment studies

Attachment studies were used to assess the microorganisms' relative affinity towards the coal, pyrite or any of the gangue minerals. The data obtained enabled the selection of microorganisms that show higher selectivity towards a specific mineral, for further testing. The relation of attachment studies to

flotation results has been demonstrated in a number of previous studies. For example, Patra and Natarajan (2003) showed that cells of *P. polymyxa* exhibited very high affinities towards sulphide minerals such as pyrite and chalcopyrite compared to gangue minerals such as quartz and calcite. These cells further enhanced the hydrophilicity of pyrite, resulting in its depression during flotation.

The selective attachment of microorganisms to either coal, pyrite or the gangue minerals was investigated using each culture individually. Attachment studies were performed separately for each mineral to evaluate the attachment behaviour of each microbial strain on a particular mineral with respect to time and initial cell concentration (Africa, et al., 2013a). All attachment studies were carried out in 250 mℓ Erlenmeyer flasks containing 100 mℓ of the medium along with select masses of the coal, sulphide or gangue minerals. Samples were removed at selected time points, and the unattached cells remaining in the solution were enumerated using direct microscopic cell counts. This was done until an approximate steady state or full attachment was observed. The cell concentration obtained was subtracted from the initial cell concentration to quantify microbial attachment. Control experiments with no mineral added were used to ensure that the cell number did not multiply significantly in the experiment time span and that the experiment conditions were sufficient to obtain a well-mixed microbial suspension (i.e. no natural settling of the microorganisms).

As discussed in Section 3.5.1.1, pH plays an important role in microbe-mineral interactions. Therefore, the effect of pH on attachment density was investigated. Attachment studies were performed at pH 2 (for acidophilic chemolithotrophs), pH 4 (circa isoelectric point for heterotrophs) and pH 7 (as per chemical two-stage flotation process) to evaluate the pH at which maximum attachment would occur. This range was chosen based on the pH ranges of process water typically found on mineral processing plants. To achieve pH control, NaOH was used for alkalinisation. For acidification, HCl was used for the heterotrophs and H_2SO_4 for the chemolithotrophs.

4.4.2 Surface chemical property measurements

Mineral beneficiation processes such as flotation and flocculation use the difference in surface chemical properties of minerals to effect efficient separation (Wills, 1997). In bioflotation, attachment of the microbe to the mineral is crucial to induce mineral surface modifications that enable the selective separation of sulphide minerals (Chandraprabha & Natarajan, 2009), as discussed in Section 3.1. Alternatively, the surface may be altered by attachment of a microbial product. Thus, to optimise the best identified bioflotation options, it is important to understand comprehensively the interactions and surface chemical changes that are a consequence of microbe-mineral or microbial product-mineral interaction before the utility of microorganisms and their by-products can be established in mineral processing. Physicochemical interactions identified as crucial to the overall attachment of bacterial cells to the mineral surface include electrostatic and hydrophobic interactions (Van der Wal, et al., 1997).

The surface charge of a mineral, quantified using zeta potential measurements, affects the likelihood of polar interactions with water molecules and is thus a key factor in flotation. The more charged a surface is (positive or negative), the less hydrophobic it becomes. This has been discussed in detail in Sections 2.3.2 and 3.1. The zeta (electrokinetic) potentials of the sulphide minerals and bacterial suspensions have been measured in order to investigate the changes that occur following bacterial interaction. This was done for the mineral prior to exposure to the microorganisms, and following microbial attachment and subsequent detachment. The final zeta potential that exists after interaction determines if the mineral floats or is depressed. The effect of pH on the surface charge was considered as part of the optimisation process, as a neutral bacterial surface charge, or opposing charges between the microbe and mineral surface, has been shown to best permit attachment.

Zeta potential measurements were made using a Malvern Zetasizer. For mineral samples, 0.2-1 wt% of sample were conditioned in 1 mM NaCl solution at the required pH value for 15 to 30 minutes prior to zeta potential measurements. For bacterial suspensions, bacterial cultures were resuspended in 1 mM NaCl solution at a cell concentration of 107 cell/mℓ and conditioned at the required pH value for 15

to 30 minutes before measurement. The parameters listed in Table 4 were used for all zeta potential experiments.

Table 4: Parameters used for the determination of the net surface electrical charge of pyrite, quartz and bacterial suspensions

Parameter	Pyrite	Quartz	Bacterial suspension
Refractive index	a = 1.730 b = 1.758 y = 1.838	nε = 1.551-1.554 nω = 1.543-1.545	1.386
Absorption	700-750 nm	467.8 nm	700 nm
Dispersant	NaCl	NaCl	NaCl
[Dispersant]	1 mM	1 mM	1 mM
pH [Dispersant]	3, 5, 7, 9	3, 5, 7, 9	3, 5, 7, 9
Refractive index dispersant	@700 nm = 1.5387	@467.8 nm = 1.56	@700 nm = 1.5387
Dielectric constant	ε = 10.9	ε = 4.2	ε = 18-19
Dielectric constant dispersant	ε = 3.0-15.0	ε = 3.0-15.0	ε = 3.0-15.0

4.5 Flotation Experiments

Following the characterisation of the bioflotation separation potential (detailed in Section 4.4), microorganisms and bioproducts which appeared suitable were tested in full flotation experiments.

The flotation experiments were carried out using a 3 ℓ Leeds bottom-driven and sub-aeration laboratory scale batch flotation cell, pictured in Figure 5. In this setup, the concentrate is scraped off the top of the float cell at specific time intervals whilst the tails exit via a pipe at the bottom of the float cell. A pulp density in the region of 6.67 % (0.2 kg per float) was used in most tests, as this is consistent with the original chemical system testing (Kazadi Mbamba, 2011). The only exception to this was for pure pyrite sample floats, for which a pulp density of 1.67 % (0.05 kg per float) was used due to mineral availability.

The desulphurisation flotation experimental methodology and procedure as presented by Kazadi Mbamba (2011) were applied. The mineral sample was pre-mixed with approximately 500 mℓ of water at that had been adjusted to the desired pH (2, 4 or 7) and decanted into the float cell. Thereafter, the float cell was filled with water to the 1 cm mark. Once the float cell was filled, the impeller was turned on and set to a speed of 1200 rpm. After a 4 minute mixing time, a 50 mℓ sample was extracted from the float cell to obtain a measure of the float feed. After a further 1 minute, the collector was added. A contacting time was then allowed, the standard time being 5 minutes. This was varied in the biotic tests to account for attachment kinetics. Thereafter, the frother (MIBC) was added if being used. After an additional 1 minute conditioning time, the airflow valve was opened and the rotameter set to 6 ℓ/min. Over the next 20 minutes, four collections of concentrate were made by scraping froth off the top of the float cell into a container every 15 seconds. The first concentrate was collected over the initial 2 minutes, the second concentrate over the following 4 minutes, the third concentrate over the next 6 minutes and the final concentrate over the last 8 minutes. The residual tails were collected at the end of the 20 minutes and the float cell thoroughly rinsed with tap water to ensure total collection.

The slurry in each container was filtered using a Buchner funnel to separate out the solids; the tailings fraction was filtered using a filter press. The filtered wet solids were then dried in a 37°C room. The dried samples were weighed to determine the mass of the solids recovered. Finally, the total sulphur of the samples was measured using LECO analysis as an indicator for pyrite deportation, and the combustible coal recovery determined using an ashing test. For the ashing test, a pre-weighed 1 g sample was burnt in a furnace for an hour at 500°C and then again for an hour at 815°C. The remaining solid after burning is weighed and classified as ash.

Abiotic experiments were performed with and without the chemical reagents to form a comparative basis for the bioflotation results. In the bioflotation tests, the microorganisms or bioproduct were added in place of the chemical reagents.

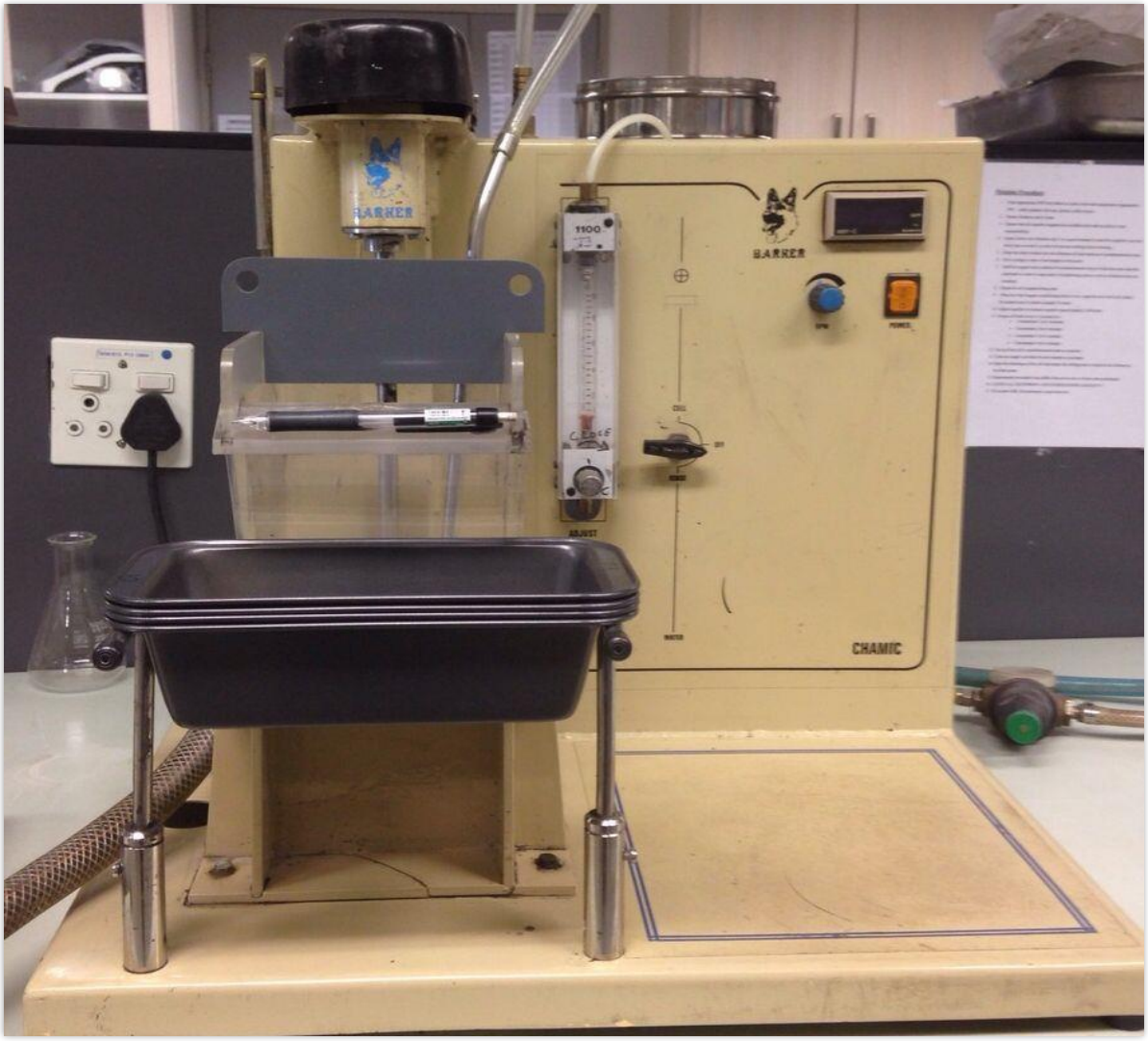

Figure 5: Leeds 3 litre bottom-driven and sub-aeration laboratory scale batch flotation cell

CHAPTER 5: Bioflotation Reagent Performance for Coal Flotation

5.1 Chemical Flotation Controls

Experiments were done using both real coal and constructed samples, the latter to ensure that there was mineral liberation to confirm the interactions of the reagents with the different minerals. The 'real' ultrafine coal waste tailings samples were from the Witbank and Waterberg regions. The compositions of the samples were 48.7% ash and 4.02% sulphur for the Witbank coal and 47.8% ash and 4.57% sulphur for the Waterberg coal. The constructed coal samples contained by mass 64% of the Witbank coal, 34% additional quartz (ash) and 2% additional pyrite, such that the final ash and sulphur content was 67.1% and 3.68% respectively.

Chemical flotation tests were performed as positive controls based on the work of Kazadi Mbamba (2011) and Iroala (2014) at neutral pH. The reagents used for this were a MIBC frother and a dodecane or oleic acid collector, as per the previous studies. Each float was performed in duplicate. A summary of the results is shown in Table 5. The results of these tests were the positive and negative controls to which the bioflotation results were compared.

- Full chemical flotation (positive control): 2.79 kg dodecane / ton and 0.28 kg MIBC / ton solid
- Full chemical flotation (positive control): 2.79 kg oleic acid / ton and 0.28 kg MIBC / ton solid
- Frother-only flotation (negative control): 0.28 kg MIBC / ton solid

Table 5: Yield and recoveries achieved in the chemical flotation separation of coal from pyrite and gangue

	Full chemical flotation	**Frother-only flotation**
Constructed sample (dodecane)		
Overall yield to concentrate (%)	28.5 ± 4.6	6.3 ± 0.1
Combustible coal recovery to concentrate (%)	58.7 ± 1.6	12.0 ± 2.3
Ash recovery to concentrate (%)	13.7 ± 2.8	4.0 ± 0.6
Sulphur recovery to concentrate (%)	15.9 ± 0.3	4.2 ± 0.1
Witbank coal (dodecane)		
Overall yield to concentrate (%)	37.8 ± 0.8	8.1 ± 3.6 *
Combustible coal recovery to concentrate (%)	52.3 ± 0.2	-
Ash recovery to concentrate (%)	22.6 ± 0.6	-
Sulphur recovery to concentrate (%)	20.4 ± 0.1	-
Waterberg coal (oleic acid)		
Overall yield to concentrate (%)	34.7 ± 0.8	1.8 ± 0.2
Combustible coal recovery to concentrate (%)	50.9 ± 0.2	-
Ash recovery to concentrate (%)	17.0 ± 0.6	-
Sulphur recovery to concentrate (%)	25.2 ± 0.1	-

* calculated based on constructed sample result, scaled to only account for Witbank coal fraction behaviour

5.2 *Mycobacterium phlei*

5.2.1 Attachment studies for *Mycobacterium phlei*

Figure 6 shows the percentage attachment of *M. phlei* cells to 1 g samples of coal, pyrite and quartz in a 100 mℓ suspension of 2×10^7 cells/mℓ as a function of time. The rate of attachment of *M. phlei* to all three solid types was rapid with a negligible rate of change of attachment found after 5 minutes of contacting time, confirmed statistically using an ANOVA analysis. After the 5 minute contact, there was a high degree of attachment of *M. phlei* to coal with an average degree of attachment of 90.2 ± 0.9%. By contrast, the degree of attachment occurring with pyrite and quartz after 5 minutes was calculated to be 17.2 ± 15.7% and 22.1 ± 9.0% respectively. The attachment achieved with all solids was found to be statistically significant using a T-test when compared to the negative test to which no mineral was added.

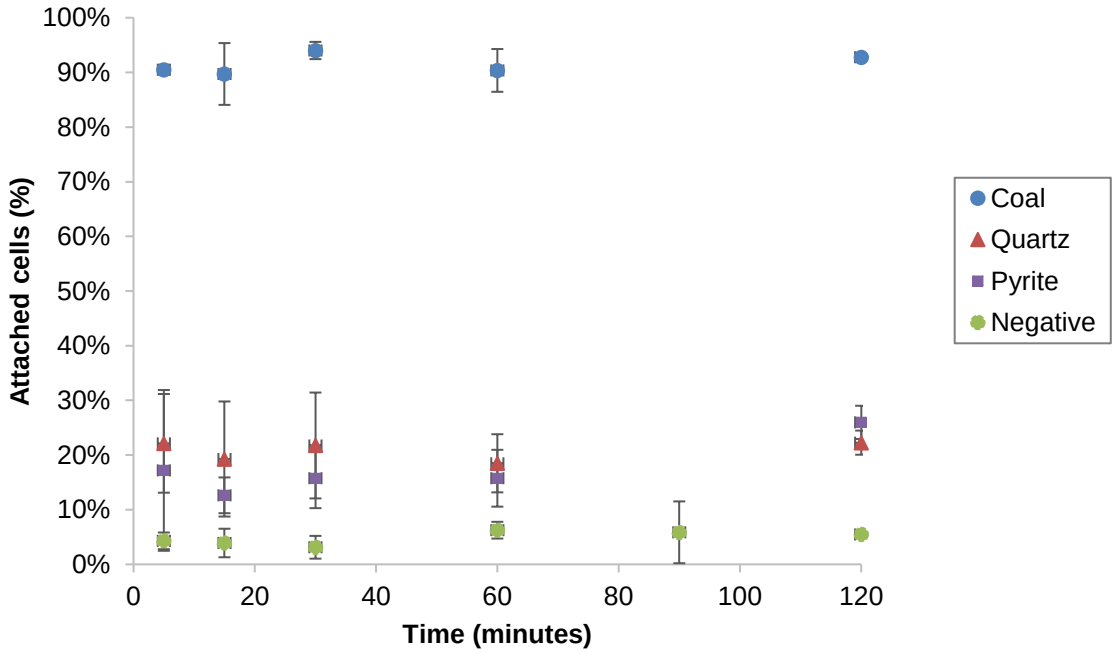

Figure 6: Attachment of *M. phlei* to coal, quartz and pyrite in a 1 g solid per 100 mℓ suspension containing a cell concentration of 2×10^7 cells/mℓ

Microscopic images were taken to confirm the interaction of *M. phlei* with the different solids. While no significant observation could be made for the pyrite and quartz samples, attachment of *M. phlei* was observed for the coal sample, as seen in Figure 7.

Thus the results of the attachment study clearly indicate a selective preference for attachment of *M. phlei* to coal compared with pyrite and quartz. This is supported by Raichur et al. (1996) who confirmed that coal and *M. phlei* both exhibit high levels of hydrophobicity and hence appreciable attachment. This result infers promising potential for the bioflotation separation using *M. phlei* since it is expected to increase the hydrophobicity of the solids to which it attaches.

The attachment results indicate that a 5 minute contacting time is adequate for sufficient attachment to occur, confirming that 5 minutes conditioning should be adequate prior to beginning flotation. This is favourable since it means that it will be easier to scale up the process to a continuous system as this is in line with chemical reagent conditioning times.

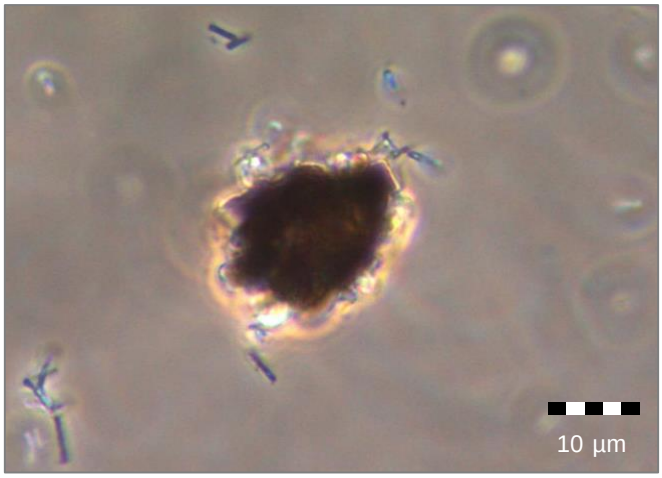

Figure 7: Microscope image of *M. phlei* attaching to coal

An adsorption isotherm was developed to determine the maximum achievable adsorption of *M. phlei* cells to the surface of coal at equilibrium. This is useful to determine the maximum concentration of cells required for the flotation tests. Figure 8 shows the number of cells adsorbed to the surface of the coal particles as a function of the equilibrium concentration of the suspension (cells/g solid), indicating the point at which the coal surface becomes the attachment limiting factor.

It is seen in Figure 8 that the adsorption of cells onto the surface of the coal particles increases with increasing availability of cells in suspension until a plateau is reached, at an equilibrium concentration of approximately 1.34 $\times 10^{10}$ cells/g attached for a supply of 2×10^{10} cells/g. This is in line with adsorption isotherms of Patra and Natarajan (2004a) who showed equilibrium concentration of the order of 10^9 cells/g for both *Bacillus polymyxa* and *Paenibacillus polymyxa* (Patra & Natarajan, 2004a; 2004b).

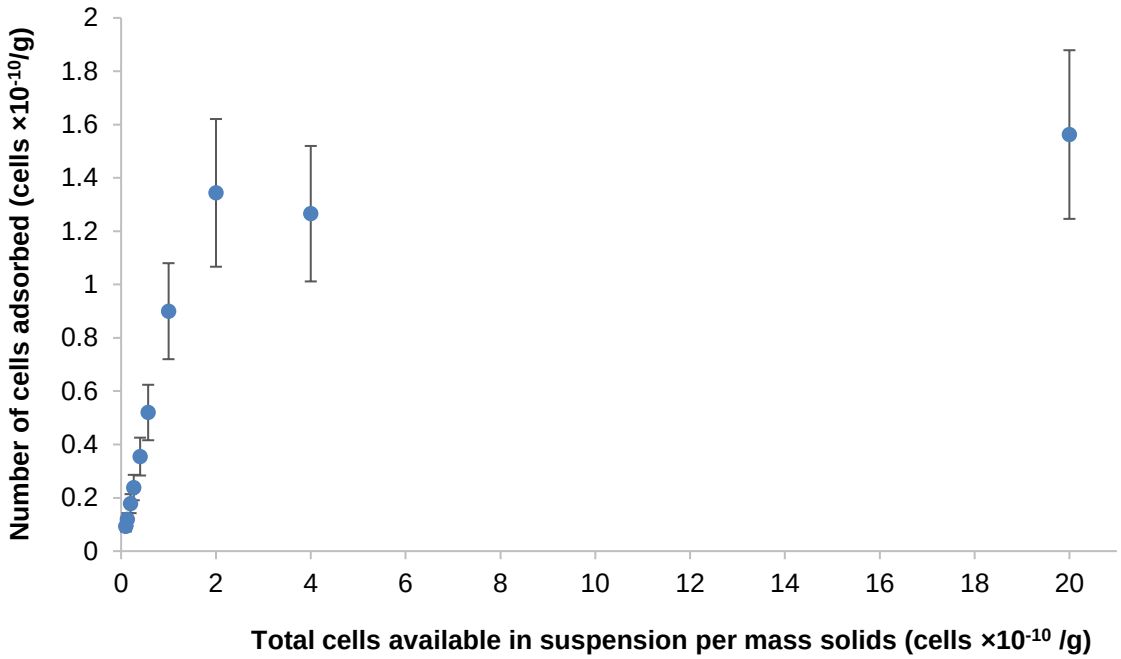

Figure 8: *M. phlei* adhesion to coal adsorption isotherm

5.2.2 Bioflotation tests for *Mycobacterium phlei*

The primary objective of the first-stage of the two-stage flotation separation process is to recover a coal product with low ash and sulphur content, thereby producing a saleable coal product with a high enough calorific value and complying with stricter environmental laws (Mittal, et al., 2012). For successful flotation separation to occur, the concentrate needs to show an improved composition of combustible coal (reduced ash content) as well as have a high recovery of combustible coal.

The attachment studies have shown that *M. phlei* exhibits appreciable and selective attachment to coal over pyrite and quartz. Flotation tests aim to develop these results further to determine whether the attachment of *M. phlei* to coal results in the coal having an increased floatability (hydrophobicity) compared to pyrite and quartz, and as a result shows preferable attachment to air bubbles and separate out through the froth.

5.2.2.1 Constructed coal waste

Figure 9 shows the cumulative concentrate yield obtained for each flotation test using the constructed coal waste. The highest yield was achieved in the full chemical float (using dodecane and MIBC as reagents), with a final accumulated yield after a 5 minute float time of 28.5 ± 4.6 %. The next highest overall yield was achieved using *M. phlei* (high concentration) with MIBC as reagents at 13.3 ± 1.3% followed by *M. phlei* (high concentration) with no frother, achieving a cumulative yield of 9.5 ± 0.6%. The tests using MIBC only and *M. phlei* (low concentration) with MIBC produced the lowest yields with cumulative yields of 6.3 ± 0.1% and 6.7 ± 0.7%. A t-test comparing the cumulative yields of the tests using MIBC only and low concentration *M. phlei* with MIBC showed the difference between the two test types to be statistically insignificant.

Observations made while conducting the flotation tests saw that the tests using dodecane and MIBC produced a consistent and stable froth, the tests containing high concentration *M. phlei* with MIBC produced a stable but low production of froth and that the remaining tests resulted in unstable and low yielding froths. This is reflected by the relative magnitudes of cumulative yields achieved.

The composition (combustibles and non-combustibles) of the feed sample and the concentrates recovered from each test are given in Figure 10. All tests achieved yields that have an increased composition of combustibles compared to that with the feed sample. The test in which the greatest combustible composition was achieved was the full chemical float (positive control) with a combustible composition of 67.9 ± 1.6%. This was followed by the negative control using MIBC only, in which a combustible composition of 59.5 ± 0.1% was achieved. The remaining tests using *M. phlei* at both a high and low concentration and with and without MIBC produced similar results to one another, all achieving combustible concentrations of around 49% compared with 33% in the feed sample.

