Desulphurisation flotation for the selective removal of pyrite from coal discards using microorganisms

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Declaration

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Synopsis

Mineral beneficiation processes such as base metal and coal mining produce large amounts of waste rock and coal discards that contain significant quantities of sulphide minerals with Acid Rock Drainage (ARD) generating potential. ARD is caused by the exposure of sulphide minerals, primarily pyrite (FeS_2), to both water and oxygen, and microorganisms. This is a naturally occurring process, but the exposure of the sulphide containing mining wastes greatly accelerates ARD formation. Thus, ARD is a major issue associated with inactive mines, waste rock dumps and tailings impoundments, which over time presents a major environmental risk.

The desulphurisation of coal discards, mine tailings and finely divided waste rock prior to their disposal has been proposed as a method of preventing ARD formation. This involves the selective separation of residual values from the waste rock, followed by selective separation of sulphide minerals – especially pyrite – from the residual waste material using a two-stage froth flotation to obtain a values stream, a low volume sulphide-rich concentrate that can be easily contained, and a high volume benign tailings fraction that can be safely disposed of. The technical feasibility of this two-stage process has been demonstrated; however, the cost of the flotation reagents used in this process are particularly high in comparison to the other operating costs, contributing as much as 75% of the operating costs for desulphurisation of coal fines. Furthermore, apart from being expensive, many of the inorganic flotation reagents are relatively toxic and could be hazardous to the environment due to their slow degradation rate.

Microorganisms and their metabolic products have been identified in literature as potential reagents that can be used in the selective separation of sulphide minerals using froth flotation. Just like conventional chemical flotation reagents, the microorganisms assist separation through surface chemical alterations that modify a mineral's hydrophobic properties, thus facilitating bioflotation. The aim of this study was to investigate the prevention of ARD formation through the desulphurisation of pyrite-containing coal discards and base metal hard rock samples using microbial cultures as alternative bioflotation reagents. In this study the feasibility of using *P. polymyxa, R. palustris, R. opacus, B. subtilis,* and *B. licheniformis* as bio-collectors for the removal of pyritic sulphur in the second stage of the two-stage desulphurisation froth flotation process was investigated.

Microbial screening tests were performed using a pyrite concentrate to assess each microbial culture's affinity to pyrite and their ability to float the mineral in a batch flotation cell. Attachment experiments and batch bioflotation tests were carried out to screen for a microbial culture that showed potential. Following attachment experiments at pH 4 and pH 7, all microorganisms except *B. licheniformis* exhibited attachment to pyrite. The level of attachment was different

for each microbial culture. *P. polymyxa* had the highest percentage attachment of 95.6 ± 1.0 % at pH 4 and 97.1 ± 0.7 % at pH 7 after 20 minutes of interaction.

Subsequent results from the pyrite-only bioflotation tests revealed that *R. opacus, R. palustris* and *B. subtilis* did not affect the floatability of pyrite. *P. polymyxa*, however, showed a significant effect on the floatability of pyrite, achieving a cumulative mass recovery of 7.0 ± 0.42 % at pH 4 and 81.3 ± 0.4 % at pH 7. Zeta-potential tests revealed that *P. polymyxa* had the most neutral net surface charge across the pH range tested, while the other microorganisms had a large net positive or negative charge. Based on this result, it was deduced that the hydrophobicity of *P. polymyxa* as a consequence of its near neutral surface strongly made it seek out a surface to attach to rather than remaining suspended in water. Hence, *P. polymyxa* was chosen as the bio-collector candidate for the bioflotation separation of pyritic sulphur from coal discard and base metal hard rock samples.

Despite the positive batch pyrite bioflotation tests, *P. polymyxa* was not successful for the flotation of pyrite from the coal discards nor did it upgrade pyritic sulphur to the concentrate, with the bioflotation results not significantly different from the negative control without collector. *P. polymyxa* did affect the floatability of the base metal hard rock, achieving cumulative mass recoveries comparable with the chemical control using PAX. However, there was no significant upgrade of pyritic sulphur content, with the biofloat achieving 22.6 % total sulphur in the concentrate which was significantly less than the 66.4 % total sulphur recovered with PAX.

The study thus yielded positive results from fundamental studies of *P. polymyxa*'s ability to enhance the flotability of pyrite. However, tests using actual samples were less successful. Although *P. polymyxa* enhanced the floatability of the base metal hard rock, it did not achieve the aim of obtaining a low volume sulphide-rich concentrate as the PAX did. Recommendations for the continuation of this work include contact angle measurements and FT-IR spectroscopy to better understand the effects of *P. polymyxa* attachment, as well as performing a kinetic study on the growth of *P. polymyxa* alongside adaptation of the microbial culture to a pyrite mineral concentrate in order to test if this can improve selective flotation of the desired mineral owing to modified surface properties.

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Acronyms and abbreviations

ACRONYMS

ARD	Acid Rock Drainage
ASTER	Activated Sludge Tailings Effluent Remediation
BM	Base metal
EPS	Exopolysaccharide
FAME	Fatty acid methyl esters
MIBC	Methyl Isobutyl Carbinol
OA	Oleic Acid
PAX	Potassium amyl xanthate
ROM	Run-of-mine
SEM	Scanning Electron Microscopy
XRF	X-ray fluorescence
vvm	Vessel Volumes per Minute

ABBREVIATIONS

Θ	Contact angle
º	Degree Celsius (centigrade)
pH	Measure of acid concentration ($-\log(H^+)$)
rpm	Revolutions per minute
mV	millivolt
t_d	Doubling time (h^{-1})

CHAPTER 1: Introduction

The long-term generation of Acid Rock Drainage (ARD) is one of the major environmental issues within the mining industry (Akcii & Koldas, 2006). ARD is perpetuated through the disposal of large volumes of sulphide-bearing waste rock tailings that are produced following the extraction and processing of sulphide-bearing ores and coal deposits (Simate & Ndlovu, 2014). Pyrite, as the most abundant sulphide mineral in waste rock tailing dumps, is the primary mineral responsible for ARD generation because of its oxidative dissolution to acid producing sulphates in the presence of water and oxygen.

Conventional ARD treatment and management strategies rely on end-of-pipe treatment technologies which are aimed at preventing the dispersion of ARD in the environment through its treatment or at delaying its onset, but do not necessarily address the problem in the long term (Broadhurst, et al., 2015). Furthermore, many of these technologies have been well researched, and shown to be efficient in handling the immediate problem at an industrial scale, but their operation is costly as they typically require constant high energy inputs and the continuous addition of costly neutralising reagents (Johnson & Hallberg, 2005). This has made the prevention of ARD a subject of considerable interest.

The pre-disposal removal of sulphide minerals from waste rock tailings and coal discards as a means of ARD prevention has been demonstrated by Hesketh et al. (2010), Harrison et al. (2010), Kazadi Mbamba et al. (2012) and Iroala et al. (2014) by using a two-stage desulphurisation froth flotation process. In the case of ultra-fine coal desulphurization, the process yields a clean coal product along with a low-volume sulphide-rich concentrate and a high-volume sulphide-lean tailings fraction that has less ARD generating potential (Kazadi Mbamba, et al., 2012). The benign tailings generated thus can be safely disposed of or used as a resource in other industries, while the sulphide concentrate can be contained and handled in an environmentally acceptable manner (Harrison et al. 2010).

Although Kazadi Mbamba et al. (2012) have demonstrated the technical feasibility of the two-stage desulphurization froth flotation process, the cost of the flotation reagents used in this process are particularly high in comparison to the other operating costs (Jera, 2013). Furthermore, the chemical reagents used in sulphide mineral flotation are relatively toxic and have been shown to have a deleterious influence on aquatic systems and living organisms (Serrano, et al., 1997 and Hammarstrom, et al., 2005), making them environmentally hazardous and unsustainable. This has motivated research in the field of flotation reagents to find environmentally sustainable and inexpensive alternatives to chemical reagents.

The main objective of this study is to investigate the prevention of ARD formation through the desulphurization of pyrite-containing coal discards and base metal tailings samples using alternative bioflotation reagents. This will focus on the use of microbial cultures in the second stage of the two-stage desulphurization froth flotation process for the recovery of sulphide minerals as a potential method to lower operating costs and offer a more environmentally sustainable process. The microorganisms chosen will be assessed for their potential as biological collectors, surface modifiers or depressants for the recovery of a low-volume sulphide-rich fraction and a high-volume benign tailings fraction in the second stage of desulphurization froth flotation.

The literature review, presented in Chapter 2, provides a more detailed overview on ARD generation and its implication on the environment. This is followed by an overview of the froth flotation and bioflotation separation processes. The microbial species selected for this investigation will be presented in the literature review. Chapter 3 details the materials, experimental methods and procedures used to fulfil the objectives of the study. Chapter 4 presents the results and discussion of the microbial culture screening tests (using attachment and batch bioflotation experiments), performed to investigate the affinity of each microbial species to pyrite and their bioflotation potential. Furthermore, the results and discussion of the surface chemical characterization study which uses zeta-potential measurements to further assess the microbial effect on mineral floatability are presented in Chapter 4. Chapter 5 provides the results and discussion of batch sulphide bioflotation tests using first-stage coal discard tails and base-metal hard rock. Overall yield and sulphur recovery results are presented and contrasted to those obtained using chemical flotation. Chapter 6 concludes this investigation with a summary of conclusions drawn, their significance and recommendations made from this study.

CHAPTER 2: Literature review

2.1 **Coal overview**

Coal is the most widely used primary fuel worldwide and accounts for about 36% of the total fuel consumption of the world's electricity generation (Eberhard, 2011). In 2011, total world coal production was 7 678.4 Mt with an average annual growth rate of 4.4 percent since 1999 (IEA, 2012). China, USA and India are ranked as the top 3 producers of coal in the world with South Africa (SA) ranked as the 7th largest producer of coal and the 5th largest producer of steam coal worldwide (IEA, 2012). Furthermore, South Africa is the 6th largest coal and 5th largest steam coal exporter in the world, exporting 71.1 Mt and 71.6 Mt of coal and steam coal respectively in 2011 (SACRM, 2011).

Economically recoverable coal reserves in South Africa are estimated to be between 15 and 55 billion tons; 96% of which are bituminous coal, 2% are metallurgical coal and the remaining 2% anthracite coal (SACRM, 2011). The Witbank, Highveld and Ermelo coalfields make up most of South Africa's coal reserves (12 000 Mt) and mines where coal of bituminous quality is largely mined. Run-of-mine (ROM) coal which is raw unprocessed coal contains non-combustible material such as stones, rocks, wood and ash forming material (carbonates, silicates and sulphides). These non-combustible materials reduce the calorific value of the coal and hence through coal processing and beneficiation, these unwanted impurities are removed to generate uniform saleable products that are suitable for commercial markets (SACRM, 2011).

To obtain good quality export grade saleable coal, the ROM coal goes through a screening stage to separate the coal into various size fractions and a washing/cleaning stage. The latter, also known as coal beneficiation, involves the reduction of ash forming mineral content from raw coal using a variety of washing techniques/technologies. Jigs and dense medium baths are commonly used for the separation of coarse coal fractions while dense medium cyclone or spiral separators are used for the separation of intermediate coal and fine coal respectively. Froth flotation is widely used for the separation of ultra-fine coal fractions but due to the relatively high moisture content of the final product, a dewatering step is usually essential to reduce the high moisture content (SACRM, 2011).

Large amounts of poor quality ultra-fine coal discards are produced as a product of coal beneficiation, most of which are disposed of in slimes dams or slurry dumps. In 2011, close to 1.5 billion tons of coal discard were estimated to be present in South Africa, produced at an excess of 60 Mt annually (Belaid, et al., 2013). These coal discards have large amounts of sulphur present in the form of pyrite and because of exposure to oxidation and chemical

weathering, are prone to generate Acid Rock Drainage (ARD). ARD contains elevated levels of hazardous trace elements and acid producing sulphate salts that are detrimental to aquatic ecosystems, and hence poses a serious environmental challenge (Akcii & Koldas, 2006).

2.2 Acid Rock Drainage (ARD)

The long-term generation of ARD is one of the major environmental challenges facing coal mining industries (Akcii & Koldas, 2006). It is perpetuated through the disposal of large volumes of poor quality sulphide-bearing coal discards following coal processing and beneficiation. The exposure of pyrite (FeS_2), which is the sulphide mineral that is primarily responsible for ARD formation, to oxidizing species such as molecular oxygen, water and ferric iron, greatly accelerates the ARD formation process (Hesketh, et al., 2010).

2.2.1 ARD formation

Acid Rock Drainage forms because of the oxidation of sulphide minerals, such as those in Table 2.1, to acid producing sulphate salts in the presence of water and oxygen (van Hille, et al., 2012). ARD formation occurs through a combination of two processes: abiotic oxidation of pyrite through chemical weathering and biotic oxidation reactions catalysed by bacteria (Fernandez, et al., 1995). The abiotic oxidation of pyrite is slow, occurs under neutral conditions and molecular oxygen is used as the primary oxidant (van Hille, et al., 2012). The presence of iron and sulphur-oxidising bacteria increases the kinetics of ARD formation a thousand-fold through the regeneration of ferric ions (Fe^{3+}), which under biotic conditions are used as the primary oxidant (Gazea, et al., 1996).

The first two abiotic reactions involved are detailed below for the case of pyrite:

Equation 1

$$2FeS_2 + 2H_2O + 7O_2 \rightarrow 2FeSO_4 + 2H_2SO_4$$

Equation 2

$$4FeSO_4 + O_2 + 2H_2SO_4 \rightarrow 2Fe_2(SO_4)_3 + 2H_2O$$

ARD generation is initiated due to chemical weathering at pH values higher than 4.5. Pyrite is oxidised to form ferrous sulphate and sulphuric acid as seen Equation 1. The ferrous sulphate formed is further oxidised to ferric sulphate as shown in Equation 2. Iron oxidising bacteria such as *Acidithiobacillus ferrooxidans, Leptospirillum ferrooxidans* and *Leptospirillum ferriphilum* increase the rate of the reaction in Equation 2, catalysing the increase in ferrous sulphate oxidation to ferric sulphate as shown in the reactions below (Loos, et al., 2000):

Equation 3

$$Fe_2(SO_4)_3 + 6H_2O \rightarrow 2Fe(OH)_3 + 3H_2SO_4$$

Equation 4

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

At pH values higher than 2.3, the ferric sulphate in Equation 3, precipitates as oxyhyroxide and releases sulphuric acid, lowering the pH and producing an acidic environment for acidophilic oxidising bacteria to thrive (Zangury, et al., 2007). In addition to the oxidative reactions, ferric iron produced in Equation 2 reacts with more pyrite shown in Equation 4, thus producing more ferrous iron to drive reaction 2. At this point, at pH values below 3, ferric iron becomes the dominant oxidant and oxidation of pyrite by ferric iron becomes the dominant reaction in ARD formation (van Hille, et al., 2012).

Table 2.1: Sulphide minerals commonly implicated in ARD formation with pyrite and marcasite being the predominant minerals responsible for ARD formation (Harrison, et al., 2013).

Metal sulphide	Chemical Formula
Pyrite	FeS_2
Marcasite	FeS_2
Pyrrhotite	$Fe_{1-x}S$
Chalcocite	Cu_2S
Covelite	CuS
Chalcopyrite	$CuFeS_2$
Molybdenite	MoS_2
Millerite	NiS
Galena	PbS
Sphalerite	ZnS
Arsenopyrite	FeAsS

In addition to iron oxidising bacteria, ARD environments typically contain microorganisms that are capable of oxidising reduced sulphur species that contribute to ARD formation (Loos, et al., 2000). Microorganisms such as *Acidithiobacillus thiooxidans* and *Acidithiobacillus caldus* make use of elemental and reduced sulphur species as the electron donor to produce

sulphates and protons, as shown in Figure 2.1. The protons contribute to the low pH acidity of the environment and further dissolution of the sulphide mineral.

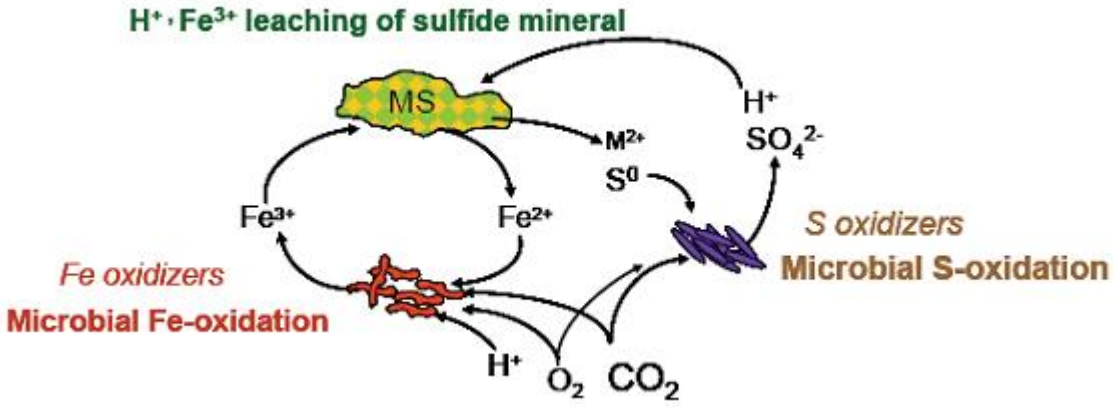

Figure 2.1: Schematic depiction of the reactions catalysed by iron and sulphur oxidising microorganisms in ARD environments (Ojumu, 2008).

2.2.1.1 ARD implications on the environment

As discussed in 2.2.1, the oxidation of sulphide minerals results in the generation of sulphuric acid which contributes to the acidity of ARD discharges. In addition to acid, ARD typically contains several heavy metals such as zinc, copper, lead, mercury and chromium which are released during the oxidative dissolution of sulphide minerals other than pyrite (Simate & Ndlovu, 2014). These toxic metals have adverse effects on the aquatic systems that get contaminated with ARD. Humans exposed to ARD contaminated areas can also be adversely affected by the toxic metals present. Table 2.2 and Table 2.3 summarize some of the major effects heavy metals have on humans and plant life in contaminated aquatic systems.

Table 2.2: Effects of heavy metals on plants (Adapted from Simate & Ndlovu, 2014).

Heavy metal	Effects
Zinc	Reduces Ni toxicity and seed germination, increases plant growth and ATP/chlorophyll ratio
Copper	Inhibits photosynthesis, plant growth and reproductive process
Lead	Reduces chlorophyll production and plant growth
Mercury	Decreases photosynthetic activity, water uptake and antioxidant enzymes; accumulates phenol and proline
Chromium	Decreases enzyme activity and plant growth; promotes cell membrane, chlorosis and root damage

Table 2.3: Effects of heavy metals on human health (Adapted from Simate & Ndlovu, 2014).

Heavy metal	Effects	Permissible level (mg/L)
Zinc	Damage to nervous system	15.0
Copper	Anaemia, liver and kidney damage, stomach and intestinal irritation	0.10
Mercury	Damage to nervous system, protoplasm poisoning, spontaneous abortion, minor physiological changes, tremors	0.01
Lead	Mental retardation in children, development delay, liver, kidney and gastrointestinal damage, acute or chronic damage to the nervous system	0.10

2.2.2 ARD treatment

Several technologies/methods are used for the treatment of ARD. These are end-of-pipe treatment technologies that are implemented as the last stage of a process before the waste

stream is discharged into the environment (Broadhurst, et al., 2015). The treatment technologies are categorised as either active or passive, as shown in Table 2.4, and typically combine physical, chemical or biological approaches to the treatment of ARD. Methods that are most commonly used are based on neutralisation, oxidation, precipitation and sedimentation (Simate & Ndlovu, 2014).

2.2.2.1 Active treatment technologies

Active treatment technologies fall into two categories which are fixed plant and *in situ*. Fixed plant treatment technologies are typically installed on site and require constant pumping of ARD, the addition of costly reagents and constant energy input. *In situ* treatment technologies are those that use portable land-based or water-based systems for the treatment of affected aquatic environments and their surroundings (Akcii & Koldas, 2006).

2.2.2.2 Passive treatment technologies

Passive treatment technologies are those that are used for post closure, low acidity load treatments and are not used for actively operating mines. They do not require continuous chemical inputs, but rather they take advantage of the naturally occurring chemical and biological reactions that occur to treat contaminated mine waters. They are largely aggregate-carbonate based and are designed to accommodate slow reaction rates (Akcii & Koldas, 2006). Aerobic wetlands are an example of passive ARD treatment technologies. These wetlands are typically associated with or in close proximity downstream an inactive mine. Essentially, aerobic wetlands are shallow ponds that lower suspended solids and provide a substrate and increased water retention time (due to reduced flow rates) for the reaction between influent alkalinity and acidity generated from ARD via metal oxidation and precipitation within the wetland. They typically contain vegetation planted in relatively impermeable sediments where precipitates are retained on plants surface. Aerobic wetlands have been utilized as a passive treatment system in Australian and South African inactive mines (Taylor, et al., 2005).

Table 2.4: Typical technologies/applications used in ARD treatment (Taylor, et al., 2005).

ARD Treatment Technologies		
Active Treatment		Passive Treatment
Fixed plant	*In situ*	
High density sludge plants	Heap leach ponds	Constructed aerobic or anaerobic wet lands
Low density sludge plants	Waste rock pile discharges	Anoxic limestone drains
Electrochemical concentration	Tailing dams	Open/Oxic limestone drains
Ion exchange/Adsorption or Absorption/Flocculation and Filtration	Ground water seepage discharges	Successive alkalinity producing systems (SAPS)

2.2.3 ARD prevention

The active and passive treatment technologies presented in Section 2.2.2 are typically well researched and have been shown to be effective at an industrial scale. However, the high-energy inputs required and continuous addition of neutralising chemicals in active operating plants make the treatment process of ARD a very costly one. Methods directed towards the prevention of ARD have become a subject of interest since they may provide a permanent solution to the ARD problem.

One of the primary routes of ARD prevention is inhibiting the oxidation of sulphide minerals by minimising exposure to water and oxygen. This could be through the flooding and sealing of underground mines if the location of all shafts and adits is known. Other methods include the removal of acid producing sulphide minerals from waste rocks (Harrison, et al., 2013), the principal focus of this thesis.

2.2.3.1 Desulphurisation of coal discards by flotation

Desulphurisation flotation is a separation technique that has been developed for the selective removal of sulphides from acid generating mine wastes and tailings. The process takes

advantage of the difference in surface properties of the sulphide minerals and the other gangue material.

The desulphurisation of coal discards can be achieved by floating the coal and depressing the sulphide mineral, in most cases pyrite, to obtain a clean coal product, or by depressing the coal and floating the pyrite to obtain a sulphide-rich concentrate (Harrison, et al., 2013). The advantage of the latter technique is that a low volume sulphide-rich concentrate is obtained and is easier to manage and control, mitigating the risk of ARD generation from coal discards (Kazadi Mbamba, et al., 2012).

