The effect of microbial load and water recycling on the flotation performance of a PGM bearing ore

Vushe Wabatagore

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ABSTRACT

Mineral processing requires large quantities of water for its operations. With the continuing move to reduce freshwater withdrawal and mine water discharge, the mineral industry has been applying water recycling and reuse to improve water use efficiency. Previous studies on the use of remediated water as supplementary point source water and water recycling within the flotation circuit have shown that these practices affect the flotation process performance. It is therefore important to understand the effect that components within recycled and reclaimed water may have on flotation performance. While research on the effects of the abiotic components such as ions on flotation is well represented in literature, the effects of biotic water components, particularly microorganisms, on flotation performance still remain understudied and poorly understood. This study aimed to contribute to our understanding of the effects microbes have on the flotation performance of a PGM bearing ore from the Bushveld IgneousComplex in South Africa. In addition, the effects of xanthate collectors such as Sodium Ethyl Xanthate (SEX) and Sodium Isobutyl Xanthate (SIBX), widely employed in sulphide mineral beneficiation, on microbial growth were also considered. Results suggest that the presence of microbial cells and recycling of flotation waters increase water and solids recovery, while the metal grade recoveries were negatively affected. The microbial community used in this study could proliferate in the presence of up to 240 ppm for SEX and 480 ppm for SIBX, with an increase in the lag phase of growth observed with increasing collector concentrations. The presence of microbes at a concentration of 10^9 cells/ml resulted in the compete removal of 60 ppm collector from solution, both SEX and SIBX, from solution within a 2-hour time period. Outcomes of this study include a method for the measurement of microbial activity within a mineral slurry, which will further facilitate studying the effects of microorganisms on flotation systems. The work presented in this dissertation revealed that the presence of microbial consortia studied here negatively affected metal (Cu and Ni) grades attesting to the detrimental effect posed by the usage of microbial laden water for flotation operations. Further, the microbial consortium showed that it can facilitate the bioremoval of xanthate from solution which could affect the recovery of valuable minerals during flotation operations. This mechanistic framework, explaining the mechanisms by which the microbes affect flotation and the detrimental effects posed by microbes found in flotation waters is an actionable (fundamental) for the mining industry. From the present study, it is recommended that researchers should assess the microbial load present in flotation waters prior to their usage for flotation operations as high microbial load proved to be detrimental as far as flotation performance is concerned on a laboratory scale. Conclusively, the knowledge generated from this study builds on the ongoing scientific efforts decoding the effects of naturally prevailing microbes in flotation waters.

ABBREVIATIONS

µm	microns
µL	microlitre
BIC	Bushveld Igneous Complex
C1	First concentrate
C2	Second concentrate
C3	Third concentrate
C4	Fourth concentrate
CeBER	Centre for Bioprocessing Engineering
CCC	Critical Coalescence Concentration
CFU	Colony Forming Unit
CMR	Centre for Minerals Research
Cu	Copper
FDA	Fluorescein Diacetate
g	grams
g/t	grams per ton
hr	hour
L/min	Litres per minute
min	minute
Ni	Nickel
OD	Optical density
PBS	Phosphate Buffered Saline
PGE	Platinum Group Element
PGM	Platinum Group Mineral
ppm	parts per million
Pt	Platinum
RFU	Relative Fluorescence Unit
rpm	revolutions per minute
SEX	Sodium Ethyl Xanthate
SIBX	Sodium Isobutyl Xanthate
SPW	Synthetic Plant Water
UCT	University of Cape Town
XRF	X-ray fluorescence

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CHAPTER 1

1 INTRODUCTION

For just over a century, froth flotation has been used as the workhorse for concentrating minerals (Wills and Napier-Munn, 2006; Ata, 2012; Wills and Finch, 2016) owing to its cost effectiveness and technical versatility in the mining industry (Wills and Finch, 2016). Mineral concentration by froth flotation is achieved by the exploitation of the differences in the surface properties of target minerals and the deleterious gangue materials. Hydrophobic particles (water repellent), typically valuable minerals, are separated from the hydrophilic (water loving) particles, usually the non- valuable material (Bradshaw, et al., 1998; Kawatra, 2009; Wills and Finch, 2016) through adhesion to air bubbles. It is noteworthy that for this process to be a success, chemical reagent treatment with flotation reagents is usually carried out in order to alter the surface properties of the target mineral for flotation and prevent the gangue material from entering the concentrate i.e depression (Bradshaw et al., 1998 and Kawatra, 2009).