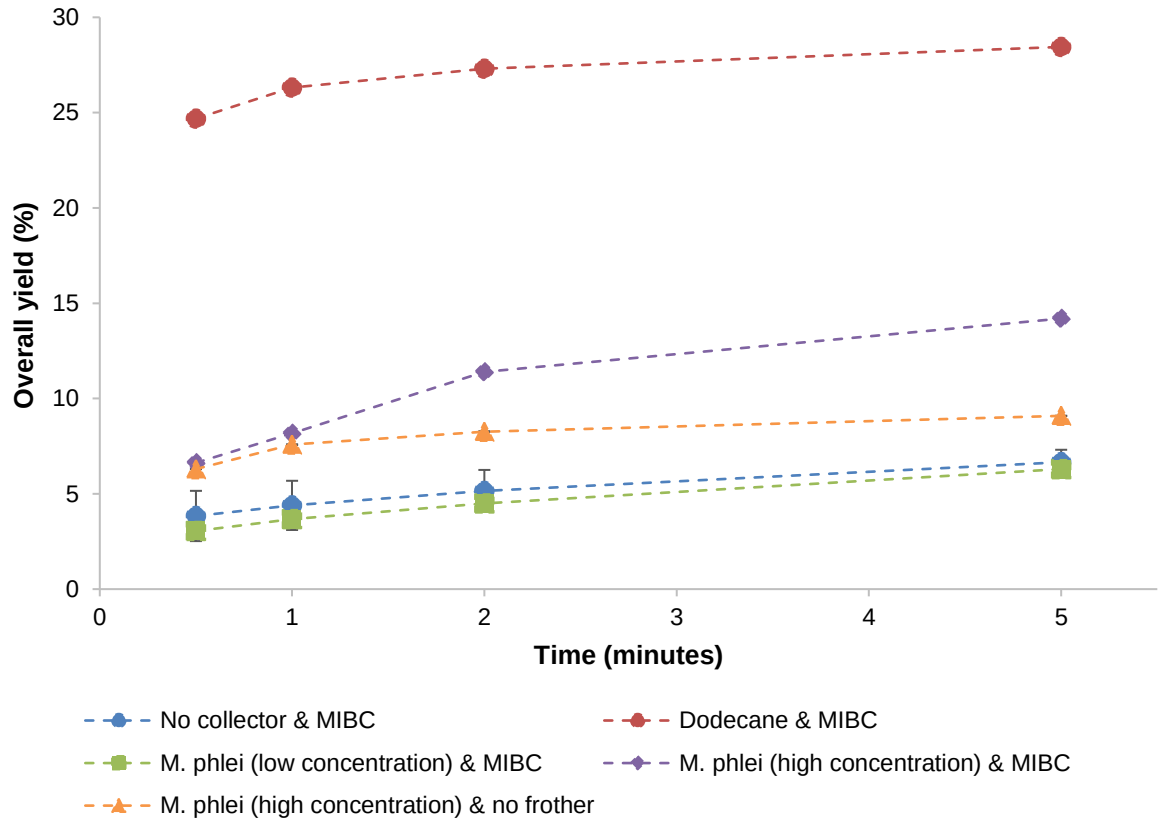

Figure 9: Overall yield achieved in the concentrate as a function of time for the constructed coal sample

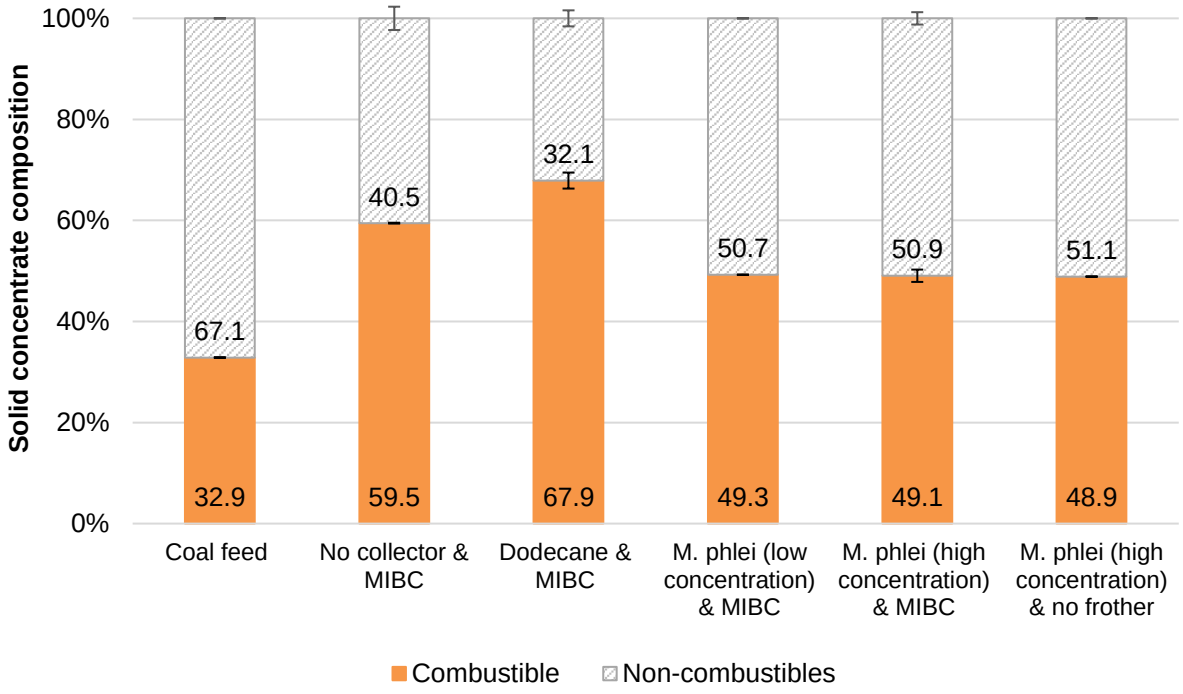

Figure 10: Composition of final cumulative solid concentrates after flotation of the constructed coal waste tailings sample

Table 6 summarises the percentage recovery of combustible coal, ash and sulphur to the concentrate in the various constructed coal float tests. Because the objective of the 1st flotation stage is to upgrade and desulphurise the coal, one would ideally like a high combustible coal recovery and as low as possible ash and sulphur recoveries. As seen in Table 6, for each test the recovery of sulphur and ash is significantly less than that of combustible coal. For example, the positive control using dodecane with MIBC recovers 58.7 ± 1.6% of the combustible coal being fed into the flotation cell whereas only 13.7 ± 2.8% and 15.9 ± 0.3% of the ash and sulphur components respectively are recovered in the overhead concentrate. There is therefore a preferential recovery of combustible coal in the overhead concentrate compared with the ash and sulphur components in the flotation with chemical collector.

Table 6: Recovery of combustible coal, ash and sulphur to the concentrate for each flotation test using a constructed coal sample feed

Test	**Recovery to concentrate (%)**		
	Combustible coal	**Ash**	**Sulphur**
No collector and MIBC	12.0 ± 2.3	4.03 ± 0.62	4.17 ± 0.08
Dodecane & MIBC	58.7 ± 1.6	13.7 ± 2.8	15.9 ± 0.3
M. phlei (low concentration) & MIBC	9.44 ± 0.01	4.76 ± 0.06	3.30 ± 0.15
M. phlei (high concentration) & MIBC	19.8 ± 1.2	10.1 ± 1.2	8.97 ± 0.08
M. phlei (high concentration) & no frother	14.1 ± 0.1	7.23 ± 0.44	6.20 ± 0.07

The tests using a high concentration *M. phlei* with and without MIBC produced low but appreciable overall yields compared to the negative test. Additionally, both achieved yields with increased combustible coal compositions indicating selective separation has occurred. This result indicates that *M. phlei* at a concentration of 6×10^9 cells/g does improve the floatability of solids in the flotation cell and that these separations show selectivity towards combustible coal. The low concentration *M. phlei* with MIBC test produced a low yield similar to the negative MIBC test therefore it can be classified as ineffective for separation. The *M. phlei* adsorption isotherm (Figure 8) shows that the maximum adsorption that can occur of the surface of the coal is approximately $15.6 \pm 3.16\times10^9$ cells/g. The flotation tests conducted using 6×10^9 cells/g (high concentration) were therefore not optimised in terms of maximum bacterial concentration and so there is potential for improved yield by increasing the bacterial concentration in the cell. Furthermore, no optimisation in terms of pH, salinity or other physicochemical effects have yet been done.

The result attained using high concentration *M. phlei* with no MIBC frother, which achieved appreciable overall yield and selectivity to combustible coal, indicates that *M. phlei* has frothing potential. The yield, however, was lower than that of the test with high concentration *M. phlei* with MIBC. Therefore, given the above results, *M. phlei* cannot conclusively replace MIBC as a frother and further analysis is required.

5.2.2.2 Witbank coal analysis

Constructed coal samples were used in order to ensure liberation of coal and sulphide to enable a proof of separation concept. While these tests provided insight into the flotation separation potential between liberated components, it is not a realistic account of the separation potential for industrial fine waste coal, which is not always completely liberated. Thus, tests were also performed using a feed of the Witbank coal, without added quartz and pyrite.

The cumulative concentrate yield obtained for each Witbank coal flotation test is shown in Figure 11. In this instance, all three tests achieve similar overall yields after five minutes of flotation. The tests done with dodecane and MIBC, high concentration *M. phlei* with MIBC and high concentration *M. phlei* with no frother achieved cumulative yields of 37.8 ± 0.8%, 37.2 ± 1.1% and 39.4 ± 2.0% respectively. The differences in yield between each test were calculated to be statistically insignificant using an ANOVA

analysis. However, the full chemical control had a quick initial concentrate recovery in the first minute whereas both tests using *M. phlei* (with and without MIBC) had more gradual initial recoveries.

Observation of the physical froths produced revealed that the froth produced in the chemical positive control had a quick and thick initial froth for the first minute, followed by a less thick and more slowly formed froth for the remaining four minutes. The froths developed in the *M. phlei* test (with and without MIBC) were consistently thick, with a fast-producing froth through the full 5 minute period. These observations support the respective cumulative yields seen in Figure 11.

Figure 12 gives the composition (combustibles and non-combustibles) of the feed sample and the concentrates recovered from each test. All tests produced concentrates with increased combustible coal compositions. The full chemical float achieved the best final composition of combustible coal with an improvement from the feed composition of 51.4 ± 1.2% to 71.0 ± 0.2%. Tests using high concentration *M. phlei* with and without MIBC respectively achieved compositions of 57.3 ± 0.1 % and 57.2 ± 0.4 %.

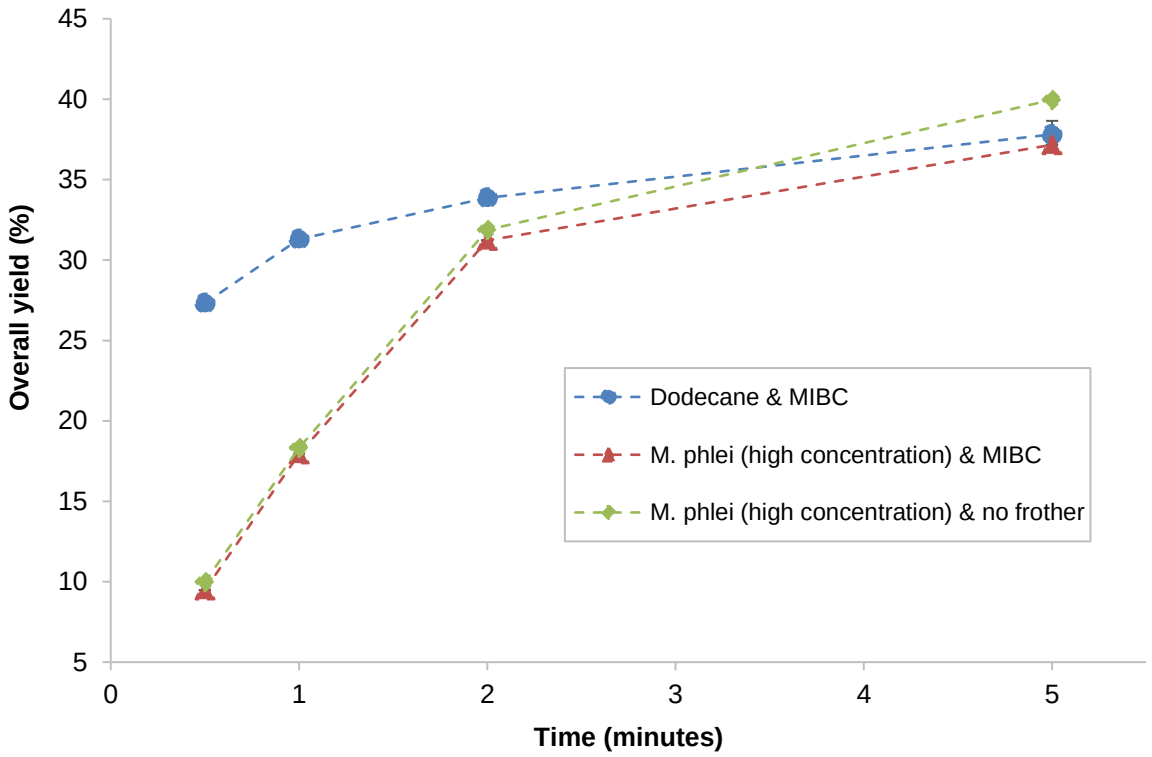

Figure 11: Overall yield achieved in the concentrate as a function of time for Witbank coal waste tailings flotation using *M. phlei*

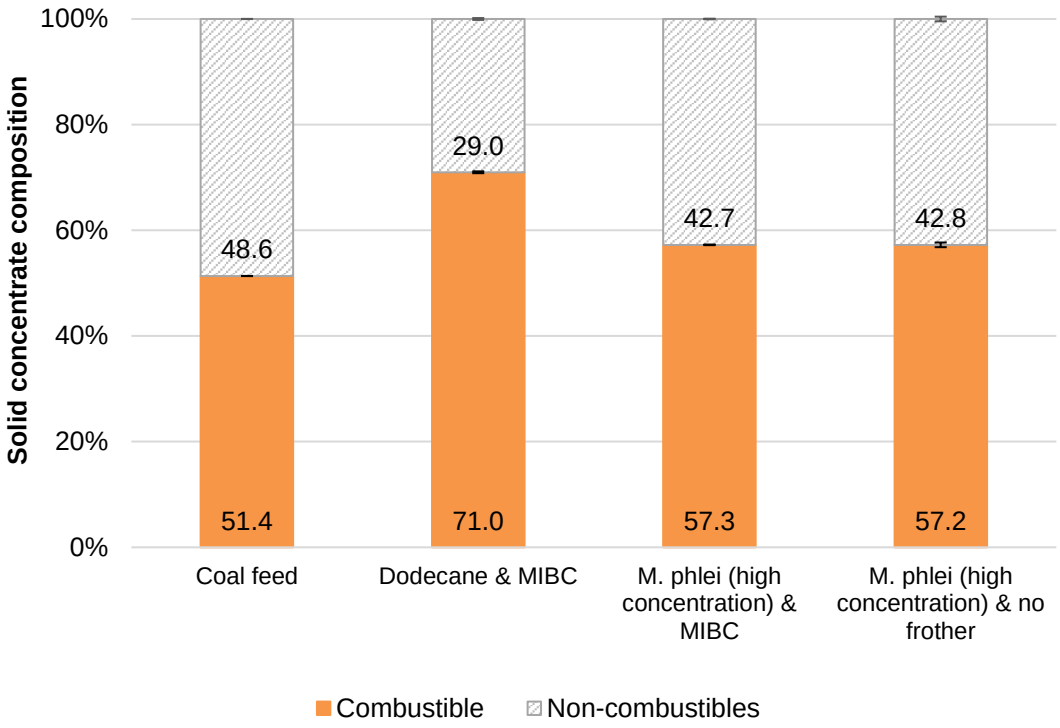

Figure 12: Composition of final cumulative solid concentrates after flotation of Witbank coal waste tailings using *M. phlei*

Table 7 summarises the percentage recovery of combustible coal, ash and sulphur from each test. The recovery of combustible coal is significantly greater than that of both ash and sulphur in all three tests. Sulphur achieves the lowest percentage recovery of all components. The difference in recoveries achieved by the tests with *M. phlei* with and without MIBC were confirmed to be statistically insignificant.

Table 7: Recoveries of combustible coal, ash and sulphur to the concentrate for each flotation test using *M. phlei* for a Witbank coal sample

Test	**Recovery to concentrate (%)**		
	Combustible coal	**Ash**	**Sulphur**
Chemical control: Dodecane & MIBC	52.27 ± 0.17	22.56 ± 0.64	20.37 ± 0.09
M. phlei (high concentration) & MIBC	41.44 ± 0.06	32.66 ± 0.97	24.09 ± 0.12
M. phlei (high concentration) & no frother	44.55 ± 044	35.13 ± 2.10	26.12 ± 0.01

Therefore, the results achieved in the test using high concentration *M. phlei* with and without MIBC showed appreciable yield and selectivity to combustible coal. Both achieved similar yields (within reasonable variance) as the full chemical float. This indicates that *M. phlei* was successful in increasing the hydrophobicity and hence the floatability of the solids in the flotation cell. The results shown in Table 7 and Figure 12 show that the recovery of solids is selective towards combustible coal. This confirms that *M. phlei* does have a selective affinity for combustible coal and therefore improves its floatability. Although the yields of the tests using *M. phlei* (with and without MIBC) were similar to those using dodecane and MIBC, the preferential affinity for combustible coal was still better in the chemical float. Despite this, the yield and recovery of the tests using *M. phlei* (with and without MIBC) were appreciable and prove the initial feasibility of *M. phlei* as a collecting reagent for the recovery of combustible coal. Further optimisation studies are recommended before final conclusions can be drawn.

The fact that the cumulative yields and froth produced by the tests using *M. phlei* with and without MIBC showed no statistically significant difference indicates that *M. phlei* was successful in producing a stable

and consistent froth without the use of MIBC frother. In order to understand why no frother is required with *M. phlei,* further research into the mechanisms used by *M. phlei* to stabilise froth should be carried out. This result indicates that it may be feasible for *M. phlei* to replace MIBC as well as dodecane or oleic acid in the first-stage of flotation for the desulphurisation and upgrading of coal waste.

5.2.2.3 Comparison between constructed coal sample and Witbank coal sample results

From the cumulative yield results, it is clear that a greater overall yield was achieved with 'as is' Witbank coal samples compared with the constructed samples in all tests conducted using different reagents. This is likely because of a larger amount of combustible coal being present in the Witbank coal waste samples compared with the constructed samples. As a result, there was more floatable (hydrophobic) mass present in the flotation cell and hence a greater recoverable mass. This further supports the conclusion that the reagents tested, specifically *M. phlei*, show preferential interaction with combustible coal and resultantly increases its hydrophobicity, incurring better flotation recovery.

For the tests using *M. phlei* as a collecting reagent (both with and without MIBC), it was observed that a significant amount more froth was produced with the 'as is' Witbank coal samples compared with the constructed samples. A higher recovery of combustible coal to the concentrate was correspondingly observed. It is speculated that this change in froth production is not the result of different levels of pyrite or quartz within the samples because the tests using dodecane and MIBC for the different coal samples produced similar amounts of froth for both samples. The significant increase in froth production with the Witbank coal and *M. phlei* is therefore likely the result of increased flocculation and flotation of combustible coal to the surface, where the attached *M. phlei* resultantly produces extra froth. However, further experimentation and research is required to better understand the mechanisms and phenomena involved.

5.2.3 Recommendation for *Mycobacterium phlei*

M. phlei was demonstrated to have significant preferential affinity for coal compared to pyrite and quartz. Bioflotation experiments performed using *M. phlei* showed an increase in overall recovery of coal to the concentrate compared to the negative control. Thus *M. phlei* shows good potential as an alternative biological collector for the selective recovery of combustible coal. It additionally shows potential as a frothing agent, therefore potentially negating the need for any chemical reagents in the 1st flotation stage.

5.3 Algal Lipids

5.3.1 Algal lipids characterisation

The lipid profile obtained using gas chromatography of the extracted FAMEs (Figure 13) showed that the majority of the lipids produced by *Scenedesmus* are poly-unsaturated fatty acids (PUFAs) with 18 carbon atoms (C18) in the chain. This corresponds to the chain length of oleic acid. A significant amount of C16 was also present in the mixture of extracted lipids. The profile closely matched that of tall oil (a by-product of the pulp and paper and industry) which was reported to float a variety of minerals including phosphates and hematite (Kou, et al., 2010), thus supporting the hypothesis that algal lipids and their derivatives are potential biocollectors (see Section 3.2.2).

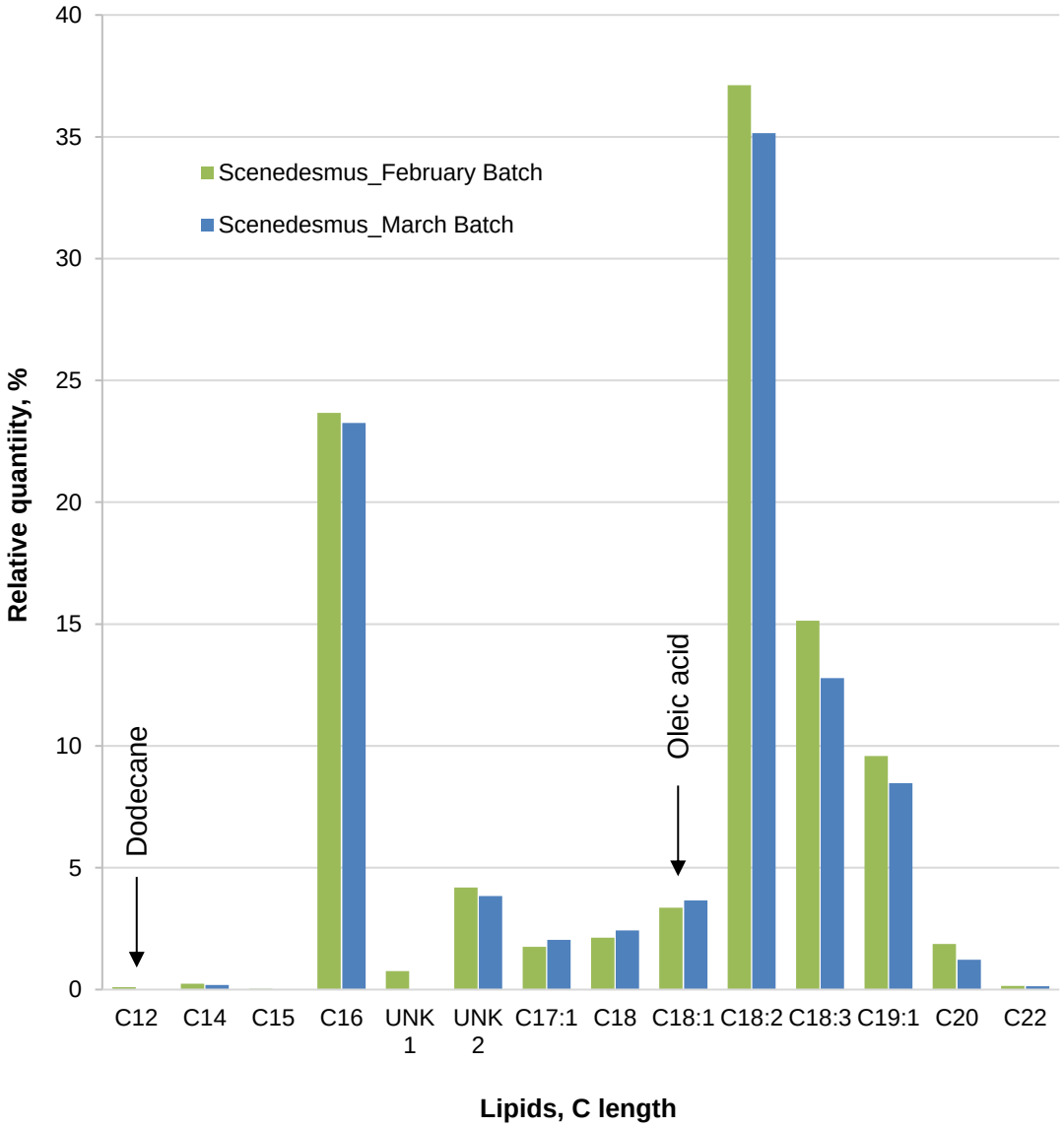

Figure 13: Lipid profile for *Scenedesmus* batches grown in February and March of 2017

5.3.2 Bioflotation tests for algal lipids

The potential of algal lipids and their derivatives as bioproducts for consideration as an alternative bioreagent was assessed directly using bioflotation tests. Figure 14 presents the cumulative concentrate yields achieved in these tests, performed on a Waterberg coal sample. Oleic acid was used as a positive control and the dosage of raw algal lipids (RAL) and FAMEs derived from the RAL was matched to that of the control (2.79 kg/ton of coal waste). The oleic acid positive control achieved the highest cumulative yield at 34.7 ± 2.8%, though this was followed closely by the FAMEs experiment which had an overall yield of coal of 30.3 ± 2.5%. RAL had the lowest yield, at 27.9 ± 0.8%. Though statistically similar results were obtained in all systems after 5 minutes' flotation, in terms of flotation kinetics, the bioflotation reagent systems had slower initial rates of concentrate recovery relative to the chemical system.

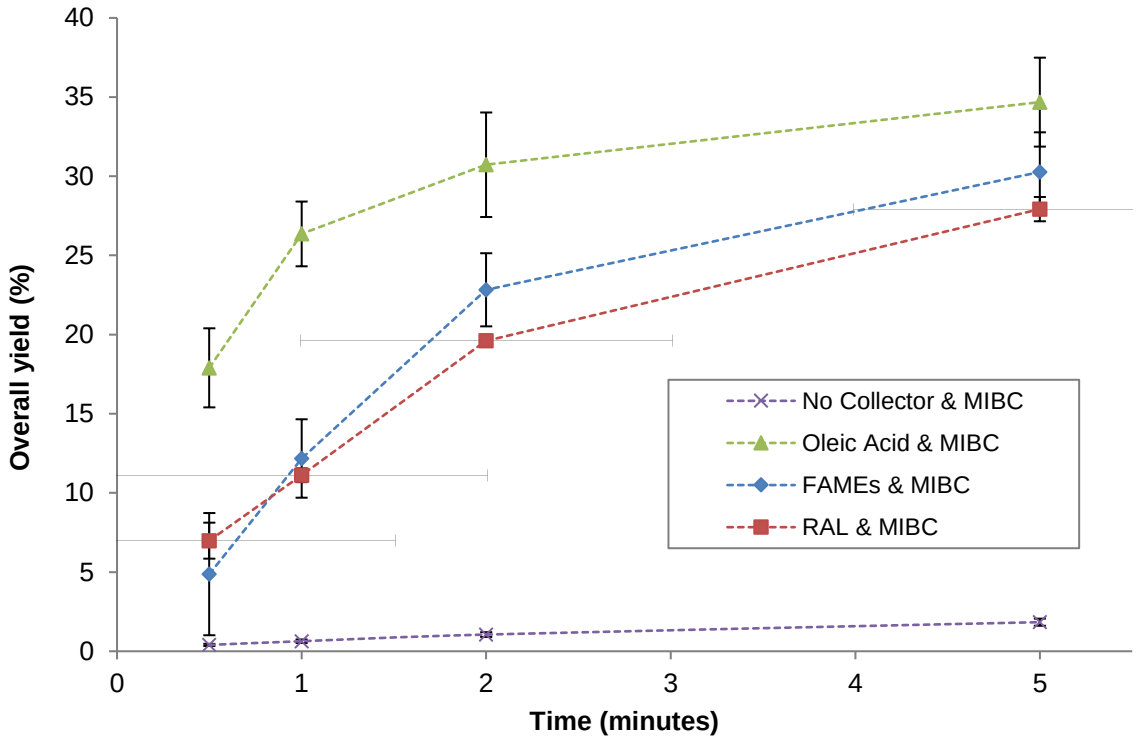

Figure 14: Overall yield achieved in the concentrate as a function of time for Waterberg coal waste tailings flotation using RAL and algal derived FAMEs at a concentration of 2.79 kg/ton of coal waste

The combustibles and non-combustibles composition of the feed sample and the concentrates recovered in each test are shown in Figure 15. All reagents were successful in being able to increase the combustible coal composition of the concentrate, thereby producing a cleaner coal product. The system with FAMEs as collector achieved the best combustible recovery with an increase from 52.1 ± 0.2% to 79.2 ± 0.6%. The oleic acid and the RAL tests achieved concentrate combustible coal compositions of 76.6 ± 2.0% and 73.3 ± 0.5%, respectively.