2.2.3.2 Two-stage coal desulphurisation flotation process

The technical feasibility of the UCT two-stage desulphurisation process has been demonstrated by Hesketh et al. (2010) and Kazadi Mbamba et al. (2012) using ultrafine copper and coal mine discards respectively. In the case of coal desulphurization, a two-stage froth flotation process is used to separate the coal discards into two separate fractions, the mechanism of which is explained in section 2.3. In the first stage of flotation, a clean coal-rich stream is obtained as a concentrate. The first stage tailings undergo a second desulphurization step where the sulphide mineral in the form of pyrite is removed to obtain a low-volume sulphide-rich concentrate and a sulphide-lean benign tailings fraction (Figure 2.2). Using a sulphide containing coal ultrafines, Kazadi Mbamba et al. (2012) showed that a lower-sulphide tailings fraction with low ARD potential could be produced. Results showed that the total amount of sulphur was reduced by approximately 75% in the benign tailings fraction relative to the feed.

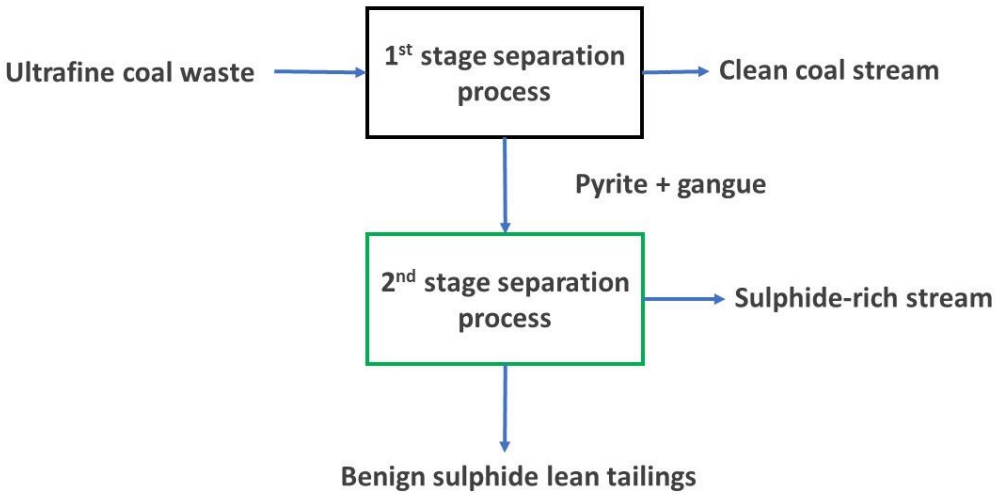

Figure 2.2**:** Conceptual approach for the two-stage desulphurisation of coal using froth flotation (Kazadi Mbamba et al, 2012).

In another study, Iroala et al. (2014) looked at combining froth flotation with reflux classification with the aim of mitigating the ARD generating potential of coal ultrafines via sulphide removal. Two process routes were investigated for coal samples from Witbank and Waterberg coal mines. Process route 1 involved coal flotation followed by reflux classification, while process route 2 involved reflux classification followed by coal flotation. Results showed that the latter was found to give better ash and sulphur recoveries than process route 1 (coal flotation followed by reflux classification). Furthermore, the reflux classifier separated ash and sulphur from coal very effectively with approximatley 90 % of the sulphur removed to the tailings in both process routes. In comparsion, the UCT two-stage flotation process removed approximatley 50 to 60 % sulphur from the feed to the tailings for both coal samples. The authors found that while reflux classification produced good results for the separation of coal from ash and sulphur, sulphide floataion produced the highest sulphur grades.

2.3 **Flotation separation**

Mineral processing and beneficiation follows mining and is essentially the preparation and extraction of valuable metals in the case of metal ores, or clean coal products from “run-of-mine” (ROM) or raw unprocessed ores. The beneficiation process is, in principle, the separation of the valuable mineral from the commercially worthless gangue mineral to obtain an enriched concentrate containing the valuable mineral and a tailings or discard fraction that is mainly made up of gangue material (Wills, 1997).

ROM coal ores contain non-combustible material such as rocks, stones, wood and ash forming material (silicates, carbonates and sulphur) that reduce the calorific value of the coal. To produce good quality saleable and export grade coal, the ROM is processed with the purpose of separating the coal from the non-combustible material. To achieve this, a variety of coal beneficiation technologies are employed for the separation of coal as shown in Table 2.5. In most cases, the separation is achieved by utilising some specific difference in physical or chemical properties between the valuable coal product and gangue material in the ore (Pryor, 1965). Coal beneficiation is mainly concerned with physical methods of separation which include:

- Separation dependant on specific gravity differences (jigs, dense medium separators)
- Separation utilizing the different surface properties of the minerals (froth flotation)

In this section of the review, separation by froth flotation utilizing the different surface properties of the minerals will be discussed further.

Table 2.5: Coal beneficiation technologies used for the separation of coal (SACRM, 2011).

Coal size	Particle size range (mm)	Separation technique
Coarse coal	12 – 250	Jigs, dense medium drums
Small/intermediate	1 – 12	Dense medium cyclones
Fine	0.1 – 1	Dense medium spirals
Ultrafine	0.1 – 0.15	Froth flotation

2.3.1 Principle of Froth Flotation

Froth flotation, a separation technique introduced over a century ago, is one of the most widely used mineral processing techniques in the mineral industry (Wills, 1997). The technique utilizes the difference in surface properties of particles of various minerals to perform a separation. The process is applied to ultrafine well-liberated particles which get conditioned with flotation reagents that adhere to the surface of the mineral. These reagents modify/alter the surface properties of the minerals and hence facilitate separation based on the relative hydrophobicity or hydrophilicity of the mineral or gangue material. As shown in Figure 2.3, the more hydrophobic mineral is transferred to the froth fraction via an air bubble to obtain a concentrate while the hydrophilic material remains in the pulp or tailings fraction (Pryor, 1965). For efficient separation to occur, the air bubble needs to displace water from the mineral surface and this can only occur if the mineral is hydrophobic and water repellent (Finkelstein and Lovell, 1972). As most minerals are not inherently hydrophobic/hydrophilic, flotation chemical reagents are usually added to the pulp to enhance flotation. The most important reagents are collectors, frothers and depressants/regulators.

Many chemical and physical variables affect the froth flotation process. Chemical factors such as the pH of the pulp affect the surface properties of the mineral. The pH of the flotation solution affects the surface electrical properties of the mineral, while the degree of ionization/hydrolysis of the surface affects the wettability and thus the degree of hydrophobicity (Johnson, 1994).

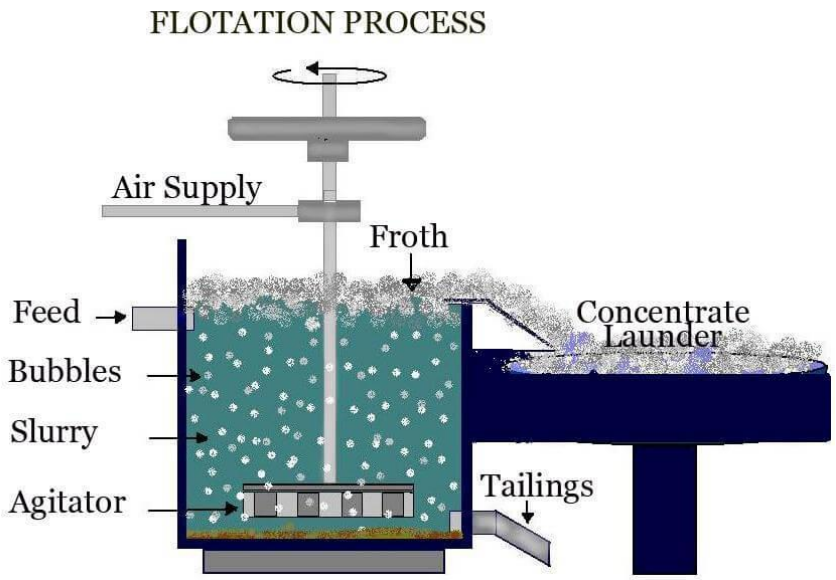

Figure 2.3: Diagrammatic representation of the flotation process (Michaud, 2009).

2.3.2 Surface properties required for flotation

Mineral beneficiation processes such as froth flotation rely largely on the differences in surface chemical properties of minerals to effect efficient separation (Wills, 1997). Physicochemical surface properties that have been identified as crucial for the overall attachment of mineral particles to air bubbles include electrostatic and hydrophobic interactions.

The surface charge of a mineral particle 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. All materials spontaneously acquire a surface electrical charge when brought into proximity with a polar medium such as water. Generally, the net surface charge of most particles is negative when deionized water is used, although some materials can acquire a net positive charge (Hunter, 1981). When a mineral particle is immersed in a liquid it can acquire charges in several ways. The most important and common mechanisms are:

1. Differences in the affinity of the two phases for electrons
2. Differences in the affinity of the two phases for ions of one charge or the other
3. Ionization of surface groups
4. Physical entrapment of non-mobile charge in one phase

A net negative surface charge typically arises by dissociating protons (H^+) from oxygen or sulphur bearing atoms while a net positive surface charge is created through the acquisition of protons on groups containing nitrogen. Additionally, at a given pH value, a surface can have a net neutral charge referred to as the point of zero charge (PCZ). However, it cannot be

inferred that the PZC corresponds to an uncharged state; this simply means there is an equal number of positive and negative charges on the surface (Hiemenz, 1977). Therefore, a given surface will have a net positive charge at solution pH values less than the PZC and thus be a surface that attracts anions. Inversely, a surface will have a net negative charge at solution pH values greater than the PZC and thus be a surface that attracts cations (Tadros, 2007).

In the case of flotation, the magnitude of the charge determines whether attraction or repulsion will occur between the charged particle and the gas bubble. As zeta-potentials are used to measure surface charge, a large positive or negative zeta-potential has been shown to be detrimental to the rate of floatation with neutral particles showing a distinct optimum (Johnson, 1994). Authors reported that the contact angle is maximized at the PZC thereby suggesting an increase in hydrophobicity and interaction with the gas bubble. Furthermore, Derjaguin and Shukakides (1961) investigated the effect of particle charge by measuring the zeta-potential of naturally hydrophobic antimonite particles. The authors found that the rate of flotation dropped sharply as the zeta-potential of the particles was increased beyond a critical value. Therefore, maximum flotation efficiencies were achieved when the zeta-potential of the particles was at a minimum.

Surface hydrophobicity affects whether a particle will attach to the gas bubble, get transported to the froth phase and be recovered in the concentrate or if it will remain in the flotation liquor to be recovered in the tails later. The wettability of a particle is typically characterised by the three-phase contact angle θ formed between the solid surface and the bubble at the point of attachment through the liquid phase, as illustrated in Figure 2.4 (Johnson, 1994). A contact angle greater than 90° generally indicates that the particle is hydrophobic, lowering its wettability and increasing its adherence to a gas bubble (Nowak, et al., 2013). A non-wettable hydrophobic surface is essential in flotation as it lacks polar ionic groups or hydrogen-bonding sites. Thus, there is no affinity between water molecules and the surface to bond together. Early experiments conducted by Blake and Kitchener (1979) have showed that long-range hydrophobic attraction forces (>10 nm) are required to establish the mineral-particle/air-bubble attachment that is necessary for the selective enrichment of an ore. However, most minerals are not naturally hydrophobic and require the use of chemical collectors and modifiers to enhance their hydrophobicity (Fuerstenau, et al., 2007).

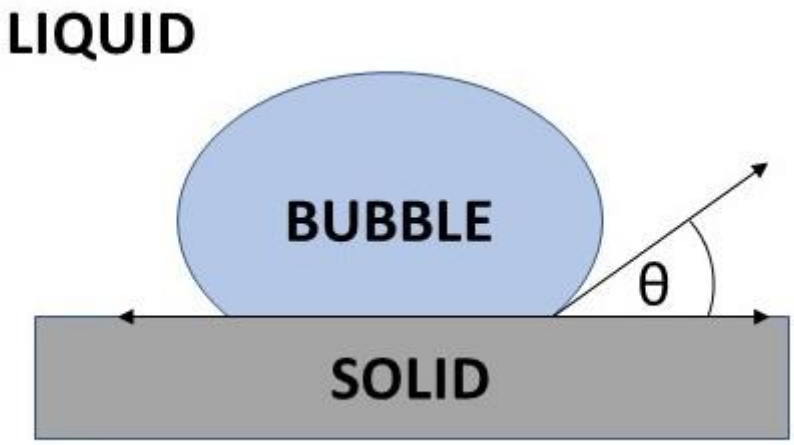

Figure 2.4: Contact angle between bubble and mineral solid in an aqueous medium.

2.3.3 Chemical reagents of flotation

2.3.3.1 Collectors

Collectors are chemical reagents that selectively adsorb onto mineral surfaces and impart the required hydrophobicity to float them (Pryor, 1965). The collectors are added to the pulp and allowed to adsorb onto the mineral surface over a given time frame known as the conditioning period. This allows the collectors to render the mineral particles water-repellent, greatly increasing the contact angle so that they attach to the air bubble (Wills, 1997).

Anionic sulfhydryl collectors are the most widely used collectors in sulphide mineral flotation. These include the xanthates and dithiophosphates which contain a polar and a non-polar group, the latter being a hydrocarbon. Although the mechanism by which the polar end of the molecule attaches to the mineral is not completely understood, the effect is to displace water molecules by anchoring the collector molecule on the mineral surface with its non-polar groups pointing outwards. When enough binding sites have become occupied, a thin monolayer film of non-polar hydrophobic hydrocarbons is formed. The hydrocarbon film is aerophilic and is thus attracted to an air-water interphase (bubble). When the particle has been sufficiently coated by the collector, flotation occurs where the air bubble carries the particle to the froth phase where it is held and collected (Farrokhpay, 2011).

2.3.3.2 Frothers

Frothers are chemical reagents that are added to produce a stable froth phase that can hold the floated mineral particles until they are recovered from the flotation cell (Farrokhpay, 2011). They are heteropolar surface-active molecules that contain both a hydrophilic group and a hydrophobic hydrocarbon chain. The more hydrophilic or polar groups are orientated toward

the water phase while the hydrophobic or non-polar portion of the molecule faces the air bubble. Frothers function in a three-phase system where the molecules concentrate between the water phase and air bubble, forming an envelope around the bubbles (Wills, 1997). This prevents the bubbles from colliding or touching one another which could cause them to break and drop their solids load to the pulp phase.

Frothers are classified depending on their properties and behaviours in solution pulp. The most effective frothers typically include hydroxyl, carboxyl, carbonyl, amino or sulpho groups in their composition. From these, the alcohols (OH) are the most widely used since they do not have any collector properties. An example of a widely-used alcohol frother is Methyl isobutyl carbinol (MIBC) (Farrokhpay, 2011).

2.3.3.3 Depressants

Depressants are flotation chemicals that are used to prevent collectors from adsorbing onto particular mineral surfaces. Essentially, they increase the selectivity of the targeted mineral by rendering the other material more hydrophilic, thus impeding the flotation of the other material (Wills, 1997).

Cyanides are commonly used in the selective separation of sulphide minerals. They are strong depressants of pyrite and are used to “deactivate” sphalerite that has been unintentionally activated in selective copper flotation. Therefore, it is for this reason that they are widely used due to their high degree of selectivity in sulphide mineral flotation. Sodium cyanide is most commonly used and hydrolyses in solution to form free alkali and insoluble hydrogen cyanide which is extremely toxic (Wills, 1997). Furthermore, apart from being toxic cyanides are also relatively expensive. Organic reagents such as starch, tannin and dextrin have been used for many years as mineral depressants. Although they have the advantage of being less toxic than most ionic depressants, they are required in large quantities for them to effectively depress minerals.

2.4 Bioflotation separation

2.4.1 Principle of bioflotation

As in chemical flotation, microorganisms can be used as collectors or depressants to enhance the hydrophobicity or hydrophilicity of minerals through surface modifications/alterations, known as bioflotation. Rapid microbial adhesion onto the mineral surface is essential to achieve surface alterations which can occur in one of three ways:

1. Attachment of the microorganisms to the surface of the mineral such that the minerals surface characteristics are determined by those of the microorganism

2. Microbial production of exopolysaccharides that form a thin biofilm layer on the surface of the mineral
3. Microbially catalysed redox reactions that occur on the surface of the mineral where the iron and sulphur present on the mineral are oxidised

The attachment of microorganisms on mineral surfaces has been postulated to occur in two steps. The first step is the initial reversible adsorption that is determined by several physico-chemical variables that will govern the microbe-mineral interaction (Yee, et al., 2000). This first step relies on the bacterial cells coming into proximity with the mineral surface either via chemotaxis or bacterial mobility (Dunne, 2002). The interactions that result upon adhesion include electrostatic interactions and hydrophobic interactions at large, and acid-base interactions and van der Waals forces. The second step is irreversible and involves the production of exopolysaccharides (EPS) that anchor the cells onto the mineral surface. Over time, the bacteria start producing more EPS that gradually leads to the formation of a biofilm on the mineral surface (Africa, et al., 2013).

Attachment of the microorganisms onto the mineral surface is also determined largely by the structure of the bacterial cell envelope. Most bacterial cell envelopes can be characterised as being either Gram positive or Gram negative depending on the cell surface composition. Gram positive cells are those that have a thick cell wall in which the main component is a large polymer called peptidoglycan that consists mainly of sugar derivatives and amino acids. In addition to peptidoglycan, other molecules such as sugar alcohols (glycerol) and amino acids (teichoic acids) are distributed throughout the cell wall. These molecules extend through the peptidoglycan layer and are responsible for the overall surface charge. Gram negative cells on the other hand have a thin peptidoglycan layer that's surrounded by an outer phospholipid membrane that contains proteins and lipopolysaccharides (Dwyer, et al., 2012). The presence of these molecules on the cell's surface also contributes to the overall surface charge and hydrophobicity (Sharma, et al., 2001). This is due to the presence of ionisable functional groups present on macromolecules such as lipopolysaccharides, lipoproteins and bacterial surface proteins embedded in the cell wall. Functional groups that are commonly observed on these molecular structures are carboxylic acids (-COOH), amine ($-NH_2$) and hydroxyl groups (-OH) whose ionization and/or dissociation (degree of charge development) and net molecular charge is dependent on the pH of the solution (Hiemenz, 1977)

2.4.2 Sulphide mineral bioflotation separation

Several microorganisms have been identified for their potential in bioflotation of sulphide minerals. These include chemolithotrophic microorganisms such as *Acidithiobacillus* species

and heterotrophic microbes such as *Mycobacteria phlei, Rhodococcus opacus, Bacillus subtilis* and *Paenibacillus polymyxa*.

2.4.2.1 *Acidithiobacillus* species

Acidithiobacillus ferrooxidans is one of the most studied microorganisms in mineral bioprocessing and has been utilized as a leaching agent of many sulphide minerals for many years. Members of the *Acidithiobacillus* genus are Gram negative chemoautotrophs that obtain the energy required for growth through the oxidation of ferrous iron and/or reduced sulphur compounds to obtain ferric iron and protons that are responsible for the dissolution of the sulphide mineral.

Coal desulphurisation flotation studies using *A. ferrooxidans* by Misra et al. (1996) showed that attachment of the bacteria onto pyrite rendered the mineral hydrophilic due to surface chemical changes that occurred. This meant that the bacteria had the ability to act as a pyrite depressant in coal desulphurisation flotation. To investigate the selectivity of *A. ferrooxidans* to pyrite, Nagaoka et al. (1999) investigated the microorganism's ability to selectively depress pyrite from other non-ferrous sulphides. Their results showed that in the absence of *A. ferrooxidans,* >90 % of the pyrite was recovered in the froth when doing a standard flotation. However, in the presence of bacteria the recovery of pyrite was reduced to 19.3 % showing that the microbe had the ability to selectively depress pyrite. Attachment of the microbe to the mineral resulted in surface chemical changes that rendered the mineral more hydrophilic. Another study using a chalcopyrite/pyrite mixture showed that preconditioning with small amounts of collector before conditioning with cells of *A. ferrooxidans* enhanced the microbe's selective adsorption onto pyrite (Chandraprabha, et al., 2004a). This was demonstrated by the selective flotation of chalcopyrite and the depression of pyrite. The selective separation of pyrite from gangue minerals such as quartz has also been demonstrated through selective bioflocculation using *A. ferrooxidans* (Natarajan & Das, 2003). Although *A. ferrooxidans* has been shown to act as a pyrite depressant, it can also promote flotation under certain conditions. Flotation of the sulphide mineral can be achieved through the biooxidation of elemental sulphur that forms on the surface of the sulphide mineral. However, prolonged bacterial interaction leads to the re-oxidation of the sulphur to form sulfoxy compounds that are ultimately oxidized to sulphates which build up on the mineral surface and impede flotation (Chandraprabha & Natarajan, 2009). While literature has shown that bacterial conditioning in the presence of *A. ferrooxidans* enhances depression of pyrite, the microbe has also been shown to enhance the floatability of other sulphide minerals such as sphalerite (Amini, et al., 2009).

2.4.2.2 *Mycobacterium phlei*

Mycobacterium phlei is a heterotrophic bacterium that is ubiquitously isolated in soil and on the leaves of plants. Unlike most bacterial cells, *M*. *phlei* cell envelopes contain mycolic acids instead of peptidoglycan. This gives the bacterium an overall negative surface charge which means that it is relatively hydrophobic. Electrostatic forces and hydrophobic interactions govern this microbe's attachment to less negatively charged surfaces (Hanumatha Rao & Subramanian, 2007) and so this has sparked interest in using *M*. *phlei* as a flocculent and flotation collector in mineral bioprocessing. Raichur et al. (1996) showed that *M*. *phlei* caused rapid flocculation and settling of coal particles. The authors postulate that the principle mechanism of attachment/adsorption of bacterial cells to the coal surface was through hydrophobic interaction. To substantiate this, the authors explained that maximum adsorption of *M*. *phlei* onto the coal surface was achieved at a pH value of 4. If the interaction between *M*. *phlei* and coal was electrostatic, the maximum adsorption and flocculation would occur at pH values between 1.5 and 2 where *M*. *phlei* has zero surface charge, offering less resistance for mutual interaction with coal particles. After flocculation with *M*. *phlei,* column flotation experiments performed using the flocs to separate the coal from pyrite revealed that *M. phlei* acted as a pyrite depressant. Bench-scale bioflotation results presented by Fagan-Endres & Harrison, (2017) showed that *M. phlei* successfully upgraded and desulphurized a South African coal tailings feed and delivered approximately the same concentrate yields as the optimized chemical flotation conditions (37 – 39%). However, a projected financial feasibility analysis that assessed the incorporation of the *M. phlei* into the two-stage flotation process found that the bioflotation process was not profitable due to the very large concentration of cells required (2×10^{16} cells/ton coal) and the associated additional equipment and growth media component costs Although *M*. *phlei* has been shown to depress pyrite, other studies suggest that the microbe acts a collector of sulphide minerals such as haematite (Smith, et al., 1993).

2.4.2.3 *Rhodococcus opacus*

Rhodococcus opacus is another hydrophobic heterotroph that has been used in the selective separation of sulphide minerals. *R. opacus* is a Gram-positive soil microbe that carries a net negative surface charge just like *M. phlei*. *R. opacus* has been used as a flotation collector in the separation of haematite from a haematite-quartz mixture (Dwyer, et al., 2012). The microbe was shown to exhibit a high affinity for haematite, adhering to the mineral over a wide pH range between 2 and 8 (de Mesquita, et al., 2003). *R. opacus* has also been used in the flotation of magnesite and calcite (Botero, et al., 2008) and in the biosorption of heavy metals such as cadmium and zinc (Vasquez & Botero, 2007).

2.4.2.4 *Paenibacillus polymyxa*

Paenibacillus polymyxa, formerly called *Bacillus polymyxa*, is a Gram-positive neutrophilic heterotroph that is found indigenously associated with many mineral deposits. Its use in mineral biobeneficiation has been investigated from as early as 1987 where it was observed to induce flocculation of haematite, coal and phosphate limes (Chandraprabha & Natarajan, 2009). The use of the bacterium and its extracellular metabolites has been investigated in sulphide mineral separation using flotation. Earlier studies using a sphalerite-galena mixture showed that interaction with *P. polymyxa* and its metabolites rendered sphalerite more hydrophobic while galena was rendered hydrophilic (Hanumatha Rao & Subramanian, 2007). However, adsorption was influenced by pH with maximum adsorption observed at pH 6 for sphalerite. Patra and Natarajan (2004) showed that the bacterium successfully achieved selective separation of sphalerite from pyrite through flocculation and flotation. Adhesion of the microbe rendered pyrite hydrophobic, where 80% of the mineral settled over a wide pH range between 3 and 9. However, increasing pH was observed to decrease the settling behaviour of sphalerite from 80% at pH 3 to 20% at pH 9. Patra and Natarajan (2004) further investigated the used of *P. polymyxa* and its metabolites on the separation of pyrite from quartz. Both bacterial cells and bacterial proteins were shown to influence the settling behaviour of pyrite. Furthermore, the interaction between *P. polymyxa* and quartz rendered the mineral more hydrophobic allowing its separation form pyrite.

2.4.2.5 *Bacillus subtilis*

Bacillus subtilis is a Gram-positive neutrophilic bacterium that is commonly found in soil and associated with mineral deposits. Zheng et al. (2001) investigated the use of *B. subtilis* as a dolomite depressant in the flotation separation of apatite from dolomite. *B. subtilis* exhibited greater adhesion to dolomite than to apatite or quartz over a wide pH range between 4 and 10. Maximum bacterial adhesion occurred when the pH was 4 and decreased with increasing pH. Both bacterial and mineral surfaces are influenced by the pH of the solution thus at pH 4, minerals such as dolomite and apatite have relatively positive surface charges, while *B. subtilis* has a negative charge. Hence maximum bacterial adsorption can be explained, in part, by the surface chemical changes that occur at acidic pH values. Flotation results also showed that *B. subtilis* was a strong depressant of dolomite when sodium oleate was used as a collector. Recently, Sarvamangala et al. (2013) showed that *B. subtilis* exhibited a higher affinity toward pyrite compared to galena at neutral pH values. Through microbially induced flocculation, interaction of *B. subtilis* cells with pyrite increased the settling rate of the mineral while bacterial interaction with galena resulted in its dispersion. *B. subtilis* rendered pyrite more hydrophilic while galena was rendered hydrophobic. Bacterial EPS and proteins also affected the settling behaviour of pyrite where settling rates were 74% after interaction with EPS and 72% after

interaction with proteins. Separation of pyrite from galena using flotation was also investigated. Selective flotation of galena from a 1:1 pyrite-galena mixture following bacterial interaction was achieved while pyrite flotation was significantly impaired. Pre-treatment with bacterial EPS and proteins also impaired pyrite flotation. These results demonstrate the potential use of *B. subtilis* and its metabolites as effective depressants of pyrite.

2.4.2.6 Bacterial metabolites as flotation reagents

Bacterial metabolites such as extracellular proteins and polysaccharides have been used for the separation of pyrite from gangue minerals such as quartz and calcite. Using EPS and bacterial proteins produced by *P. polymyxa*, Patra and Natarajan (2003) showed that bacterial proteins significantly affected the settling behaviour of pyrite. More than 90% of the pyrite settled 10 minutes after interaction with bacterial proteins at pH 3 and 6 using 20 ppm bacterial protein. Flotation results also showed that extracellular proteins produced by *P. polymyxa* act as strong depressants of pyrite. Following interaction with a 1:1 pyrite-quartz mixture for 15 minutes, the recovery of quartz was 82% compared to the 59% that was recovered without the use of a collector. This result showed that the bacterial proteins enhance the hydrophobicity and dispersion of quartz in the aqueous solution while significantly depressing pyrite. In a similar study, Patra and Natarajan (2003) also investigated the use of bacterial EPS from *P. polymyxa* on the settling behaviour and separation of pyrite. Their results showed that the EPS did not have a significant effect of the settling behaviour of pyrite or the dispersion of quartz as the results obtained were the same as the control.

2.4.3 Bioflotation implementation considerations

As with the chemical flotation process, a number of factors influence the effectiveness of the bioflotation process. These include the pH of the flotation pulp, particle size, flotation time and the density of the bacterial suspension.

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). Thus, 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 (de 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 to pH 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 values, the microbial cultures tend to coagulate, thus inhibiting their effectiveness during bioflotation (Vilinska & Hanumantha Rao, 2008).

The effect of particle size and bacterial suspension on biological flotation has been demonstrated in literature. Ohmura & Saiki (1996) investigated the desulphurization of different-sized coal fractions using *Acidithiobacillus ferrooxidans*. The coal sample was divided into two fractions: a small-size coal between 38 µm and 53 µm and a large-size coal between 53 µm and 75 µm. The authors found that the pyritic sulphur content decreased from 2.88 % of the feed coal to 0.98 % of the product coal for the large-size coal and from 2.77 % of the feed coal to 1.12% of the product coal for the small-size coal using microbial flotation. This led to the conclusion that microbial column flotation was more effective for the desulphurization of coal particles that are between 53 µm and 75 µm. Additionally, it was proven that finer coal grains required the use of a greater density of *A ferrooxidans* bacterial suspension. For the large-size coal the best results were achieved at a cell concentration of 0.5×10^9 cells/cm^3 while in the case of the small-size coal, best results were obtained after using a cell concentration of 1.5×10^9 cells/cm3.

2.5 **Summary**

Conventional collector-assisted flotation requires the use of expensive reagents. Furthermore, collectors such as xanthates may be toxic to aquatic organisms that are exposed to high concentrations of the collector downstream the mining process. Tailings that are produced from mining activities usually contain large quantities of inorganic ionising chemicals used to depress the gangue material. These inorganic chemicals are relatively unstable and have the potential to harm the environment.

The research studies presented in this review have demonstrated the potential of using microorganisms as flotation collectors or depressants. This has been exhibited through their ability to adhere selectively to specific mineral surfaces and alter their surface chemical properties. These alterations can render the minerals hydrophobic or hydrophilic, allowing for their subsequent separation using froth flotation.

Based on the findings in literature, a selection of microorganisms to be investigated as bioflotation reagents was made. The microorganisms would be used to investigate the separation of pyrite from gangue minerals using flotation. The microorganisms selected fall into two subcategories:

- Microorganisms that have already been applied in pyrite bioflotation studies successfully
- Microorganisms that have not been reported in previous bioflotation studies, but are known to exhibit pyrite affinity, as this has been demonstrated to be a requirement for bio-beneficiation

Chemolithotrophic microorganisms, such as *Acidithiobacillus* species, have been shown to act as pyrite depressants (Natarajan & Das, 2003). This does not make them eligible for this study as the aim is to produce a sulphide-lean tailing fraction with a low ARD generating potential. Furthermore, if co-disposed with tailings that contain residual amounts of sulphur, there is a risk of generating and perpetuating ARD in the environment.

Heterotrophic microorganisms that have been chosen for the flotation separation of pyrite from gangue minerals in the second stage of desulphurization flotation are:

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

These microorganisms are indigenously associated with most minerals and grow under neutral conditions, making them ideal candidates. They have all been reported in the literature to show selective affinity for pyrite, other sulphide minerals or gangue, thereby facilitating the bioflotation separation of these substances.

Two microorganisms that have not been tested before for their bioflotation potential will be considered in this study. 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 suggested that *R. palustris* may have potentially interesting interactions and affinities to the different mineral species present in the flotation process and motivated 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.

The concept of bioflotation separation could potentially be applied in the prevention of ARD through the microbially assisted separation of pyrite from mineral discards and tailings using froth flotation. This could lead to the development of an economically and environmentally sustainable desulphurization process for waste tailings.

2.6 Objectives, hypothesis and key questions of this study

2.6.1 Objectives

The purpose of this study is to investigate the prevention of ARD formation through the desulphurization of pyrite-containing coal discards and base metal or hard rock tailings, addressing the following objectives:

- To investigate the use of microorganisms as bioflotation reagents to recover pyrite in the two-stage desulphurisation froth flotation process
- To screen for specific microorganisms that can be used as biological collectors, surface modifiers or depressants in pyrite flotation (second stage)
- To produce a low-volume sulphide-rich concentrate and a high-volume benign tailings fraction from coal discards and hard rock tailings using bioflotation
- To gain a comprehensive understanding of the surface interactions between the successful microbe and the mineral that lead to alterations of the mineral surface.

2.6.2 Hypothesis

Microbial cultures can be used as alternative biological collectors, surface modifiers or depressants for the recovery of a low-volume sulphide-rich concentrate and a high-volume benign tailings fraction in the two-stage sulphide mineral desulphurization froth flotation

process. This is due to selective attachment of the microorganisms onto the sulphide mineral surface that result in surface chemical alterations, enhancing the mineral's hydrophobicity or hydrophilicity and thus facilitating bioflotation separation

2.6.3 Key questions

- Which microbial species selectively attach to or show more affinity toward pyrite and gangue materials?
- Which physico-chemical interactions are responsible for the selective attachment of the microbes to the mineral surface?
- What are the optimal conditions that will affect bacterial attachment onto the mineral surface and achieve flotation?
- Will using microorganisms in place of conventional flotation reagents still achieve acceptable recoveries and yields of pyrite?.

CHAPTER 3: Materials and Methods

3.1 Introduction

As presented in Chapter 1, the main objective of this study is to investigate the prevention of ARD formation through the desulphurization of pyrite-containing coal discards. This will be done using microbial cultures as bioflotation reagents in the second stage of the two-stage desulphurization froth flotation process.

A description of the experimental work and techniques employed to investigate the bioflotation potential of the microorganisms is presented in this chapter. This includes mineral preparation, microbial culture preparation and maintenance, attachment studies, surface chemical characterization tests, batch flotation tests and analytical techniques employed throughout the study.

3.2 Mineral sample preparation

Mineralogical characterization previously done on several ultrafine waste coal samples from different coal mining regions in South Africa 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 ($CaCO_3$), as shown in Table 3.1. The analysis further showed that together quartz and kaolinite represented, at the minimum, about 80% of the coal samples. These results provided the basis for the selection of minerals for which microbe-mineral interaction should be tested. These were pyrite (acid-generating sulphide mineral), and quartz and kaolinite (gangue minerals). 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.

Table 3.1: Mineralogical characterization of South African coal ultrafine waste [adapted from Kazadi Mbamba et al. (2012) and Iroala et al. (2014)].

Mineral	Mineral composition	Concentration in coal (wt%)		
		Middleburg (Site 4)	Witbank (Site 5)	Waterberg (Site 3)
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

3.2.1 Sulphide mineral preparation

A pyrite (95.3 wt%) mineral concentrate (Agnes mine, courtesy of BHP Billiton) was used to assess the bioflotation potential of the different microorganisms prior to testing with an actual ultrafine coal sample. A summary of the major elemental composition of the concentrate, determined using X-ray fluorescence (XRF) analysis (Analytical laboratory, Department of Chemical Engineering, University of Cape Town (UCT)), is presented in Once the desired particle size distribution was attained, the sample was passed through a Dickie and Stockler rotary splitter, dividing it into ten equal portions. Two samples from adjacent positions were combined and further divided into smaller samples. This procedure was repeated until the samples were grouped into bags of approximately 250 g suitable for flotation experiments.

Table 3.2. The evaluation of the interaction between the microbial cultures and the mineral enabled the assessment of the microorganisms' bioflotation separation potential. The optimal particle size for flotation is generally in the range of 10 – 100 µm (Rahman, et al., 2012). The preparation of the raw samples was performed at the Centre for Minerals Research (CMR) laboratory of the Department of Chemical Engineering at UCT as follows. The procedure began with pulverising the samples to a fine powder for 1 minute followed by dry sieving. Using an automatic shaker, a 106 µm sieve was used to separate the -106 µm fraction from the coarser material. The -106 µm fraction was then passed through a 38 µm sieve to obtain a

fraction that was between 38 and 106 µm. The minerals were additionally washed with an acidified water solution to remove any soluble secondary precipitates prior to use. Particle size analysis was performed using a Malvern Mastersizer 2000 (Malvern instruments – model number MAL1033911) and the resulting particle size distribution is given in Figure 3.1. Once the desired particle size distribution was attained, the sample was passed through a Dickie and Stockler rotary splitter, dividing it into ten equal portions. Two samples from adjacent positions were combined and further divided into smaller samples. This procedure was repeated until the samples were grouped into bags of approximately 250 g suitable for flotation experiments.

Table 3.2: Major elemental composition of the Agnes pyrite concentrate.

Mineral concentrate	Iron (%)	Sulphur (%)
Pyrite	27.38	20.92

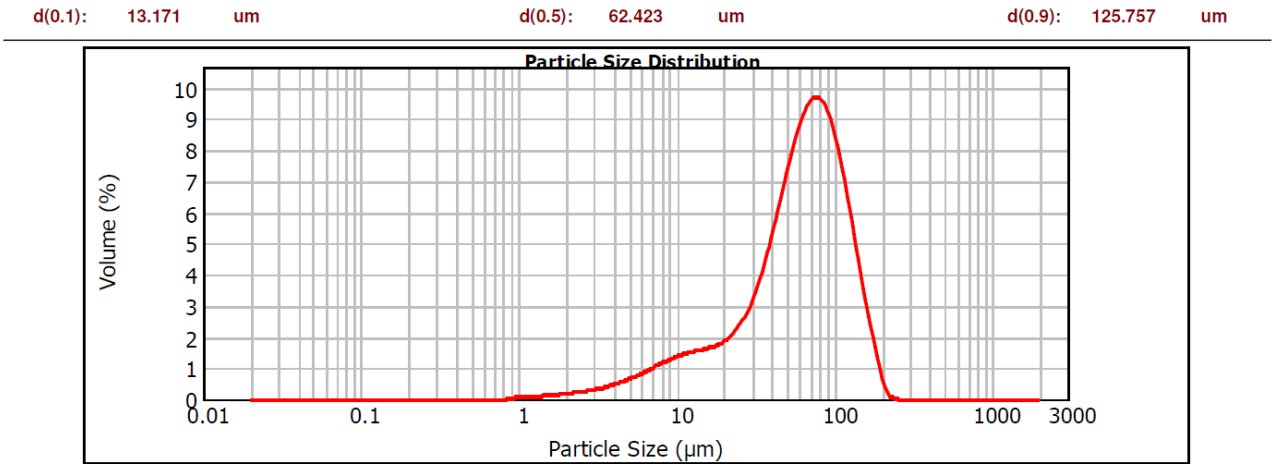

Figure 3.1: Pyrite concentrate particle size distribution.

3.2.2 Coal discard sample preparation

The coal discard sample used in this study originated from the Waterberg coalfields (Site 1) and is described by Chiodza (2018). The 'as-received' sample was dry sieved using an automatic shaker. The screen sizes used were 850, 425, 300, 212, 150, 106 and 75 µm. All 7 sieves were arranged in a stack with the coarsest sieve on the top and the finest sieve at the bottom. All fractions that were greater than 212 µm were milled using a rod mill with 10 rods for 7 minutes followed by dry sieving to obtain a sample with a particle size distribution that ranged between -25 µm and +212 µm. This sample was mixed with the initial screened 'as received' fraction to obtain a final nominal PSD of -25+212 µm. LECO analysis indicated that the sulphur content of the coal discards was 4.57 ± 0.31 %.

The fine coal waste sample was initially treated with both chemical and biological collectors in the first-stage coal flotation process. This stage was carried out as part of the study of Chiodza (2018) and was done to obtain a clean coal stream with reduced sulphur, and a high sulphide-containing tails stream as feed for the second-stage sulphide flotation process. Oleic acid (May & Baker LTD Dagenham England) was used as a control reagent, dosed at 2.79 kg/t, the optimum found byKazadi Mbamba, (2011) while fatty acid methyl esters -FAMEs- (UCT) were used as the biological collector dosed at 1.2 – 3.7 kg/t (Chiodza, 2018).

Flotation tests were done in batches at room temperature using tap water. The mass of coal required to achieve a 6% solids loading was calculated based on the bulk density of the coal and the amount of water required was based on the mass of coal used. At the start of the experiment, the cell was filled with about 20 mL of water. The impeller was switched on, at 170 rpm, followed by addition of the coal sample. The rest of the water was then added, followed by the appropriate amount of collector. The pulp was conditioned for 5 min to allow for even distribution throughout the pulp, at the end of which MIBC was added and left to condition for a further 1 min. After the conditioning period was over, a feed sample was collected and then 5 L/min of air was supplied to the cell to induce froth formation. The froth produced was collected at regular intervals in concentrate basins. Four concentrates were collected: initial concentrate was collected in the first 30 s, concentrate 2 collected in the next 30 s, concentrate 3 collected over the subsequent 1 minute, and concentrate 4 collected over the last 3 minutes. This gave a total flotation time of 5 min. The cell was drained to collect the tails at the end of each batch. The feed sample, concentrates and tails were filtered on filter papers of a known mass and dried in an oven set at 80°C for 24 hours (Memmert, Laboratory and Scientific Equipment Co. (LASEC)). The dried feed samples, concentrates and tails were weighed for yield determination (Chiodza, 2018).

The tails from the first-stage oleic acid and FAME flotation tests were subsequently used as feed for the second-stage sulphide mineral flotation tests,the focus of this study. The sulphur content of the first-stage coal tailings sample was 5.4 % after treating with 2.79 kg/t oleic acid and 4.9 % following treatment with 3.7 kg/t FAMEs. The resulting particle size distribution of the sample after the first-stage flotation treatment is given in Figure 3.2. The tailings were within the particle size range typically required for the flotation of fine coal particles – an intermediate particle size of 74 – 250 µm (Liang, et al., 2015). The first-stage tailings had an average median diameter of 240 µm.

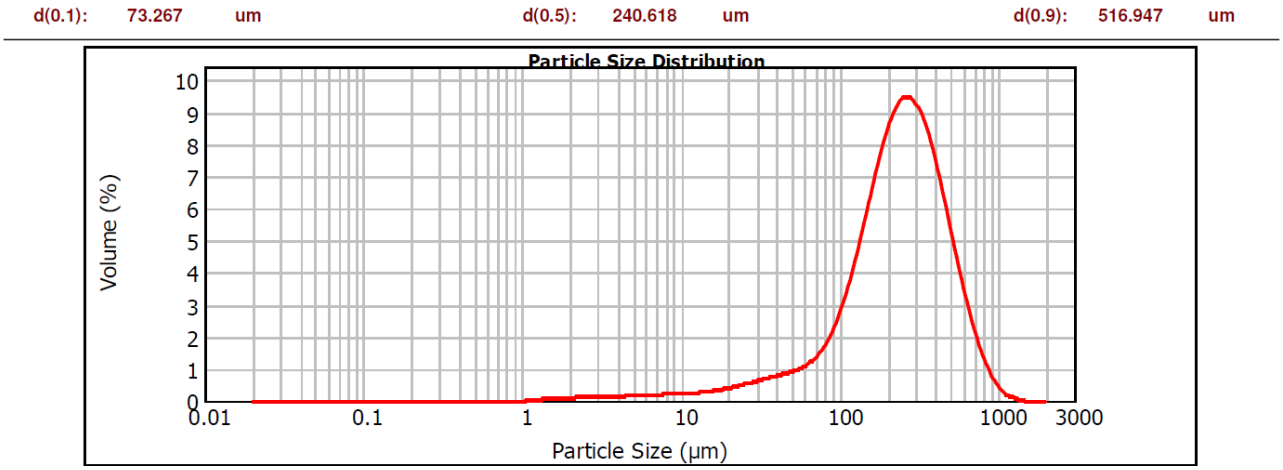

Figure 3.2: Particle size distribution of first-stage coal tails

3.2.3 Base metal hard rock sample preparation

The low-grade hard rock sample used in the experiments originated from a zinc mine. Following QEMSCAN analysis, the bulk mineralogical composition of the sample presented by Makaula et al. (2018) showed that sulphur was largely present in the form of pyrite (33.4 wt. %) and pyrrhotite (3.9 wt. %), both acid generating minerals. Acid consuming minerals present in the sample consisted of predominantly dissolving calcite (0.03 wt. %), fast and intermediate weathering minerals present were predominantly garnet (4.52 wt. %) and slow weathering minerals present in the sample consisted predominantly of muscovite. The largest component of the waste rock consisted predominantly of inert quartz mineral (34.76 wt. %). The 'as-received' sample was prepared in the same way as the pyrite concentrate to obtain a particle size distribution suitable for flotation as shown in Figure 3.3. LECO analysis indicated that the sulphur content of the sample was 1.7 % total sulphur mass.