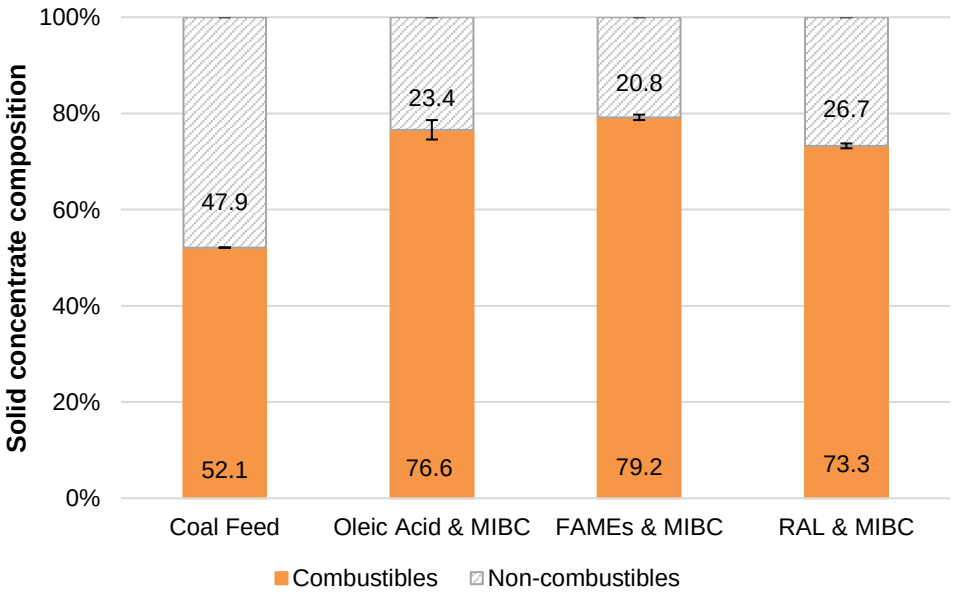

Figure 15: Composition of final cumulative solid concentrates after flotation of Waterberg coal waste tailings using RAL and algal derived FAMEs at a concentration of 2.79 kg/ton of coal waste

The percentage recovery of combustible coal, ash and sulphur are summarised in Table 8. Coal recovery in all three tests was significantly greater than both ash and sulphur. In terms of ash rejection, the test with FAMEs outperformed the chemical flotation and the RAL result, with an ash rejection of 86.4 ± 1.8% compared to 84.6 ± 2.0% for the RAL test and 83 ± 0.6% for the oleic acid test. With regards to sulphur, the FAMEs float had the lowest sulphur recovery, at 19.7 ± 0.1%, compared to the oleic acid and RAL floats which had 25.2 ± 0.1% and 28.1 ± 0.1%, respectively. Hence, the FAMEs were best able to desulphurise the coal waste tailings.

Table 8: Recoveries of combustible coal, ash and sulphur to the concentrate for each flotation using RAL and algal derived FAMEs test for a Waterberg coal sample

Test	Recovery to concentrate (%)		
	Combustible coal	Ash	Sulphur
Chemical control: Oleic acid & MIBC	50.88 ± 0.17	17.00 ± 0.64	25.17 ± 0.06
FAMEs & MIBC	44.73 ± 0.32	13.57 ± 1.84	19.73 ± 0.07
RAL & MIBC	39.61 ± 0.27	15.44 ± 1.96	28.13 ± 0.12

The results presented prove the technical feasibility of algal lipids as bioflotation reagents, with comparable concentrate yields achieved relative to the chemical system. Table 8 and Figure 14 show that the recovery of solids is selective towards combustible coal. The low ash recovery (high ash rejection) achieved by FAMEs indicate that they have a higher affinity for coal compared to RAL and oleic acid. A similar observation has been made by other researchers who used FAMEs from vegetable oil to recover coal (Dube, 2012; Vasumathi, et al., 2013; Yi, et al., 2015). This higher affinity for combustibles is due to the various functional groups provided by a mixture of different carbon chain length fatty acids. The added advantage of additional functional groups that are present in FAMEs, but not in RAL, is also noticed in the higher recovery of combustibles to the concentrate in the FAMEs test.

An additional valuable observation made in preliminary tests that excluded MIBC, was that the system with RAL alone produced a stable froth. As seen in Figure 16, the froth bubbles produced by the RAL were generally evenly distributed and relatively small compared to those produced in the system with FAMEs (Figure 17). This observation supports the hypothesis that algal lipids could work both as collector and frother in coal flotation.

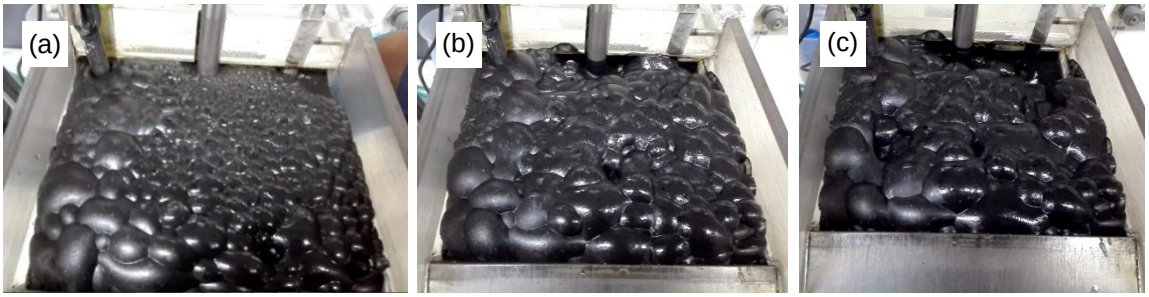

Figure 16: Froth produced in a float using only RAL (a) 2 seconds, (b) 7 seconds and (c) 15 seconds after air on

Figure 17: Froth produced in float using only FAME (a) 2 seconds, (b) 7 seconds and (c) 15 seconds after air on

5.3.3 Recommendations regarding algal lipids

The tests carried out with algal lipids successfully demonstrated their ability to recover coal from fine waste tailings containing gangue and pyrite preferentially to a degree comparable to the chemical reagents. From a technical standpoint, FAMEs are clearly a better option between the two bioflotation reagents. They produced a good quality concentrate at higher recoveries compared to those obtained using RAL. The difference between RAL and FAMEs is that the latter is a derivative of the former, a product of transesterification. Therefore, the final assessment of which bioflotation reagent is best cannot be made solely on technical data. An economic analysis is required in order to develop an informed conclusion as to which reagent is a better replacement of chemical reagents.

CHAPTER 6: Bioflotation Reagent Performance for Sulphide Separation by Flotation

6.1 Chemical Flotation Controls

6.1.1 Pyrite samples

Chemical flotation experiments of pyrite-only samples were used as a base case to which the collection of pyrite to the concentrate using bioflotation could be compared. Two sets of experiments were carried out:

- Full chemical flotation (positive control): 2.33 kg PAX / ton and 0.75 kg MIBC / ton solid
- Froth-only flotation (negative control): 0.75 kg MIBC / ton solid

The MIBC concentration was set to higher than the 0.11 kg/ton used by Kazadi Mbamba et al. (2011; 2012) as the froth was observed to be unstable, likely due to the pure pyrite sample at low solids loading versus an actual tailings from the first-stage float. Dextrin was omitted as a flotation reagent because it is a coal depressant and is therefore not required. The full chemical float was done at pH 7 (as per the chemical flotation tests) while the froth only float was done at pH 7 and pH 4. Table 9 summarises the achieved cumulative mass recovery of pyrite in the concentrate.

Table 9: Cumulative mass recovery of pyrite in the concentrate using chemical flotation

	Full chemical flotation (pH 7)	**Froth-only flotation (pH 7)**	**Froth-only flotation (pH 4)**
Pyrite recovery (%)	95.3	0.1	0.2

In the full chemical flotation, the recovery of pyrite was immediately observed with 94.1% recovered in the first concentrate due to the high sulphide selectivity of PAX. Virtually no collection was observed in froth-only flotation tests at both pH 4 and pH 7.

6.1.2 Constructed pyrite-gangue samples

Chemical flotation experiments conducted using constructed pyrite-gangue samples (1.1 wt% sulphur) were used in select instances as a base case on which the efficiency of sulphide separation from quartz using bioflotation could be compared. As with the pure pyrite samples, two sets of tests were performed:

- Full chemical flotation (positive control): 2.33 kg PAX / ton and 0.18 kg MIBC / ton solid
- Froth-only flotation (negative control): 0.18 kg MIBC / ton solid

The MIBC concentration was set to ensure a stable froth. The froth-only flotation was done at both pH 7 and pH 2, the latter because a lower pH is required for the acidophilic chemolithotroph bioflotation tests. The lower pH was not tested for the full chemical flotation as the PAX collector releases a toxic gas under acidic conditions. Table 10 summarises the sulphur grade (weight percent of the final cumulative concentrate) and recovery achieved in the concentrate.

In the full chemical float, 96 ± 9.3% of the sulphur was recovered to the concentrate as expected due to the hydrophobic nature of pyrite (Diao, et al., 2014). The addition of PAX enhances the hydrophobicity of pyrite due to the high sulphide selectivity of PAX, thus facilitating an efficient separation of pyrite from the quartz-pyrite sample (Kazadi Mbamba, 2011). Removal of the PAX collector therefore resulted in a significant decrease in the sulphur recovery to 49 ± 1.2%. This decreased even further at the lower pH to 13 ± 1.2%. Thus poor pyrite flotation was achieved at low pH. According to Gupta et al. (2007), this may be as a result of a decrease in the froth stability under acidic conditions. A decrease in froth stability

led to the bursting of the bubbles reaching the top of the float cell, resulting in the minerals dropping back into the pulp (Wright, 1999). A similar trend was seen for the concentrate grade which decreased from 38 ± 3.7% for the full chemical float to less than 10% when PAX was omitted.

Table 10: Grade and recovery of sulphur in the concentrate for chemical flotation separation of pyrite and gangue

	Full chemical flotation (pH 7)	Frother-only flotation (pH 7)	Frother-only flotation (pH 2)
Sulphur recovery (%)	96 ± 9.3	49 ± 1.2	13 ± 1.2
Concentrate grade (%)	38 ± 3.7	8 ± 0.5	5 ± 0.5

6.2 Chemolithotrophs

6.2.1 Attachment studies for chemolithotrophs

The affinity of the mixed chemolithotrophic culture to pyrite was assessed using attachment studies at pH 2 and solids loading to give 1.1 wt% sulphur in suspension. The results are presented in Figure 18 as percentage cell attachment with respect to the initial inoculum population (1×10^7 cells/mℓ). The percentage attachment of the microorganisms onto the pyrite increased with increasing contact time, with the rate slowing as the contact time increased. After an hour, more than 80% of the microorganisms had attached. This confirmed that the mixed culture had a strong affinity for pyrite.

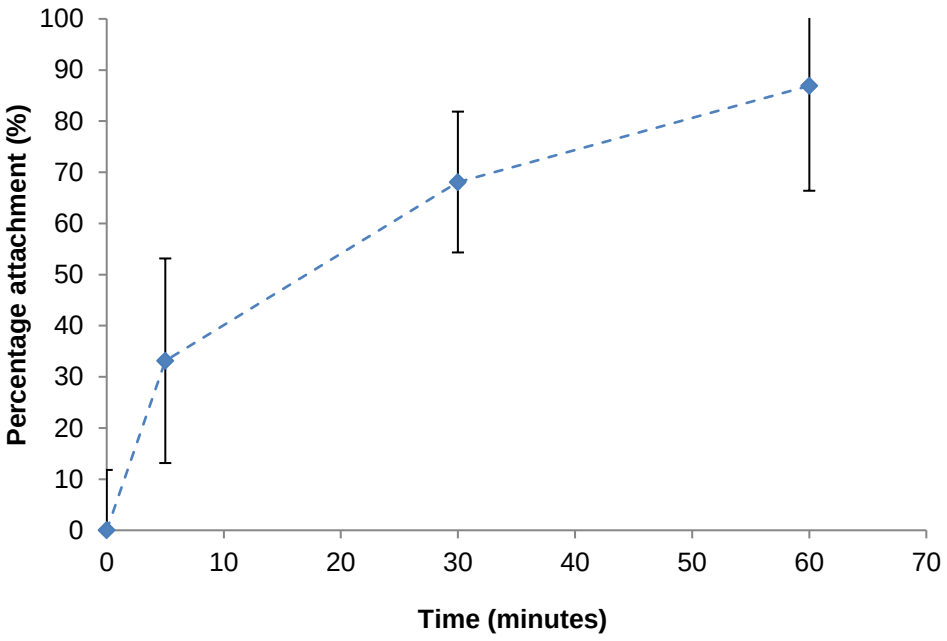

Figure 18: Attachment of a mixed mesophilic culture to pyrite over time. Error bars show a standard deviation variation across triplicate repeats.

6.2.2 Bioflotation tests for chemolithotrophs

Bioflotation tests using the mixed mesophilic culture were performed using a constructed pyrite-gangue sample, as reported in Section 6.1.1 and the same conditions as the frother-only flotation at pH 2, with an inoculum of the microorganisms added in place of the PAX. Two factors were varied: the cell concentration and the contacting time.

The cell concentration tests aimed to ensure that the efficacy of the bioflotation was not compromised due to the bacterial numbers being too low relative to the mineral. Two microbial concentrations were

trialled: 1 × 10^8 cells / g pyrite and 2 × 10^9 cells / g pyrite. A 30 minute contacting time was allowed to ensure that interaction of the cells and mineral was not constrained by time limitations of contacting of the cells with the pyrite, based on the attachment lag time seen in Figure 18.

The results on the grade and recovery of pyrite to the concentrate are presented in Figure 19. No statistical change in grade and recovery was observed at the higher cell concentration. This confirms that maximum attachment of the microbial cultures to pyrite had occurred at 1×10^8 cells / g pyrite.

The concentrate grades achieved in both cases were higher than that of the feed (1.1%) and an increase in both grade of the concentrate and recovery of pyrite was achieved relative to the abiotic controls. The increases in grade indicate that the microbial cultures improved on the natural floatability of the pyrite and that there was a preferential effect on the pyrite compared to the silica. This is supported by a previous study by Harneit et al. (2006) which found that the microbial attachment to silica was only 18% as compared to 90% for pyrite. Similarly, Africa et al. (2013a) showed that attachment of *A. ferrooxidans* to quartz and low grade ore rich in quartz was low (< 32%), compared with >90% attachment to pyrite and chalcopyrite. Similar levels of attachment were demonstrated with *A. ferrooxidans* and *L. ferriphilum*. However, according to Diao et al. (2013) *L. ferrooxidans* has similar adhesion forces for both silica and pyrite. The increase in recovery with bioflotation indicates that the microbial culture was acting as a collector or that froth stability was improved. The first possibility is in contradiction with previous studies which found that *L. ferrooxidans, A. ferrooxidans* and *A. thiooxidans* behave as depressants (Nagaoka, et al., 1999; Vilinska & Hanumantha Rao, 2008; Mehrabani, et al., 2011).

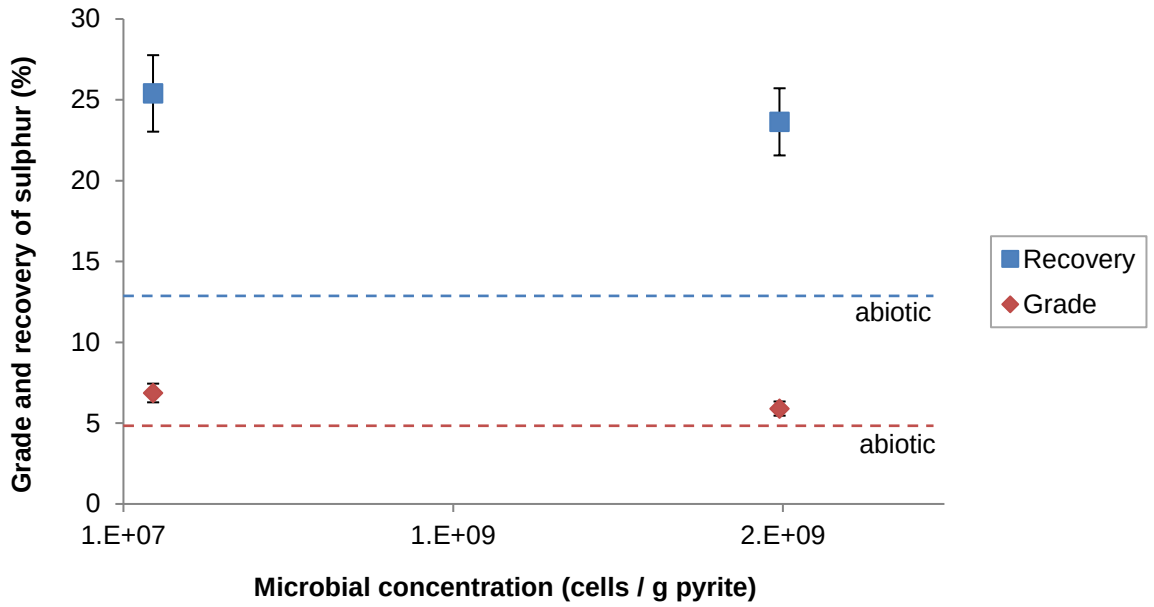

Figure 19: Grade and recovery of sulphur in the concentrate achieved using low and high microbial concentrations, following a 30 minute contacting time. Error bars show a standard deviation variation across two repeats. The dashed lines show the baseline for the negative control.

The effect of contacting time on the separation of pyrite from the constructed sample using bioflotation was investigated for times of 5, 30 and 60 minutes. This was done using an inoculum which gave 1×10^8 cells/g pyrite. The results of these tests are presented in Figure 20.

The recovery of pyrite to the concentrate in the bioflotation experiments decreased with increasing contact time: from 30.3 ± 2.5% after 5 minutes, to 25.4 ± 2.4% after 30 minutes, and finally to 21.1 ± 2.0% after an hour. The grade of the concentrate achieved in the bioflotation experiments also decreased slightly with increasing contacting time: from 7.46 ± 0.58% after 5 minutes, to 6.86 ± 0.58% after 30 minutes, and finally to 5.81 ± 0.48% after an hour. Thus the relative mass of quartz to pyrite in the concentrate increased with increasing contacting time. Furthermore, the final value at an hour is

very close to the abiotic control in which a grade of 5.0 ± 0.5% was achieved. These results indicate that prolonged exposure to the mixed chemolithotrophic culture reduces the preferential recovery of the pyrite. This may be due to attachment of the microorganisms to the silica occurring at the later times, in agreement with Diao et al. (2013).

The best sulphide bioflotation results, both with respect to grade and recovery, were therefore achieved using a contacting time of 5 minutes. The higher recovery relative to the control suggests that the microbial cultures may have increased the stability of the froth, thereby improving recovery of the froth concentrate. This is because an increase in froth stability prevents the bubbles from bursting upon reaching the top of the float cell thereby ensuring a reduced sulphide mineral fall-back into the float cell. This theory is supported by Khoshdast and Sam (2011) who found that froth stability under acidic conditions increases with the introduction of microbial cultures due to their high molecular weights resulting in more surface water interactions. The fact that the recovery decreased with contacting time indicates that planktonic (unattached) cells have a more pronounced effect than attached cells, supporting the theory of contribution to frother stability.

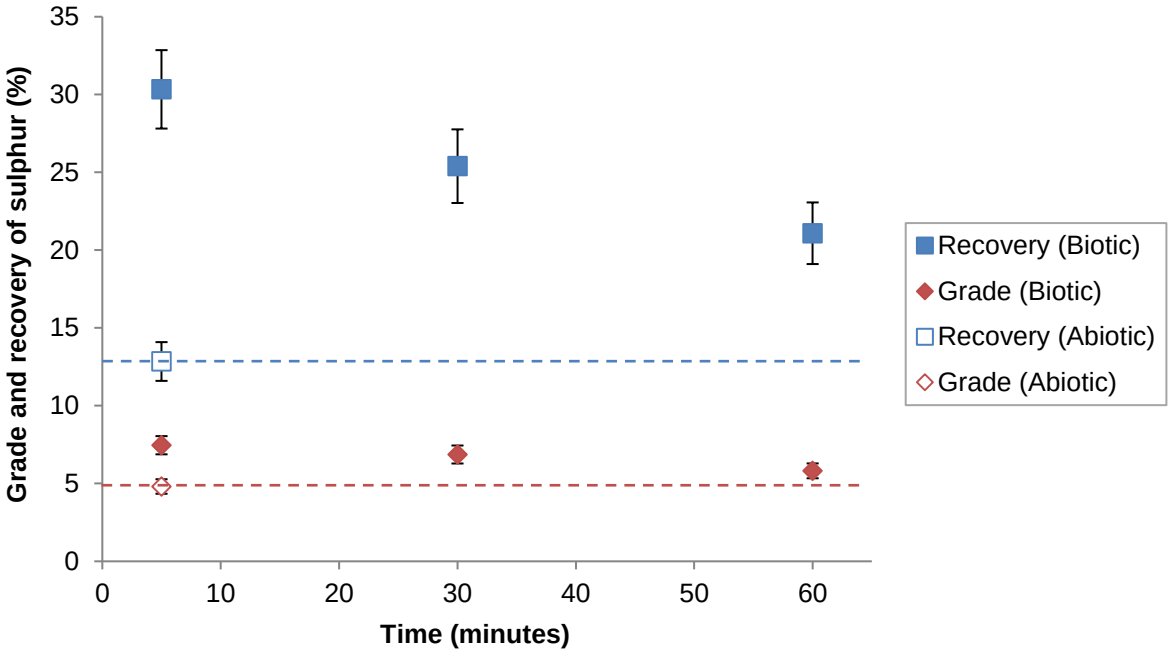

Figure 20: Grade and recovery of sulphur in the concentrate with increased contact time, using 1×10^8 cells/g pyrite. Error bars show a standard deviation variation across two repeats.

6.2.3 Recommendation for chemolithotrophs

Despite the best bioflotation experiments using the mixed acidophilic chemolithotrophic culture having a better recovery and grade than the abiotic equivalents, the best results of 30.3 ± 2.5% and 7.46 ± 0.58% are far below the full chemical equivalents of 96 ± 9.3% and 38 ± 3.7% respectively. This indicates that the chemolithotrophs are not suitable for the second flotation stage, but may still be effective as pyrite depressants in the 1st flotation stage in which pyrite and gangue are separated from coal.

The acidic operating conditions that this selection of chemolithotrophs require introduces an environment that is favourable for ARD reactions, thereby increasing the potential risk associated with the streams that would leave this flotation stage. This would not be problematic if a concentrated sulphide stream was achieved, as this could be treated. However, the poor separation of the pyrite makes this undesirable. Therefore *L. ferriphilum, A. ferrooxidans* and *A. thiooxidans* are not recommended as potential bioflotation reagents for the second-stage of the desulphurisation flotation process.

6.3 Heterotrophs

6.3.1 Attachment studies for heterotrophs

A suite of heterotrophic microorganisms was tested for their affinity and attachment to pure pyrite, namely *Bacillus subtilis, Bacillus licheniformis, Paenibacillus polymyxa, Rhodococcus opacus* and *Rhodopseudomonas palustris*. Attachment experiments were carried out at pH 4 (around the heterotrophic microbes' isoelectric points) and pH 7 (as per chemical two-stage flotation process) to evaluate the extent of attachment over the range of potential conditions. An initial cell concentration of 1×10^7 cells/mℓ was used for all experiments.

Control attachment tests which had no mineral added were performed for each microorganism. The concentration of suspended cells was observed to remain practically unchanged over the duration of the control experiment for all microbes, confirming no natural settling of the microbes occurred at the test conditions and so that any change in microbial concentration during the actual experiments was due to mineral-microbe interaction.

Results obtained for the attachment experiments at pH 7 are presented in Figure 21. The plot shows the percentage of cells attached to the pyrite surface over time. Attachment was observed for all cultures except for *B. licheniformis. P. polymyxa* attached the most rapidly, having achieved the highest percentage attachment after 1 minute of 93.0 ± 2.0%. At the same time, *R. palustris* and *R. opacus* exhibited an attachment of ± 70%, followed by *B. subtilis* at 51.6 ± 5%. After 5 minutes, there was a significant increase in attachment observed for *R. opacus* cells to 94.5 ± 0.9% attachment. Over the total duration of the experiment, *P. polymyxa* and *R. opacus* demonstrated near complete attachment of 97%. A slight decrease in attachment was observed for *R. palustris* cells at 5 minutes, after which the percentage attachment remained the same to 10 minutes, with a highest observed attachment of 75 ± 1.1%. The highest percentage attachment for *B. subtilis* cells was achieved at 10 minutes at 82.3 ± 1.7%.

Results of the attachment experiments at pH 4 are presented in Figure 22, excepting that for *B. licheniformis* for which no attachment was observed. After 1 minute of interaction, attachment was detected for all cultures, though the degree was less than at pH 7 for all microbes, with *R. opacus* and *P. polymyxa* cultures having the lowest and highest percentage attachment of 41.4 ± 8.6% and 70.1 ± 1.6% respectively. After 5 minutes, there was a significant increase in attachment observed for all cultures, with *R. opacus* and *P. polymyxa* cells both obtaining an attachment of ± 88%. After 10 minutes, both *R. opacus* and *P. polymyxa* cultures achieved more than 95% attachment, while *R. palustris* achieved 86.2 ± 0.7%, higher than that found at pH 7.

These results show that *B. subtilis*, *R. opacus* and *P. polymyxa* cells have a high affinity for pyrite with most cells attached after 5 minutes of interaction. Previous authors have demonstrated the successful attachment of *P. polymyxa* cells onto pyrite (Patra & Natarajan, 2004a and Chandraprabha & Natarajan, 2009) while the results showing the percentage attachment of *R. opacus and R. palustris* cells onto pyrite are presented for the first time in this study. All microorganisms investigated (besides *B. licheniformis)* were observed to attach to pyrite in varying degrees. When comparing the percentage attachment between pH 4 and 7, all microorganisms besides *R. palustris,* showed a higher affinity for pyrite at the neutral pH conditions, with the highest percentage attachment of 97.4% and 97.1% obtained for *R. opacus* and *P. polymyxa* respectively. *R. opacus* and *P. polymyxa* cells showed the highest affinity for pyrite under both neutral and acidic conditions, with the most rapid attachment at both pH conditions observed for *P. polymyxa*. The results provide sufficient motivation for the continued investigation of the flotation separation of pyrite using *R. palustris, R. opacus, B. subtilis* and *P. polymyxa*.

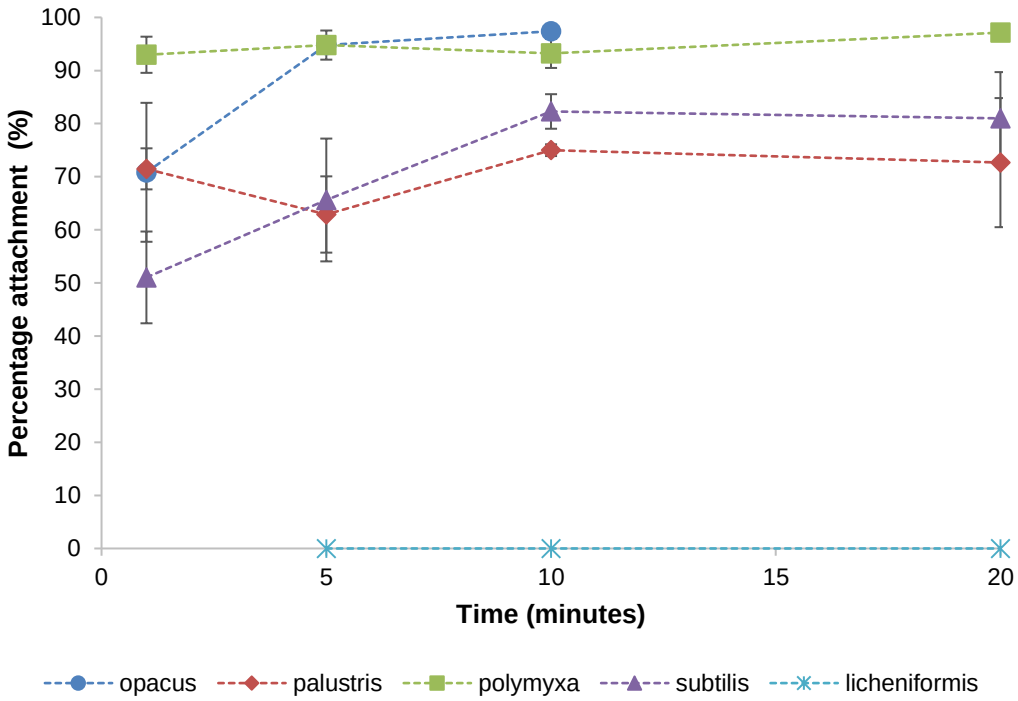

Figure 21: Percentage attachment of *R. opacus, R. palustris, P. polymyxa, B. subtilis* and *B. licheniformis* cells onto pyrite in a 1 g solid per 100 mℓ suspension containing a cell concentration of 1×10^7 cells/mℓ at pH 7. Error bars show a standard deviation variation across triplicate repeats.

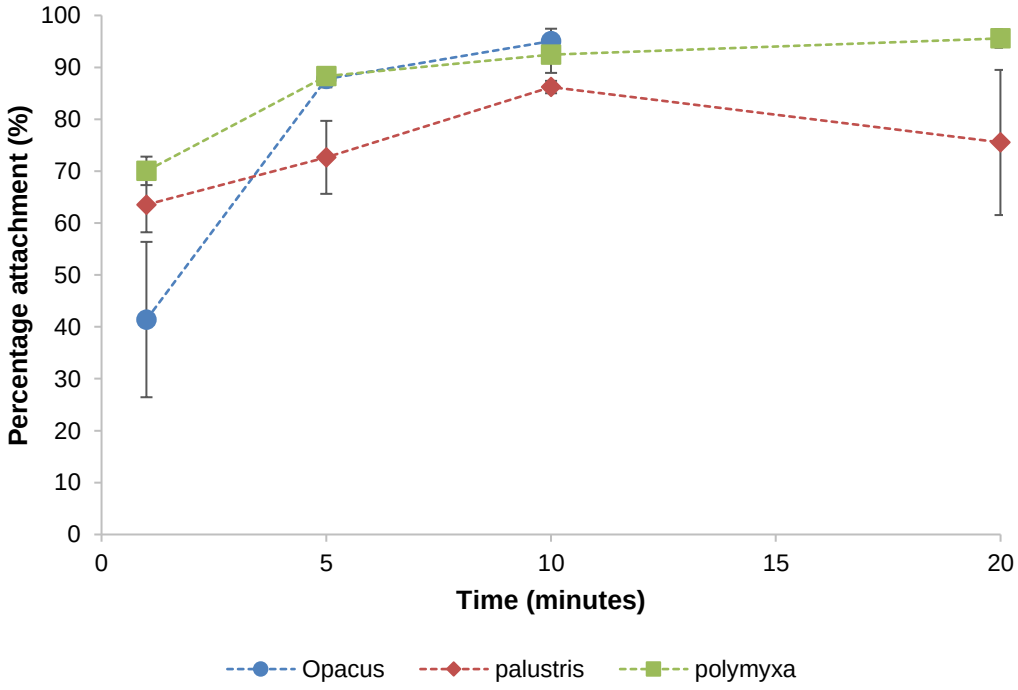

Figure 22: Percentage attachment of *R. opacus, R. palustris* and *P. polymyxa* cells onto pyrite in a 1 g solid per 100 mℓ suspension containing a cell concentration of 1×10^7 cells/mℓ at pH 4. Error bars show a standard deviation variation across triplicate repeats.

6.3.2 Zeta potential tests of pyrite and microbial cultures

Zeta potentials were measured to assess the changes in the surface chemical charge of both bacterial cells and the mineral, the results of which are shown in Figure 23 for pyrite and *R. opacus* and *P. polymyxa*.

The pyrite mineral was found to have a net positive charge in the acidic pH range, only recording a net negative charge (-73 mV) at neutral pH before interaction. The trend indicates that the pyrite's point of zero surface charge (PZC) is around pH 6. The aim of a collector would be to adapt the pyrite surface to have a more neutral surface charge at the flotation pH, thereby facilitating hydrophobicity.

The *R. opacus* bacterial cells had a large net negative charge over a wide pH range, with a net positive charge only observed at pH 3, indicating a PZC between pH 3 and pH 4. This agrees with literature values obtained where most bacterial PZC's are between pH 2 and pH 4 (Somasundaran, et al., 2001) and that of *R. opacus* is between pH 3.2 and pH 3.7 (Kim, et al., 2015). By contrast, the zeta potential magnitude of the *P. polymyxa* was near zero at all pH's tested, with magnitudes 100-fold less than the *R. opacus* cells' surface charges. Though the error bars for the *P. polymyxa* measurements do overlap, it appears that a marginally more neutral surface charge exists at pH 7.

This indicates that the *P. polymyxa* is the more hydrophobic of the two microorganisms across the pH range tested as its surface charge is closer to neutral and so less attracted to water. This is an indicator that *P. polymyxa* is more likely to be floatable than the *R. opacus*.

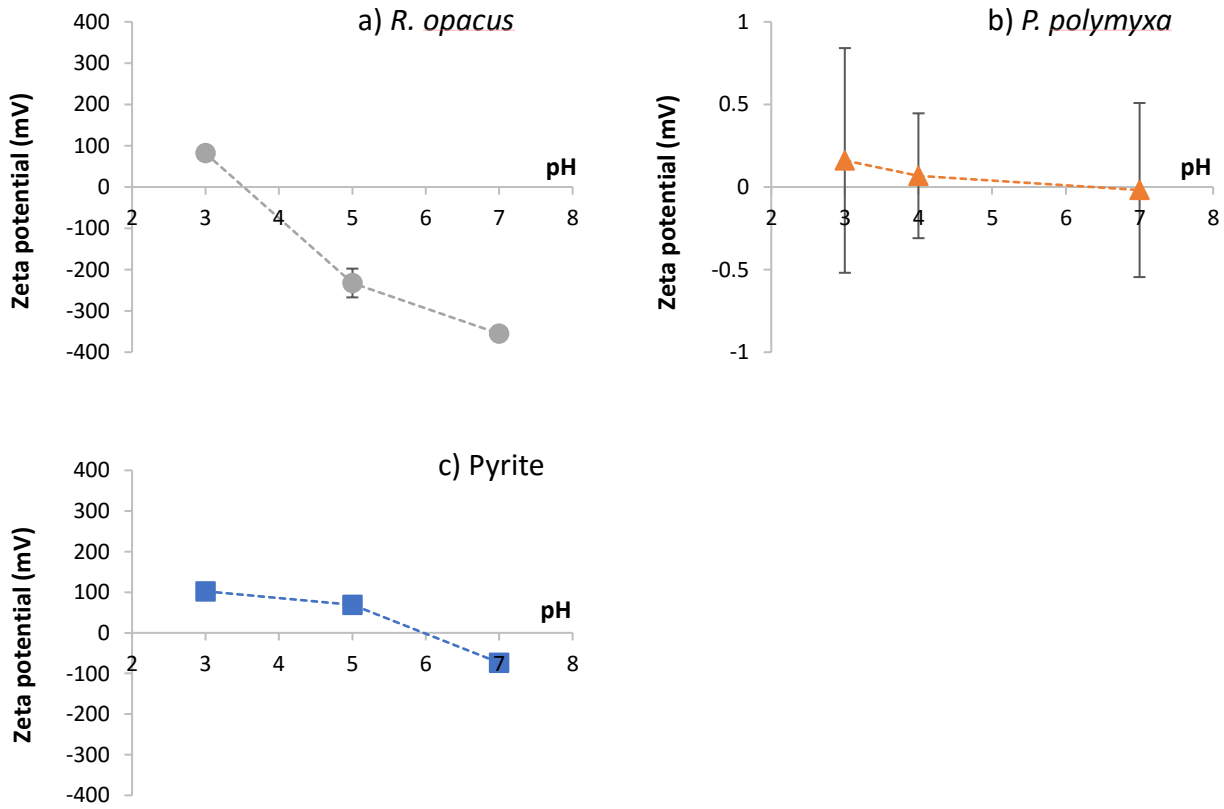

Figure 23: Measured zeta potential as a function of pH for (a) *R. opacus*, (b) *P. polymyxa* and (c) pyrite mineral

6.3.3 Bioflotation tests for heterotrophs

Pure pyrite bioflotation tests, under the same conditions as the froth-only flotation at pH 4 and pH 7, were performed for the four microorganisms identified in Section 6.3.1 as being able to attach to pyrite. As only the pure mineral was used, the floats were evaluated based on the cumulative mass recovery of pyrite in the concentrate.

Figure 24 shows the cumulative percentage mass recovery of pyrite to the concentrate after 50 g was individually treated with a *R. opacus, R. palustris, B. subtilis* or *P. polymyxa* culture at pH 4. In the positive chemical control using PAX, a highly selective sulphide mineral collector, 94.1% of the pyrite was recovered in the first concentrate and a cumulative total of 95.3% was recovered after 20 minutes. In contrast, the microbial cultures did not perform well under the acidic conditions, as the highest achieved cumulative recovery was 7 ± 0.4% using *P. polymyxa.* All other microorganisms achieved recoveries of less than 2% under the acidic pH 4 condition.

Figure 25 shows the recoveries achieved when the bioflotation test were conducted at pH 7. The same result was observed under neutral conditions compared to pH 4 for *R. opacus, R. palustris* and *B. subtilis*, for which there was little to no recovery. However, the *P. polymyxa* bioflotation cumulatively recovered 81.3 ± 0.4% of the pyrite, only marginally below that of the chemical control.

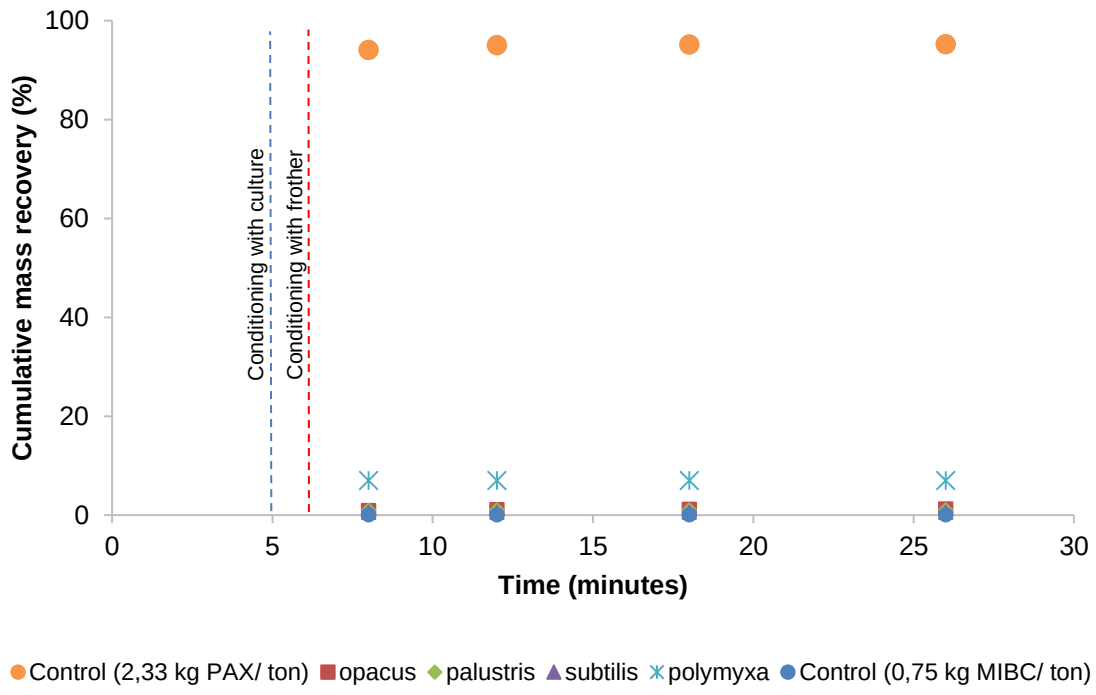

Figure 24: Cumulative mass recovery of pyrite after floating with the *R. opacus, R. palustris, B. subtilis* and *P. polymyxa* culture at pH 4 with a cell concentration of 4.2×10^9 cells/g for all cultures excepting 2.45×10^9 cells/g for *P. polymyxa*

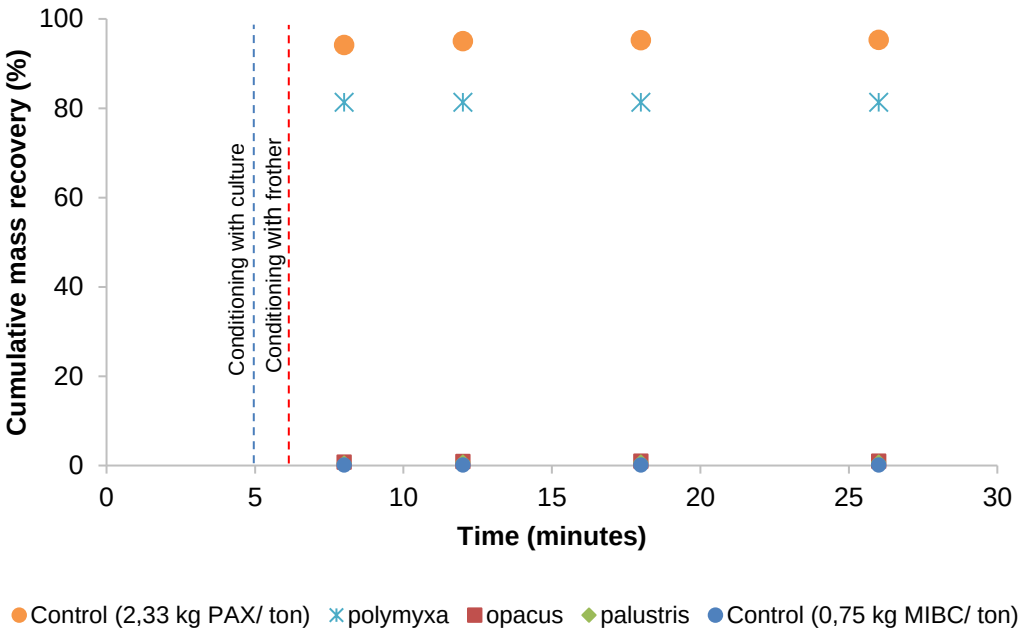

Figure 25: Cumulative mass recovery of pyrite after floating with the *R. opacus, R. palustris, B. subtilis* and *P. polymyxa* culture at pH 7 with a cell concentration of 4.2×10^9 cells/g for all cultures excepting 2.45×10^9 cells/g for *P. polymyxa*

6.3.4 Recommendation for heterotrophs

Although a high degree of attachment was observed for all the microorganisms tested, this did not lead to surface modifications that enhanced the floatability of pyrite when using *R. opacus, R. palustris* and *B. subtilis* cultures as collectors. This result was found even though zeta potential measurements confirmed a change in the pyrite surface charge at pH 7 following microbial exposure. These results suggest that *R. opacus, R. palustris* and *B. subtilis* do not demonstrate sufficient promise to be investigated further as bioflotation reagents for this application.

P. polymyxa showed the greatest potential as a pyrite collector in sulphide mineral flotation. The recovery obtained was comparable to that using a chemical collector (Table 9) with a difference of only 14% between the two. These results show that following mineral-microbe interaction, there is a modification of the mineral surface that changes its properties, making it hydrophobic enough to attach to the air bubble and be collected in the concentrate froth. The success of *P. polymyxa* compared to the poor flotation performance of the other microbes may be linked to its comparatively very rapid attachment at pH 7, suggesting a strong preference for these surfaces.

Based on these results, *P. polymyxa* has been chosen as the heterotrophic candidate for the bioflotation of pyrite from coal waste tailings. Challenges with its growth kinetics (specific in Section 4.3.1.7) should be noted, as this predicts challenges in its scale-up.

6.4 Mixed Culture Flotation Tests

A series of additional flotation tests were carried out using mixed cultures associated with pyrite systems to identify microorganisms not currently included in the bioflotation literature for pyrite flotation. Mixed cultures that were used included a thiocyanate (SCN^-) degrading mixed culture containing autotrophic and heterotrophic species from an Activated Sludge Tailings Effluent Remediation (ASTER™) reactor, a mixed mesophilic sulphur oxidizing culture and a mixed culture of the microorganisms that were initially proposed for this project. Because most bacterial surfaces have PZC's below pH 4, these tests were done at a pH value of 4.

6.4.1 Sulphur oxidizers flotation tests

A mixed mesophilic culture of microorganisms that oxidize elemental sulphur was used. The culture was grown using acidified 0K basal salt medium (BSM) (pH 2.0) (see Appendix A.2.1) and maintained on a shaking platform in a 30°C incubator.

Figure 26 shows the cumulative mass recoveries of pyrite after floating with the sulphur oxidizing mixed culture. Initially, 50 g of pyrite was treated with a mixed culture with a concentration of 1×10^9 cells/g. Although there was collection observed in comparison to the control, only 0.54% of the mineral was recovered in the concentrate, thus the culture did not have a significant effect on the floatability of pyrite. To investigate whether the cell number was a limiting factor, the amount of pyrite used was reduced to 20 g and the pulp was treated using a culture with a concentration of 2×10^9 cells/g. In this case, only 0.2% of the pyrite was collected in the concentrate, indicating that there still no significant effect on the floatability of pyrite. These results suggest that the microorganisms in the sulphur oxidizing mixed culture do not have an impact on the floatability of pyrite. This was an unexpected result because the microorganisms use elemental sulphur as a source of energy for their growth and thus it was expected that the sulphur present in pyrite would attract the microorganisms.

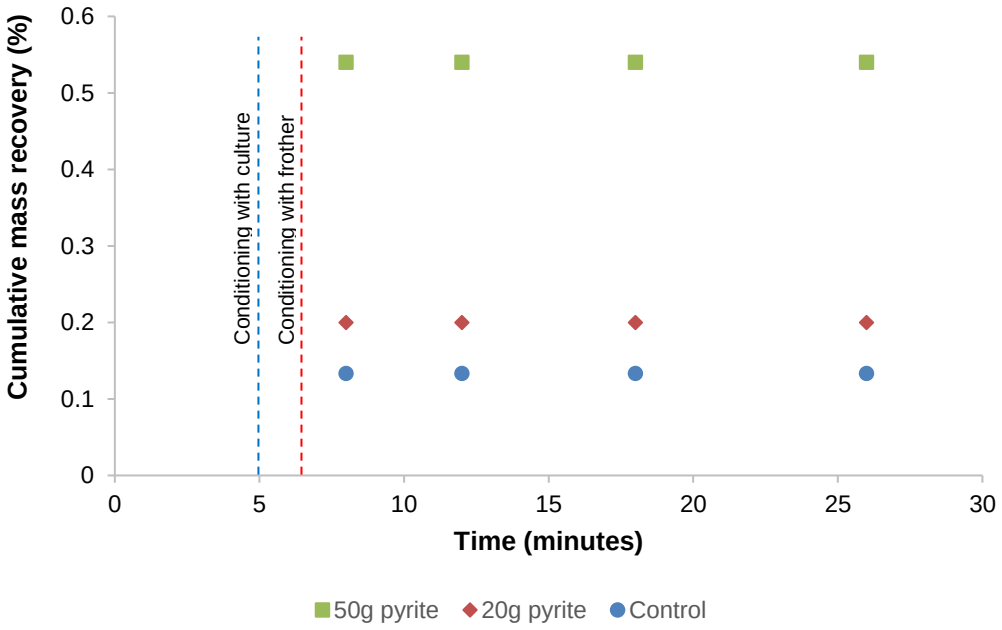

Figure 26: Cumulative mass recovery of pyrite after floating with sulphur oxidizing mixed culture: 50 g of pyrite was treated with a culture containing a cell concentration of 1×10^9 cells/g, and 20 g of pyrite was treated with a culture containing a concentration of 2×10^9 cells/g

6.4.2 Autotrophic thiocyanate (SCN^-) degrading ASTER™ mixed culture floats

A mixed autotrophic culture from thiocyanate (SCN^-) degrading ASTER™ reactors was used to investigate its effect on the floatability of pyrite. The culture was grown using acidified 0K basal salt medium (BSM) at pH 2.0 (see Appendix A.2.1) supplemented with thiocyanate and maintained on a shaking platform in a 30°C incubator.

Figure 27 shows the cumulative mass recovery of pyrite after floating with the autotrophic SCN^- degrading mixed culture. To investigate the effect this culture would have on the floatability of pyrite, the amount of mineral was reduced to 20 g and treated with the highest possible concentration of cells that was obtained from this culture which was 4×10^8 cells/g. This was because cell concentration

became a limiting factor with this culture as the cells grow preferentially in a matrix of exopolysaccharide rather than in liquid suspension. A total of 0.63% of pyrite was recovered in the concentrate. This suggests that this culture does not have a significant effect on the floatability of pyrite when compared with the negative control. However, the autotrophic culture performed better than the sulphur oxidizing culture in terms of the pyrite recovered in the concentrate.

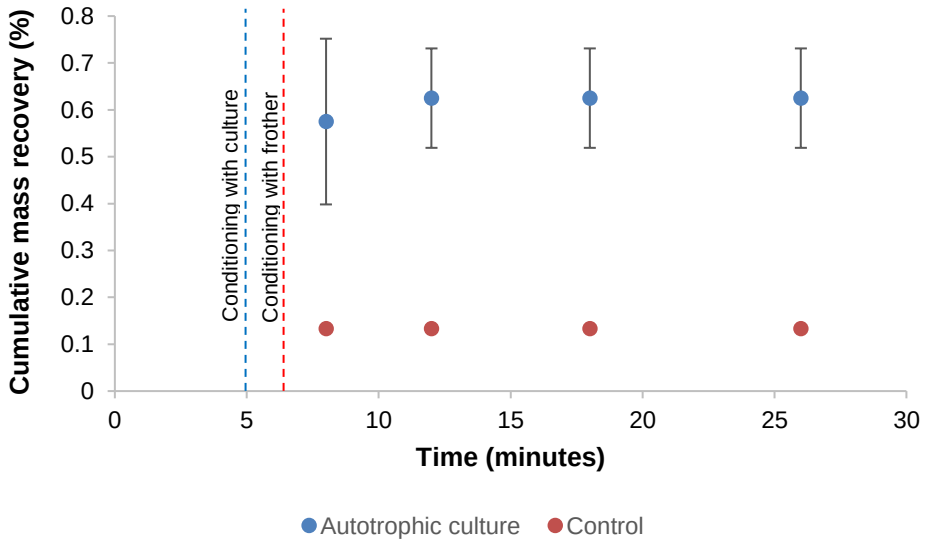

Figure 27: Cumulative mass recovery of pyrite after floating with autotrophic SCN^- degrading mixed culture; 20 g of pyrite was treated with a culture containing a concentration of 2×10^9 cells/g. Error bars show a standard deviation variation across duplicate repeats.

6.4.3 Recommendation for mixed cultures

Both the mixed mesophilic culture of sulphur oxidisers and the mixed autotrophic culture from thiocyanate (SCN-) degrading ASTER TM reactors demonstrated improved pyrite concentrate recoveries compared to the negative control, thus confirming that association with and subsequent alteration of the pyrite surface properties did occur. However, the recoveries were too low to recommend them for further investigation.

CHAPTER 7: Environmental and Economic Assessment of Potential Bioflotation Reagents

The microbial screening and preliminary bioflotation test work presented in Chapters 5 and 6 successfully demonstrated the technical feasibility of four biological options, namely:

- *M. phlei*, algal lipids or algal derived FAMEs as coal collectors in the first flotation (coal) stage
- *P. polymyxa* as a pyrite collector in the second flotation (sulphides) stage

In all cases, the performance of the bioflotation systems was comparable to the chemical equivalents, both in terms of yield and sulphide recovery. However, the environmental and economic impacts of using a biological system must be quantified before bioflotation to be considered a viable, practical alternative.

7.1 Environmental Assessment of Potential Bioflotation Reagent Use

The life cycle assessment on the chemical two-stage flotation system (Fundikwa, 2016) demonstrated multiple environmental advantages of using the process for coal desulphurisation, including: a more than 80% decrease in human-toxicity and eco-toxicity owing to less zinc leaving with the tailings and thus entering the environment, as well as more efficient land usage and less wastage. Furthermore, Kazadi Mbamba (2011) stated that the two-stage flotation process should result in improved energy efficiency and significantly reduce the amount of ultrafine waste that requires processing.

The environmental impact of using biological flotation reagents instead of chemical ones in the context of the two-stage flotation process changes needs to be considered from two key perspectives, considering the main aspects of the process that would have changed:

- Toxicity of the bioflotation reagents versus the chemical equivalents
- ARD generating potential of the product streams in comparison to the product streams of chemical flotation

7.1.1 Toxicity of the bioflotation reagents

The toxicity of the (bio)flotation reagents used in the two-stage process must be evaluated with respect to the final uses of the product streams. In the first-stage, the upgraded coal recovered in the concentrate will be sold as an energy resource and therefore combusted. The second-stage produces a low-volume, high-sulphide pyrite concentrate and a higher-volume gangue containing tails, the final usage of which are not fixed, though may include either further processing (Harrison, et al., 2013) or disposal of the streams. The latter requires consideration of potential effects of the release of the (bio)flotation reagents into the environment.

The proposed chemical flotation reagents include MIBC, oleic acid or dodecane, and PAX. The first three have low reported toxicity levels and do not pose a significant threat if released into the environment along with the solid mineral products. They are also standard coal flotation reagents and do not pose a risk if combusted with the coal product. The PAX used in the second-stage for pyrite recovery, however, is highly toxic if ingested (LD_{50} (rats) 470 mg/kg; LC_{50} (fish) 10-100 mg/l), for both to humans and other life forms, and so cannot be allowed to contaminate water sources. This may prove highly problematic with respect to final use of the second-stage product streams, whether in disposal, reuse or further processing.

To be considered as a bioflotation reagent, all microorganisms were required to be non-pathogenic and non-toxic (Section 3.4). Thus, environmental release of any of the four identified bioflotation reagents

does not pose an environmental risk. Combustion of the microorganisms or algal products with combustion of the coal produced in the first-stage is also non-problematic and potentially beneficial to the heating value of the coal as they will contribute to the organic material content of the coal product. Thus the biological approach is better than the chemical route with respect to the toxicity of reagents.

7.1.2 ARD generating potential of the product streams

The final locations of the pyrite (the main sulphide mineral) in the product streams was relatively unchanged in the biological flotation systems relative to the chemical equivalents (positive controls), as shown in Table 7, Table 8 and Figure 25. The ARD generation risk associated with the products produced in the chemical and biological systems will therefore be approximately the same for both systems. Hence, no further investigation into this aspect was deemed necessary at this preliminary stage.

7.2 Economic Assessment of Potential Bioflotation Reagent Use

The chemical two-stage flotation process is designed primarily as a waste treatment technology to allow for mitigation of the risk associated with fine coal wastes, but with the additional advantage of producing some saleable products, chiefly coal. It therefore has the multiple economic benefits of reducing the liabilities associated with the raw wastes, reducing the volume of the wastes that need to be disposed of and being a potentially cost neutral or even profitable treatment option.

An economic analysis was performed by Jera (2013) (also presented in Harrison et al (2013). This was expanded to additional example separations and further discussed in Harrison et al (2015)) which considered the construction and operating costs of implementing a chemical two-stage flotation process for the desulphurisation treatment of a South African fine coal waste, given the production of a saleable coal product. It was found that the treatment process could generate a positive net present value (NPV) under certain coal pricing conditions, thus making it an attractive treatment option compared to those that offer only the benefit of liability and/or waste volume reduction. The main operating cost of the process was the flotation reagents, accounting for up to 64% of the total (Harrison, et al., 2015). The oleic acid collector used in the first-stage contributed to this most, accounting for 82% of the reagent cost, largely since it was required at a flowrate of approximately ten times that of the other reagents (Harrison, et al., 2015). Thus, the replacement of the chemical reagents with biological options is expected to have a significant effect on the economics of the process.