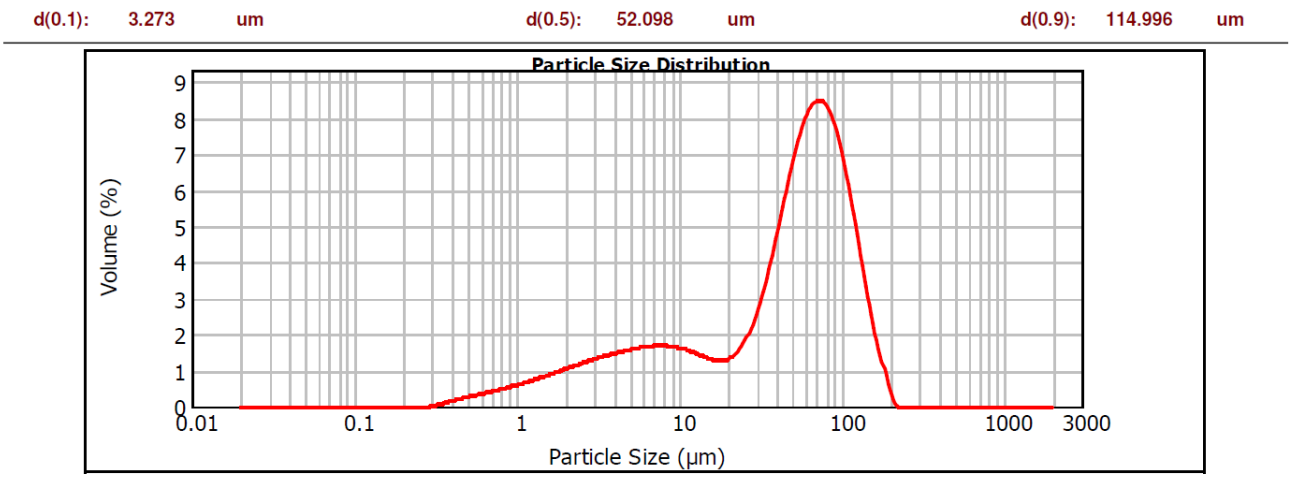

Figure 3.3: Base metal hard rock particle size distribution.

3.2.4 Quartz sample mineral preparation

The quartz mineral used in the attachment experiments was acquired from a garden nursery in the form of quartz pebbles. The pebbles were milled using a rod mill with 10 rods for 7 minutes to reduce the sample to a coarse powder. The powder was subsequently pulverised for 1 minute to obtain a fine powder followed by dry sieving. Using an automatic shaker, a 106 µm sieve was used to separate the -106 µm fraction from the coarser material. The -106 µm fraction was then passed through a 38 µm sieve to obtain a fraction that was between 38 and 106 µm. The mineral was additionally washed with an acidified water solution to remove any soluble secondary precipitates prior to use. Particle size analysis was performed using a Malvern Mastersizer 2000 (Malvern instruments – model number MAL1033911) and the resulting particle size distribution is given in Figure 3.4. Once the desired particle size distribution was attained, the sample was passed through a Dickie and Stockler rotary splitter, dividing it into ten equal portions. Two samples from adjacent positions were combined and further divided into smaller samples. This procedure was repeated until the samples were grouped into bags of approximately 250 g to be used for attachment experiments.

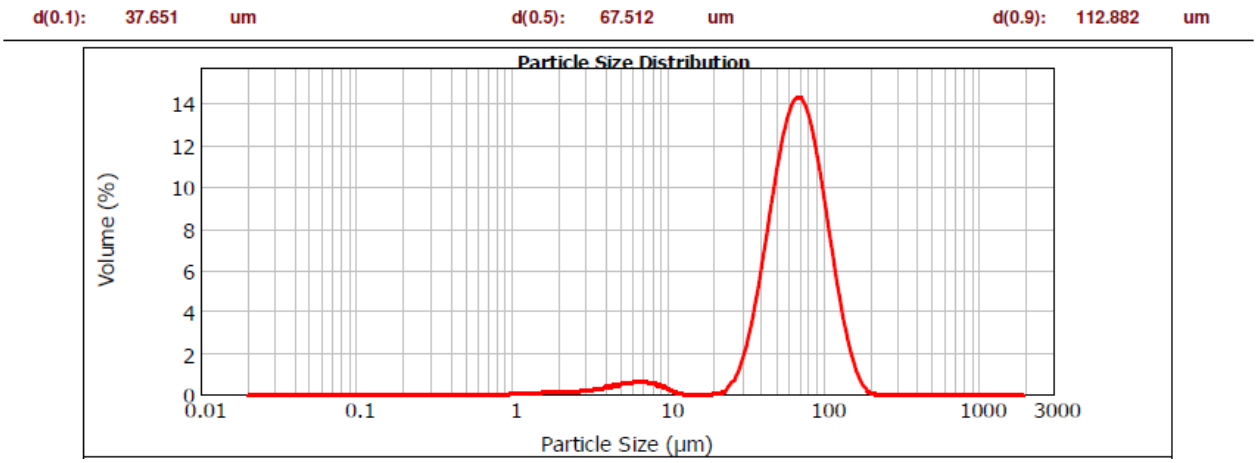

Figure 3.4: Particle size distribution of the quartz sample.

3.3 Microbial cultures: maintenance, culture conditions and inoculum preparation

The microorganisms identified as potential bioflotation reagents were cultured by inoculating a 10 % volume of an actively growing culture in fresh growth medium (appropriately chosen for each microorganism) and grown under conditions that allowed optimum growth for each strain, based on literature. These conditions are detailed in Section 3.3.1. Stationary phase cultures were used for the bioflotation tests.

3.3.1 Pure culture growth conditions

3.3.1.1 Growth conditions for *Bacillus licheniformis* JCM2505

Bacillus licheniformis JCM 2505 cultures [Centre for Bioprocess Engineering Research (CeBER) culture collection, Department of Chemical Engineering, UCT] were grown using Tryptic-Soy liquid medium (see Appendix A) in 500 mL Erlenmeyer flasks containing 250 mL of culture and incubated at 30 °C on a shaking platform for 48 hours.

3.3.1.2 Growth conditions for *Bacillus subtilis*

Bacillus subtilis cultures DMB55 (CeBER culture collection, Department of Chemical Engineering, UCT) were grown aerobically using Tryptic-Soy liquid medium (see Appendix A) in 500 mL Erlenmeyer flasks containing 250 mL of culture and incubated at 30 °C on a shaking platform for 48 hours.

3.3.1.3 Growth conditions for *Rhodopseudomonas palustris* ATCC 17007

Rhodopseudomonas palustris ATCC 17007 cultures (CeBER culture collection, Department of Chemical Engineering, UCT) were grown anaerobically using a nitrogen gas sparged medium, modified *Rhodospirillaceae* medium (see Appendix A). The culture was grown in a

2 L Schott glass bottle containing 2 L of culture at pH 7.4 and 32 °C under illumination with tungsten filament lamps.

3.3.1.4 Growth conditions for *Rhodococcus opacus* DSM43205

Rhodococcus opacus DSM 43205 cultures (DSMZ culture collection) were grown aerobically using GYM *Streptomyces* medium (see Appendix A) in 500 mL Erlenmeyer flasks containing 250 mL of culture and incubated at 30 °C on a shaking platform.

3.3.1.5 Growth conditions for *Paenibacillus polymyxa*

Paenibacillus polymyxa ATCC 842 cultures (DSMZ culture collection) were grown aerobically using modified Bromfield medium (see Appendix A) in a 5 L New Brunswick bioreactor using a radial flow impeller. Batch cultures was grown at 30 ºC, 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.

3.3.2 Mixed microbial culture growth conditions

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

The maintenance and growth conditions of the autotrophic thiocyanate (SCN^-) degrading microbial consortium used in this study are described in detail by van Zyl et al. (2017). The culture was grown using acidified 0K basal salt medium (BSM) at pH 2.0 (see Appendix A) supplemented with thiocyanate and maintained on a shaking platform in a 30 °C incubator.

3.3.2.2 Sulphur oxidizers flotation tests

A mixed mesophilic culture dominated by *Acidithiobacillus caldus,* a lesser percentage of *Leptospirillum ferriphilum* and Archaea was used. The maintenance and growth conditions of the culture are described in Ngoma et al. (2017). The culture was grown on elemental sulphur in acidified 0K basal salt medium (pH 2.0) and maintained on a shaking platform in a 30 °C incubator.

3.3.3 Inoculum preparation

The microbial culture was centrifuged at 10 000 rpm (JA-10 rotor, Beckmann centrifuge) at room temperature for 10 – 15 minutes to pelletise the cells. The supernatant was decanted and the harvested cells were washed two to three times using tap water at pH 4 or pH 7. The cells were re-suspended in tap water, after which the cell concentration was enumerated, as described in Section 3.7.2. The inoculum was diluted accordingly using tap water to obtain the desired cell concentration for the following experiments.

3.4 Batch attachment experiments

The attachment of each of the microbial cultures to the pyrite concentrate was investigated. All the attachment experiments were carried out in 250 mL Erlenmeyer flasks containing 1 wt% of pyrite and made up to 100 mL using tap water. The flasks were agitated on a platform shaker at 200 rpm to ensure that the mineral remained in suspension. The attachment studies were performed to evaluate the attachment behaviour of each bacterial strain onto the mineral with respect to time and initial cell concentration (Africa, et al., 2013a). The microbial inoculum was added at time zero, following which samples were removed at select time points. The unattached planktonic cells remaining in the solution were enumerated using direct microscopic cell counting. The cell concentration obtained was subtracted from the initial cell concentration to quantify the number of cells attached to the mineral.

As discussed in Section **Error! Reference source not found.**, pH plays an important role in microbe-mineral interactions. Therefore, the effect of pH on attachment efficiency was investigated at pH 4 and 7 to evaluate the pH at which maximum attachment would occur. The value of pH 4 was selected on the basis that most bacterial PZC's are between pH 2 and pH 4 where the bacterial surface is neutral and the force of repulsion is less, as discussed in Section 2.3.2. Furthermore, a pH of 6.5 – 7.5 is used conventionally for the flotation of low pyrite-copper ores (Fuerstenau, et al., 2007). Therefore, microbial attachment to pyrite was investigated at pH 7, as well as pH 4.

3.5 Surface chemical characterisation

Zeta potential measurements were made using a Malvern Zetasizer Nano Series (NS) ZN3600 instrument. For mineral samples, 0.2 – 1 wt% of sample was conditioned in 1 mM NaCl solution at the required pH value for 15 to 30 minutes prior to zeta potential measurements. For bacterial suspensions, 10^7 cell/mL of bacterial culture in deionised water was added to 1 mM NaCl solution and conditioned at the required pH value for 15 to 30 minutes prior to zeta potential measurements. The parameters listed in Table 3.3 were used for all zeta potential experiments.

Table 3.3: 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	700nm - 750nm	467.8nm	700nm
Dispersant	NaCl	NaCl	NaCl
[Dispersant]	1mM	1mM	1mM
pH [Dispersant]	3, 5, 7, 9	3, 5, 7, 9	3, 5, 7, 9
Refractive index dispersant	@700nm = 1.5387	@467.8nm = 1.56	@700nm = 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

3.6 Flotation experiments

Laboratory batch flotation tests were carried out on pyrite, tailings from the first-stage flotation of fine coal discards and low-grade base metal samples. Chemical flotation tests were carried out as a base case to provide a comparison for flotation with microbial cultures. This was followed by flotation using microbial cultures to evaluate their bioflotation potential.

3.6.1 Flotation cells

3.6.1.1 Pyrite concentrate flotation

All flotation experiments using pyrite were carried out using a 3 L Leeds bottom-driven and sub-aeration laboratory scale batch flotation cell, pictured in Figure 3.5. In this setup, the concentrate was scraped off the top of the float cell at specific time intervals whilst the tails exited via a pipe at the bottom of the float cell at the end of the run. A pulp density in the region of 1.67 % (50 g per float) was used in all tests. Experiments were conducted at pH 4 or pH 7 to evaluate the pH at which maximum recovery would occur.

Figure 3.5: Leeds 3 litre bottom-driven and sub-aeration laboratory scale batch flotation cell.

3.6.1.2 First-stage coal discard tailings flotation

Due to a limitation in the sample size available for tests, a smaller 0.5 L Leeds bottom-driven and sub-aeration laboratory scale batch flotation cell (pictured in Figure 3.6) was used for the experiments with coal discard tailings that had already gone through the first flotation separation stage to remove the coal. The setup described in Section 3.6.1.1 was applied in all tests at the same solids loading of 1.67 % (8.35 g per float).

3.6.1.3 Base metal hard rock flotation

The setup described in Section 3.6.1.2 was applied in all tests at the same solids loading of 1.67 % (8.35 g per float).

Figure 3.6: Leeds 0.5 litre bottom-driven and sub-aeration laboratory scale batch flotation cell.

3.6.2 Flotation conditions

3.6.2.1 Chemical flotation

The experimental method and procedure as presented by Kazadi Mbamba (2011) for desulphurisation flotation were applied for positive chemical control flotation experiments. The samples were pre-mixed with approximately 500 mL of tap water at the desired pH and decanted into the float cell. Thereafter, the float cell was filled with water to a volume of 3 L. Once the float cell was filled, the impeller was turned on and set to a speed of 1200 rpm. The pulp was conditioned with the sulphide collector Potassium Amyl Xanthate (PAX, Senmin®) for 5 minutes while keeping a constant pH value of 7 to ensure that the conditions remained neutral throughout the conditioning period. This was followed by the addition of the frothing agent Methyl Isobutyl Carbinol (MIBC, Sigma-Aldrich) and an additional 1 minute of conditioning. After conditioning with the frother, compressed air was introduced in the cell at a flow rate of 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 an 80°C room. The dried samples were then weighed to determine the mass of the solids recovered. Finally, the total sulphur of the samples was measured using LECO analysis.

The same procedure was followed using the 0.5 L float cell except the conditions were 3 L/min air flow rate and 140 pm impeller speed.

3.6.2.2 Biological flotation

The bioflotation procedure was the same as the chemical flotation procedure except microbial cultures were used as a collector in place of PAX. In the bioflotation tests, a microbial culture inoculum of the microbe being tested was added to the pulp (to a final volume of 3 L) and conditioned together for 5 minutes while keeping a constant pH value of 4 or pH 7. This was done to ensure that the conditions remained acidic or neutral throughout the conditioning period to allow for maximum association. After conditioning, MIBC frother was added and an additional 1 minute of conditioning was allowed after which four concentrates were collected over 20 minutes as in the chemical flotation.

3.7 Analytical techniques

3.7.1 Determination of pH

All pH measurements were conducted using a Metrohm 704 pH meter and probe. The probe was calibrated at pH 7.0, pH 4.0 and pH 1.0 before use.

3.7.2 Enumeration of cell concentration

The cell concentration required for all bacterial experiments was determined using a *Thoma* counting chamber and an *Olympus* CX41 microscope under 1000X magnification using oil immersion. The formula used for calculating cell concentration per mL is given below:

Volume of one small square = depth*area

$$= \frac{0.02\ mm * (0.05\ mm * 0.05\ mm)}{1000}$$

$$= 5 \times 10^{-8}\ cm^3$$

Concentration total (cells mL^{-1}) $= \frac{\left(cell\ count * \frac{N}{n}\right)}{volume\ one\ square * total\ number\ of\ small\ squares} * dilution\ factor$

where N is the total number of big squares (16) and n is the number of squares counted (4).

3.7.3 (Bio)flotation sample analysis

Feed, concentrate and tails samples from each experiment were filtered, dried overnight at 75 °C and weighed before analysis. The total yield was reported as the % mass recovery to the concentrate, calculated as shown below:

$$Yield(\%) = \frac{mass\ of\ concentrate}{mass\ of\ feed} \times 100$$

3.7.4 Sulphur analysis

The total sulphur content in the feed, concentrates and tails from all flotation experiments was analysed used a LECO sulphur analyser in the analytical laboratory at the University of Cape Town. LECO combustible analysis is a reliable method for determining the concentration of Carbon, Sulphur, Nitrogen, Oxygen and Hydrogen in a variety of metal/alloy combinations. LECO analysis measures the infrared absorption of combustion gases from a sample to accurately determine the presence and concentration of these elements. LECO analysis successfully detects low levels of C, O, S, N and H (from 0.0002 to 0.05 percent depending on the sample) (Holmes, 1963). The sulphur recovery to the concentrate was calculated as:

$$Sulphur\ recovery\ (\%) = \frac{(sulphur\ content\ of\ concentrate) \times (mass\ of\ concentrate)}{(sulphur\ content\ of\ feed) \times (mass\ of\ feed)} \times 100$$

3.7.5 Particle size analysis

The particle size distribution was determined by laser diffraction using a Malvern Mastersizer 2000 particle size analyser (Malvern instruments – model number MAL1033911) at the Analytical laboratory in the Department of Chemical Engineering at UCT.

CHAPTER 4: Microbial screening tests

4.1 Introduction

As stated in Chapter 1, the main objective of this study was to investigate the prevention of ARD formation through the desulphurization of pyrite-containing coal discard and low-grade base metal hard rock samples using alternative bioflotation reagents. To achieve this, microbial culture screening tests using attachment experiments, batch bioflotation experiments and surface characterization tests were performed to investigate the affinity of each microbial species to pyrite and their resultant bioflotation potential.

The microbial growth curves for *B. subtilis, R. opacus, P. polymyxa, B. licheniformis* and *R. palustris* are presented in Section 4.2. The information obtained from these curves was used to inform the age (growth phase) at which each culture was used for all attachment and bioflotation experiments. The growth curves were used to determine the time it took for the cultures to get to stationary phase. The trends of microbial attachment to a pyrite concentrate were assessed for each species using batch agitated attachment tests and the results are presented in Section 0. The attachment data provides an indication of the microorganisms' affinity to pyrite and thus forms the initial screening step in the selection of candidates to be taken forward to bioflotation testing. Section 4.4 provides the results of the batch bioflotation experiments using each of the selected microbial species to investigate their ability to enhance the floatability of pyrite. Elucidating the influence of each microorganism on the floatability of pure pyrite enabled the selection of microorganisms with potential to separate pyrite from the gangue in coal discard or base metal sulphide mineral samples. Section 4.5 provides the results of the surface characterization tests using zeta-potential measurements to characterize surface charge. This is of essential importance in flotation separation as the surface charge on a mineral or bacterial cell affects the likelihood of polar interactions with water molecules and is thus a key factor in flotation. A discussion of the results is given is Section 4.6 followed by conclusions which highlight, inter alia, the selection of a candidate microorganism to be carried forth for testing with pyrite-containing coal discards and the base metal sulphide mineral samples.

4.2 Microbial culture growth conditions and inoculum preparation

Microbial growth curves were produced to determine the specific growth rate (μ) and doubling time (t_d) for each microbial species. Cultures in the stationary phase of growth were used for all experiments. During stationary phase, the net reproduction rate is zero with the number of cells undergoing division equal to the number undergoing cell death. Finally, the bacteria stop

dividing completely and the onset of the death phase occurs. In stationary phase, the growth rate is stabilized and the assumption is that there will be no significant variation in the initial cell concentration. Furthermore, all attachment experiments were conducted within a single doubling time of the microbe. However, it is worth noting that the environmental conditions for cultivation such as the nutrients available in the medium, pH, dissolved oxygen concentration and temperature are all factors that affect μ and t_d (Verster, et al., 2013). Hence, the values obtained in literature vary with cultivation practices.

The specific growth rate (μ) and the doubling time (t_d) were calculated using the exponential phase data of a growth curve. The following formulae were used to calculate μ and t_d.

$$Specific\ growth\ rate\ (h^{-1}): \mu = \frac{ln(X) - ln(X_0)}{t - t_0}$$

$$Doubling\ time\ (h^{-1}): 2 = e^{\mu t_d}$$

The curves were constructed using absorbance readings (as a measure of cell concentration) as a function of incubation time for each microorganism as displayed in Figure 4.1 and Figure 4.2. *P. polymyxa* was grown using a stirred tank bioreactor due to low biomass concentrations that were achieved in shake flasks, requiring increased culture volume. The latter occurs as a result of organic acids produced by the microorganism which decrease the pH of the medium below that at which *P. polymyxa* grows optimally. Hence, controlling the pH using a bioreactor ensured that the pH remained neutral, thereby achieving maximum biomass concentrations as shown in Figure 4.1.

Table 4.1 summarises the specific growth rate (μ) and doubling time (t) values calculated from the growth curves. *B. subtilis* and *B. licheniformis* exhibited a similar growth rate where both cultures had reached stationary phase after 24 hours of incubation. Based on the results, all subsequent experiments using *B. subtilis* and *B. licheniformis* were conducted using stationary phase cultures grown for 48 hours. *P. polymyxa* cultures grown for 12 hours were used for all experiments as the cultures reached stationary phase at this time. *R. palustris* cultures grown for 1 week (after reaching stationary phase and achieving the required biomass concentration) were used for all experiments.

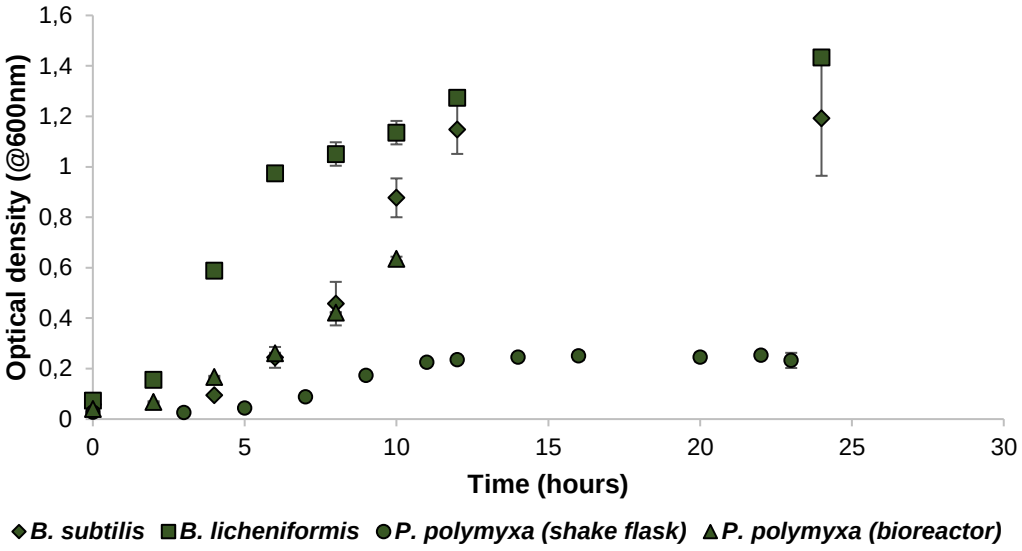

Figure 4.1: Bacterial growth curves depicting the growth trends of *B. subtilis, B. licheniformis* and *P. polymyxa*. Error bars represent variation between triplicate flasks.