Because the oleic acid for the first-stage flotation was the most significant reagent cost, the economic effect of replacing this reagent with a biological one, namely *M. phlei*, was considered for investigation. The RAL or FAME options were not investigated at this stage since further research is required into methods of production at a large enough scale, as bubble-columns (per Section 4.3.2) are not suitable for full production volumes. The relatively slow growth rate (not yet optimised) of the *P. polymyxa* means that it too requires further investigation into suitable systems for scale up to full production volume. Only then will accurate costing estimates be possible.

The *M. phlei* production could be achieved using standard bioprocess units and processes and so lent itself for relatively accurate initial costing. The process flowsheet and detailed economic analysis by Jera (2013) (also presented in Harrison et al (2013)) was used as a basis and adapted to incorporate the *M. phlei* production and usage replacing purchase and usage of oleic acid in the first-stage flotation. All previously obtained 2013 costs were inflated to 2016 equivalents to maintain an accurate comparison.

It should be noted that the production of *M. phlei* on site (as part of the flotation flowsheet) was chosen due to ease of incorporation into the existing chemical process technoeconomic proposal. However, an alternative option would be to design the *M. phlei* production as a stand-alone process, with the culture then purchased by the plant, in the same way as the chemical reagents. This approach may present advantages with respect to production scale as well as enabling process efficiencies, leveraged from

bioprocess focused expertise and complementary co-located bioindustries. It should also be noted that optimisation of *M. phlei* cultivation (including medium composition, growth rate and maximum cell density) was beyond the scope of this investigation and may still present significant productivity improvement and resultant cost reduction.

7.2.1 Process flowsheet and description

The process flowsheet for the chemical two-stage coal flotation process, described in Jera (2013), was adapted to make provision for the preparation and growth of the *M. phlei* cells required in the bioflotation process, which replaces the chemical reagent preparation in the first-stage of flotation. The bioprocessing addition to the flowsheet makes use of standard well-established processing techniques. The proposed bioflotation flowsheet is presented in Figure 28 and a process description now follows.

The flowsheet assumes that the ultrafine coal waste feed stream enters the system in the form required for flotation. Hence, the ultrafine waste stream will enter with a solids content of 6 wt%, within a particle size range of 75-200 µm. The ultrafine waste stream is fed directly to the first conditioning tank at a rate of 100 t solids/h, under atmospheric conditions.

The growth and preparation of the *M. phlei* required for the process is accounted for in the adapted flowsheet. The cells are cultivated in a liquid phase bioreactor using a glycerol-soil medium, the components for which include peptone, meat extract, soil extract, glycerol and deionised water (see Appendix A.2.6). The components enter the bioreactor in an excess of 10%, except for glycerol, the carbon source. Glycerol is, therefore, the limiting substrate and enters the system in the stoichiometric amount, ensuring the exact number of bacterial cells required for flotation. The growth medium components are combined in a mixing tank before being sent through an autoclave for sterilisation. The autoclave is heated continuously using steam to a temperature of 121°C (Pele & Cimpeanu, 2012). The sterilised growth medium is then transferred to a continuous bioreactor (fermenter), in which the bacteria cells are grown. The bioreactor is cooled, using cooling water, to maintain a temperature of 37°C (ATCC, 2016). Agitation of the bioreactor ensures a well-mixed bacterial suspension, with a microbial concentration equivalent to a maximum possible concentration (obtained in this study) of 3.7×10^8 cells/mℓ. This maximum cell concentration is noted as lower than expected for bacterial cultures where 10^9 to 10^{10} cells/mℓ is typical on average.

The medium containing *M. phlei* cells is withdrawn from the bioreactor and sent to a centrifuge in which it is assumed that 100% separation occurs. The excess growth medium is recycled back to the mixing tank, after 10% (amounting to 5 t/h) of the stream is purged. The recovered *M. phlei* cells are combined with the ultrafine coal waste, in conditioning tank 1.

Once combined in conditioning tank 1, the *M. phlei* cells are allowed a conditioning time of five minutes to ensure attachment to the coal, as per the bioflotation tests (Chapter 4) and prior chemical work (Kazadi Mbamba, 2011). MIBC is added to the tank, at a dosage rate of 0.28 kg/t solids to ensure froth stability during flotation. A further minute is then allowed in the conditioning tank before the material is sent to the first-stage flotation cell.

During this first-stage flotation, coal is selectively floated, achieving a combustible coal recovery of 19.8%, as per the bioflotation results in Table 6, Section 5.2.2. Along with combustible component, this recovered stream also comprises sulphide material (2%), ash (29%) and water (15%). This stream is sent through a plate and frame filter press, producing a coal concentrate which exits the system at 15 t/h. This coal concentrate forms the only product stream (in this study) which is sold for revenue. Additionally, water is recovered (2.58 t/h) as a potential recycle stream. The tailings from this first flotation cell forms the sulphide-containing stream, sent to the conditioning tank 2 for further processing.

For this initial economic assessment, it is assumed that the second-stage flotation operates using conventional chemical flotation reagents. Therefore, the chemical reagent dosage rates are added as follows: 2.33 kg/t PAX as the collector, 0.11 kg/t MIBC as the frother and 0.93 kg/t dextrin as the depressant (Kazadi Mbamba, et al., 2012). The depressant is deemed necessary since only 19.8% of

the coal entering the system is recovered in the first-stage. Once the chemical reagents are added to conditioning tank 2, and sufficient time has been allowed for conditioning, the material flows to the second-stage flotation cell. It is here that pyrite is selectively floated. It is assumed that all the pyrite entering the cell is floated and that the floated pyrite makes up 2.68% of the overall recovered stream (Kazadi Mbamba, 2011). The remainder of the stream was assumed to consist of water only.

This recovered stream is sent through a plate and frame filter press, where 100% separation is assumed to occur. Here, a sulphide concentrate stream is produced, exiting the system at a rate of 3.85 t/h. Once again, a water stream is recovered and forms the second potential recycle stream, exiting at a rate of 132 t/h. Uses for the sulphide concentrate product are still under research and so was considered beyond the scope of this project. It is therefore assumed that the sulphide concentrate is sent to a stockpile for disposal and does not act as a source of revenue. The tailings from the second-stage flotation form a benign stream which is first dewatered before it is filtered. It is assumed, that these solids (81.8 t/h) are sent to a stockpile for disposal, whereas the water recovered, from both the thickener and filter presses, can be recycled back to the plant for reuse (1389 t/h and 43 t/h, respectively).

It should be noted that for creating the bubble phase to which the hydrophobic particles attach, air is sparged through the flotation units rather than oxygen.

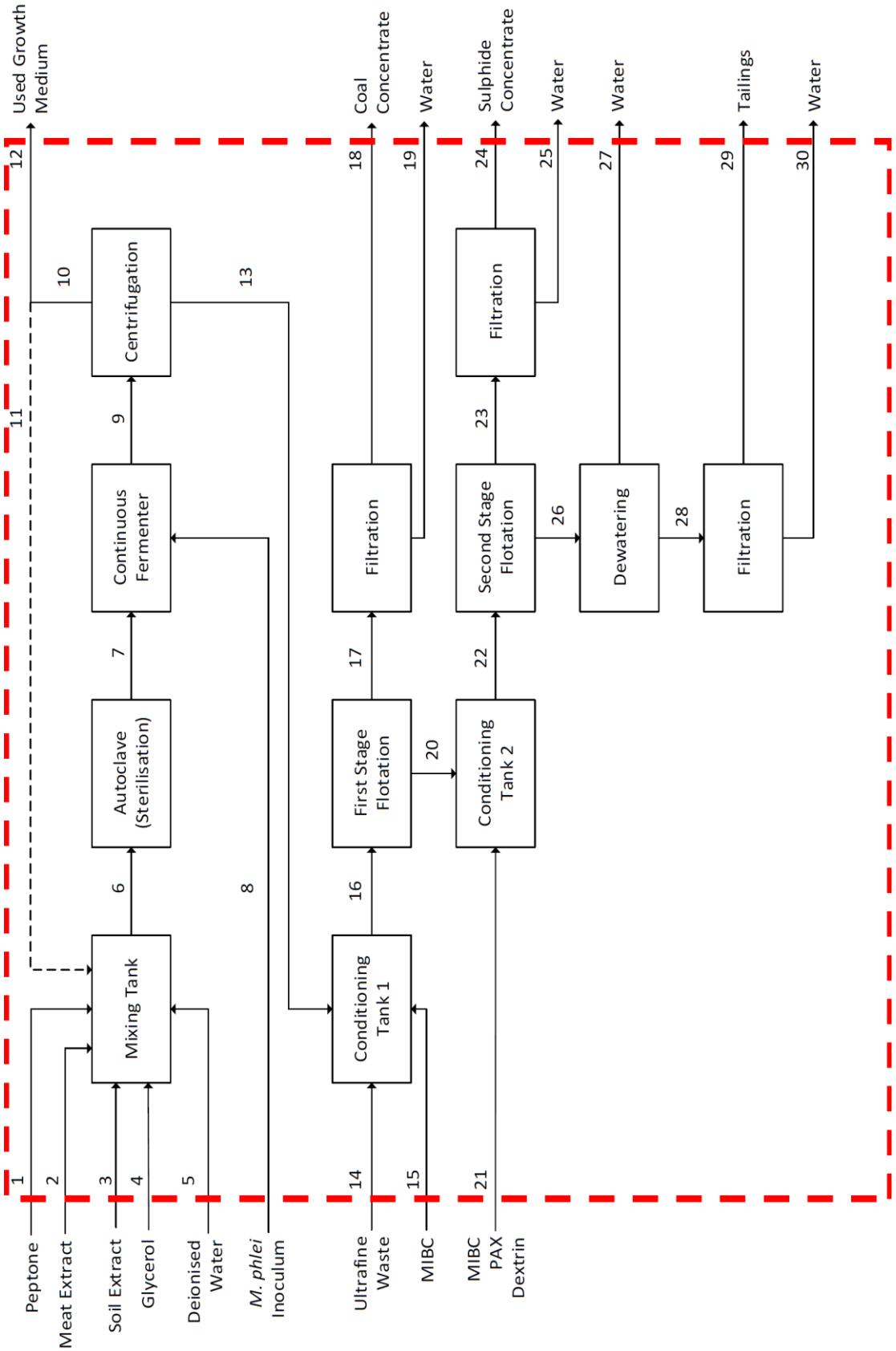

Figure 28: Bioflotation process flowsheet for *M. phlei* usage in the first-stage of the two-stage process

7.2.2 Material balance development

The material balance was solved in two parts beginning with the coal flotation and subsequently using this information to work backwards to the bacteria preparation.

7.2.2.1 First-stage coal bioflotation

A basis of 100 t/h of ultrafine coal waste (solids) was assumed as the primary feed, entering the process in the state necessary for flotation: within the particle size range of 75-200 µm, at 6 wt%. It was further assumed that the solids in this stream consists only of combustible coal, sulphides and ash, with the solids composition the same as the Witbank coal waste tailings described in Section 5.1 (48.7 wt% ash and 4.02 wt% sulphur). It was assumed that water accounted for the remaining 94% of the feed stream.

The recovery achieved in the first-stage flotation was assumed to be that of the highest recovery obtained in the *M. phlei* bioflotation tests (20% combustibles, 10% ash and 9% sulphur recovery to concentrate). It was assumed that the water recovery was the same as that in Jera (2013), calculated to be 15%. It was further assumed that perfect separation occurs in the filtration of the first-stage concentrate.

7.2.2.2 Second-stage pyrite flotation

The sulphide-containing tailings from the first-stage flotation cell are sent to the chemical second-stage of flotation. The recovery for the second-stage flotation was based on the results obtained by Kazadi Mbamba (2011). Per these results, sulphide material makes up 2.68% of the overall recovered stream, with the balance assumed to be water. Additionally, it was assumed that all the sulphide material entering the second flotation cell was recovered in the froth phase.

Perfect separation was assumed to occur in the filtration of the second-stage concentrate. The tailings stream contained a substantial amount of water (roughly 95%) and was therefore sent through dewatering before filtration. It was assumed that 97% of the water in the stream entering the thickener reported to the overflow and was removed from the system, based on Jera (2013). Finally, the thickener underflow, sent for further filtration in a filter press, is once again assumed to be perfectly separated.

7.2.2.3 M. phlei preparation

The material balance across the bacterial preparation units was performed by first calculating the amount of bacteria required for optimal flotation. The absorption isotherm for *M. phlei* (Figure 8) predicts the saturation of the coal surface at a microbial concentration of 2×10^{10} cells/g. Thus the number of *M. phlei* cells required in the first-stage of flotation was determined to be 2×10^{18} cells/h. Furthermore, based on an approximate cell mass of 1.18×10^{-10} mg/cell (that of *A. ferrooxidans* (Moon, 1995)), it was determined that the mass of cells required in the first-stage of flotation is 0.236 t/h.

Knowing the number and mass of cells required in flotation, it is possible to calculate the amount of growth medium required in the continuous bioreactor to achieve a growth rate that would ensure the bacteria's availability. The flowrate of growth medium required was determined by first calculating the amount of carbon (and therefore the amount of glycerol) required to grow 2×10^{18} cells/h. Using a general formula for bacteria ($CH_{1.8}O_{0.5}N_{0.2}$), the percentage carbon making up both the bacterial cells and glycerol ($C_3H_8O_3$), was determined. As the bacterial cells and glycerol are made up of 49% and 39% carbon, respectively, the amount of carbon required to produce 2×10^{18} cells/h was assumed to be 49% of the moles of the bacteria required:

$$C_{required} = 49\% \times \frac{Mass_{bacteria}}{Molar\ mass_{bacteria}} = 49\% \times \frac{0.236\ t/h \times 10^6\ g/t}{24.6\ g/mol} = \mathbf{4680\ mol/h}$$

Using the fact that glycerol contains 39% carbon, the mass of glycerol required was calculated as:

$$Glycerol_{required} = \left(\frac{4680\ mol/h}{39\%}\right) \times 92 g/mol = 1100262\ g/h = \mathbf{1.10\ t/h}$$

The media component flowrates to the bioreactor during continuous operation, incorporating the 10% excess glycerol required, were thus calculated and presented in Table 11.

Table 11: Flowrates of media components to the continuous bioreactor for *M. phlei* cultivation at a rate of 2×10^{18} cells/h, without and with recycle

Continuous bioreactor media component	Flowrate (t/h) for normal continuous operation	Flowrate (t/h) for continuous operation, including recycle
Peptone	0.28	0.28
Meat extract	0.17	0.17
Soil extract	8.25	8.25
Glycerol	1.10	1.10
Deionised water	46.76	5.14

It was further assumed that the feed entering the bioreactor under continuous operation conditions was sterile, with the *M. phlei* cells already present in the bioreactor. This means that stream 8 in the process flowsheet (Figure 28) is only necessary at start-up. Furthermore, it was assumed that no water is taken up by the bacteria during its growth. Therefore, 100% of the water entering the reactor exits with the reactor product. Except for this water and the excess glycerol, it was assumed that all material is consumed in the bioreactor, during the bacterial growth.

From the reactor, the exiting material moves to the centrifuge, where it is assumed that perfect separation is achieved. While the solid bacteria are sent to conditioning tank 1, the excess growth medium is recycled to join the fresh medium entering the system. Before the stream is recycled, however, it is assumed that 10% of the stream is purged and removed from the system to avoid the risk of accumulation. Knowing the amount of growth medium recycled and that required in the bioreactor, the amount of fresh growth medium required could be calculated as the difference between the two streams, the results of which are presented in the last column of Table 11.

7.2.2.4 Overall material balance

To ensure that the law of conservation of mass holds across the system, a material balance check was performed by summing the flowrates of the incoming and outgoing streams. The result is shown in Table 12. The analysis indicates a minor loss of 0.57% of the total mass flowing through the system and was therefore deemed insignificant. This loss was found to occur across the bioreactor. This can possibly be explained by the assumptions that were made in order to solve the mass balance across this unit, such as: that the water taken up by the bacteria during growth is negligible, the general chemical formula of a cell used, the mass of a cell used, the exclusion of the formation of metabolites, etc. All these assumptions contribute to the uncertainty of the material balance, which most likely contributes to the error.

Table 12: Material balance check over whole process flowsheet

Stream	IN (t/h)	OUT (t/h)
Total	1682	1672
Difference	–10	
Error	–0.57%	

7.2.3 Equipment sizing

This section details the methods undertaken to size the equipment required for the bioflotation process.

7.2.3.1 Flotation cells

To determine the size of the flotation cells used, as well as the number of cells required in each flotation bank, a similar method was adopted to that used by Jera (2013). This introduces a factor of consistency in the comparison between the two studies. The total required flotation cell volume, for both the first and second-stage flotation, was calculated using the equation:

$$V = \frac{(Q)(T_r)(S)}{60(Ca)} \tag{7}$$

where V is the total required flotation volume (m^3), Q is the volumetric flow rate of the feed (m^3/h), T_r is the retention time (min), S is the scale-up factor and Ca is the aeration factor (accounting for the air in the pulp phase).

For the coal flotation stage, Jera (2013) split the total volume required into several 30 m^3 cells, thus determining the number of cells required in the coal flotation bank. The same calculations were performed for the sulphide flotation bank, using 42 m^3 cells, instead. For the sake of consistency, the same principle was applied for the flotation cells in this study. The total required volume and number of cells in each flotation stage are listed in Table 6.1 and compared to that obtained in Jera (2013). It shows that the volume, as well as the number of cells, required for this study is larger than that required for a purely chemical process.

Table 13: Flotation cell banks comparison for the biological and chemical processes

	Biological process	**Chemical process** (Jera, 2013)
Coal flotation (stage 1)		
Total required volume (m^3)	314	192
Volume of each cell in bank (m^3)	30	
Number of cells per bank	11	7
Sulphide flotation (stage 2)		
Total required volume (m^3)	1242	474
Volume of each cell in bank (m^3)	42	
Number of cells per bank	29	11

7.2.3.2 Plate and frame filter presses

To determine the size of the filter presses required, a similar method was once again adopted to that used by Jera (2013). The volumes required were calculated using the filter's volumetric capacity and the number of filtration cycles per hour. Calculations show the method followed to determine the data for the coal filter press:

Table 14: Data used for the sizing of plate and frame filter presses

Data		**Reference**
Coal bulk weight (t/m^3)	0.8	(Jera, 2013)
Flotation cycle time (min)	8	(Jera, 2013)
Solids feed (t/h)	15	Mass balance
Safety factor	10 %	(Jera, 2013)

$$Volumetric\ capacity = \frac{Mass\ flow\ rate}{Bulk\ weight} \times Safety\ factor = \frac{15\ t/h}{0.8\ t/m^3} \times 1.1 = \mathbf{20.4\ m^3/h}$$

$$Cycles\ per\ hour = \frac{Minutes\ per\ hour}{Flotation\ cycle\ time} = \frac{60\ min/h}{8\ min/cycle} = \mathbf{7.5}$$

$$Required\ filter\ volume = \frac{Volumetric\ capacity}{Cycles\ per\ hour} = \frac{20.4\ m^3/h}{7.5\ cycles/h} = \mathbf{2.72\ m^3}$$

The same approach was applied to determine the capacity of the remaining two filter presses, resulting in volumes of 0.67 m^3 and 14.99 m^3 for the sulphide and tailings filter presses, respectively.

7.2.3.3 Thickener

As with the previous equipment thus far, the sizing of the thickener followed the approach by Jera (2013). The required thickening area and thickener diameter was calculated as follows:

Table 15: Data used for the sizing of thickeners

Data		Reference
Hydraulic loading (m^{3}h/m^2)	2.1	(Jera, 2013)
Thickener feed flow rate (t/h)	1514	Mass balance
Density (kg/m^3)	1000	Assumed that of water
Safety factor	10 %	(Jera, 2013)

$$Volumetric\ flowrate, Q = \frac{Mass\ flow\ rate}{Density} = \frac{1514\ t/h \times 1000\ kg/t}{1000\ kg/m^3} = \mathbf{1514\ m^3/h}$$

$$Thickener\ area, A = \frac{Q}{Hydraulic\ loading} \times Safety\ factor = \frac{1514\ m^3/h}{2.1\ m^3h/m^2} \times 1.1 = \mathbf{793\ m^2}$$

$$Thickener\ diameter, D = \sqrt{\frac{4A}{\pi}} = \sqrt{\frac{4(793\ m^2)}{\pi}} = \mathbf{32\ m}$$

7.2.3.4 Conditioning tanks, mixing tank and autoclave

As Jera (2013) did not provide sample calculations for the conditioning tanks, or make provision for biological reagents in the flotation procedure, the remaining equipment in this study had to be determined through alternate means. The conditioning tanks, mixing tank and autoclave were sized using storage tank sizing heuristics. A sample calculation for conditioning tank 1 is detailed below.

Table 16: Data used for the sizing of conditioning tanks, mixing tanks and the autoclave

Data		Reference
Feed flow rate (t/h)	1667	Mass balance
Density (kg/m^3)	1000	Assumed that of water
Safety factor	10 %	(Jera, 2013)
Holding time (h)	0.1	Assumed holding time of 6 min: 5 min after collector addition + 1 min after frother addition

$$Volumetric\ flowrate, Q = \frac{Mass\ flow\ rate}{Density} = \frac{1667\ t/h \times 1000\ kg/t}{1000\ kg/m^3} = \mathbf{1667\ m^3/h}$$

$$Volume, V = Q \times Holding\ time \times Safety\ Factor = 1667\frac{m^3}{h} \times 0.1\ h \times 1.1 = \mathbf{183\ m^3}$$

Repeating these calculations across the second conditioning tank, results in a required volume of 181 m^3. The same can be said for the sizing of the mixer and autoclave, both of which are required for the preparation of the bacterial growth medium. With 62 m^3/h entering each tank and assuming a holding time of 20 min, the required volume amounts to 23 m^3, each.

7.2.3.5 Continuous bioreactor

The continuous bioreactor was assumed to operate as a steady-state mixed stirred tank reactor, as is conventionally used bioreactor in industry (Moo-Young, 2011). Defining the system thus means that it can be assumed that the mixing within the bioreactor is sufficiently vigorous to ensure a well-mixed, essentially homogeneous system. Consequently, it can be assumed that the composition of the material throughout the reactor is uniform, and that there are no temperature gradients throughout the reactor. For the purpose of this study, the bioreactor will operate isothermally at a temperature of 37°C (ATCC, 2016) and a pH of 7.

The "black box model" was used to design the bioreactor. It is known as the simplest design model, where all the occurring biological reactions are grouped into a single, overall reaction (Ratledge & Kristiansen, 2001). This proves beneficial to this study as not much information is available surrounding the design of an industrial size bioreactor, for the growth of *M. phlei*. The generation of metabolites and extracellular macromolecules was not considered in this study and it is therefore assumed that the mass balance across the bioreactor only defines the volume needed to grow the required *M. phlei* cells.

The steady-state system mass balance describing the bioreactor is given as:

$$D(C_{i,in} - C_{i,out}) + r_i = 0 \tag{8}$$

where D is the dilution rate (h^{-1}) and is equal to the volumetric flowrate divided by the reactor volume, $C_{i,in}$ is the concentration of component i in the feed (g/L or mol/L), $C_{i,out}$ is the concentration of component i in the reaction mixture and effluent stream (g/L or mol/L), and r_i is the specific rate of substrate consumption or net generation (g/g.h or mol/g.h)

The rate of reaction, r_i, is often defined as the product of the specific growth rate (μ) and the cell mass per unit volume (x) (Bailey & Ollis, 1986). Substituting this for cell mass in the equation (8) gives:

$$Dx_{i,in} = (D - \mu)x_{i,out} \tag{9}$$

It is further assumed that a sterile feed enters the reactor i.e. it is assumed that the process is already in operation and inoculum addition is therefore unnecessary. Therefore, $x_{i,in} = 0$ and thus:

$$D = \mu \tag{10}$$

The required capacity of the bioreactor can now be determined through the calculation of the dilution rate which, in turn, can be determined through the calculation of the specific growth rate. It must be noted that the yield of biomass was assumed to be constant and any endogenous metabolism effects were ignored (Ratledge & Kristiansen, 2001).

The μ_{max} for *M. phlei* was experimentally determined to be 0.202 hr^{-1}, with the specific growth rate then typically calculated through a correlation such as Monod's equation, defined below:

$$\mu = \mu_{max}\left(\frac{S}{K_s + S}\right) \tag{11}$$

where S is the limiting substrate concentration (g/L) and K_s is the limiting substrate concentration where $\frac{\mu}{\mu_{max}} = 0.5$. However, to determine the K_s value, a plot of the specific growth rate versus the limiting

substrate concentration is required. Due to a lack of information, this plot could not be found or generated and, consequently, the use of the Monod equation was not suitable. The specific growth rate was therefore determined through its relationship with the doubling rate of *M. phlei* (Ratledge & Kristiansen, 2001). The doubling rate was experimentally determined to be 3.5 hours, and the specific growth rate could therefore be calculated as follows:

$$\mu = \frac{\ln(2)}{doubling\ time} = \frac{\ln(2)}{3.5\ h} = \mathbf{0.198\ h^{-1}}$$

Thus, the specific growth rate is roughly 98% of the maximum growth rate. Assuming the density of the medium can be approximated as that of water, the volumetric flow rate through the reactor can be determined:

$$Volumetric\ flow\ (F) = \frac{Mass\ flow}{Density} = \frac{62\ t/h}{1\ t/m^3} = 62\ m^3/h$$

Therefore, the required volumetric capacity of the bioreactor can be determined according to:

$$V = \frac{F}{\mu} = \frac{62\ m^3/h}{0.198\ h^{-1}} = \mathbf{314\ m^3}$$

7.2.3.6 Centrifuge

The bioreactor product was centrifuged using a disc-stack centrifuge, the most appropriate option for a continuously produced cell suspension of this volume. The size of the centrifuge required was estimated using equations detailed by Van der Linden (1987):

$$Volumetric\ flowrate, Q = \frac{5008\ t/h \times 1000\ kg/t}{1000\ kg/m^3} = 5008\frac{m^3}{h} \times \frac{h}{3600s} = \mathbf{1.39\ m^3/s}$$

The Stokes' velocity (v_g) of each particle was then calculated to be 5.5×10^{-8} m/s. This, along with the volumetric flowrate determined above, allowed for the calculation of the centrifuge's sigma value through the relationship:

$$Q = 2v_g\Sigma \tag{12}$$

It was therefore determined that:

$$\Sigma = \mathbf{1.28 \times 10^7 m^2}$$

The sigma value for a disc-stack centrifuge is defined as:

$$\Sigma = \frac{2\pi N_s \omega^2 (R_2^2 - R_1^2)}{3gtan(\theta)} \tag{13}$$

where N_s is the number of discs, $R_2\ and\ R_1$ are the distances from the centre of the centrifuge to the external and internal edges of the discs, θ is the half-angle of inclination of the discs and ω is the angular velocity (rad/s) of the centrifuge.

The calculation of the centrifuge size was calculated as follows:

Table 17: Data used for the sizing of disc-stack centrifuge

Data		Reference
N_s, number of discs	100	Average between the typical 50-150 discs (Green & Perry, 2008)
ω, angular velocity (rpm)	10 000	Assumption based on the statement in Van der Linden (1987) that disc centrifuges can handle a higher number of revolutions
θ, half-angle	47.5°	Average between the typical 40°-55° (Green & Perry, 2008)

$$(R_2^2 - R_1^2) = \frac{3\Sigma g tan(\theta)}{2\pi N_s \omega^2} = \frac{3 \times (1.28 \times 10^7 m^2) \times 9.81 \frac{m}{s^2} \times \tan(47.5°)}{2\pi \times 100 \times \left(10000 \frac{rev}{minute} \times \frac{minute}{60\ s} \times \frac{2\pi rad}{rev}\right)^2} = \mathbf{0.22\ m^2}$$

Due to a lack of information regarding either R_1 or R_2, an assumption needed to be made as to the ratio between the two radii. It was therefore assumed that R_1 is a quarter of the length of R_2. Therefore:

$$\left(R_2^2 - \frac{R_2^2}{4^2}\right) = 0.22\ m^2$$

$$R_2 = \mathbf{0.48\ m}$$

If it is then assumed that the diameter of the centrifuge is approximately equivalent to twice the length of R_2:

$$D = 2R_2 = \mathbf{0.96\ m}$$

7.2.4 Process economics

7.2.4.1 Equipment costing

The purchase cost of the equipment (PCE) was calculated for all equipment sized in Section 7.2.3 as well as estimated for additional auxiliary equipment. In each case the individual PCEs were scaled to 2016 prices using the Chemical Engineering Plant Cost Indices (CEPCI). A summary of the PCEs is given in Table 18. It shows that the unit contributing the majority of the cost is the second-stage flotation cell. This is followed by the bioreactor and thickener as the second and third most expensive units, respectively. This is both because these units process large volumes of material and they hold the material for longer periods of time.

Table 18: Summary of the PCE for the bioflotation system

Equipment item	PCE	Costing method/reference
Mixer	R 222 719	(Sinnott, 2005)
Autoclave	R 1 674 193	(Seider, et al., 2010)
Bioreactor	R 11 163 903	Costed as an agitated, jacketed reactor (Sinnott, 2005)
Centrifuge	R 6 471 648	(Towler & Sinnott, 2008)
Conditioning tank 1	R 701 507	(Sinnott, 2005)
Conditioning tank 2	R 697 524	
Float cell 1 (1st stage)	R 5 624 080	Cost per cubic meter of R17 308/m^3 (Jera, 2013)
Float cell 2 (2nd stage)	R 20 757 967	
Filter press 1 (coal)	R 3 113 805	Filter costing curves (Loh, et al., 2002)
Filter press 2 (sulphide)	R 2 529 966	
Filter press 3 (tailings)	R 3 892 256	
Pumps/other equip.	R 5 669 000	As per Jera (2013)
Thickener	R 7 050 048	(Seider, et al., 2010)
Total	R 69 568 614	

7.2.4.2 Required capital investment

The required fixed capital investment was calculated using Lang factors from Sinnott (2005), in combination with the following equations:

$$Physical\ Plant\ Cost, PPC = PCE \times 3.15 = R\ 69\ 568\ 614 \times 3.15 = \boldsymbol{R\ 219\ 141\ 135}$$

$$Fixed\ capital, FC = PPC \times 1.40 = R2\ 19\ 141\ 135 \times 1.40 = \boldsymbol{R\ 306\ 797\ 589}$$

Thus, estimating the working capital as 10% of the fixed capital investment gives a total capital investment of:

$$Total\ capital\ investment = FC + 0.10(FC) = R\ 306\ 797\ 589 + 0.10(R\ 306\ 797\ 589) = \boldsymbol{R\ 337\ 477\ 348}$$

Jera (2013) states that the total capital investment required for the chemical flotation process amounts to approximately R146 million. Therefore, the capital required to implement the use of *M. phlei* instead of oleic acid in the first-stage of the two-stage coal flotation process is 2.4 times larger than that required for conventional chemical flotation. It must be noted that optimisation the processes included has not been conducted prior to this costing.

7.2.4.3 Operating costs

The operating costs are split into fixed and variable operating costs. The categorisation made by Jera (2013) is given in Table 19.

Table 19: Classification of fixed and variable operating costs (Jera, 2013)

Item	Variable costs	Factor
1	Raw materials	Flowsheet data
2	Miscellaneous	10% of maintenance
3	Utilities	Flowsheet data
4	Sub-total A	-
5	**Fixed costs**	**Estimation**
6	Maintenance	5-10% of fixed capital
7	Labour	Manning estimates
8	Laboratory	20-23% of labour
9	Supervision	20% of labour
10	Plant overheads	50% of labour
11	Capital charges	10% of fixed capital
12	Insurance	1% of fixed capital
13	Taxes	2% of fixed capital
14	Royalties	1% of fixed capital
15	Sub-total B	-
16	Direct production cost	A + B

7.2.4.3.1 Fixed costs

After calculating the labour costs, the fixed operating costs of the process can be calculated using known values. For the sake of this study, the cost of labour was taken as that indicated by Jera (2013) and scaled using CEPCI values to represent the labour costs in the present time:

$$Labour_{2016} = Labour_{2013} \times \frac{CEPCI\ 2016}{CEPCI\ 2013} = R\ 16\ 800\ 000 \times \frac{587}{578} = \boldsymbol{R\ 16\ 542\ 419}$$

The remaining fixed operating costs were calculated accordingly and are given in Table 20. In total, the fixed operating costs amounts to R104 236 362/year.

Table 20: Summary of the fixed operating costs for the bioflotation system

Fixed Capital: R306 797 589	
Fixed Costs Based on Fixed Capital	**Calculated Value (R/year)**
Maintenance	R25 310 801
Capital charges	R33 747 735
Insurance	R3 374 773
Taxes	R6 749 547
Royalties	R3 374 773
Labour Costs: R16 542 419 (per annum)	
Fixed Costs Based on Labour Costs	**Calculated Value (R/year)**
Laboratory costs	R3 556 620
Supervision	R3 308 484
Plant overheads	R8 271 210

7.2.4.3.2 Variable costs

For this study, the variable costs include the cost of raw materials, utilities and miscellaneous costs (Table 19), in addition to the disposal cost of the purged growth medium (stream 12 in the material balance).

The raw material costs comprise the cost of the chemical reagents required for flotation, as well the components which make up the growth medium required for bacteria preparation. It is assumed that the ultrafine coal waste feed is received from within the company and thus, is not included as a raw material cost. These costs were calculated assuming 24 hours in a day, with 365 days in a year.

The costs of the chemical reagents required were calculated using scaled values of the cost prices provided in Jera (2013). In knowing the cost prices of each reagent, as well as the total required flowrates, the total cost could be determined. These costs are listed in Table 21.

Table 21: Summary of chemical reagent costs for bioflotation process

Chemical Reagent	Cost (R/h)	Cost (R/year)
MIBC	503	4 407 488
PAX	4 564	39 984 657
Dextrin	723	6 335 869

The growth medium components were cost in the same manner as that of the chemical reagents. This time, however, using the referenced listed in Table 22. In all cases, the prices used were the lowest prices that could be found for the corresponding components.

Table 22: Summary of growth medium component costs for bioflotation process

Chemical reagent	Cost (R/h)	Cost (R/year)	Reference / assumption
Peptone	5 562	48 722 072	Shandong Guanghao Biological Products (2016)
Meat extract	35 794	313 557 890	Xi'an Rongsheng Biotechnology (2016)
Glycerol	4 401	38 553 187	(Coronado, 2013)
Deionised water	-	-	Assumed received on site
Soil extract	-	-	Assumed received on site

The raw material costs, alone, amount to a total of approximately R452 million (R451 561 163). The largest individual raw material cost is the cost of meat extract, contributing 69% to the total raw material costs. The second highest cost is the cost of peptone (11%). The contribution of the remaining raw materials all fall below 10%, which suggests that attempts to reduce raw material costs should focus on these two reagents.

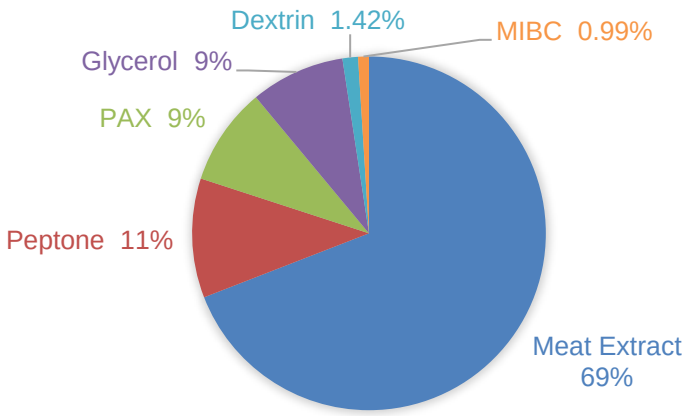

Figure 29: Distribution of raw material costs

7.2.4.3.3 Utility costs

Three different types of utility are required for the operation of this process: low pressure (LP) steam, cooling water and electricity.

Electricity is required to power the agitators, as well as the pumps throughout the process. As the cost for the pumps and ancillary equipment (including agitators) was taken from Jera (2013), it was similarly assumed that the cost of electricity in this study could be estimated as a scaled value of that stated in Jera (2013) report. Therefore, the cost of electricity is estimated at approximately R21 million per annum (R20 678 024/year).

It was assumed that LP steam, used to heat the material within the autoclave to a temperature of 121°C for sterilisation purposes, enters at 618 kPa and 160°C. Assuming that the stream entering the autoclave is at a temperature of 25°C, the amount of heat required by the stream is calculated as:

Table 23: Factors used for calculation of heating cost

Data		**Reference**
Mass flow rate (m)	62.1 t/h	Mass balance
Specific heat (Cp)	4.180 kJ/kg.°C	(Engineering Toolbox, 2016)
Specific enthalpy of evaporation	2193 kJ/kg	(Engineering Toolbox, 2016)

$$Q = mCp\Delta T = \left(62.1\,\frac{t}{h} \times 1000\,\frac{kg}{t} \times \frac{h}{3600\ s}\right) \times 4.180\,\frac{kJ}{kg.℃} \times (121\ ℃ - 25\ ℃) = \mathbf{6922\ kW}$$

Using this value to calculate the amount of steam required gives:

$$Steam = \frac{Q}{Specific\ enthalpy\ of\ evaporation}\ \frac{6922\ kJ/s \times 3600\ s/h}{2193\ kJ/kg} \times \frac{t}{1000kg} = \mathbf{11.4\ t/h}$$

The cost of steam required was therefore calculated to be R10 325 581 per year. It is noted that heat integration, typical of a continuous steriliser, is not included; however the steam cost I a small fraction of the variable costs, hence this refinement is not included here.

Cooling water is used as a cooling medium for the material flowing from the autoclave to the bioreactor. This stream requires cooling from a temperature of 121°C, to the optimum *M. phlei* growth temperature of 37°C. Therefore:

Table 24: Factors used for calculation of cooling cost

Data		Reference
Mass flow rate (m)	62.1 t/h	Mass balance
Specific heat at 121°C (Cp)	4.248 kJ/kg.°C	(Engineering Toolbox, 2016)
Specific heat at 37°C (Cp)	4.178 kJ/kg.°C	(Engineering Toolbox, 2016)
Cooling water temperature in	30°C	Assumed
Cooling water temperature out	45°C	Assumed

$$Q = mCp\Delta T = \left(62.1\frac{t}{h} \times 1000\frac{kg}{t} \times \frac{h}{3600\ s}\right) \times 4.248\frac{kJ}{kg.℃} \times (121\ ℃ - 37\ ℃) = \mathbf{6155\ kW}$$

Using this value to calculate the amount of cooling water required gives:

$$m = \frac{Q}{Cp\Delta T} = \frac{6155\ kJ/s \times 3600 s/h}{4.178 kJ/kg℃ \times (45\ ℃ - 30\ ℃)} = \mathbf{353\ 580\ kg/h}$$

Assuming a density of 1000 kg/m^3, the cost of cooling water required was then calculated to be R21 681 506.

Although the rest of the system operates at an assumed temperature of 25°C, it was assumed that no effort was made to recover the heat lost from the bioreactor to the centrifuge (i.e. as the stream naturally cools from 37°C to 25°C). This was because a heat exchanger was required between the two pieces of equipment, which had not been included in the developed flowsheet and was consequently not included in the capital costing process. However, in order quantify the amount of energy that would be lost, the above calculations were once again employed, assuming that cooling water would once again be used to cool the stream. It was calculated that, should no heat exchanger be included between the bioreactor and centrifuge, 733 kW would be lost to the environment. A cooling water flowrate of 42.1 t/h would be required to capture this energy, amounting to a utility cost of R2.5 million. This cooling water cost would contribute a mere 5% to the overall utility cost and it was for this reason that its potential inclusion in this study was deemed insignificant. This, combined with the added capital cost of the heat exchanger required for this transaction, served as justification for excluding this loss of heat from the feasibility study.

The summation of the costs required for electricity, LP steam and cooling water amounts to approximately R53 million per annum (R52 685 111/year). This amount could be decreased through the incorporation of heat integration across the units. This would form part of the process optimisation, which was not considered within the scope of this project.

7.2.4.3.4 Miscellaneous costs

The miscellaneous costs were estimated as 10% of the maintenance costs and calculated to be R2 646 129. It was assumed that this cost would covered aspects such as spare parts for the equipment on site.

7.2.4.3.5 Disposal costs

The disposal-cost of the purged growth medium was calculated using data from Seider et al. (2010) to be R1 700 145 per annum.

Therefore, the variable operating costs amounts to a total of R508 476 726/year, of which the meat extract media component accounts for 62%.

7.2.4.3.6 Total operating costs

The summation of the fixed and variable operating costs brings the total operating costs to a value of R613 million per annum (R612 713 088/year), with the contribution of each amounting to 17% and 83%, respectively. This is four times the R150 million/year determined by Jera (2013) for the chemical system. The high variable cost component suggests that it would prove most beneficial to investigate ways in which these can be reduced.

The pie chart breakdown of the costs, presented in Figure 30, shows that the cost of raw materials contributes the majority (74%) towards the overall operating costs. This is consistent with the results of Jera (2013) which found that, for a coal flotation plant processing 250 t/h of coal, the cost of raw materials contributes 72% of the overall costs. Thus, no matter whether the flotation is chemical or biological, the reagents required contribute approximately the same fraction towards the overall operating costs of the operation.

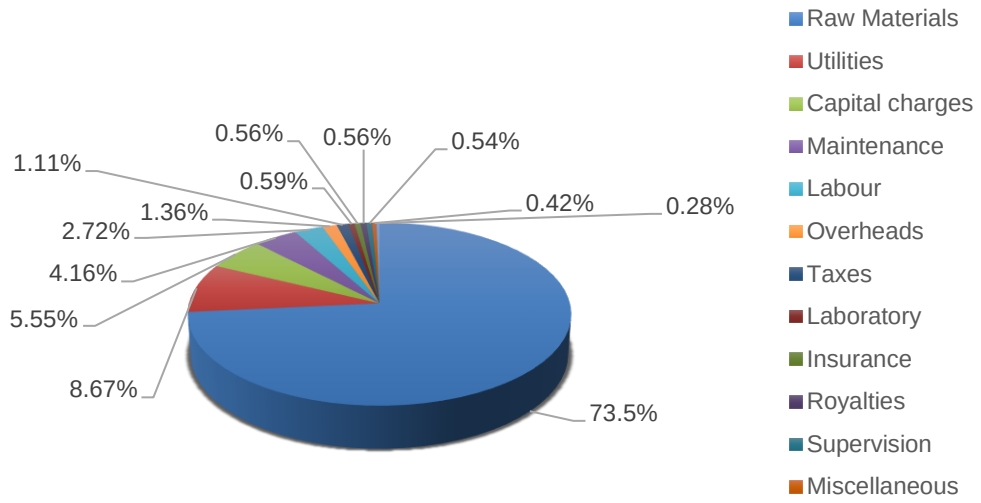

Figure 30: Distribution of total operating costs

7.2.4.4 Profitability analysis results and discussion

The potential profitability of the process was analysed through the determination of several key performance indicators, across a process lifetime of 15 years (Jera, 2013). Before any analysis could be performed, a few parameters needed to be established. These parameters are listed in Table 25.

Table 25: Profitability analysis parameters

Parameter	Value	Reference
Depreciation period	5 years	Assumption
Scrap value	10%	Assumption
Tax rate	28%	Assumption
Escalation rate	5%	Assumption
Discount rate	14%	(Jera, 2013)
Coal selling price	R344/ton coal	(Jera, 2013) scaled to 2016 price
Plant availability	82%	(Jera, 2013)

The financial profitability of the process was assessed through determination of the revenue generated by the process, followed by its gross and net profit. Knowing the net profit, the cumulative and discounted cumulative cash flow projections were calculated, to determine the cash value of the process at the end of its lifetime. This is the value upon which the profitability of the process was determined. Furthermore, the return on investment and payback period were calculated.

Those factors that are evaluated across each subsequent year of operation were scaled in order account for the time value of money. The factors that must take into account the time value of money (e.g. the revenue, operating costs, etc.) were scaled using Equation (14), for which i is the discount or escalation rate and n is the number of periods (days, months or years):

$$Present\ value = \frac{Future\ value}{(1+i)^n} \quad (14)$$

7.2.4.4.1 Revenue Generated and Operating Costs

The revenue generated by the process was calculated by multiplying the coal selling price by the amount of coal produced per annum (stream 18) while taking into account the plant availability. Therefore:

$$Revenue = 15\frac{t}{h} \times \frac{R\ 344}{t} \times 24\frac{h}{day} \times \frac{365 days}{year} \times 82\% = \boldsymbol{R\ 36\ 726\ 012/year}$$

As the operating costs had already been determined, they were multiplied by 82% to account for the plant availability. This brought the yearly operating costs to R502 424 732/year.

The revenue and operating costs were escalated across the 15-year period. The gross profit, earned by the process over its lifetime, could then be calculated as the difference between the revenue and operating costs.

7.2.4.4.2 Depreciation and net profit

The net profit of the process is defined as the profit after tax. However, since the yearly depreciation of equipment is exempt from taxation, this annual amount must first be subtracted from the gross profit.

Depreciation is defined as the decrease in the value of an asset (the process equipment, in this case), over time. The depreciable amount for this process was calculated as follows:

$$Yearly\ depreciation = \frac{Fixed\ capital \times (1 - Scrap\ value)}{Number\ of\ years\ over\ which\ depreciation\ occurs} \quad (15)$$

$$Yearly\ depreciation = \frac{R\ 306\ 797\ 589 \times (1 - 0.1)}{5\ years} = \boldsymbol{R\ 55\ 223\ 566/year}$$

Therefore, for the first five years of operation, R55 million was deducted from the gross profit before the net profit was determined. After five years of operation, the full gross profit amount was taxed. The net profit was therefore calculated as follows, where the text in red was valid for the first five years of operation, only:

$$Net\ profit = (Gross\ profit - depreciation) \times (1 - tax\ rate) \quad (16)$$

7.2.4.4.3 (Discounted) cash and cumulative cash flows

It was assumed that the cash flow calculations begin at year zero (the present time), first depicting the use of the capital investment to bring the plant to a state in which it can begin generating revenue. This takes place across a two-year period. Year three through to year seven then constitutes the first five years of operation.

During these first five years, the cash flow of each year is determined through the addition of the net profit to the depreciation amount, previously subtracted. Thereafter, the cash flow simply constitutes the net profit. The cumulative cash flow sums the amount of cash either earned or lost by the project, across each consecutive year. The discounted cash and cumulative cash flows must account for the time value of money.

7.2.4.4.4 Key Performance Indicators

The amount calculated for the discounted cumulative cash flow in year fifteen corresponds to the value of the process at the end of its lifetime – the net present value (NPV) of the process. This value is highlighted in Figure 31.

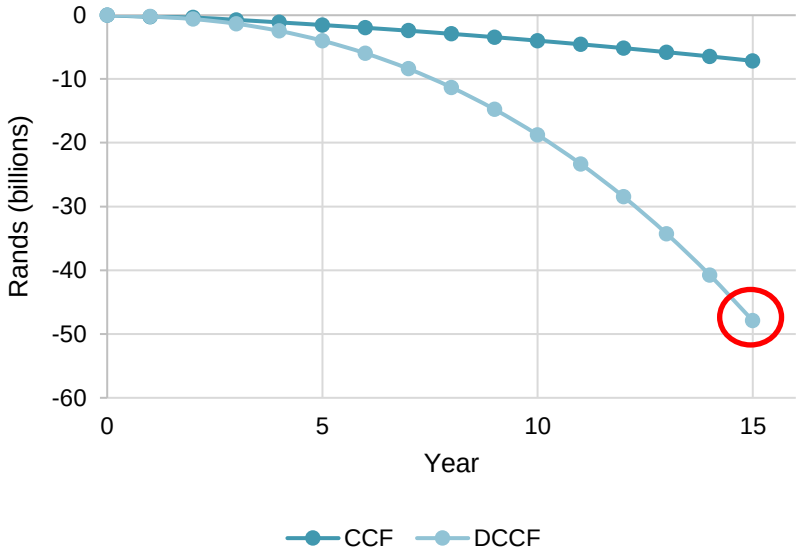

Figure 31: The discounted (DCCF) and cumulative cash flow (CCF) projections across 15 years

According to Figure 31 and the financial projection calculations, the net present value for this process amounts to approximately –R48 billion. This negative NPV indicates that the process is unable to achieve the minimum acceptable rate of return (14%) and, owing to the magnitude of the value, the process is highly unfeasible across a 15-year period if it was required to be self-sustaining.

The return on investment (ROI) and payback period (PBP) were calculated using the following equations:

$$ROI = \frac{Average\ annual\ net\ profit}{Total\ capital\ investment} \quad (17)$$

$$PBP = \frac{Fixed\ capital\ investment}{Average\ annual\ cash\ flow} \quad (18)$$

An internal rate of return (IRR) value was not calculated as the process did not “break even” within 15 years. The key performance indicators are summarised in Table 26, alongside those available for the chemical process in Jera (2013).

Table 26: Summary of the key performance indicators

Indicator	**Current Study**	**Jera** (2013)
NPV (R)	(-) 48 billion	50 million
ROI (%)	(-) 161%	(not mentioned)
PBP (years)	(-) 0.59	(not mentioned)
IRR (%)	-	19

The calculated key performance indicators indicate that bioflotation may not yet be a concept ready for implementation in industry. The negative payback period indicates that the process will not be able to pay back the fixed capital investment within its lifetime. Furthermore, this study confirms that the

bioflotation process incurs a loss on investment of 161% i.e. potential investors would “lose more money” than that invested in the process. Comparing the two NPVs in Table 26 suggests that, at this point in time, chemical flotation is more economically viable than bioflotation as a solid waste treatment process as it is potentially self-sustaining. The high annual operating costs required to run the biological process is the cause behind the largely negative NPV. It is therefore highly unlikely that a change in coal recovery would be enough to ensure project profitability. This is, however, investigated further in the sensitivity analyses.

7.2.4.4.5 Sensitivity analyses

The effect of two key factors were investigated with respect to their effect on the large, negative NPV of the process:

1. the coal yield/recovery
2. the largest operating cost

The effect of changing coal recovery was investigated across a range of 0-100%, increasing the recovery in increments of 20%, the result of which is plotted in Figure 32. A linear relationship is evident in the figure: as coal recovery increases, so does the NPV (the NPV becomes less negative). An increase in recovery of 20% results in an increase in NPV of R2.6 billion. However, even if all the coal in the system were to be recovered, it would still not be enough to ensure the profitability of the process. At a coal recovery of 100%, the value of the process at the end of 15 years would amount to –R38 billion. Therefore, unless the process can generate additional revenue, the ultrafine waste stream does not contain enough coal to generate a revenue which is large enough to offset the high operating costs of the biological process. This confirms that the NPV will not attain a positive value above a coal recovery of 69%, unlike the case presented in Jera (2013) for pure chemical flotation.

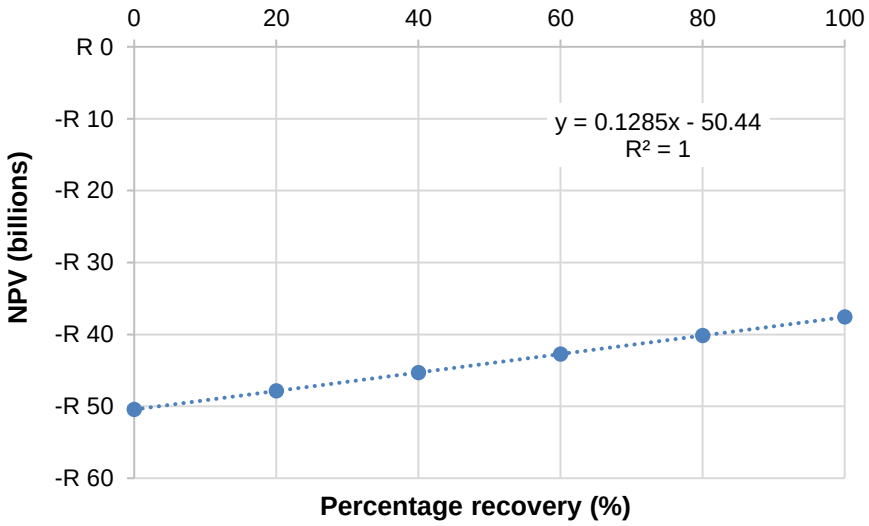

Figure 32: Analysis of the sensitivity of the NPV to coal recovery

According to Figure 29 and Figure 30, the raw materials contribute the majority of cost (74%) towards the total annual operating expenses, with 69% of this cost attributed to the cost of meat or beef extract Figure 29. The cost price of meat extract thus plays one of the largest roles in the unprofitability of the process. Its price influence was investigated through varying the cost price within a range of R0/kg-R20 000/kg, increasing the cost in increments of R4 000/kg. The resulting relationship between the cost price of meat extract and the NPV is of a linear nature (Figure 33), with the NPV increasing as the cost price of meat extract decreases (at a rate of R5.9 billion per decrease of R4 000/kg).

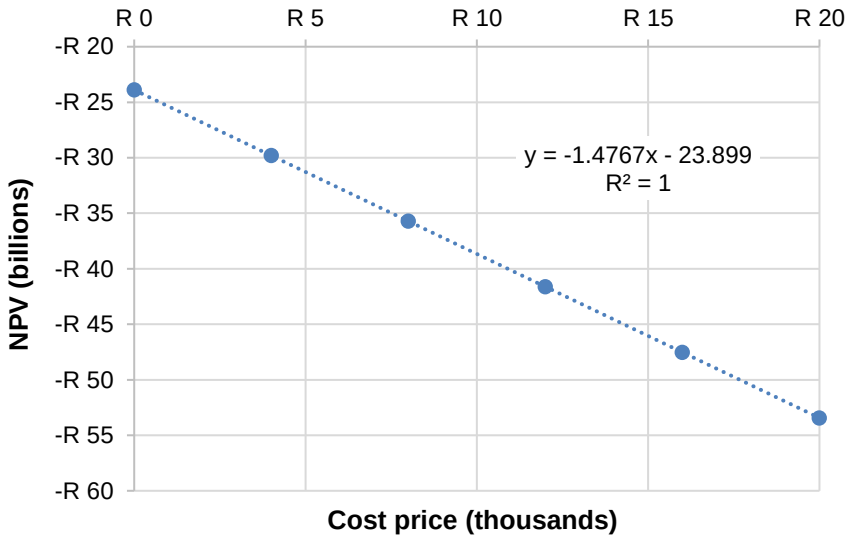

Figure 33: Analysis of the sensitivity of the NPV to the cost price of meat extract

The cost price of raw materials and the selling price of product streams, are factors which cannot be controlled by the process (except for finding a less expensive supplier or a client willing to pay more). The cost price of raw materials and reagents is an aspect of business which is typically determined by the markets, influenced by supply and demand. Therefore, although a reduction in this cost can significantly improve the economics of the process, it is the least likely change to readily occur.