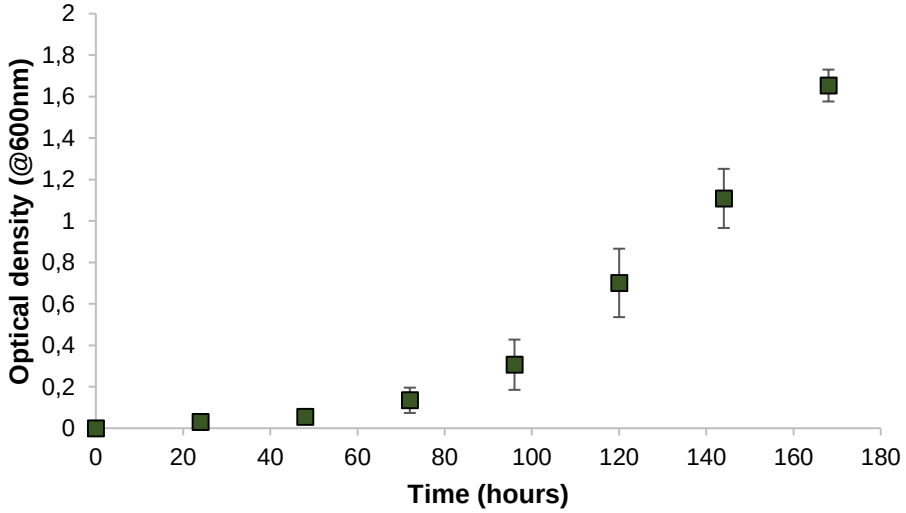

Figure 4.2: Bacterial growth curve for *R. palustris* which uses optical density of the suspension as a proxy for cell concentration.

Table 4.1: Summary of the specific growth rate (μ) and doubling time (*t*) values of all microorganisms used in this study.

Microorganism	μ (h^{-1})	t_d (h)
B. subtilis	0.3	2.3
B. licheniformis	0.2	3.4
P. polymyxa (shake flask)	0.3	2.3
P. polymyxa (bioreactor)	0.2	3.3
R. palustris	0.028	24.8

4.3 Batch agitated attachment experiments

Attachment experiments were carried out at pH 4 (around heterotrophic microbes' general isoelectric point) and pH 7 (as per the 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. Data was reported as an average of triplicate results and the error bars represent a single standard deviation in the results.

Control attachment tests which had no mineral added were performed for each microorganism. The concentration of suspended cells was observed to remain unchanged over the duration of the control experiment for all microbes, confirming no natural settling of the microbes occurred at the test conditions nor any significant growth and so that any change in microbial concentration in the liquid during the actual experiments was due to mineral-microbe interaction.

4.3.1 Attachment results for *B. subtilis*

The attachment data of *B. subtilis* to pyrite at pH 4 and pH 7 is presented in Figure 4.3. After 1 minute of interaction, 51.0 ± 5.0 % attachment was obtained at pH 7 while a lower percentage attachment of 49.2 ± 3.3 % was achieved at pH 4, indicating microbe-mineral interaction. The percentage of attachment increased after 5 minutes to 65.6 ± 6.7 % at pH 7 and 60.7 ± 2.3 % at pH 4. The highest percentage attachment attained at pH 7 was 82.3 ± 1.9 % at pH 7 after 10 minutes, with a slight, but not significant decrease to 81.0 ± 5.0% after 20 minutes. The same increase in attachment over time trend was observed at pH 4 with the highest percentage attachment of 76.0 ± 1.9 % achieved after 20 minutes.

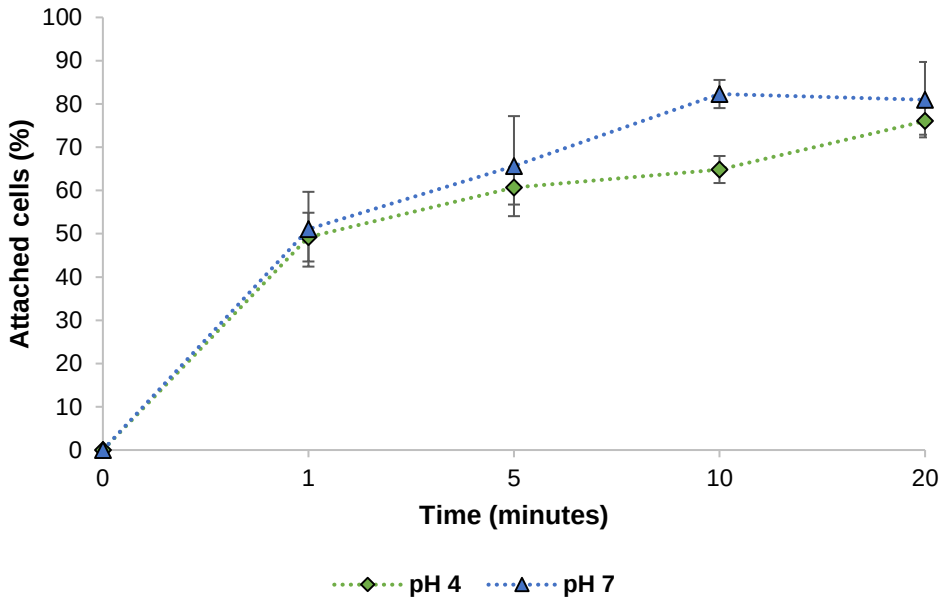

Figure 4.3: Percentage attachment of *B. subtilis* cells to pyrite in a 1 g pyrite per 100 mL suspension containing an initial cell concentration of 1×10^7 cells/mL.

4.3.2 Attachment results for *R. opacus*

Figure 4.4 shows the percentage attachment of *R. opacus* cells to pyrite at pH 4 and pH 7 over time. Attachment of *R. opacus* to pyrite was evident at both pH conditions. A percentage attachment of 70.8 ± 13.1 % was achieved after 1 minute of interaction at pH 7 while a lower degree of attachment of 41.4 ± 8.6 % was observed at pH 4. The level of attachment improved significantly at pH 4 after 5 minutes where 87.8 ± 0.9 % was obtained. This was still lower than the 94.8 ± 0.6 % achieved at pH 7 after 5 minutes. The levels of attachment attained after 10 minutes increased only marginally from 94.8 ± 0.6 % to 97.4 ± 0.7 % at pH 7 while an increase to 95.1 ± 1.3 % attachment was obtained at pH 4.

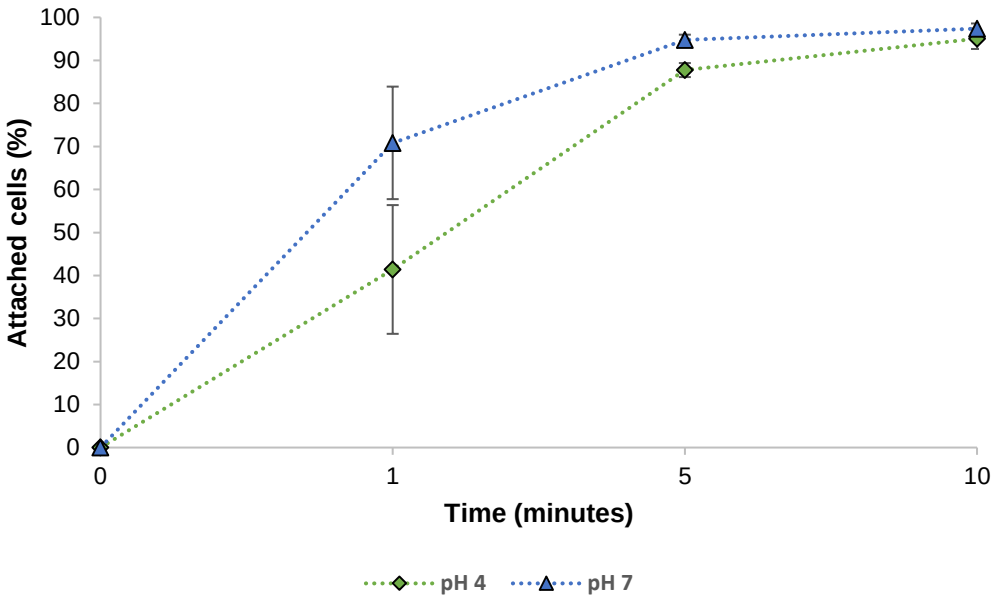

Figure 4.4: Percentage attachment of *R. opacus* cells to pyrite in a 1 g pyrite per 100 mL suspension containing an initial cell concentration of 1×10^7 cells/mL.

4.3.3 Attachment results for *P. polymyxa*

Figure 4.5 shows the percentage attachment of *P. polymyxa* cells to pyrite at pH 4 and pH 7 over time. A rapid and high percentage of attachment was obtained at pH 7 where, after 1 minute, 93.0 ± 2.0 % attachment was attained. At pH 4, the level of attachment was high at 70.1 ± 1.6 % after 1 minute, but not as rapid as that observed at pH 7. The percentage of attachment attained after 10 and 20 minutes increased only marginally from 92.4 ± 2.0 % to 95.6 ± 1.0 % at pH 4 and 93.2 ± 1.6 % to 97.1 ± 0.7 % at pH 7, respectively.

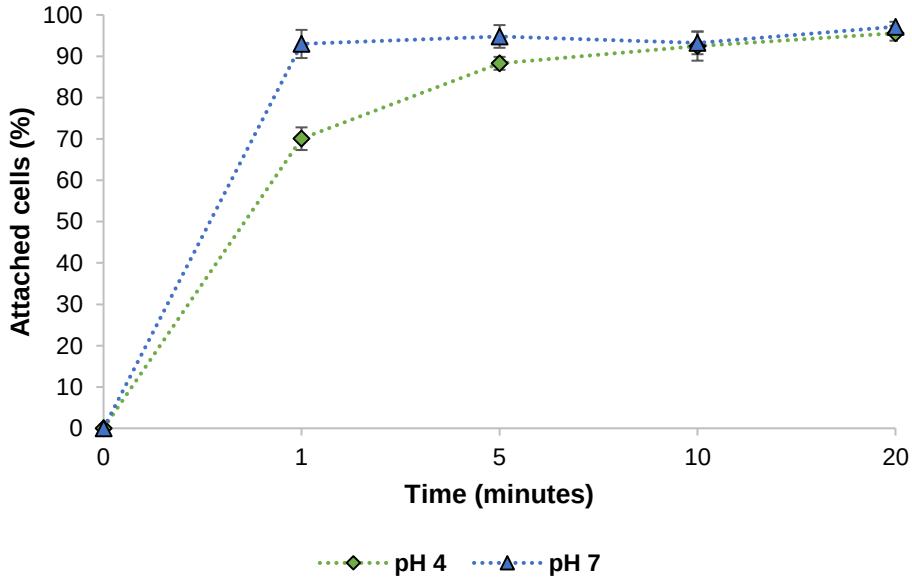

Figure 4.5: Percentage attachment of *P. polymyxa* cells to pyrite in a 1 g pyrite per 100 mL suspension containing an initial cell concentration of 1×10^7 cells/mL.

4.3.4 Attachment studies for *B. licheniformis*

Results obtained from the attachment experiments using a *B. licheniformis* culture are presented in Figure 4.6. Instead of presenting the data as percentage attachment, the plot shows the planktonic cell numbers enumerated in the samples containing pyrite as well as in the negative control to which no mineral was added. There was no significant difference between the planktonic cell numbers in the control and the experiments. This result means that *B. licheniformis* cells did not have the affinity to attach to pyrite at pH 7. Based on this result, their affinity to pyrite at pH 4 was not investigated. This represents the first study reported to consider *B.licheniformis* as a flotation reagent.

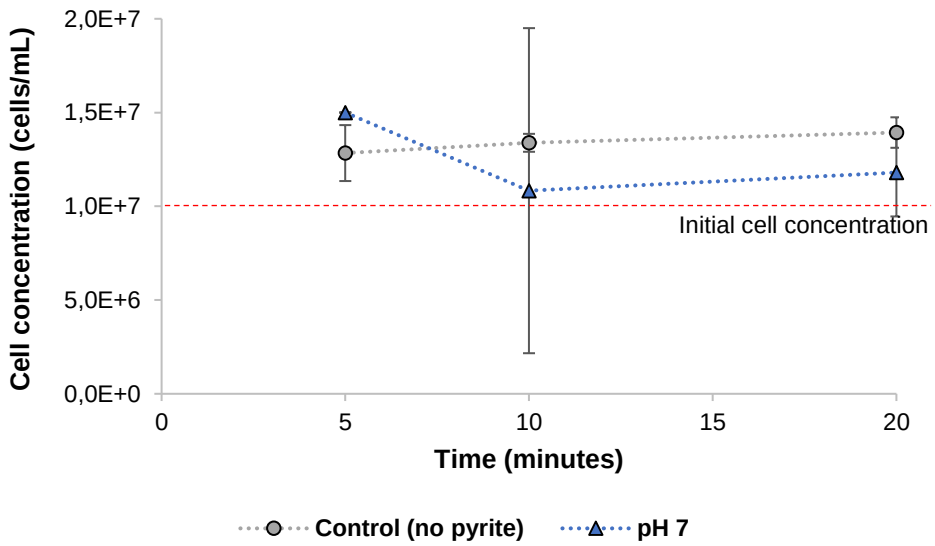

Figure 4.6: Cell numbers retained in the liquid over time (unattached cells) in the control experiment (no mineral) as well as in the presence of pyrite. Experiments were done using a 1 g solid per 100 mL suspension containing 1×10^7 cells/mL at pH 7.

4.3.5 Attachment results for *R. palustris*

Figure 4.7 shows the percentage attachment of *R. palustris* cells to pyrite at pH 4 and pH 7 over time. Attachment of the cells to pyrite occurred rapidly, with 63.5 ± 3.1 % of the initial cell population having attached to the mineral after 1 minute at pH 4 and 71.5 ± 2.2 % at pH 7. After 5 minutes, the percentage of attachment at pH 4 of 72.7 ± 4.1 %was higher than that at pH 7 of 62.9 ± 4.1 %. The highest degree of attachment was observed at 10 minutes with 86.2 ± 0.7% of the initial cell population attached to the mineral at pH 4 and 75.0% ± 0.6 attachment at pH 7, after which a slight but not significant decrease was observed at 20 minutes.

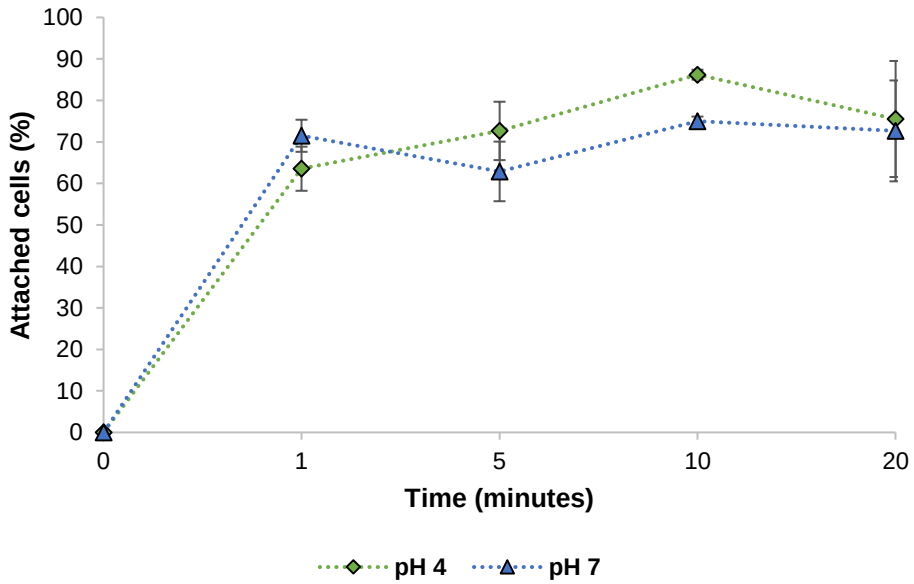

Figure 4.7: Percentage attachment of *R. palustris* cells to pyrite in a 1 g pyrite per 100 mL suspension containing an initial cell concentration of 1×10^7 cells/mL.

4.4 Batch (bio)flotation experiments

4.4.1 Chemical flotation controls

Chemical flotation experiments using samples containing only pyrite were used as a base case against which to compare the collection of pyrite to the concentrate using bioflotation. 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 at the lower concentration, likely due to the use of a pure pyrite sample instead of actual tailings from the first-stage float. Dextrin was omitted as a flotation reagent because it is a coal depressant and was therefore not required. The positive control full chemical float was done at pH 7 (as per the chemical flotation tests) while the negative control 'frother only' float was done at pH 7 and 4. A full chemical float was not done at pH 4 as PAX is not liquid-bound at low pH and hence presented a health and safety risk. Table 4.2 summarises the cumulative mass recovery of pyrite achieved in the concentrate.

Recovery of pyrite was immediately observed in the full chemical control with 94.1% recovered in the first concentrate due to the high sulphide selectivity of PAX. Negligible collection was observed in froth-only flotation tests at both pH 4 and 7.

Table 4.2: 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

4.4.2 Pure culture bioflotation experiments

Pyrite concentrate bioflotation tests were performed under the same conditions as the 'froth-only' flotation at pH 4 and pH 7. The tests were performed using the microorganisms that displayed an affinity for pyrite in the attachment experiments. *B. licheniformis* which did not attach to the mineral as shown in Section 4.3.4 was not included. Because cultures grown for 48 hours were used for *B. subtilis* and *R. opacus* attachment tests, as discussed in Section 4.2, a maximum cell concentration of 4.2×10^9 cells/mL - achieved after 48 hours of incubation - was used for all flotation experiments excluding experiments with *P. polymyxa*. This concentration was achieved by the *R. palustris* culture after a week of incubation. Bioflotation tests with *P. polymyxa* were performed using a cell to mineral ratio of 2.45×10^9 cells/mL as this was the maximum cell concentration achieved after 12 hours of incubation. For each test, 50 g of pyrite was treated with the appropriate microbial culture where the cell to mineral ratio for the test was 4.2×10^9 cells/g in general and 2.45×10^9 cells/g with *P. polymyxa*. Because only pure pyrite mineral was fed to the system, the floats were evaluated based on the cumulative mass recovery of pyrite in the concentrate only.

4.4.2.1 Bioflotation results for *B. subtilis*

The results presented in Figure 4.8 show that there was no significant collection of the mineral in the concentrate over the duration of the experiments at pH 4 and pH 7. The total cumulative mass recovered was 0.7 ± 0.7 % at pH 4 and 0.7 ± 0.4 at pH 7, only marginally higher and insignificantly than that of the negative control values of 0.2 and 0.1 % respectively. This showed that *B. subtilis* had no significant positive influence on the floatability of pyrite under the pH conditions investigated.

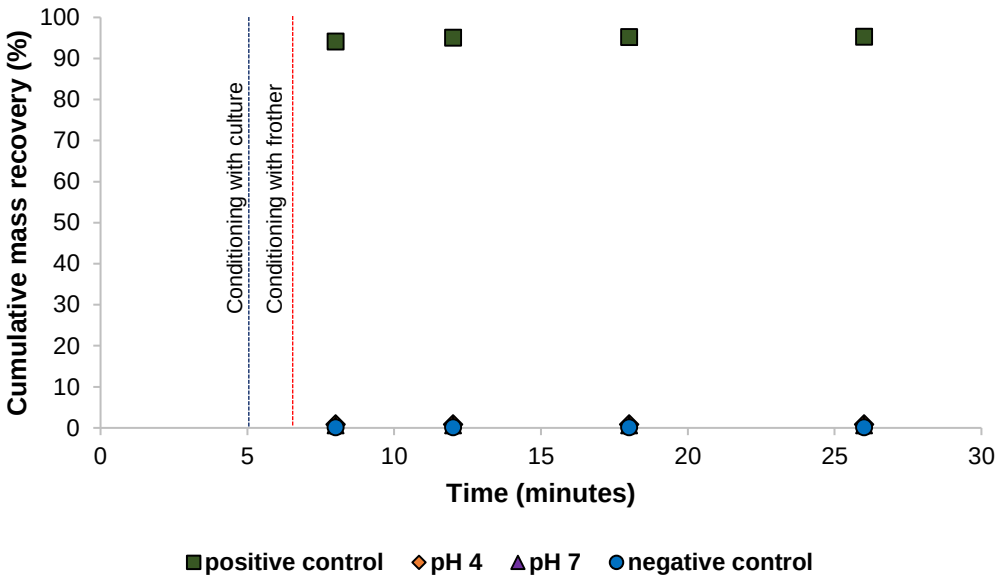

Figure 4.8: Cumulative mass recovery of pyrite after floating with the *B. subtilis* culture at a cell concentration of 4.2×10^9 cells/g.

4.4.2.2 Bioflotation results for *R. opacus*

Figure 4.9 shows the cumulative mass recovery of pyrite after treating with a *R. opacus* culture. There was no significant amount of pyrite recovered into the concentrate at both pH 4 and pH 7. Low cumulative mass recoveries of 1.79 ± 0.01% and 0.99 ± 0.04% were attained at pH 7 and pH 4 respectively. This indicated that *R. opacus* had no significant positive influence on the floatability of pyrite under the pH conditions investigated.

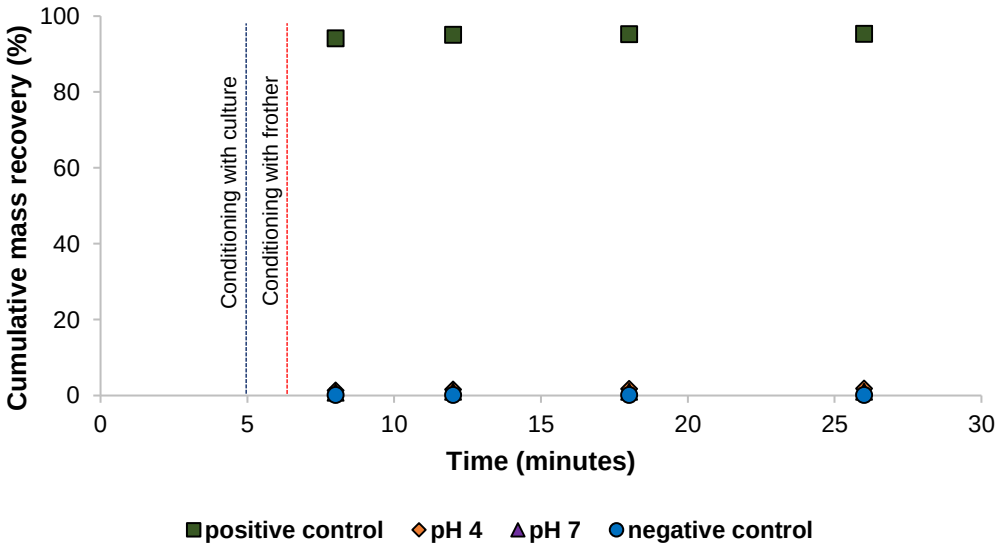

Figure 4.9: Cumulative mass recovery of pyrite after floating with the *R. opacus* culture at a cell concentration of 4.2×10^9 cells/g.

4.4.2.3 Bioflotation results for *P. polymyxa*

The results observed for the bioflotation of pyrite using a *P. polymyxa* culture are presented in Figure 4.10. At pH 4, 7.0 ± 0.42 % of the mineral was collected while 81.3 ± 0.4 % was collected at pH 7, only marginally below that of the chemical control. This observed behaviour indicated that *P. polymyxa* positively influenced the floatability of pyrite at pH 4 and pH 7. At pH 7, its performance approached the standard chemical protocol.

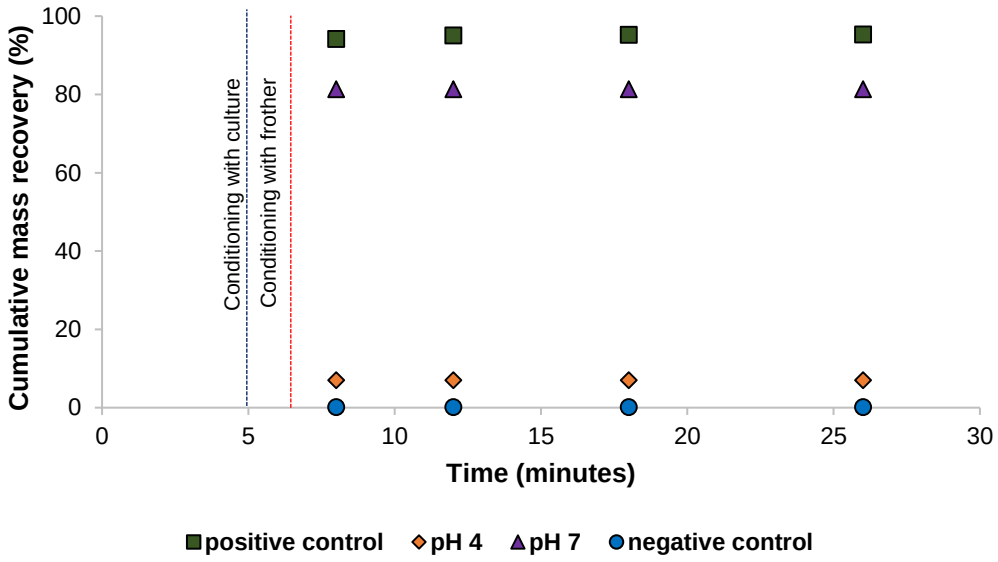

Figure 4.10: Cumulative mass recovery of pyrite after floating with the *P. polymyxa* culture at a cell concentration of 2.45×10^9 cells/g.

4.4.2.4 Bioflotation results for *R. palustris*

Figure 4.11 shows the cumulative mass recovery of pyrite after treating with a *R. palustris* culture. Low cumulative mass recoveries of 0.37 ± 0.01 % and 1.10 ± 0.04 % were attained at pH 7 and pH 4 respectively. There was no significant amount of pyrite recovered in the concentrate at both pH 4 and pH 7. This indicated that *R. palustris* had no significant positive influence on the floatability of pyrite under the pH conditions investigated.

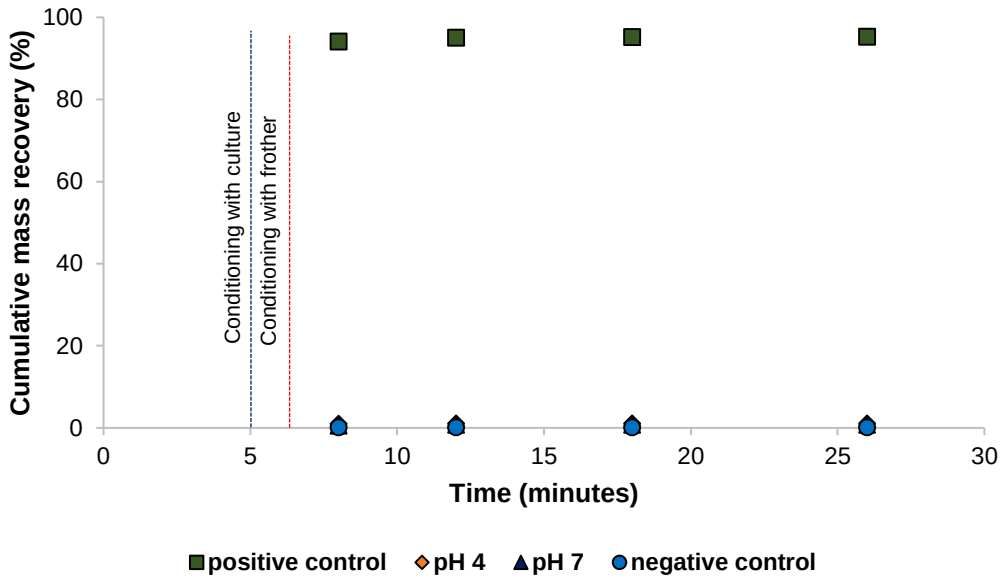

Figure 4.11: Cumulative mass recovery of pyrite after floating with the *R. palustris* culture containing a cell concentration of 4.2×10^9 cells/g.

4.4.3 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 and a mixed mesophilic sulphur oxidizing culture. Because most bacterial surfaces have PZC's below pH 4, these tests were done at a pH value of 4.

4.4.3.1 Sulphur oxidizers flotation tests

Figure 4.12 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. Furthermore, If

the mixed culture had shown an effect on the flotation of pyrite, the concentrate would have been plated on a suitable growth medium to try and isolate and identify the strain(s) responsible for upgrading the mineral to the concentrate. This would be followed by attachment tests to elucidate the attachment efficiencies of the responsible microorganism.

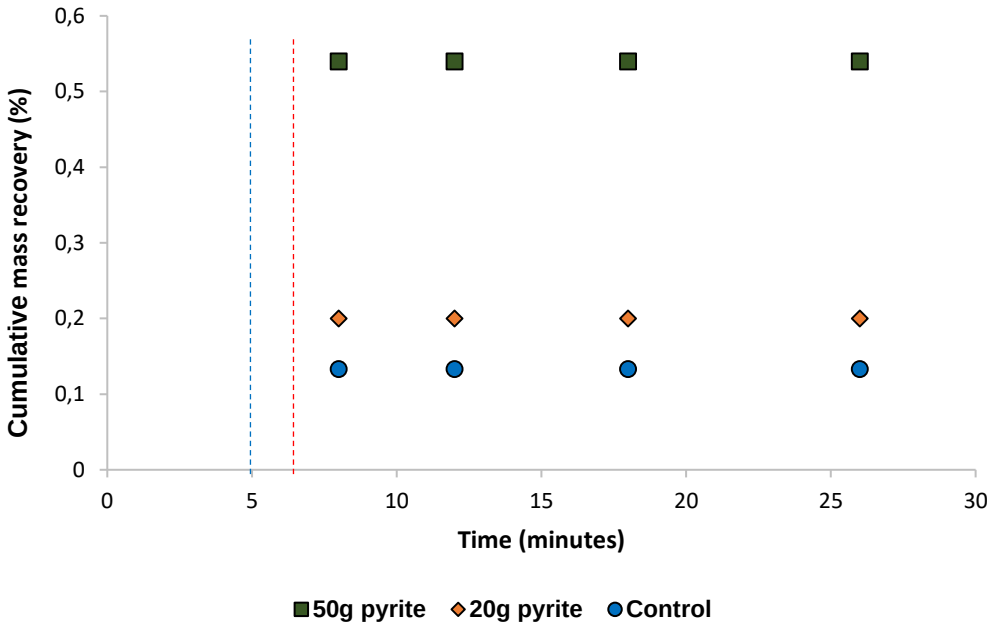

Figure 4.12: Cumulative mass recovery of pyrite after floating with sulphur oxidizing mixed culture: 50 g of pyrite was treated with a culture containing a cell concentration of 1×10^9 cells/g, and 20 g of pyrite was treated with a culture containing a concentration of 2×10^9 cells/g.

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

Figure 4.13 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.

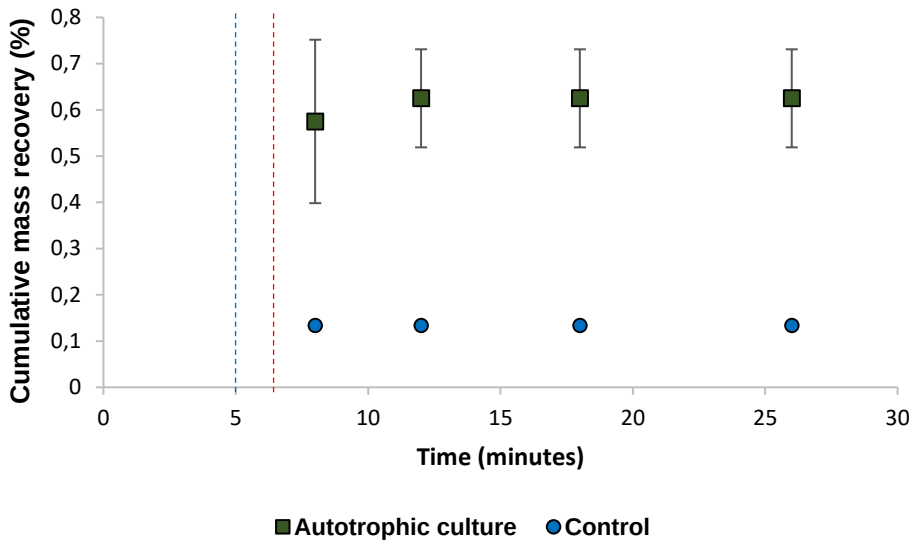

Figure 4.13: Cumulative mass recovery of pyrite after floating with autotrophic SCN^- degrading mixed culture; 20 g of pyrite was treated with a culture containing a concentration of 2×10^9 cells/g. Error bars show a standard deviation variation across duplicate repeats.

4.5 Surface characterization tests

The zeta-potential profile of the pyrite mineral concentrate before interaction with microbial cultures is presented in Figure 4.14, displaying a PZC of approximately pH 6. In the pH range 3 – 6, pyrite exhibited a net positive charge, only recording a slightly net negative charge of -73 mV at neutral pH.

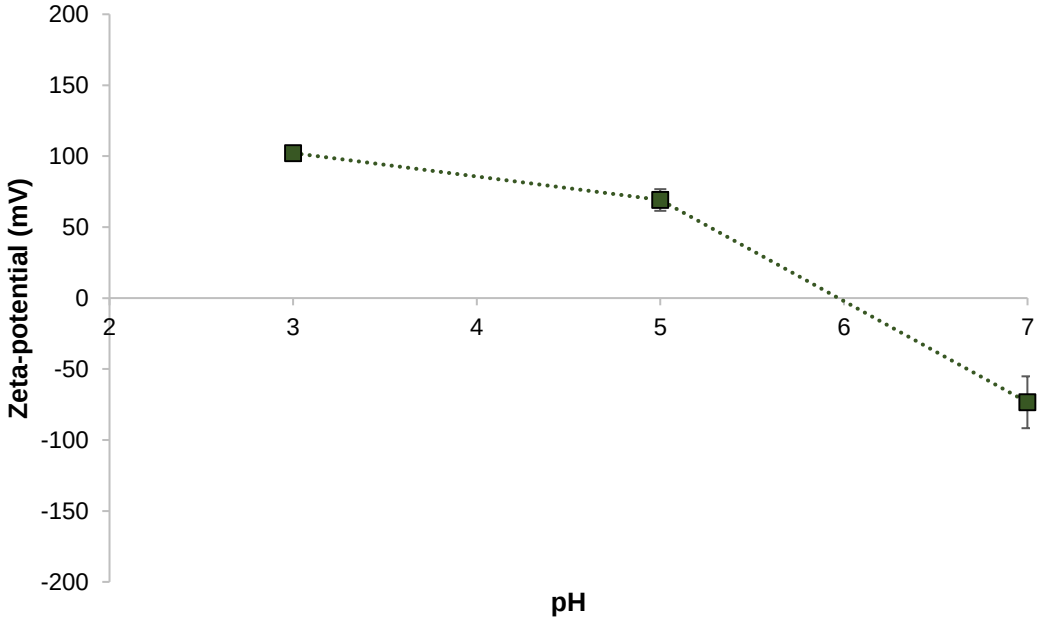

Figure 4.14: Measured zeta-potential of pyrite as a function of pH.

The zeta-potential profile for each microbial culture is presented in Figure 4.15. *B. subtilis, R. opacus* and *R. palustris* displayed PZCs around pH 3.2, pH 3.5 and pH 3.6 respectively, and were mostly negatively charged across the pH range tested. *B. licheniformis* on the other hand exhibited a net positive charge in the acidic pH range, recording a net positive charge of 242.1 mV at pH 3. A net negative surface charge was exhibited at pHs above its PZC at pH 4.6. *P. polymyxa* was the only microorganism that exhibited a near neutral surface charge across the pH range tested, sustaining zeta-potential measurements (magnitude of charge) close to the PZC.

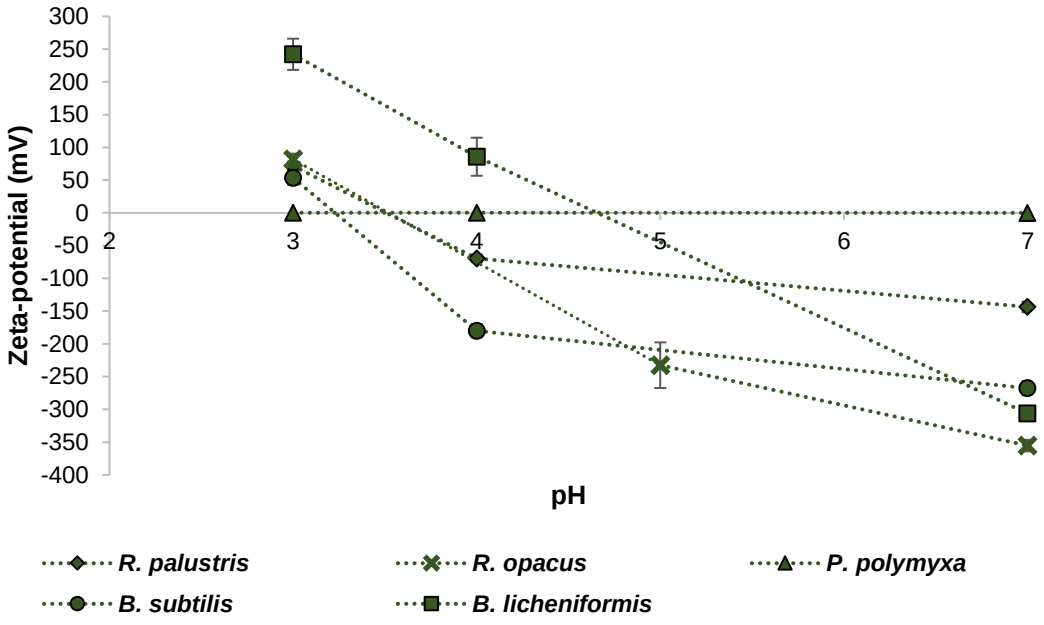

Figure 4.15: Measured zeta-potential of *R. palustris, R. opacus, B. subtilis, B. licheniformis* and *P. polymyxa* as a function of pH.

4.6 Discussion and conclusions

A set of comprehensive screening experiments was used to identify microorganisms that showed the potential to be used as bio-collectors in flotation. The initial screening test involved exposing the microorganisms to pyrite in suspension and observing the change in microbial concentration over time. A decrease in cell numbers in suspension indicated microbe-mineral interaction and attachment onto the mineral surface. Several authors have demonstrated the selective attachment of heterotrophic microorganisms to pyrite under the conditions investigated in this study (Patra and Natarajan, 2003, 2004, 2008; Sharma, et al., 2001).

Attachment was observed for all microorganisms except for *B. licheniformis* at pH 4 and pH 7. When comparing the level of attachment to pyrite for all microbial cultures under neutral conditions, *P. polymyxa* had a substantially higher and more rapid percentage attachment, achieving 93.0 ± 2.0 % attachment after 1 minute. After the same time, *R. palustris* and

R. opacus attained an attachment of ± 70.0 %, followed by *B. subtilis* at 51.6 ± 5.0 %. 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 97.0 %. 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 75.0 ± 1.1%. The highest percentage attachment for *B. subtilis* cells was achieved at 10 minutes at 82.3 ± 1.7%.

At pH 4, attachment was detected for all cultures after 1 minute of interaction, 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 95.0% attachment, while *R. palustris* achieved 86.2 ± 0.7%, higher than that found at pH 7.

These results show that *B. subtilis*, *R. opacus, P. polymyxa* and *R. palustris* cells have an 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 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 difference in attachment between pH 4 and pH 7 could be attributed by the ionization of the mineral surface as a consequence of differing degrees of the oxidation of pyrite under different pH conditions, and the influence this process has on the minerals surface characteristics. Oxidation leads to the development of precipitated layers on the surface of the pyrite particle that have different chemical compositions depending on the pH of the solution. In a study investigating the oxidation of pyrite under alkaline conditions, Caldeira et al. (2003) showed that in the presence of NaOH, the particles were initially covered by a thin oxide layer which eventually disinteragted after longer reaction times. In other studies investigating the electrochemical behaviour of pyrite and chalcopyrite under leaching conditions, Sen et al. (2014) and Carneiro & Leao. (2007) showed that in the presence of NaCl, a porous and

somewhat crystalline sulphur layer was formed on the sulphide minerals due to the presence of chloride ions. These studies showed that the surface characteristics of the sulphide minerals changed under different pH conditions and acquired different chemical compositions. Therefore, the changes that occur on the pyrite mineral surface as a result of the pH of the solution are thought to be responisble for the different attachment trends observed for each microorganism at pH 4 and pH 7.

Bioflotation of pyrite concentrate was performed for the four microorganisms identified 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 4.16 summarises the cumulative percentage mass recovery of pyrite to the concentrate after 50 g was treated individually 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 4.17 summarises 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 at pH 7, only marginally below that of the chemical control.

Primarily, three microbial adhesion mechanisms are thought to be responsible for the deposition of microbial cells on the surface of a mineral. These are initial attachment, firm attachment and surface colonization (Sharma, 2001). Due to the short time interval of the experiments (in the order of minutes), it can be postulated that initial attachment was the relevant mode of attachment as the cells did not have enough time to form either firm attachment or colonization. Initial attachment is generally characterised as reversible and non-specific and occurs as a result of net attractive and repulsive forces present on the microbial and mineral surfaces (Sharma, 2001). Physico-chemical forces that result in initial attachment include acid-base interactions, hydrophobic interactions, van der Waals and electrostatic interactions.

It is well established in bioflotation literature that the role of the microorganism, just like that of a chemical collector, is to impart sufficient hydrophobicity to the mineral surface so that the probability of particle-bubble interaction is increased. It is also reported that for the microorganism to accomplish this function, it has to attach on the mineral surface. Attachment of the microbial cells to the mineral surface is possible only if the attractive forces are stronger

than the electrostatic repulsive forces hindering attachment. In particular, oppositely charged particles have stronger electrostatic attractive forces which are able to overcome repulsive forces acting against attachment. Therefore, electrostatic interactions play a governing role in the attachment process of oppositely charged particles in flotation systems.

The surface charge present on the microbe-mineral particle has an effect on flotation behaviour. Charged particles are more hydrophilic due to their interaction with polar water molecules and are therefore less likely to float. Furthermore, large positive or negative zeta-potentials have a detrimental effect on the flotation behaviour of a mineral. Therefore, it is essential that the zeta-potential of the microbe-mineral particle is close to zero – the point of zero charge – to reduce electrostatic repulsion and maximize the hydrophobic properties of the mineral.

Following zeta-potential measurement tests, *R. opacus, B. subtilis* and *R. palustris* exhibited PZCs at a lower pH than that of pyrite and displayed a large net negative charge at both pH 4 and 7 as shown in Figure 4.15. Although the microorganisms attached to pyrite, the interaction did not result in the recovery of the mineral to the concentrate following the 'pyrite-only' flotation screening tests as presented in Section 4.4.2. The magnitude of the potential indicates the degree of electrostatic repulsion between adjacent, similarly charged particles in a dispersion medium (O'Brien, et al., 1990). When the zeta-potential is small (negative or positive), attractive forces may exceed repulsion and cause the particles to either flocculate or attach to the gas bubble, with potential recovery into the concentrate, or both. The large zeta-potential values indicate that the microorganisms did not alter the mineral surface characteristics sufficiently to make the mineral particles hydrophobic enough to attach to the gas bubbles.

Of all the microorganisms tested, *P. polymyxa* had the most neutral net surface charge across the pH range tested while the other microorganisms had either a net positive or negative charge as shown in Figure 4.15. Assuming that *P. polymyxa* attachment to the mineral surface imparts this near neutral charge to the mineral particles, this suggests that it should strongly enhance the hydrophobicity and hence floatability of pyrite. This is supported by the screening results of the 'pyrite-only' bioflotation presented in Sections 4.3.3 and 4.4.2.3. Therefore, it can be deduced that the hydrophobicity of *P. polymyxa* as a consequence of its near neutral surface makes it seek out a surface to attach to, rather than remain suspended in water. This behaviour indicates that the bacterial cells should also attach to coal discard tails and base metal mineral sulphide particles and enhance their hydrophobicity.

Pyrite is classified as a polar type mineral as it has strong covalent and ionic surface bonding which reacts strongly with water molecules, making it naturally hydrophilic (Fuerstenau, et al.,

2007). Thus, a collector acts as a surface-active reagent that makes hydrophilic particles hydrophobic enough to attach to the gas bubble in the pulp phase and get carried to the froth phase where they are concentrated. It can therefore be deduced that at both pH 4 and pH 7, *R. opacus, R. palustris* and *B. subtilis* did not exhibit the potential to control or modify the surface properties of pyrite to make it hydrophobic. Assuming that the cells formed a homogenous monolayer on the surface of the particle, this result suggests that the mineral-microbe interaction did not maximize the mineral's contact angle significantly to allow bubble-particle contact. This could be attributed to forces acting between the bubble and the microbe-mineral particle that are not hydrophobic in nature, resulting in repulsion.