7.2.4.5 Sources of uncertainty

Each decision made in the economic analysis introduces a level of uncertainty. Various sources of uncertainty could be specifically identified as:

- Assumptions made in solving the mass balance (resulting in a loss in mass)
- Information and data used throughout the study was obtained from a number of sources. As each source makes use of different assumptions in their respective study, this introduces inconsistencies and uncertainty in the results obtained for this study.
- Size and dimensions required for the equipment units did not always fall within the ranges over which the cost correlations and charts were developed
- Order of magnitude estimation techniques was used, introducing an uncertainty of 30-50%

Finally, there were several uncertainties in comparison between this study and Jera (2013). These include:

- Different particle size ranges. Jera (2013) analysed the flotation of coal within a particle size range of 80% passing 150 µm, whereas this study used coal passing 200 µm
- Jera (2013) assumed a pulp density of 10 wt% in the flotation cells, whereas in this study 6 wt% was used
- Pre-treatment versus no pre-treatment of the coal waste tailings

7.2.4.6 Process economics conclusions

The large negative NPV of the two-stage flotation process with *M. phlei* used as a bioflotation reagent, contrasted with the marginally positive NPV with oleic acid, indicates that while using microorganisms is technically feasible, it is not economically profitable based on our current knowledge. This holds even given the uncertainties in the estimates. The underlying reason for this, is the massive number of cells (2×10^{16} cells/ton) that are required to float the incoming stream of ultrafine coal waste tailings (100

ton/h) as well as the low concentration of *M. phlei* resulting from the microbial production process. This causes large equipment costs for the *M. phlei* production (R19 532 463) and high operating costs for growth medium components, accounting for 89% of the total raw material costs compared to the 82% for the oleic acid chemical case. Thus, bioflotation using *M. phlei* could only be considered from an economic perspective if additional revenue is available for the other exit streams (sulphide concentrate and gangue tailings from the second-stage).

This economic analysis was based on the lab-scale growth data for the *M. phlei* which was not optimised with respect to medium composition, growth rate and maximum cell density nor were these factors tested at larger scale continuous cultivation. Thus it is recommended that further investigation into these aspects is undertaken to identify cost-savings. The cultivation of *M. phlei* in a stand-alone facility, potentially co-located with other bioindustries, should also be considered. This could be beneficial from technical know-how, process operation efficiency and economies of scale perspectives.

Other bioflotation options using microorganisms are anticipated to have similar issues as similar equipment and growth medium factors would need to be included in the plant design. Therefore, thorough optimisation of their cultivation should be undertaken prior to costing. In the case of algal lipids, this is mostly dependent on the availability of large facilities for their growth such as are currently under development in Upington, South Africa.

CHAPTER 8: Conclusions and Recommendations

8.1 Key Findings and Conclusions

The overarching aim of this project was to investigate the technical, environmental and economic potential of using microorganisms or biologically synthesised products as alternative flotation reagents in the two-stage flotation treatment process for ultrafine sulphide-containing coal waste tailings.

Potential microorganisms and bioproducts were identified from the general microbial and specific bioflotation literature, based on evidence that they are attracted to (attach/modify) surfaces of either coal or sulphide minerals, especially pyrite. This led to the selection of *M. phlei*, algal lipids and derived FAMEs as potential coal bioflotation reagents. The heterotrophs *B. subtilis*, *B. licheniformis*, *P. polymyxa*, *R. opacus* and *R. palustris* as well as the chemolithotrophic *A. thiooxidans*, *A. ferrooxidans* and *L. ferriphilum* were identified as potential pyrite bioflotation reagents.

All the microorganisms, with the only exception of *B. licheniformis*, demonstrated strong selective attachment to the targeted mineral phase (coal or pyrite respectively). The most rapid attachment was observed for *P. polymyxa* to pyrite at pH 7, for which near complete attachment occurred within the first minute. *M. phlei* attachment to coal at pH 7, *R. opacus* attachment to pyrite at both pH 4 and 7 and *P. polymyxa* attachment to pyrite at pH 4 were also observed to occur to completion relatively rapidly, within 5 minutes. The slowest to attach were the chemolithotrophs, which took circa an hour to approach complete attachment.

Coal waste bioflotation experiments in a 3 ℓ Leeds batch flotation cell were successful for *M. phlei*, the RALs and FAMEs. In the best case for *M. phlei* coal flotation separation for a Witbank coal ultrafine waste tailings feed, the same overall flotation yield (between 37 and 40%) was achieved by the microbial system (with or without an MIBC frother) as the chemical reagent equivalent. The *M. phlei* culture was successful in upgrading the coal feed from 51.4% combustibles to 57.3 ± 0.1%. This is encouraging, but still below the performance of the chemical system which upgraded the coal to 71% combustibles. The sulphur recovery to the concentrate was low in all cases (between 20 and 26%), and so the biological system was successful in desulphurising the coal. The RALs and FAMEs, extracted from *Scenedesmus sp.*, were tested in a bioflotation system using an ultrafine Waterberg coal waste tailings feed. The yield to the concentrate was slower in the bioproduct experiments than the chemical reagent float using oleic acid, but after 5 minutes the bioproduct facilitated concentrate yields of 28 ± 1% for the lipids and 30 ± 2% for the FAMEs was only marginally less than the 35 ± 3% achieved by the chemical float. The system with FAMEs as collector achieved the best combustible recovery with an increase from 52.1 ± 0.2% to 79.2 ± 0.6%, compared to 73.3 ± 0.5% for the RAL system and 76.6 ± 2.0% for the chemical system. The FAMEs were also the most successful at desulphurising the coal, with only 19.7 ± 0.1% sulphur being recovered to the concentrate, compared to 28.1 ± 0.1% for the lipids and 25.2 ± 0.1% for the chemical system. Thus, the transformation of the RALs to the FAMEs resulted in a more efficient and selective coal biocollector. One advantage of the RALs bioproduct over the FAMEs was that it was able to create a stable froth in the absence of MIBC, thereby potentially allowing for replacement of both the chemical collector and frother. Additionally, extraction of RALs is expected to be cheaper than for FAMEs due to fewer reagents required in production. Thus, a cost-benefit analysis needs to be performed to compare the RAL and FAME options fully.

Despite the promising good microbe-pyrite attachment results, only *P. polymyxa* yielded competitive pyrite bioflotation results in the experiments in the 3 ℓ Leeds batch flotation cell. All other microorganisms resulted in very poor pyrite recoveries, especially compared to the upwards of 95% sulphur (pyrite) recovery achieved by the chemical float. In all cases an improvement in recovery to the concentrate was observed relative to the negative control (to which no collector was added), confirming that all the species tested did increase the hydrophobicity of the pyrite in the bioflotation. In the most successful

bioflotation with *P. polymyxa*, performed at pH 7, an 81% recovery of pyrite was achieved in the first 2 minutes of flotation. Notably, at this pH the *P. polymyxa* had demonstrated near immediate and complete attachment in the attachment experiments, the highest and most rapid degree of attachment witnessed in this study, as well as an essentially zero surface charge. The pyrite recovery by *P. polymyxa* at pH 4 was lower at only 7%, at which pH a slower rate of attachment was also observed. This is despite the fact that *P. polymyxa* was only marginally positively charged at this pH (+ 0.16). This may indicate that rapid attachment kinetics driving factors and not only surface charge are the critical factors influencing bioflotation.

The financial practicality of producing and of implementing a bioflotation reagent was examined by considering the *M. phlei* case. It was chosen to investigate the financial effects of producing and using this microorganism (rather than the algal bioproducts and *P. polymyxa*) on-site because the previous profitability analyses on the chemical two-stage flotation system showed oleic acid, the first-stage collector, to be the most expensive single operating cost, motivating the investigation of a coal biocollector first. In addition, large scale production of algal lipids is still under development in South Africa. The production of *M. phlei* as part of the process required the inclusion of a mixing tank for the glycerol-soil growth medium, autoclave, continuous bioreactor and disc-stack centrifuge. Together these additional units accounted for 28% of the total purchase cost of equipment. The additional units were designed to produce *M. phlei* at a rate of 2×10^{18} cells/h, a very large flow rate owing to the high cell loading used in the flotation and the low cell concentration displayed to date in the bioreactor. This was then sent to the first-stage pre-conditioning tank, after which the process units were unchanged from the chemical two-stage flotation process. The cost of the growth medium components accounted for 89% of the total raw material costs, with the meat extract (which alone accounted for 69%) having the largest single influence on the net present value (NPV) of the plant. The NPV after 15 years, starting forecasting under current (2016) market conditions, was found to be negative R48 billion, compared to the chemical process which was predicted to have a NPV of R50 million after the same period. The NPV remained negative even for the best-case coal price and a zero cost for meat extract. Thus at this stage, from a profitability perspective, the *M. phlei* biological process does not compete with the chemical process. However, this assessment is made based on unoptimized lab-scale cultivation data for *M. phlei* and thus should only be seen as a preliminary result.

8.2 Recommendations for Future Work

Our main recommendation for future research is, therefore, that investigation into reduction of the financial implications of implementing the use of a bioflotation reagent should be pursued. This should consider the location of the bioreagent production (on-site or off-site), the cost of equipment as well as operating costs (especially growth media). It should be informed by optimisation of both the production of the flotation reagent and performance of the flotation system.

We recommend that optimisation and scale up testing for the growth of both *M. phlei* and *P. polymyxa* is undertaken. The production of RALs and FAMEs should also be explored further, potentially incorporating local sources of carbon (e.g. waste CO_2 sequestration) and other nutrients (e.g. from waste resources). This would need to account for the local land and climatic conditions and the appropriate method for algal growth for the required RAL or FAME feed flowrate. Both on-site production and the sourcing of the algal bioproducts from other large scale producers should be investigated. The potential for integration of multiple products from the algal facility may influence economic profitability, though competition for the lipid-based bioproducts with the bioenergy sector will need to be considered.

Following a standard economic analysis, the cost/benefit of replacing the toxic PAX with *P. polymyxa* should also be fully assessed with respect to long-term environmental risk and hence liability considerations.

REFERENCES

Africa, C.-J., Harrison, S., Becker, M. & van Hille, R., 2010. In situ investigation and visualisation of microbial attachment and colonisation in a heap bioleach environment: The novel biofilm reactor. *Minerals Engineering,* 23(6), pp. 486-491.

Africa, C.-J., van Hille, R., Sand, W. & Harrison, S., 2013b. Investigation and in situ visualisation of interfacial interactions of thermophilic microorganisms with metal-sulphides in a simulated heap environment. *Minerals Engineering,* Volume 48, pp. 100-107.

Africa, C.-J., van Hille, R. & STL, H., 2013a. Attachment of Acidithiobacillus ferrooxidans and Leptospirillum ferriphilum cultured under varying conditions to pyrite, chalcopyrite, low-grade ore and quartz in a packed column reactor. *Applied Microbiology and Biotechnology,* Volume 97, pp. 1317-1324.

Alonso, M., Castaño, C. & Garcia, A., 2000. Performance of vegetable oils as flotation collectors for the recovery of coal from coal fines wastes. *Coal Preparation,* 21(4), pp. 411-420.

Alonso, M., Valdés, A., Martínez-Tarazona, R. & Garcia, A., 2002. Coal recovery from fines cleaning wastes by agglomeration with colza oil: a contribution to the environment and energy preservation. *Fuel Processing Technology,* 75(2), pp. 85-95.

Amini, E., Oliazadeh, M. & Kolahdoozan, M., 2009. Kinetic comparison of biological and conventional flotation of coal. *Minerals Engineering,* 22(4), pp. 344-347.

Aplan, F., 1976. *Coal flotation.* New York, American Institute of Mining, Metalurgical, and Petroleum Engineers, pp. 1235-1262.

ATCC, 2016. *ATCC M. phlei Product Sheet.* s.l.:s.n.

Atkins, A., Bridgwood, E., Davis, A. & Pooley, E., 1987. A study of the suppression of pyritic sulfur in coal froth flotation by Thiobacillus ferrooxidans. *Coal Preparation,* Volume 5, pp. 1-13.

Axelsson, M. & Gentili, F., 2014. A Single-Step Method for Rapid Extraction of Total Lipids from Green Microalgae. *PLoS ONE,* 9(2), p. 6.

Bailey, J. & Ollis, D., 1986. *Biochemical Engineering Fundamentals.* 2nd ed. s.l.:McGraw Hill International Editions.

Benzaazoua, M. et al., 2008. Integrated mine tailings management by combining environmental desulfurization and cemented paste backfill: Application to mine Doyon, Quebec, Canada. *Minerals Engineering,* Volume 21, pp. 330-340.

Benzaazoua, M., Bussière, B. & Kongolo, M., 2000. Environmental desulphurization of four Canadian mine tailings using froth flotation. *International Journal of Mineral Processing,* 60(1), pp. 57-74.

Blake, R. & Griff, M., 2012. In situ spectroscopy on intact Leptospirillum ferroxidans reveals that reduced cytochrome 579 is an obligatory intermediate in the aerobic iron respirator chain. *Frontiers in Microbiology,* Volume 3, pp. 136-144.

Booth, R. & Freyberger, W., 1962. Froths and frothing agents. In: D. Fuerstenau, ed. *Froth Flotation.* New York: AIME, p. 258.

Bromfield, L., Africa, C.-J., Harrison, S. & van Hille, R., 2011. The effect of temperature and culture history on the attachment of Metallosphaera hakonensis to mineral sulphides with application to heap bioleaching. *Minerals Engineering,* 24(11), pp. 1157-1165.

Chandraprabha, M. & Natarajan, K., 2006. Surface chemical and flotation behaviour of chalcopyrite and pyrite in the presence of *Acidithiobacillus thiooxidans*. *Hydrometallurgy,* 83(1-4), pp. 146-152.

Chandraprabha, M. & Natarajan, K., 2009. Microbially Induced Mineral Beneficiation. *Mineral Processing and Extractive Metallurgy Review,* 31(1), pp. 1-29.

Chandraprabha, M., Natarajan, K. & Modak, J., 2004. Selective separation of pyrite and chalcopyrite by biomodulation. *Colloids and Surfaces. B: Biointerfaces,* 37(3-4), pp. 93-100.

Coram, N. J. & Rawlings, D. E., 2002. Molecular relationship between two groups of the genus Leptospirillum and the finding that *Leptospirillum ferriphilum* sp. nov. dominates South African commercial biooxidation tanks that operate at 40°C. *Applied Environmental Microbiology,* 68(2), pp. 838-845.

Curl, H. & O'Donnell, K., 1977. *Chemical and Physical Properties of Refined Petroleum Products,* Boulder, Colorado: Marine Ecosystems Analysis Program.

Das, A. et al., 1999. Surface chemical and adsorption studies using *Thiobacillus ferrooxidans* with reference to bacterial adhesion to sulfide minerals. *Process Metallurgy,* Volume 9, pp. 697-707.

Das, T., Panchanadikar, V. & Chaudhry, R., 1998. Short Communication: Bio-oxidation of iron using *Thiobacillus ferrooxidan*s. *World Journal of Microbiology and Biotechnology,* Volume 14, pp. 297-298.

Demirbas, A., 2002. Demineralization and desulfurization of coals via column froth flotation and different methods. *Energy Conversion and Management,* Volume 43, pp. 885-895.

Deo, N. & Natarajan, K., 1997. Interaction of Bacillus polymyxa with some oxide minerals with reference to mineral beneficiation bacterial strain and growth conditions. *Minerals Engineering,* 10(12), pp. 1339-1354.

Deo, N. & Natarajan, K., 1998. Studies on interaction of *Paenibacullus polymyxa* with iron ore minerals in relation to beneficiation. *International Journal of Mineral Processing,* 55(1), pp. 41-60.

Diao, M. et al., 2014. Differences in adhesion of *A. thiooxidans* and *A. ferrooxidans* on chalcopyrite as revealed by atomic force microscopy with bacterial probes. *Minerals Engineering,* Volume 61, pp. 9-15.

Diao, M., Taran, E., Mahler, S. & Nguyen, A., 2013. *Scanning force spectroscopy and physicochemical analyses of interactions of bacteria with sulphide mineral surfaces.* Brisbane, Australia, Barton, ACT: Engineers Australia.

Dube, R., 2012. *Collectors for enabling flotation of oxidised coal. Unpublished thesis..* s.l.:University of Kentucky.

Dwyer, R., Bruckard, W., Rea, S. & Holmes, R., 2012. Bioflotation and bioflocculation review: microorganisms relevant for mineral beneficiation. *Mineral Processing and Extractive Metallurgy,* 121(2), pp. 65-71.

Eginton, P. et al., 1995. Quantification of the ease of removal of bacteria from surfaces. *Journal of Industrial Mircobiology,* 15(4), pp. 305-310.

Emerson, D., Roden, E. & Twining, B., 2012. *The microbial ferrous wheel: Iron cycling in terrestrial, freshwater and marine environments.* 1st ed. Oregon: Frontiers Media SA.

Engineering Toolbox, 2016. *The Engineering Toolbox.* [Online]
Available at: http://www.engineeringtoolbox.com
[Accessed 01 November 2016].

EPA, 1994. *Extraction and beneficiation of ores and minerals,* Washington DC: U.S. Environmental Protection Agency.

European Biofuels Technology Platform, 2011. *Fatty Acid Methyl Esters (FAME).* [Online]
Available at: http://www.biofuelstp.eu/factsheets/fame-fact-sheet.pdf
[Accessed 1 June 2016].

Faille, C. et al., 2014. Presence and function of a thick mucous layer rich in polysaccharides around Bacillus subtilis spores. *Biofouling,* 30(7), pp. 845-858.

Farahat, M. et al., 2008. Adsorption of SIP E. coli onto quartz and its applications in froth flotation. *Minerals Engineering,* Volume 21, pp. 389-395.

Fazaelipoor, M., Khosahdast, H. & Ranjbar, M., 2009. Coal flotation using a biosurfactant from *Pseudomonas aeruginosa* as a frother. *Korean Journal of Chemical Engineering,* Volume 27, pp. 1527-1531.

Florian, B. M., 2012. *Investigation of initial attachment and biofilm formation of mesophilic leaching bacteria in pure and mixed cultures and their efficiency of pyrite dissolution. Unpublished Thesis.* s.l.:University of Duisburg-Essen.

FTM, 2014. *Flotation Cell (Fote Heavy Machinery).* [Online]
Available at: http://www.chinafote.com/pro/p21.html

Fundikwa, B., 2016. *Environmental Performance Assessment of Froth Flotation for Coal Recovery and Sulfur Removal from Ultrafine Coal Waste.* Unpublished thesis ed. s.l.:University of Cape Town.

Geldenhuis, S. & Bell, F. G., 1998. Acid mine drainage at a coal mine in the Eastern Transvaal, South Africa. *Environmental Geology,* 34(2/3), pp. 234-242.

Govender, Y. & Gericke, M., 2011. Extracellular polymeric substances (EPS) from bioleaching systems and its application in bioflotation. *Minerals Engineering,* 24(11), pp. 1122-1127.

Green, D. W. & Perry, R. H., 2008. *Perry's Chemical Engineers' Handbook.* 8th ed. New York: McGraw Hill.

Griffiths, M., Hille, R. & Harrison, S., 2012. Lipid productivity, settling potential and fatty acid profile of 11 microalgal species grown under nitrogen replete and limited conditions.. *Journal of Applied Phycology,* Volume 24, pp. 989-1001.

Griffiths, M. J., Hille, R. P. v. & Harrison, S. T. L., 2010. Selection of Direct Transesterification as the Preferred Method for Assay of Fatty Acid Content of Microalgae. *Lipids,* 45(11), p. 8.

Gupta, A. K., Banerjee, P. K., Mishra, A. & Satish, P., 2007. Effect of alcohol and polyglycol ether frothers on foam stability, bubble size and coal flotation. *International Journal of Mineral Processing,* 82(3), pp. 126-137.

Hamid, K. & Abbas, S., 2011. Flotation frothers: review of their classifications, properties and preparation. *The Open Mineral Processing Journal,* Volume 4, pp. 25-44.

Han, C., 1983. *Coal cleaning by froth flotation. PhD Thesis.* s.l.:Iowa State University.

Hancock, I. C., 1991. Microbial cell surface architecture. In: N. Mozes, P. S. Handley, H. J. Busscher & R. P. G, eds. *Microbial Cell Surface Analysis.* New York: VCH, pp. 22-59.

Hanumantha Rao, K. & Subramanian, S., 2007. Bioflotation and bioflocculation of relevance to minerals bioprocessing. In: E. R. Donati & W. Sand, eds. *Microbial Processing of Metal Sulfides.* Netherlands: Springer, pp. 267-286.

Harneit, K. et al., 2006. Adhesion to metal sulfide surfaces by cells of *Acidithiobacillus ferrooxidans*, *Acidithiobacillus thiooxidans* and *Leptospirillum ferrooxidans*. *Hydrometallurgy,* 83(1-4), pp. 245-254.

Harrison, S. T. L., 2011. Cell disruption. In: M. Moo-Young & C. Webb, eds. *Engineering Fundamentals of Biotechnology, Comprehensive Biotechnology.* 2 ed. s.l.:s.n., pp. 619-640.

Harrison, S. T. L. et al., 2015. *An Industrial Ecology Approach to Sulphide-containing Mineral Wastes to Minimise ARD Formation. Part 2: Design for Disposal and Extraction of Products of Value. Unpublished Interim Report 5,* Pretoria: Water Research Commission, South Africa.

Harrison, S. T. L. et al., 2010. *A systematic approach to sulphidic waste rock and tailings management to minimise ARD formation,* Pretoria: Water Research Commission.

Harrison, S. T. L. et al., 2013. *Evaluating approaches to and benefits of minimising the formation of acid rock drainage through management of the disposal of sulphidic waste rock and tailings,* Pretoria: Water Research Commission, South Africa.

Harris, T. A., 2001. *The development of a flotation simulation methodology towards an optimisation study of UG2 platinum flotation circuits. Unpublished Thesis.* s.l.:University of Cape Town.

Hesketh, A. H., Broadhurst, J. L. & Harrison, S. T. L., 2010. Mitigating the generation of acid mine drainage from copper sulfide tailings impoundments in perpetuity: A case study for an integrated management strategy. *Minerals Engineering,* Volume 23, pp. 225-229.

Hikmet, S., Ozbayoglu, G. & Sarikaya, M., 2004. Utilization of fine coal tailings by flotation using ionic reagents. *Energy Sources,* 26(10), pp. 941-949.

Hines, J., Dopico, P. & Kennedy, D., 2011. *Novel coal collector derived from pine tree pulping,* Denver, CO: SME Annual Meeting.

Hosseini, T. R. et al., 2005. Bioflotation of Sarcheshmeh copper ore using *Thiobacillus ferrooxidans* bacteria. *Minerals Engineering,* 18(3), pp. 371-374.

Iroala, O., Franzidis, J.-P. & Harrison, S., 2014. *Combining froth flotation with reflux classification to mitigate ARD generating potential of the Waterberg and Witbank coal ultrafines via sulphide removal,* Cape Town: University of Cape Town.

Jera, M. K., 2013. *An economic analysis of coal desulphurisation by froth flotation to prevent acid rock drainage (ARD) and an economic review of capping covers and ARD treatment processes. Unpublished Thesis.* s.l.:University of Cape Town.

Jiang, C. L., Wang, X. H., Parekh, B. K. & Leonard, J. W., 1998. The surface and solution chemistry of pyrite flotation with xanthate in the presence of iron ions. *Colloids and Surfaces A: Physicochemical and Engineering Aspects,* Volume 136, pp. 51-62.

Johnson, B. D., 2003. Chemical and microbiological characteristics of minerals spoils and drainage waters at abandoned coal and metal mines. *Water Air & Soil Pollution: Focus,* Volume 3, pp. 47-66.

Kawatra, S. K., 2009. *Froth Flotation – Fundamental Principles,* Michigan: Michigan Technological University.

Kazadi Mbamba, C., 2011. *Using froth flotation to mitigate acid rock drainage risks while recovering valuable coal from ultrafine colliery wastes. Unpublished Thesis.* s.l.:University of Cape Town.

Kazadi Mbamba, C., Harrison, S. T. L., Broadhurst, J. L. & Franzidis, J.-P., 2012. Mitigating acid rock drainage risk while recovering low-sulphur coal from ultrafine colliery waste using froth flotation. *Minerals Engineering,* Volume 29, pp. 13-21.

Khoshdast, H. & Sam, A., 2011. Flotation frothers: review of their classifications, properties and preparation. *The Open Mineral Processing Journal,* Volume 4, pp. 25-44.

Kim, D.-J.et al., 2008. Effect of pH and temperature on iron oxidation by mesophilic mixed iron oxidizing microflora. *Materials Transactions,* 49(10), pp. 2389-2393.

Kim, G. et al., 2015. Bioflotation of malachite using different growth phases of Rhodococcus opacus: Effect of bacterial shape on detachment by shear flow. *international Journal of Mineral Processing,* Issue 143, pp. 98-104.

Knothe, G., 2006. Analyzing biodiesel: standards and other methods. *Journal of the American Oil Chemists' Society,* Volume 83, p. 11.

Kolmert, A. & Johnson, D. B., 2001. Remediation of acidic waste waters using immobilised acidophilic sulfate-reducing bacteria. *Journal of Chemical Technology and Biotechnology,* Volume 76, pp. 836-843.

Konstantine, K., Matis, K. & Stalidis, G., 1992. Cationic Flotation of Pyrites. *Journal of Colloid and Interfacial Science,* Volume 155, pp. 409-414.

Kou, J., Tao, D. & Xu, G., 2010. Fatty acid collectors for phosphate flotation and their adsorption behavior using QCM-D. *International Journal of Mineral Processing,* 95(1), pp. 1-9.

Kumari, P., Kumar, M., Reddy, C. & Jha, B., 2013. *Algal lipids, fatty acids and sterols.* s.l.:Woodhead Publishing Limited.

Larimer, F. W. et al., 2004. Complete genome sequence of the metabolically versatile photosynthetic bacterium *Rhodopseudomonas palustris*. *Nature Biotechnology,* 22(1), pp. 55-61.

Laskowski, J., 2013. Surface Chemistry Fundamentals in Fine Coal Processing. In: *The Coal Handbook: Towards Cleaner Production Volume 1 – Coal Production.* s.l.:s.n.

Leja, J., 1982. *Surface chemistry of froth flotation.* s.l.:Springer Science & Business Media.

Lens, P. & Pol, L. H., 2000. *Environmental technologies to treat sulfur pollution: Principles and Engineering.* 1 ed. London: IWA.

Liu, J. S., Wang, Z. H., Chen, H. & Zhang, Y. H., 2006. Interfacial electrokinetic characteristics before and after bioleaching microorganism adhesion to pyrite. *Transactions of Nonferrous Metals Society of China,* Volume 16, pp. 676-680.