By contrast, *P. polymyxa* displayed collector properties at pH 4 and pH 7 that controlled and modified the surface properties of pyrite to make it hydrophobic. However, there was a significant difference in the amount of mineral collected under the pH conditions investigated. This may have been caused by shifts in the surface charge of the *P. polymyxa* under different pH, which would have affected both its attraction and adhesion to the pyrite and bubble surfaces. This difference could also have been caused by the dissolution of pyrite under acidic conditions and subsequent impact on the surface chemistry of the mineral and the chemistry of the flotation liquor. Park et al. (2015) showed that under acidic conditions, pyrite undergoes oxidation which results in the formation a SO_4^{2-} layer on the surface of the mineral through the reaction of S with oxygen in the pulp. Therefore, the presence of this layer changes the surface chemistry of the mineral, affecting how it interacts with both the microbe and the gas bubble. In the case of the *P. polymyxa* attachment experiment, it can be postulated that the attachment in the shake flask was possible at pH 4 as there was no oxygen being sparged into the system, and hence there was a limited amount of oxygen available to react with S. Attachment in the flotation cell pulp would have been inhibited as a result of the formation of a SO_4^{2-} layer on the pyrite mineral due to the constant oxygen supply via the aeration. This layer would have changed the surface chemistry of the mineral (electrostatic behaviour), altering its contact angle (hydrophobicity) and the potential to interact with a gas bubble. This motivates a test using pure nitrogen to sparge as opposed to air.

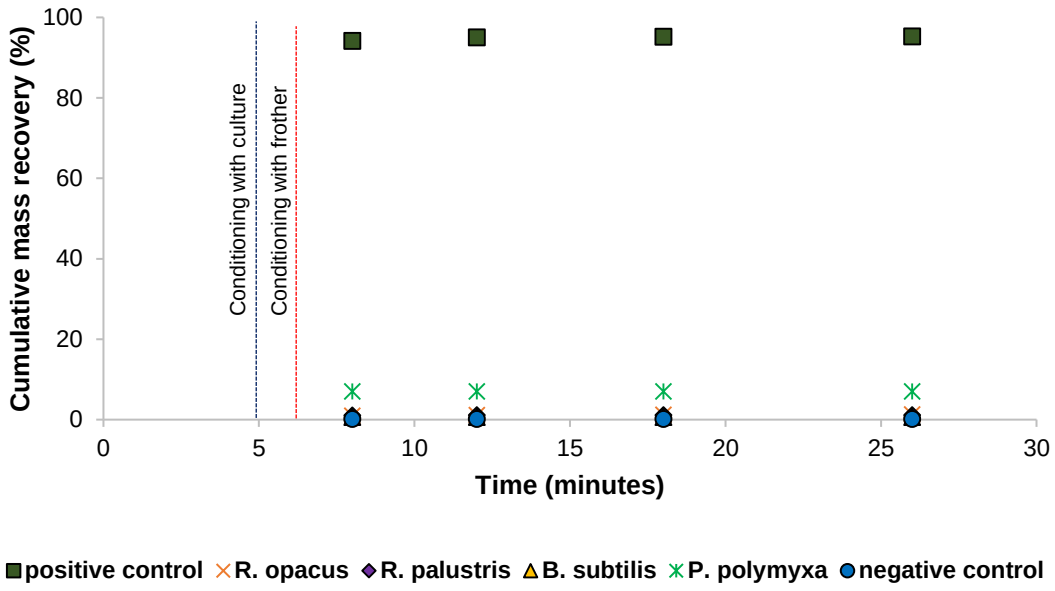

Figure 4.16: Cumulative mass recovery of pyrite after floating with the *R. opacus, R. palustris, B. subtilis* and *P. polymyxa* culture at pH 4 with a cell concentration of 4.2×10^9 cells/g for all cultures excepting 2.45×10^9 cells/g for *P. polymyxa.*

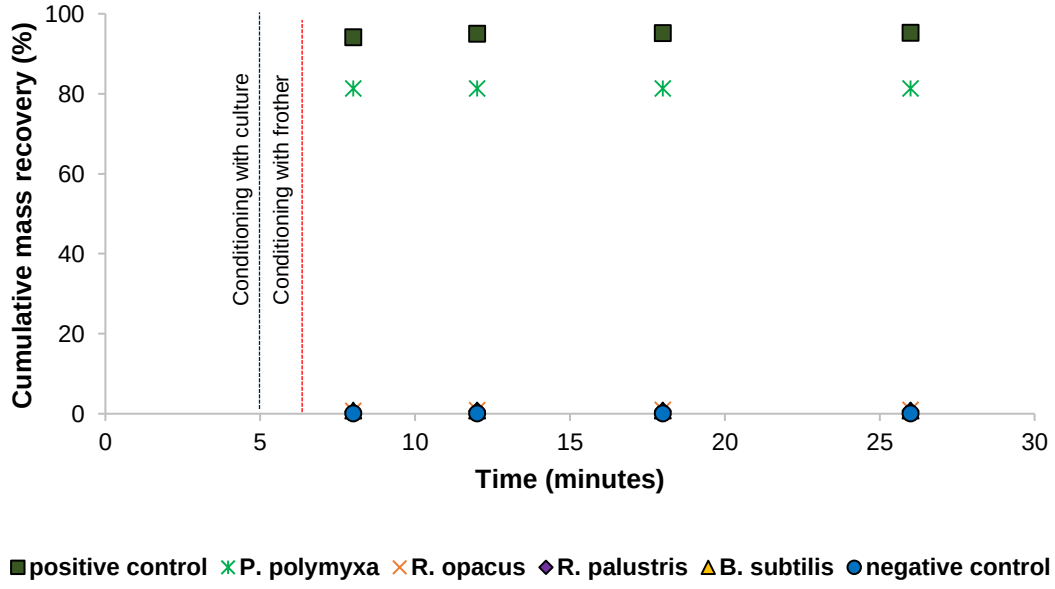

Figure 4.17: Cumulative mass recovery of pyrite after floating with the *R. opacus, R. palustris, B. subtilis* and *P. polymyxa* culture at pH 7 with a cell concentration of 4.2×10^9 cells/g for all cultures excepting 2.45×10^9 cells/g for *P. polymyxa.*

Although a high degree of attachment was observed for all the microorganisms in pure culture used in 'pyrite only' flotation tests, this did not lead to surface modifications that enhanced the floatability of pyrite when using *R. opacus, R. palustris* and *B. subtilis* cultures as bio-collectors. These results recommend 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 at pH 7. The recovery obtained was comparable to that using a chemical collector (Table 4.2) where both systems achieved recoveries of greater than 80 mass %. Furthermore, the microorganism displayed a near neutral surface charge across the pH range tested. These results show that following mineral-*P. polymyxa* interaction, there is a modification of the mineral surface that changes its properties, making it sufficiently hydrophobic to attach to the air bubble and be collected in the concentrate froth, particularly under neutral pH conditions. The success of *P. polymyxa* compared to the poor flotation performance of the other microbes may be linked to its comparatively rapid attachment at pH 7.

Based on these results, *P. polymyxa* has been chosen as the bio-collector candidate for the bioflotation separation of pyrite from coal discards and finely milled mineral sulphide samples.

CHAPTER 5: Bioflotation tests

5.1 Introduction

The microbial screening and surface characterisation tests, detailed in Chapter 4, recommend *P. polymyxa* as the best candidate microorganism to further investigate the bioflotation separation of pyrite from discards, tailings and finely divided mineral preparations. The selection was based on the microorganism's rapid rate of attachment to pyrite, the high cumulative mass recovery obtained which was comparable with the chemical positive control, and its near neutral surface charge which should impart hydrophobic properties to the mineral samples and therefore improve floatability.

The suitability of using *P. polymyxa* for the flotation separation of pyrite from other mineral components was assessed using tests to quantify the relative attachment to different mineral samples and bioflotation experiments. The bioflotation experiments were carried out according to the procedure outlined in Sections 3.6.1.2 and 3.6.1.3. In all tests, the impeller speed was 140 rpm, the aeration was set at 3 L/min and the pulp was maintained at pH 7 throughout the conditioning step. Two sample types were considered as representative feeds: finely divided base metal mineral sulphide samples and first-stage treated coal discard tails.

5.2 Results

5.2.1 Attachment of *P. polymyxa* to pyrite, quartz, first-stage coal discard tails and base metal mineral sulphides

The percentage of the *P. polymyxa* cell population which attached to the mineral samples as a function of time is shown in Figure 5.1. Rapid attachment to pyrite and first-stage coal discard tails was observed after 1 minute of interaction, where 93.0 ± 2.0 % and 86.2 ± 2.2 % attachment was attained respectively. After 10 minutes of interaction, a similar level of attachment was observed for the two mineral samples with no significant difference between the two. Both mineral samples achieved more than 95 % attachment after 20 minutes of interaction. Attachment of *P. polymyxa* to quartz and the base metal mineral sulphide was significantly lower, where 46.1 ± 3.2 % and 49.5 ± 2.1 % attachment was obtained respectively after 1 minute of interaction. After 10 minutes of interaction, 79.7 ± 3.4 % attachment was attained for quartz after which a slight but insignificant decrease was observed. The percentage attachment of the microorganism to the base metal mineral sulphide after 20 minutes was the lowest of all the mineral samples with 59.9 ± 4.1 % attachment obtained.

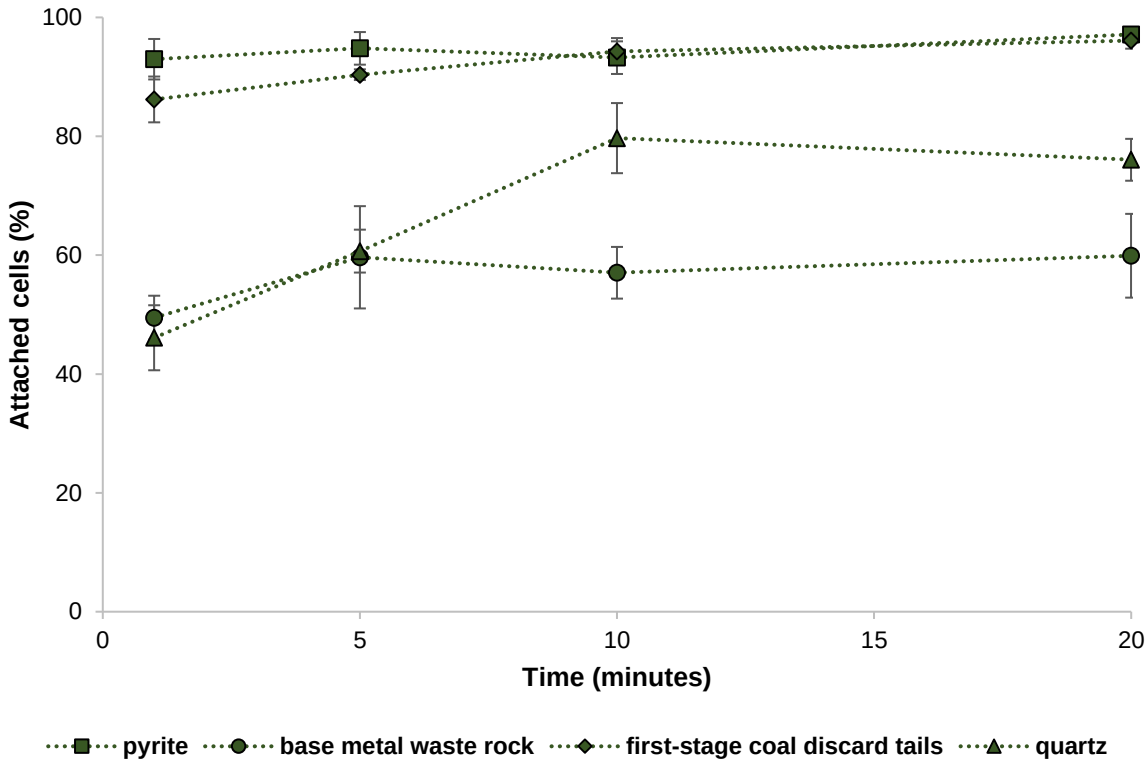

Figure 5.1: Percentage attachment of *P. polymyxa* cells to pyrite, first-stage treated coal discard tails, base metal hard rock or quartz in a suspension containing 1 g mineral per 100 mL suspension containing an initial cell concentration of 1×10^7 cells/mL. All experiments were performed at pH 7. Error bars represent variation between triplicate experiments.

5.2.2 Mineral surface characterization tests

The zeta-potential profiles of first-stage coal discard tails and base metal mineral sulphides samples before bioflotation tests are presented in Figure 5.2. The base metal mineral sulphide sample exhibited a net negative charge across most of the pH range tested, recording a slightly net positive charge of 54.7 mV at pH 3. With regards to the magnitude of the zeta-potentials, the first-stage coal discard tails displayed the largest net positive and net negative values, recording 169 mV at pH 3 and -186 mV at pH 7. The base metal mineral sulphide sample exhibited a net negative charge across most of the pH range tested, recording a slightly net positive charge of 54.7 mV at pH 3.

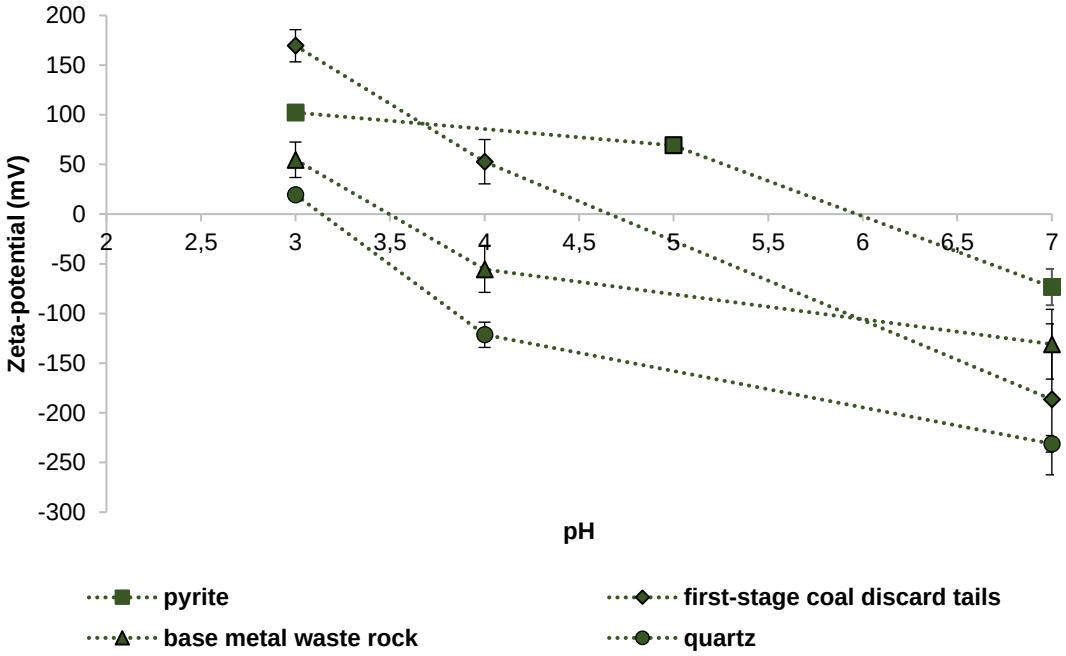

Figure 5.2: Measured zeta-potential of first-stage coal discard tails and base metal mineral sulphide as a function of pH.

5.2.3 First-stage treated coal discard tails bioflotation

Figure 5.3 shows the cumulative mass recovery of first-stage coal discard tails following chemical flotation using PAX and biological flotation using *P. polymyxa.* Table 5.1 further shows results of the sulphur analysis conducted on feed and concentrate samples following the second-stage sulphide flotation tests. The coal discard samples had previously been treated with oleic acid or Fatty Acid Methyl Esters (FAMEs) to recover coal in the first-stage separation process, as described by Chiodza (2018), with the tails used in this experiment.

After chemical flotation of first-stage 'oleic acid treated coal discard tails' using PAX, a cumulative mass yield of 49.2 ± 6.4 % was obtained after 20 minutes of flotation. In the absence of a collector, only 20.7 ± 2.8 % was recovered to the concentrate at the end of the experiment. *P. polymyxa* bioflotation of 'oleic acid treated coal discard tails' yielded a cumulative mass yield of 15.9 ± 1.1 %, significantly lower than the yield obtained for the negative control. In the bioflotation of 'FAME-treated coal discard tails' using *P. polymyxa*, there was no significant difference when compared to the negative control, clearly depicted in Figure 5.3.

The feed sample contained 0.50 % total sulphur mass before flotation. When using the chemical collector PAX, 76.27 % of the total sulphur mass reported to the concentrate. This was significantly higher than the 18.45 % total sulphur mass that was recovered to the concentrate in the absence of a collector. For 'oleic acid' and 'FAME treated' samples that were floated using *P. polymyxa*, 9.45 % and 21.25 % total sulphur mass reported to the

concentrate respectively. Therefore, the sulphur recoveries obtained in the bioflotation tests using *P. polymyxa* were similar or worse than the negative control, indicating no specificity of *P. polymyxa* for pyrite over the other minerals, primarily quartz and kaolinite, present.

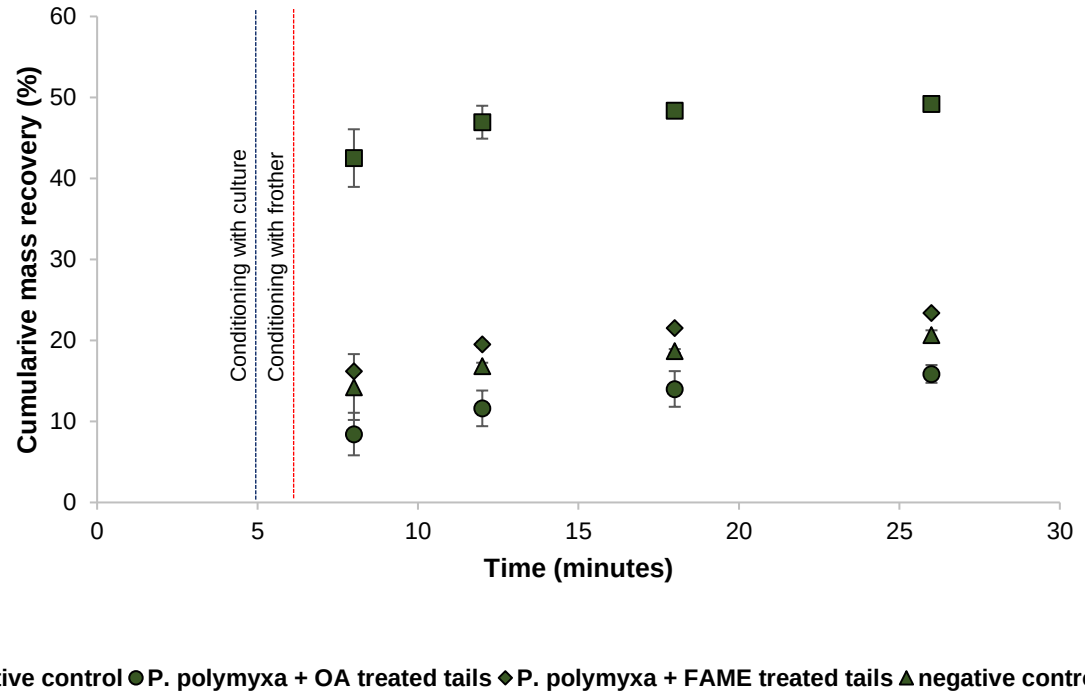

Figure 5.3: Cumulative mass recovery to the concentrate of first-stage coal discard tails in the absence of a collector (negative control), after treating with 2.33 kg/ton PAX (positive control), and after treating with a *P. polymyxa* culture at a cell concentration of 2.45 × 10^9 cells/g. Oleic acid and FAME treated coal discard tails samples were used.

Table 5.1: Results of sulphide flotation tests carried out on the first-stage coal discard tails using PAX as a chemical collector and *P. polymyxa* as a biological collector.

Sample treatment	Mass yield (g)	Concentrate sulphur (g)	S recovery to concentrate (%)
Feed	100.0	0.50	0.0
Oleic acid treated tails + no collector	20.7 ± 2.8	0.09 ± 0.02	18.45
Oleic acid treated tails + PAX	49.2 ± 6.4	0.38 ± 0.01	76.27
Oleic acid treated tails + *P. polymyxa*	15.9 ± 1.1	0.05	9.45
FAME treated tails + *P. polymyxa*	23.4 ± 0.3	0.08 ± 0.02	21.25

5.2.4 Base metal mineral sulphide bioflotation

The recovery of the base metal mineral sulphide to the concentrate over time is given as a percentage cumulative mass recovery in Figure 5.4. There was no collection of the mineral to the concentrate in the absence of a collector. Following chemical treatment with PAX, 8.2 ± 4.1 % was recovered within 2 minutes of flotation. The mass yield increased sharply to 24.6 ± 3.53 % after 4 minutes, with 28.4 ± 1.3 % recovered after 20 minutes of flotation. The mass recovery of the mineral sulphide to the concentrate was even more rapid when using *P. polymyxa* compared to PAX, as 21.7 ± 1.7 % was collected within the first 2 minutes of flotation. A cumulative mass recovery of 30.2 ± 1.7 % was attained after 20 minutes of flotation.

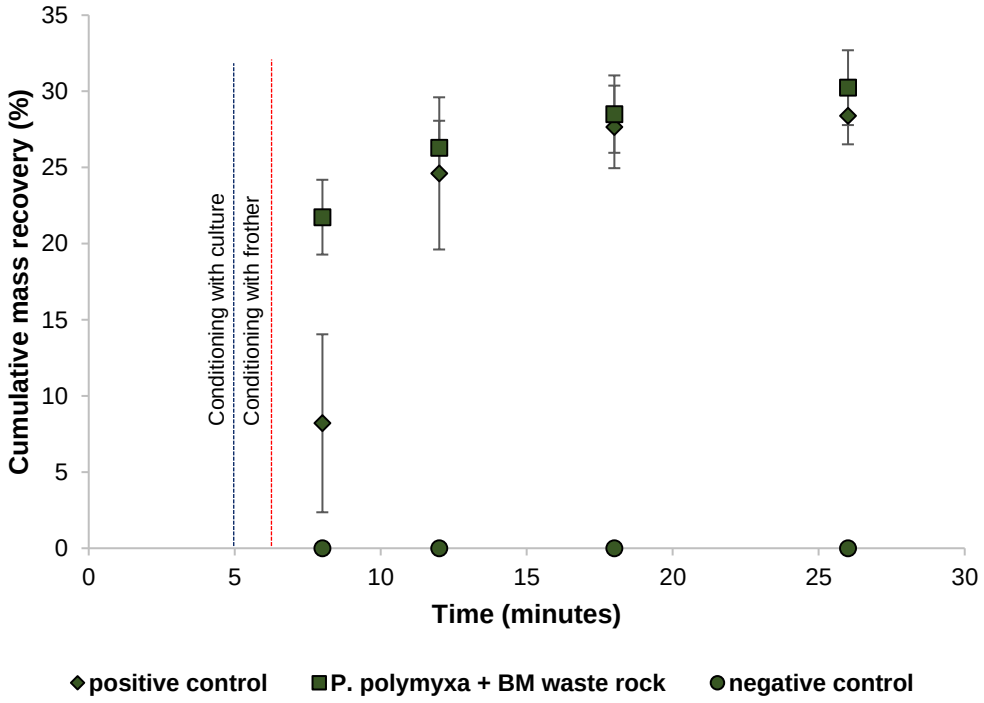

Figure 5.4: Cumulative mass recovery to the concentrate of base metal hard rock after treating with 2.33 kg/ton PAX, in the absence of a collector and after treating with the *P. polymyxa* culture at a cell concentration of 2.45×10^9 cells/g.