Loh, H. P., Lyons, J. & White III, C. W., 2002. *Process Equipment Cost Estimation – Final Report.* USA: s.n.

Lottermoser, B., 2003. *Mine Wastes: Characterization, Treatment and Environmental Impacts.* s.l.:Springer.

Mandal, S. & Mallick, N., 2009. Microalga Scenedesmus obliquus as a potential source. *Applied Microbiology and Biotechnology,* 84(2), p. 2.

Meadows, P., 1971. The attachment of bacteria to solid surfaces. *Archives of Microbiology,* Volume 75, pp. 374-381.

Mehrabani, J. V., Mousavi, S. M. & Noaparast, M., 2011. Evaluation of the replacement of NaCN with *Acidithiobacillus ferrooxidans* in the flotation of high-pyrite, low-grade lead-zinc ore. *Separation and Purification Technology,* Volume 80, pp. 202-208.

Mesquita, L., Lins, F. & Torem, M., 2003. Interaction of a hydrophobic bacterium strain in a hematite-quartz flotation system. *International Journal of Mineral processing,* Issue 71, pp. 21-44.

Miller, M. B. & Bassler, B. L., 2001. Quorum sensing in bacteria. *Annual Review of Microbiology,* Volume 55, p. 165 199.

Mishra, M., Smith, R., Dubel, J. & Chen, S., 1993. Selective flocculation of fine coal and with hydrophobic Mycobacterium phlei. *Mineral and Metallurgy Processing,* Volume 10, pp. 20-23.

Misra, M., Bukka, K. & Chen, S., 1996. The effect of growth medium of *Thiobacillus ferrooxidans* on pyrite flotation. *Minerals Engineering,* Volume 9, pp. 157-168.

Misra, M. & Chen, S., 1995. The effect of growth medium of *Thiobacillus ferrooxidans* on pyrite and galena flotation. In: D. S. Holmes & R. W. Smith, eds. *Minerals Bioprocessing II.* Pennsylvania: The Minerals, Metals and Materials Society, pp. 313-322.

Mittal, M. L., Sharma, C. & Singh, R., 2012. Estimates of emissions from coal fired thermal power plants in India. In: *2012 International Emission Inventory Conference.* s.l.:s.n.

Moon, J., 1995. *Quantification of biomass in a biooxidation system.* Unpublished thesis ed. s.l.:University of Cape Town.

Moo-Young, M., 2011. *Comprehensive biotechnology.* 2nd ed. Netherlands: Elsevier.

Murphy, R. & Strongin, D., 2009. Surface reactivity of pyrite and related sulfide. *Surface Science Reports,* 64(1), pp. 1-45.

Nagaoka, T., Ohmura, N. & Saiki, H., 1999. A novel mineral flotation process using *Thiobacillus ferrooxidans*. *Applied and Environmental Microbiology,* 65(8), pp. 3588-3593.

Naicker, K., Cukrowska, E. & McCarthy, T. S., 2003. Acid mine drainage arising from gold mining activity in Johannesburg, South Africa and environs. *Environmental Pollution,* Volume 122, pp. 29-40.

Natarajan, K. A., 2008. Microbial aspects of acid mine drainage and its bioremediation. *Transaction of Nonferrous Metals Society of China,* Volume 18, pp. 1352-1360.

Natarajan, K. A. & Das, A., 2003. Surface chemical studies on *Acidithiobacillus* group of bacteria with reference to mineral flocculation. *International Journal of Mineral Processing,* Volume 72, pp. 189-198.

Nordstrom, D. K. & Alpers, C. N., 1999. Geochemistry of acid mine waters. In: G. S. Plumlee & M. J. Logsdon, eds. *The Environmental Geochemistry of Mineral Deposits. Part A: Processes, Techniques and Health Issues.* s.l.:Reviews in Economic Geology, pp. 133-160.

Olson, G. J., 1991. Rate of pyrite bioleaching by *Thiobacillus ferrooxidans*: results of an interlaboratory comparison. *Applied and Environmental Microbiology,* 57(3), pp. 642-644.

Oner, E. T., 2013. Microbial production of extracellular polysaccharides from biomass. In: Z. Fang, ed. *Pretreatment Techniques for Biofuels and Biorefineries.* Berlin: Springer, pp. 23-56.

Patra, P. & Natarajan, K., 2003. Microbially-induced flocculation and flotation for pyrite separation from oxide gangue minerals. *Minerals Engineering,* Volume 16, pp. 965-973.

Patra, P. & Natarajan, K., 2004a. Microbially enhanced removal of pyrite and chalcopyrite from oxide gangue minerals with reference to desulphurization of tailings. *Minerals and Metallurgical Processing,* 21(4), pp. 169-178.

Patra, P. & Natarajan, K. A., 2004b. Microbially induced flotation and flocculation of pyrite and sphalerite. *Colloids Surfaces B,* Volume 36, p. 91-99.

Patra, P. & Natarajan, K. A., 2008a. Role of mineral specific bacterial proteins in selective flocculation and flotation. *International Journal of Mineral Processing,* 88(1-2), pp. 53-58.

Patra, P. & Natarajan, K. A., 2008b. Microbially-induced separation of chalcopyrite and galena. *Minerals Engineering,* 21(10), pp. 691-698.

Pearse, M. J., 2005. An overview of the use of chemical reagents in mineral processing. *Minerals Engineering,* 18(2), pp. 139-149.

Pele, M. & Cimpeanu, C., 2012. *Biotechnology: An Introduction.* Boston: WIT Press.

Pesic, B. & Kim, I., 1993. Electrochemistry of *Thiobacillus ferrooxidans* interactions with pyrite. *Metallurgical Transactions B,* 24(5), pp. 717-727.

Platt, T. G. & Fuqua, C., 2010. What's is a name? The semantics of quorum sensing. *Trends in Microbiology,* Volume 18, pp. 383-387.

Rahman, R. M., Ata, S. & Jameson, G. J., 2012. The effect of flotation variables on the recovery of different paricle size fractions in the froth and the pulp. *International Journal of Mineral Processing,* 106-109(1), pp. 70-77.

Raichur, A. M., Misra, M., Bukka, K. & Smith, R. W., 1996. Flocculation and flotation of coal by adhesion of hydrophobic *Mycobacterium phlei*. *Colloids and Surfaces B: Biosurfaces,* Volume 8, pp. 13-24.

Rao, M. K. Y., Natarajan, K. & Somasundaran, P., 1991. Effect of bacterial conditioning of sphalerite and galena with *Thiobacillus ferrooxidans* on their floatability. In: S. R. W & M. M, eds. *Mineral Bioprocessing.* Pennsylvania: The Minerals, Metals and Materials Society, pp. 105-120.

Ratledge, C. & Kristiansen, B., 2001. *Basic Biotechnology.* 2nd ed. Canada: Cambridge University Press.

Razatos, A., Ong, Y. L., Sharma, M. M. & Georgiou, G., 1998. Molecular determinants of bacterial ahdesion monitored by atomic force microscopy. *Proceedings of the National Academy of Sciences of the United States of America,* 95(19), pp. 11059-11064.

Reddick, J., 2006. *An investigation of cleaner production opportunities in the South African coal mining industry. Unpublished Thesis.* s.l.:University of Cape Town.

Rey, M. W. et al., 2004. Complete genome sequence of the industrial bacterium *Bacillus licheniformis* and comparisons with closely related Bacillus species. *Genome Biology,* 5(10), p. R77.

Rohwerder, T., Gehrke, T., Kinzler, K. & Sand, W., 2003. Bioleaching review part A: Progress in bioleaching: fundamentals and mechanisms of bacterial metal sulphide oxidation. *Applied Microbiology and Biotechnology,* Volume 63, pp. 239-248.

Sarvamangala, H., Natarajan, K. A. & Girisha, S. T., 2013. Microbially-induced pyrite removal from galena using *Bacillus subtilis*. *International Journal of Mineral Processing,* Volume 120, pp. 15-21.

Schippers, A., 2007. Microorganisms involved in bioleaching and nucleic acid-based molecular methods for their identification and quantification. In: E. R. Donati & W. Sand, eds. *Microbial Processing of Metal Sulfide.* s.l.:Springer, pp. 3-33.

Schippers, A. & Sand, W., 1999. Bacterial leaching of metal sulphides proceeds by two indirect mechanisms via thiosulphate or via polysulphides and sulphur. *Applied and Environmental Microbiology,* Volume 65, pp. 319-321.

Schlagermann, P. et al., 2012. Composition of algal oil and its potential as biofuel. *Journal of Combustion,* 2012(14).

Seider, W. D., Seader, J. D., Lewin, D. R. & Widagdo, S., 2010. *Product and Process Design Principles: Synthesis, Analysis, and Evaluation.* 3rd ed. s.l.:John Wiley & Sons.

Sharma, P., 2001. *Surface studies relevant to microbial adhesion and bioflotation of sulphide minerals,* Lulea: Lulea University of Technology.

Sharma, P. & Hanumantha Rao, K., 1999. Role of heterotrophic *Paenibacillus polymyxa* bacteria in the bioflotation of some sulphide minerals. *Minerals and Metallurgical Processing,* 16(4), pp. 35-41.

Sharma, P., Hanumantha Rao, K., Forssenberg, K. & Natarajan, K., 2001. Surface chemical characterisation of *Paenibacillus polymyxa* before and after adaptation to sulphide minerals. *International Journal of Mineral Processing,* Volume 62, pp. 3-25.

Shen, W. Z., Fornasiero, D. & Ralston, J., 1997. Effect of collectors, conditioning pH ad gases in the separation of sphalerite from pyrite. *Minerals Engineering,* 11(2), p. 145-158.

Shih, I. L. et al., 2001. Production of a biopolymer flocculant from Bacillus licheniformis and its flocculation properties. *Bioresource Technology ,* Volume 78, pp. 267-272.

Sinnott, R. K., 2005. *Coulson & Richardson's Chemical Engineering Volume 6: Chemical Engineering Design.* 4th ed. Oxford: Elsevier Butterworth-Heinemann.

Sisa, H., Ozbayoglub, G. & Sarikaya, M., 2003. Comparison of non-ionic and ionic collectors in the flotation of coal fines. *Minerals Engineering,* Volume 16, pp. 399-401.

Smith, R. W. & Misra, M., 1991. Mineral bioprocessing – an overview. In: R. W. Smith & M. Misra, eds. *Mineral Bioprocessing.* Pennsylvania: The Minerals, Metals and Materials Society, pp. 3-26.

Smith, R. W., Misra, M. & Raichur, A., 1995. Microorganisms in minerals processing. In: D. S. Holmes & S. R. W, eds. *Minerals Bioprocessing II.* Pennsylvania: The Minerals, Metals and Materials Society, pp. 313-322.

Somasundaran, P., Deo, N. & Natarajan, K., 2001. Utility of Bioreagents in Mineral Processing. In: S. a. N. K. Kawatra, ed. *Mineral Biotechnology: Microbial Aspects of Mineral Beneficiation, Metal Extraction and Environmental Control.* Littleton: Society for Mining, Metallurgy and Exploration, pp. 221-229.

Stanley, S., Ananadhi Padmanabhan, M. & Anitha, A., 2010. Studies on the extraction and characterisation of microalgal oil. *National Journal on ChemBiosis,* 1(2), pp. 1-3.

Stewart, M., 1999. *Environmental decision-making in minerals processing design. Unpublished Thesis.* s.l.:University of Cape Town.

Subramanian, S., Santhiya, D. & Natarajan, K., 2003. Surface modification studies on sulphide minerals using bioreagents. *International Journal of Mineral Processing,* 72(1-4), pp. 175-188.

Suzuki, I., 2001. Microbial leaching of metals from sulfide minerals. *Biotechnology Advances,* 19(2), pp. 119-132.

Talaro, K. P. & Talaro, A., 1999. *Foundations in Microbiology.* 3 ed. New York: McGraw Hill.

Towler, G. & Sinnott, R. K., 2008. *Chemical Engineering Design – Principles, Practice and Economics of Plant and Process Design.* 2nd ed. s.l.:Elsevier.

Van der Linden, J. P., 1987. *Liquid-Liquid Separation In Disc-Stack Centrifuges.* Unpublished thesis ed. s.l.:University of Delft.

Van der Wal, A., Norde, A., Zehender, A. & Lyklema, J., 1997. A determination of the total charge in the cell walls of gram positive bacteria. *Colloids and Surfaces B: Biointerfaces,* Volume 9, pp. 81-100.

Van Loosdrecht, M. C. M. et al., 1987. The role of bacterial cell wall hydrophobicity in adhesion. *Applied and Environmental Microbiology,* 53(8), p. 1893 1897.

Vasumathi, V. et al., 2013. Eco-friendly and cost-effective reagent for coal flotation. *International Journal of Engineering Research,* 2(7), pp. 418-423.

Verster, B. et al., 2013. *Introducing the wastewater Biorefinary concept: A scoping study of poly-glutamic ascid production from Bacillus rich mixed culture using municipal water,* Pretoria: Water Research Council.

Vijayalakshmi, S. P. & Raichur, A. M., 2003. The utility of *Bacillus subtilis* as a bioflocculant for fine coal. *Colloids and Surfaces B: Biointerfaces,* Volume 29, p. 265-275.

Vilinska, A., 2007. *Bacteria-sulfide mineral interactions with reference to flotation and flocculation. Licentiate Thesis.* s.l.:Luleå University of Technology.

Vilinska, A. & Hanumantha Rao, K., 2008. *Leptospirillum ferrooxidans* – sulfide mineral interactions with reference to bioflotation and bioflocculation. *Transactions of Nonferrous Metals Society of China,* 18(6), pp. 1403-1409.

Watling, H. R., 2006. The bioleaching of sulphide minerals with emphasis on copper sulphides – A review. *Hydrometallurgy,* Volume 84, pp. 81-108.

Wills, B., 1997. *Mineral Processing Technology.* 6th ed. Oxford: Butterworth-Heinemann.

Wright, B. A., 1999. *The development of a vision-based flotation froth analysis system. Unpublished Thesis.* s.l.:University of Cape Town.

Xia, W., Yang, J. & Liang, C., 2013. Improving Oxidized Coal Flotation Using Biodiesel as a Collector. *International Journal of Coal Preparation and Utilization,* Volume 33, pp. 181-187.

Xu, G., Zhang, J. & Song, G., 2003. Effect of complexation on the zeta potiental of silica powder. *Powder Technology,* Volume 134, pp. 218-222.

Yi, Q. et al., 2015. Tech-economic evaluation of waste cooking oil to bio-flotation agent technology in the coal flotation industry. *Journal of Cleaner Production,* Volume 95, pp. 131-141.

Yi, Q. et al., 2015. Tech-economic evaluation of waste cooking oil to bio-flotation agent technology in the coal flotation industry. *Journal of Cleaner Production,* 95(2011), p. 9.

Zheng, X. & Smith, R. W., 1997. Dolomite depressants in the flotation of apatite and cellophane from dolomite. *Minerals Engineering,* Volume 10, pp. 537-545.

APPENDIX A: **METHODOLOGIES**

A.1 Sample Particle Size Distributions

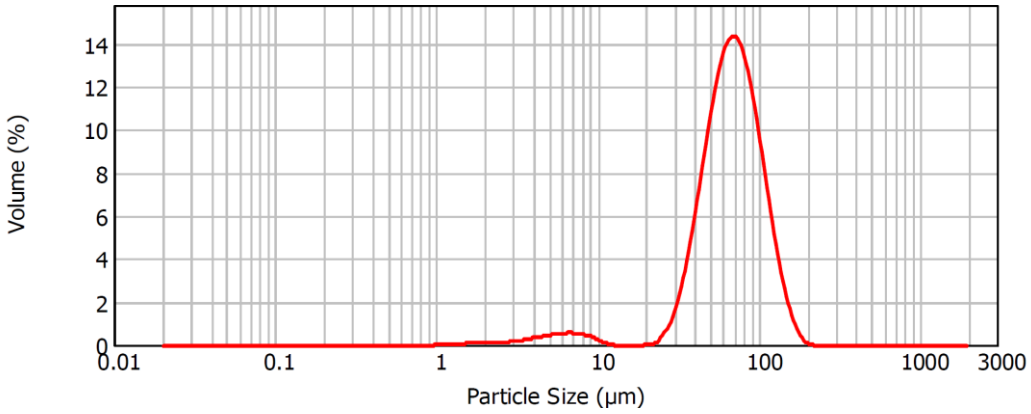

Figure 34: Particle size distribution for quartz sample, generated through a Malvern analysis

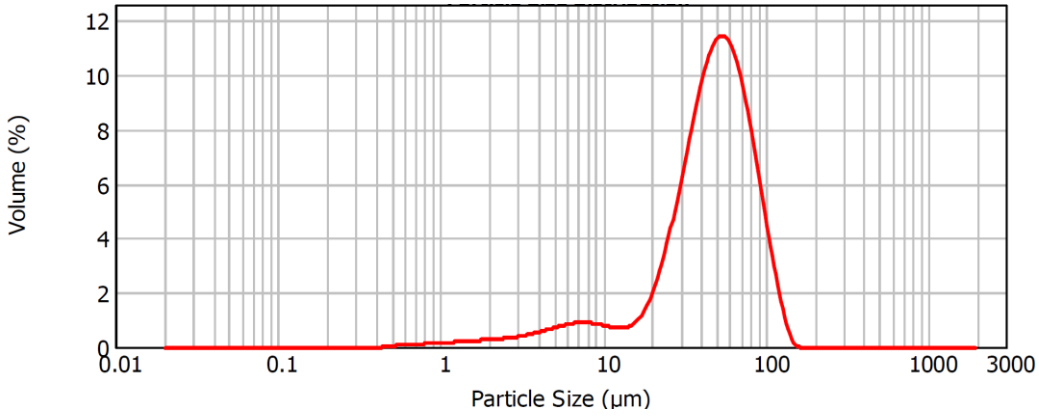

Figure 35: Particle size distribution for pyrite sample, generated through a Malvern analysis

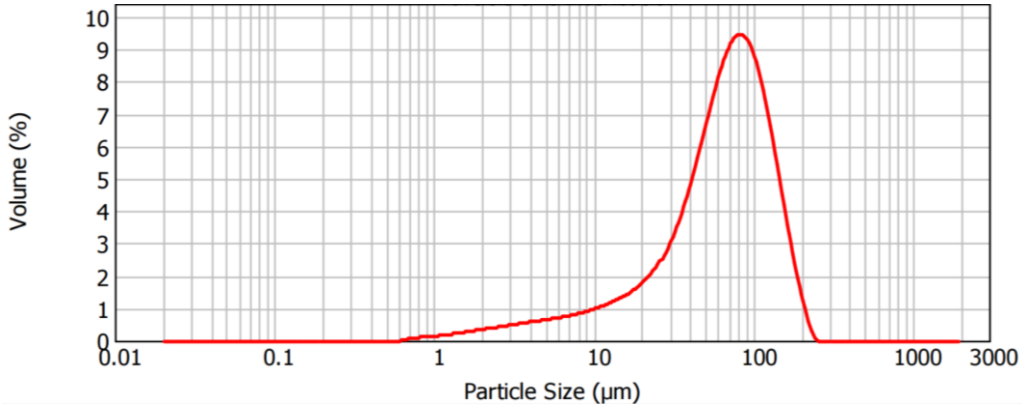

Figure 36: Particle size distribution for Witbank ultrafine coal sample, generated through a Malvern analysis

A.2 Growth media

A.2.1 0K BSM medium

The 0K basal salt medium (BSM) is recommended for the growth of mesophilic bioleaching microorganisms. The salts listed in Table 27 are dissolved one at a time in 800 mℓ of deionised water which has been acidified to the selected pH (generally 1.6-1.8) with H_2SO_4. This is then supplemented with a trace element solution (Kolmert & Johnson, 2001) and made up to the final 1 ℓ volume.

Table 27: 0K BSM recipe

Substance	Chemical formula	Quantity (g/ℓ)
Ammonium sulphate	$(NH_4)_2SO_4$	3.0
Potassium chloride	KCl	0.1
Dipotassium phosphate	K_2HPO_4	0.5
Magnesium sulphate	$MgSO_4.7H_2O$	0.5
Calcium nitrate	$Ca(NO_3)_2$	0.01

A.2.2 Glucose Yeast Malt (GYM) Streptomyces medium

Glucose Yeast Malt (GYM) Streptomyces medium was prepared according to a DSMZ 65 recipe designed for *R. opacus* cultivation. The components listed in Table 28 are dissolved in 800 mℓ of deionised water, adjusted to a pH of 7.2, made up to a final volume of 1 ℓ and autoclaved at 121°C for 15-20 minutes.

Table 28: Glucose Yeast Malt (GYM) Streptomyces medium composition (g/ℓ)

Substance	Chemical formula	Quantity (g)
Glucose	$C_6H_{12}O_6$	4
Malt extract	-	10
Yeast extract	-	4
Deionised water	H_2O	1000

A.2.3 Tryptic-soy medium

Tryptic-soy medium was used for the cultivation of *B. licheniformis.* According to the preparation instructions 30 g of the Tryptic-soy is dissolved in 800 mℓ of deionised water, adjusted to a pH of 7.3, made up to a final volume of 1 ℓ and autoclaved at 121°C for 15-20 minutes.

A.2.4 *Rhodospirillaceae* medium

A modified *Rhodospirillaceae* medium was used for the cultivation of a *R. palustris* culture. The components listed in Table 29 are dissolved in 1800 mℓ of deionised water, adjusted to a pH of 7.4, made up to a final volume of 2 ℓ and autoclaved at 121°C for 15-20 minutes. Once the medium has cooled down 1 mℓ/ℓ glycerol, 1 mℓ/ℓ vitamin solution, 1 mℓ/ℓ trace element solution and 0.5 mℓ/ℓ glutamate are added, after which the medium is inoculated and nitrogen gas is sparged to create an anaerobic condition for cultivation. The components used to make the vitamin and trace element solutions are listed in Table 30 and Table 31 respectively.

Table 29: Modified *Rhodospirillaceae* medium composition (g/ℓ)

Substance	Chemical formula	Quantity (g)
Potassium di-hydrogen orthophosphate	KH_2PO_4	3.4
Di-potassium hydrogen phosphate	K_2HPO_4	3.4
Yeast extract	-	0.4
Sodium thiosulphate	NaS_2O_3	0.3162
Sodium chloride	NaCl	0.8
Iron citrate	$C_6H_5FeO_7$	0.01
Magnesium sulphate heptahydrate	$MgSO_4 \cdot 7H_2O$	0.4
Calcium chloride dihydrate	$CaCl_2 \cdot 2H_2O$	0.1
Glycerol	$C_3H_8O_3$	1
Glutamate	$C_5H_9NO_4$	0.5
Deionised water	H_2O	2000

Table 30: Vitamin solution composition (g/100mℓ)

Substance	Chemical formula	Quantity (g)
Thiamine hydrochloride	$C_{12}H_{17}ClN_4OS \cdot HCl$	0.12
Vitamin B12		0.001
Deionised water	H_2O	100

Table 31: Trace element solution composition (mg/ℓ)

Substance	Chemical formula	Quantity (mg)
Zinc chloride	$C_{12}H_{17}ClN_4OS \cdot HCl$	70
Manganese chloride tetrahydrate	$MnCl_2 \cdot 4H_2O$	100
Boric acid	H_3BO_3	60
Cobalt chloride hexahydrate	$CoCl_2 \cdot 6H_2O$	200
Copper chloride dihydrate	$CuCl_2 \cdot 2H_2O$	20
Nickel chloride hexahydrate	$NiCl_2 \cdot 6H_2O$	20
Sodium molybdate dihydrate	$NaMoO_4 \cdot 2H_2O$	40
Deionised water	H_2O	1000

A.2.5 Modified Bromfield medium

A modified Bromfield medium was used for the cultivation of a *P. polymyxa* culture. The components listed in Table 32 are dissolved in 800 mℓ of deionised water, adjusted to a pH of 7.0, made up to a final volume of 1 ℓ and autoclaved at 121°C for 15-20 minutes. Patra & Natarajan 2004a, investigated cell growth with increase in percentage of sucrose and found that using 2 wt% increased the maximum cell number. Hence in this study the sucrose concentration was increased from 0.5 wt% to 2 wt% to achieve maximum cell number.

Table 32: Modified Bromfield medium composition (g/ℓ)

Substance	Chemical formula	Quantity (g)
Potassium di-hydrogen orthophosphate	KH_2PO_4	0.50
Magnesium sulphate heptahydrate	$MgSO_4 \cdot 7H_2O$	0.20
Ammonium sulphate	$(NH_4)_2SO_4$	1.00
Sucrose (0.5 wt%)		5.00
Yeast extract		0.15

A.2.6 Glycerol-soil medium

The glycerol-soil medium was prepared according to a DSMZ 80 recipe designed for *M. phlei*. Soil extract is prepared by sieving garden soil through a 1 mm aperture sieve and then autoclaving 400 g of this mixed with 960 mℓ of deionised water at 121°C for 1 hour. After the mixture has cooled and settled, the supernatant is decanted, filtered and autoclaved again in 200 mℓ batches and left to cool and settle. Finally, the substances found in Table 33 are mixed into a 1 ℓ Schott bottle, adjusted to a pH of 7 and autoclaved at 121°C for 15-20 minutes.

Table 33: Glycerol-soil medium composition (1 ℓ)

Substance	Chemical formula	Quantity (g)
Peptone	-	5
Beef extract	-	3
Glycerol	$C_3H_8O_3$	20
Soil extract	-	150
Deionised water	H_2O	850

A.2.7 Bold's basal medium (3N BBM)

The medium for algae growth was made according to the 3N BBM recipe. Combine 3 mℓ of each macro-element stock in deionised water (Table 34) with 6 mℓ of the PIV metal solution detailed below. Make up to 1 ℓ with deionised water and autoclave. Once cooled, add 1 mℓ of each of the two vitamin stocks. (Note: do not autoclave the vitamins as they degrade when heated).

This recipe was modified by lowering the nitrogen concentration from the recommended 750 mg/ℓ to 150 mg/ℓ to induce higher lipid production.

Table 34: 3N BBM composition (1 ℓ)

Substance	Chemical formula	Quantity (g)
Sodium nitrate	$NaNO_3$	25.0
Calcium chloride	$CaCl_2.2H_2O$	2.5
Magnesium sulphate	$MgSO_4.7H_2O$	7.5
Potassium phosphate (dibasic)	$K_2HPO_4.3H_2O$	7.5
Potassium phosphate (monobasic)	KH_2PO_4	17.5
Sodium chloride	NaCl	2.5

The PIV solution should look yellow, be autoclaved and stores in the dark. It is made as follows:

- Add to 1 ℓ of deionised water 0.75 g Na_2EDTA and the minerals in Table 35 in exactly the following sequence:

Table 35: Trace elements to make the PIV solution (1 ℓ)

Substance	Chemical formula	Quantity (mg)
Iron (III) chloride	$FeCl_3.6H_2O$	97.0
Manganese chloride	$MnCl_2.4H_2O$	41.0
Zinc Chloride	$ZnCl_2$	5.0
Cobalt chloride	$CoCl_2.6H_2O$	2.0
Sodium molybdate	$Na_2MoO_4.2H_2O$	4.0

The vitamin stocks are made as follows:

- Add 0.12 g vitamin B1 (Thiamine HCl) to 100 mℓ deionised water and filter sterilise.
- Add 0.1 g vitamin B12 (cyanocobalamin) to 1 ℓ deionised H_2O. Take 1 mℓ of this and add to 99 ml of distilled water. Filter sterilise and store in the dark when not in use.