The results of the sulphur analysis conducted on feed and concentrate samples are presented in Table 5.2. The hard rock mineral sulphide feed sample contained 1.7 % total sulphur mass before flotation. Although the cumulative mass yield results were comparable between PAX and *P. polymyxa,* it is clear that PAX produced the best sulphur recovery at 66.4 %, significantly higher than the 22.6 % obtained on using *P. polymyxa.* These results suggest that the attachment of P. polymyxa to pyrite and other mineral sulphides is not selective over the attachment and flotation of gangue minerals such as quartz.

Table 5.2: Results of sulphide flotation tests carried out on a base metal hard rock sample using PAX as the chemical collector and *P. polymyxa* as the biological collector.

Sample treatment	Mass yield (g)	Concentrate sulphur (g)	S recovery to concentrate (%)
Base metal hard rock + no collector	0.0	0.0	0.0
Base metal hard rock feed	100.0	1.7	0.0
Base metal hard rock + PAX	28.4 ± 1.3	1.1 ± 0.1	66.4
Base metal hard rock + *P. polymyxa*	30.2 ± 1.7	0.4 ± 0.02	22.6

A Nova Nano Scanning Electron Microscope (SEM) was used to take images of the surface of an untreated base metal hard rock sample and the concentrate obtained after flotation with *P. polymyxa*. The results are shown in Figure 5.5, where (a) is an image of the untreated particle surface, and (b), (c) and (d) are images of the surfaces of particles in the concentrate following flotation using *P. polymyxa* as collector. Images (b) and (c) were taken in the same region under different magnification.

The letters A, B and C on Image (b) denote three different locations where attached microbial cells are obervable. The magnification was increased from 20 000 × to 50 000 × to provide a better resolution of the microbe-mineral particle attachment sites B and C, shown in image (c). The presence of cell debris was also observed on the surface of the mineral, as illustrated at location D in image (d), suggesting cell lysis or cellular metabolites.

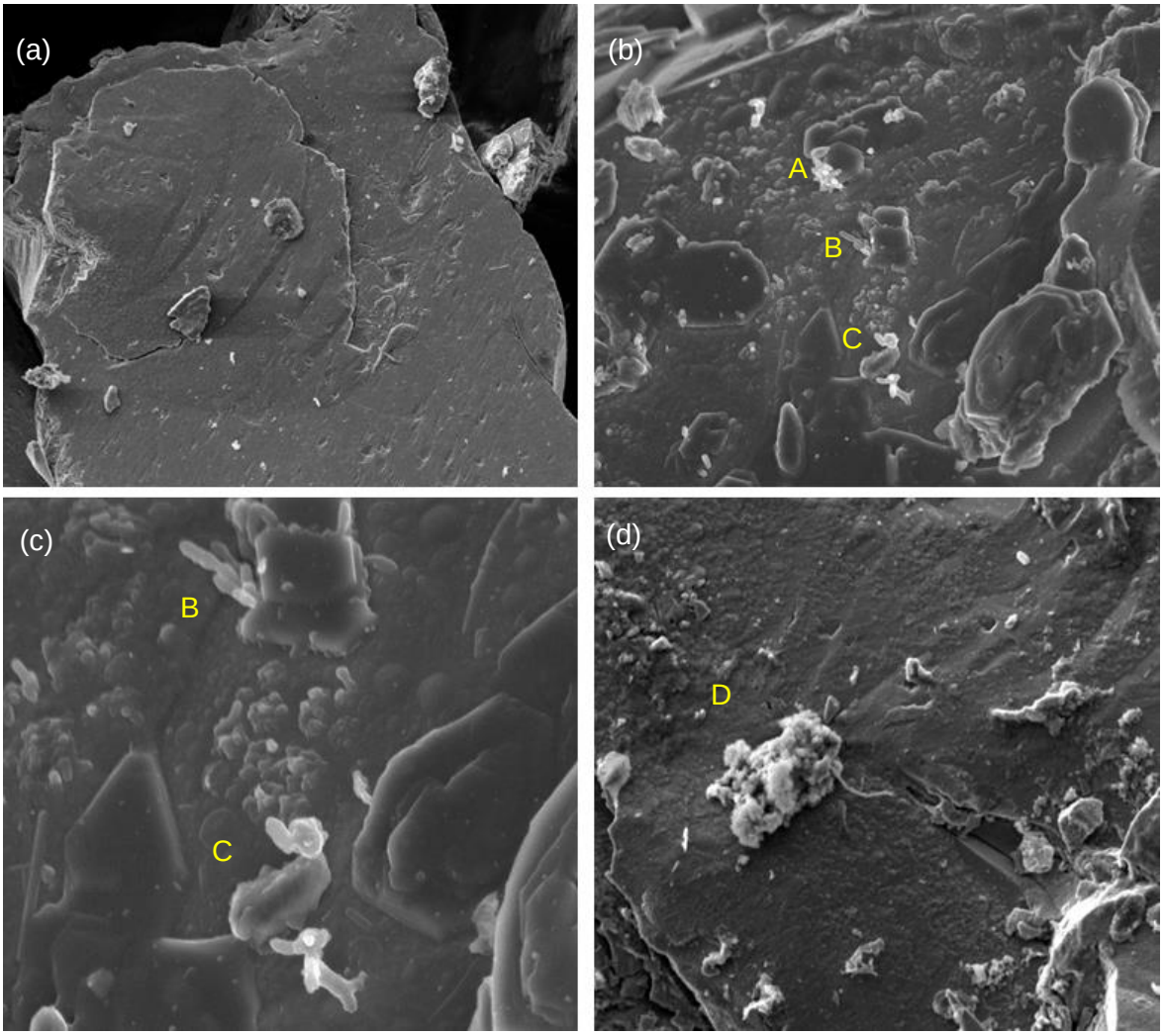

Figure 5.5: SEM Images of various regions on the surfaces of the base metal hard rock before and after flotation. Image (a) depicts the surface of an untreated particle viewed under 5000 × magnification. Image (b) and (c) display the same region viewed under 20 000 × and 50 000 × magnification respectively. Image (d) displays the surface of a particle on a different region under 10 000 × magnification.

5.3 Discussion

Bioflotation tests were performed on first-stage treated coal discard tails and base metal hard rock using *P. polymyxa* as a bio-collector. The potential of the microorganism as a bioflotation reagent was demonstrated in Chapter 4 after screening five microbial cultures using a pyrite mineral concentrate. The microorganism exhibited a near neutral surface charge which led to the modification of the mineral's surface properties – enhancing its hydrophobicity and improving floatability. In this chapter, *P. polymyxa's* potential to separate pyrite from gangue minerals was investigated.

Attachment experiments were performed at pH 7 to investigate the selective attachment of *P. polymyxa* to pyrite, first-stage coal discard tails, base metal hard rock and quartz; the latter which was used to represent gangue. Similar attachment profiles were obtained for pyrite and the coal discard tails over the duration of the experiment. However, the microorganism

exhibited a rapid rate of attachment to pyrite after 1 minute of interaction, achieving more than 90.0 % attachment compared to the 86.2 ± 2.22 % obtained for the coal discard. Thereafter, there was no significant difference in attachment after 5 minutes of interaction. A slower rate of attachment was observed for the base metal hard rock and quartz minerals. Similar attachment profiles were obtained for the first 5 minutes of interaction where more than 60.0 % of the cell population was attached to the mineral. However, after 10 minutes of interaction, the percentage attachment increased to 79.7 ± 3.4 % for quartz and remained constant for the base metal hard rock, remaining unaltered thereafter for the duration of the experiment. These were low relative to the attachment percentage obtained for pyrite and coal discard tails after 1 minute of interaction. These results demonstrated the selective attachment of *P. polymyxa* between the four mineral samples where attachment was selective in the following manner:

pyrite > first-stage coal discard > quartz > base metal hard rock

The minerals exhibited different surface charge characteristics across the pH range tested. Pyrite and first-stage coal discard tails displayed a large net positive charge under acidic pH conditions, recording 102.2 mV and 169.44 mV respectively at pH 3. Compared to the other minerals, quartz and the base metal hard rock exhibited a smaller net positive charge of 19.57 mV and 54.7 mV respectively at pH 3. Furthermore, the results suggested that the PZC values were around pH 3 - 3.5 after which they both displayed a net negative charge across most of the pH range tested. All minerals displayed a net negative charge at pH 7. Pyrite displayed a less charged negative surface with a zeta-potential of -73.41 mV, followed by base metal hard rock, first-stage coal discard tails and quartz displaying the highest zeta-potential of -231.2 mV as shown in Figure 5.2.

Pyrite and the first-stage coal discard tails displayed the same percentage attachment at pH 7. However, the coal discard exhibited a larger net negative value of -186.47 mV compared to the less negatively charged pyrite surface shown in Figure 5.2. Furthermore, the base metal hard rock particles displayed a less net negative charge relative to the highly charged coal discard and quartz particles but had the lowest percentage attachment to *P. polymyxa* out of all four minerals. This suggests a different and/or a combination of mechanisms could be responsible for microbial attachment depending on the type of mineral and its surface characteristics. Although selective attachment was observed between the minerals, electrostatic interactions alone could not adequately describe the differences in attachment as the microorganism displayed variable attachment between the minerals irrespective of the magnitude of the charge. In an aqueous system, particles with a low charge density tend to be hydrophobic and water repellent in contrast to those with a high charge density (Wang, et al., 2010). Based on this, it was expected that *P. polymyxa* – given its hydrophobic nature as

a consequence of its near neutral charge – would preferentially attach to a surface with a low charge density and show the following attachment trend based on the zeta-potential results:

pyrite > base metal hard rock >first-stage coal discard > quartz

However, this assumes that the interacting particles are ideally shaped, smooth and have a uniform charge distribution. In real systems, the influence of surface roughness, heterogeneity and charge non-uniformity has a profound effect on the interaction of particles and their stability in an aqueous environment (Czarnecki, 1986). These irregularities influence charge distribution on the surface of the mineral particles and lead to regions with a low charge density that tend to be hydrophobic (Velegol & Thwar, 2001). These regions might allow strong hydrophobic interactions with other hydrophobic colloid particles. Hence *P. polymyxa's* attachment to highly charged particles could be due to charge non-uniformity where the microbe, through hydrophobic interactions, adheres to the hydrophobic and less charged regions on the mineral particle.

P. polymyxa was used as a biological collector to investigate its bioflotation potential for the desulphurization of pyrite-containing coal discards and a base metal hard rock. The bioflotation results were evaluated based on cumulative mass yields and total sulphur recovered in the concentrate. Figure 5.3 shows the cumulative mass recovery of first-stage coal discard tails following chemical flotation using PAX and biological flotation using *P. polymyxa.* Flotation with PAX yielded a cumulative mass percentage of 49.2 ± 6.4 % while bioflotation with *P. polymyxa* yielded mass percentages that were not significantly different from the negative control as depicted in Figure 5.3. This result suggested that the culture did not have a significant effect on the floatability of the coal discard, despite the promising attachment results. Sulphur analysis was performed to evaluate the total amount of sulphur in the concentrate for each sample. As shown in Table 5.1, PAX produced the best recovery of sulphur with 76.26 % total sulphur mass recovered. *P. polymyxa* recovered 9.45 % sulphur for oleic acid treated tails and 21.25 % for FAME treated tails. These values were not significantly different from the value obtained for the 'no-collector' sample, suggesting that the culture did not have an effect on the selective flotation and separation of sulphide minerals like it did with the pure pyrite. The sulphur that reported to the concentrate after treating with the culture, and in the 'no-collector' control, could be associated with the naturally hydrophobic coal particles; hence the effect seen may have not been caused by the microorganisms.

For the experiments conducted with the base metal hard rock, the cumulative mass recoveries obtained after chemical and bioflotation tests are presented in Figure 5.4 and Table 5.2. It was evident that PAX and *P. polymyxa* both demonstrated a similar effect on the floatability of the hard rock, both recovering ~30.0 % cumulative mass after 20 minutes of flotation. However,

the microbial culture displayed a rapid rate of collection after 2 minutes of flotation, achieving 21.7 ± 1.7 % while PAX achieved a slower recovery of 8.2 ± 4.1 %. In terms of sulphur recovery, PAX demonstrated a high selectivity for the sulphide mineral recovering 66.4 % total sulphur to the concentrate. In contrast, *P. polymyxa* managed to recover 22.6 % total sulphur in the concentrate which was significantly less than the recovery achieved after using PAX.

Attachment tests showed that *P. polymyxa* had greater affinity for the first-stage coal discard – due to rapid attachment and a high percentage of attachment – compared to the base metal hard rock. Based on this result, it was expected that the microbe would enhance the floatability of the coal discard tails as was the case with pyrite concentrate. However, the microbial culture did not affect the floatability of the tails but rather affected the floatability of the hard rock by making it hydrophobic enough to attach to the gas bubble. Between the two samples, the hard rock had the highest amount of sulphur in the feed as presented in Table 5.1 and Table 5.2. Pyrite concentrate bioflotation tests demonstrated the culture's affinity for pyrite and the effect on the minerals floatability. Therefore, it can be deduced that the microorganism had the same affinity for the pyritic sulphur associated with the hard rock.

As can be seen in the SEM micrographs of hard rock particles from the concentrate (Figure 5.5), no bacteria were observed on the surface of the untreated hard rock particle shown in (a). Aggregates of rod-shaped bacteria were clearly discernible on the surface of the particle denoted by A, B and C in (b). Upon zooming in on regions B and C, the attached populations were observed to be clustered around smaller particles, providing visual confirmation of attachment. A particle covered with a polymer-like substance was observed in (d), unlike in (c) where the morphology of the cell was clearly distinguishable. This suggested that cell metabolites such as extracellular or intracellular polymers/proteins might also be interacting with the particles and causing them to attach to the gas bubble.

The results presented in this chapter show that *P. polymyxa* did not demonstrate the potential to be used as a biological collector for the removal of pyritic sulphur from first-stage treated coal discard tails. The culture showed the potential to recover pyrite and sphalerite containing minerals associated base metal hard rock based on the cumulative mass yield and total sulphur it achieved. However, the total sulphur recovered was not comparable with the chemical control; hence a low-volume sulphide rich concentrate was not achieved, indicating reduced efficiency of separation of the sulphide minerals and the gangue.

CHAPTER 6: Summary, Conclusions and Recommendations

6.1 **Summary**

This chapter consolidates the findings of this feasibility study looking to investigate the potential use of *R. opacus, R. palustris, B. subtilis, B. licheniformis* and *P. polymyxa* as sulphide mineral flotation reagents. The objective of this research was to investigate the prevention of ARD formation through the desulphurization of pyrite-containing coal discards and base metal hard rock samples using alternative bioflotation reagents. The experimental results reported in Chapters 4 and 5 can be summarized as follows:

- Attachment to a pyrite mineral concentrate was observed for all microorganisms except for *B. licheniformis* at pH 4 and pH 7. All microorganisms, besides *R. opacus,* showed a higher affinity for pyrite under neutral pH conditions. When comparing the level of attachment to pyrite for all microbial cultures, *P. polymyxa* had a substantially higher and more rapid percentage attachment than the other microbes, achieving more than 95.0 % attachment after 5 minutes of interaction at both pH 4 and pH 7.
- Pyrite concentrate bioflotation tests were performed using the microorganisms that displayed an affinity for pyrite in the attachment experiments, and so did not include *B. licheniformis* which did not attach to the mineral. At both pH 4 and pH 7, *R. opacus, R. palustris* and *B. subtilis* did not exhibit the potential to control or modify the surface properties of pyrite to make it hydrophobic for flotation. By contrast, *P. polymyxa* displayed collector properties at pH 4 and pH 7 that controlled and modified the surface properties of pyrite to make it hydrophobic. After using this microorganism, the recovery obtained was comparable to that using a chemical collector where both systems achieved cumulative mass recoveries of greater than 80 % under neutral pH conditions.
- Following surface charge characterization tests, *R. opacus, B. subtilis* and *R. palustris* exhibited PZCs at a lower pH than that of pyrite's and displayed a large net negative charge at both pH 4 and 7. It was deduced that the large magnitude of the charge present on the surface of the microbes was responsible for electrostatic repulsion from the air-bubble as increased surface charge affects the likelihood of polar interactions with water molecules, therefore impacting the floatability of the mineral. Of all the microorganisms tested, *P. polymyxa* had the most neutral net surface charge across

the pH range tested. As a result, the microbe's hydrophobic surface strongly made it seek out a surface to attach to rather than remain suspended in water. This increased the likelihood of the mineral-microbe interaction as well as attachment of the mineral-microbe aggregate to the air-bubble and hence collection in the froth.

- The bioflotation of pyrite-containing coal discard tails and base metal hard rock was performed using *P. polymyxa.* The selection was based on the microorganism's rapid rate of attachment to pure pyrite, the high cumulative mass recovery obtained which was comparable with the PAX chemical positive control, and its near neutral surface charge. Following bioflotation tests, the culture did not demonstrate the potential to be used as a biological collector for the separation of pyritic sulphur from first-stage treated coal discard tails. However, the culture did demonstrate the ability to separate pyritic sulphur or sphalerite or both from gangue in the hard rock although a lower-quality sulphide rich concentrate was obtained compared to the chemical route.

6.2 Conclusions and recommendations

The findings in this research do not fully sustain the hypothesis which stated that microbial cultures can be used as alternative biological collectors, surface modifiers or depressants for the recovery of a low-volume sulphide-rich concentrate and a high-volume benign tailings fraction in the second stage of the two-stage coal desulphurization froth flotation process. Out of the five microbial cultures investigated, four of them attached to pyrite at pH 4 and pH 7. These were *B. subtilis, R. opacus, R. palustris* and *P. polymyxa*. The latter was the only one that exhibited the potential to upgrade pyritic sulphur when it achieved a cumulative mass recovery that was comparable to the chemical control following flotation screening tests using a mineral sulphide concentrate. Surface characterization tests using zeta-potentials confirmed that this was possible as the microorganism had a near neutral surface charge which gave it "hydrophobic like" properties that modified the surface of the mineral, allowing it to attach to gas bubbles and get recovered in the concentrate. The total sulphur collected in the concentrate after using the culture was not comparable to the chemical control. Hence the feasibility of using a microbial culture to obtain a low-volume sulphide-rich concentrate and a high-volume benign tailings fraction was not demonstrated.

However, optimisation of aspects such as the *P. polymxa* characteristics and mineral interactions can still be sought. Based on the findings in this study, the following recommendations can be made:

- Contact angle measurements or aqueous two-phase partitioning tests should be performed to characterize the surface hydrophobic properties of the microbial cultures and the mineral samples. These additional tests could provide a better understanding

of the type of interactions that occur between the bacteria and mineral particles upon initial attachment. Furthermore, understanding the hydrophobic properties of the cultures and specific minerals will also help predict their behaviour in a flotation cell as this process relies on manipulating this property to achieve the desired separation.

- Fourier-transform Infrared (FT-IR) spectroscopy should be carried out for the purpose of elucidating the functional groups present on the bacterial cell wall and mineral surfaces. Knowing the surface chemical composition of the bacteria and mineral particles might offer insight into the chemical and functional groups that are responsible for interaction, overall surface charge and degree of hydrophobicity.
- The effect of increasing the *P. polymyxa* culture cell concentration on the recovery of pyritic sulphur should be carried out. In this study, a cell concentration of 2.45×10^9 cells/g was used as this was the maximum biomass concentration achieved when using a batch bioreactor. The culture showed potential at this cell concentration as presented in Sections 4.4.2.3 and 5.2.4. Performing a kinetic study on the growth of *P. polymyxa* might provide useful information that can be used to optimize its growth requirements.
- The effect of adapting the *P. polymyxa* culture to a pyrite mineral concentrate prior to bioflotation usage should be investigated. Several studies have demonstrated the selectivity of a pyrite-adapted culture versus a non-adapted culture. Pyrite-adapted cultures were more selective and were shown to achieve higher recoveries compared to the non-adapted cultures.
- Evaluation of the DLVO and X-DLVO theories in order to have a better understanding of the interaction between the microbial cell surface and the mineral surface
- Bioflotation experiments using microbial metabolites should be performed. Bacterial metabolites such as proteins and polymers have various functional groups that are either hydrophobic or hydrophilic. Their use as flotation reagents has been investigated in literature and some studies have shown that they perform better than bacterial cells as bio-collectors. Unlike chemical reagents, bacterial metabolites are biodegradable and their use as bio-flocculants has been demonstrated in literature and established in industries such as waste water treatment.
- Technoeconomic assessment to evaluate the economic feasibility/viability of the second-stage bioflotation process. The information obtained will help to predict the anticipated cost of production (input costs), future cash flows and the likely return on investment.

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Appendices

Appendix A: Media composition

Glucose Yeast Malt (GYM) Streptomyces medium

Glucose Yeast Malt (GYM) Streptomyces medium was prepared per a DSMZ 65 recipe designed for *R. opacus* cultivation. The components listed in Table 7.1 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 7.1: 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

Tryptic-soy medium

Tryptic-soy medium was used for the cultivation of *B. licheniformis* and *B. subtilis.* Per 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.

Rhodospirillaceae medium

A modified *Rhodospirillaceae* medium was used for the cultivation of a *R. palustris* culture. The components listed in Table 7.2 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 7.3 and Table 7.4 respectively.

Table 7.2: 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 7.3: 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 7.4: 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

Modified Bromfield medium

A modified Bromfield medium was used for the cultivation of a *P. polymyxa* culture. The components listed in Table 7.5 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 7.5: Modified Bromfield medium composition (g/L)

Substance	Chemical formula	Quantity (g)
Potassium di-hydrogen orthophosphate	KH_2PO_4	0.5
Magnesium sulphate heptahydrate	$MgSO_4 \cdot 7H_2O$	0.2
Ammonium sulphate	$(NH_4)_2SO_4$	1
Sucrose (0.5 wt%)		5
Yeast extract		0.15

0K BSM medium

The 0K basal salt medium (BSM) is recommended for the growth of mesophilic bioleaching microorganisms. The salts listed in Table 7.6 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 7.6: 0K BSM medium composition (g/L)

Substance	Chemical formula	Quantity (g)
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