Flotation is an intricate process (Wills and Finch, 2016) which is affected by several parameters and among these is water (Rao and Finch, 1989; Levay et al., 2001; Slatter et al., 2009; Muzenda, 2010; Liu et al., 2013a; Muzinda and Schreithofer, 2018) which is used both as a transport and process medium (Slatter et al., 2009). In the continuing move to refrain from consuming freshwater in the minerals processing industry, particularly in froth flotation, two strategies are being implemented in order to tackle this problem; recycling water within the concentrator and assessing other alternatives to freshwater such as treated domestic water and borehole water (Slatter et al., 2009 and Liu et al., 2013a). The use of different water types and recycling on mineral concentrators results in benefits such as cost-effectiveness and water sustainability within the mining space (Slatter et al., 2009; Muzenda, 2010; Muzinda and Schreithofer, 2018). In this regard, it is worthwhile mentioning that water reuse tends to address profound problems linked with wastewater generation and disposal together with fresh-water consumption (Akhoundi and Nazif, 2018). However, these water saving benefits go hand in hand with changes in water chemistry which in most cases introduce complications to the separating conditions and have, in some cases, proven to be detrimental to flotation performance (Rao and Finch, 1989; Levay et al., 2001; Seke and Pistorius, 2006). The changes in water chemistry reside in the fact that, with recycling, there is an accumulation of organics and inorganics (Rao and Finch, 1989; Slatter et al., 2009; Liu et al., 2013). The impacts associated with abiotic components/inorganics, particularly ions, on flotation performance is currently well researched (Liu et al, 2013a; Manono et al., 2013 and Dzingai et al., 2020). Conversely, the impacts of biotic components/organics, microbes in particular, remain understudied.

The utilisation of microorganisms in mineral beneficiation operations, such as bioleaching, where microorganisms are used to solubilise the metal within the mineral, has been exploited at a commercial

scale (Rao et al., 2010). Moreover, the use of microorganisms in bioflotation/bioflocculation where microorganisms are employed in place of the traditional flotation reagents has been highlighted in literature although not commercially exploited (Vilinsk et al., 2008; Rao et al, 2010; Behera; Mulaba-Bafubiandi, 2017; Msipa, 2018 and Chiodza et al., 2020) owing to the complexity of the pulp even in the absence of the microbes (Rao et al., 2010). However, the mechanisms by which the presence of microbes and other organic components, that naturally prevail in flotation waters, affects flotation performance are poorly understood. In previous work where the effects of microbial load have been investigated, it has been concluded that microbial cells which prevail in wastewaters are generally detrimental to flotation performance (Evdokimova et al., 2012; Liu et al., 2013a, 2013b; Liu et al., 2016; Bormberg et al., 2020; Mhonde et al., 2020). However, this is largely dependent on the organism itself, its concentration and extracellular products, and how long it has been in contact with the minerals. Some studies have used model bacteria in place of the microbes endemic to flotation waters to elucidate the effect of microbes on flotation performance. Furthermore, some studies have used pure single minerals in place of real ore (Evdokimova et al., 2012; Liu et al., 2013a, 2013b; Liu et al., 2016; Mhonde et al., 2020). As a result, studies which closely mimic the real microbial community and load associated with mineral concentrators are limited. It is therefore of great importance to gain insight on microbial impacts affecting flotation performance, especially as the use of treated wastewaters, including treated domestic wastewater, is becoming recognized as a potential way to improve water efficiency regionally through the application of 'fit-for-purpose' water streams for mineral processing, and these water sources may introduce microbial consortia (Liu et al., 2013a). Moreover, there is some acknowledgement of the potential for microbial cells to be introduced into the flotation circuit through the ore material (Smith and Miettinen, 2006); while not commonly acknowledged, it is in keeping with the observation of indigenous microbial communities on ores increasingly in leaching studies (Tupikina et al., 2013).

It is appreciated that xanthate, a commonly used collector in flotation systems, is toxic to aquatic life including microorganisms (Webb et al., 1975; Alto et al., 1977; Okibe and Johnson, 2002). Research has shown that some microbial strains develop tolerance towards xanthate toxicity and can effectively degrade xanthate in wastewaters by utilising xanthate as an energy source (Deo and Natarajan, 1998; Chockalingam et al., 2003; Natarajan and Prakasan, 2013).

Aspects of these microorganism studies are largely interested in using these strains for bioremediation or metal extraction, rather than investigating their effect on flotation performance (Chen et al., 2011a; Cheng et al., 2012). As such, there is a lack of scientific evidence as to how microbial cells respond when exposed to xanthate in flotation circuits. The same is true for the response of xanthate to the presence of microbial cells.

CHAPTER 1

In the present study, the effect of a mixed microbial consortium, originating from water in a South African platinum concentrator, on flotation performance was considered. Attachment studies were carried out to ascertain microbial attachment onto ore particles. Microbial growth studies were conducted in the absence and presence of ore particles to quantify the effect of ore particles on microbial cells using a Fluorescein Diacetate (FDA) assay validated during this study. To quantify the effect of the microbial consortium on flotation performance, recycling flotation experiments were conducted in the absence and presence of deliberately added microbial cells into the flotation system. The effects of the added microbial cells and recycling on flotation were investigated by comparing water and solids recoveries, metal recoveries and grades in the absence and presence of microbial cells.

The interaction of xanthate collectors, Sodium Ethyl Xanthate (SEX) and Sodium Isobutyl Xanthate (SIBX), which are widely employed for sulphide mineral beneficiation, and the microbial cells were considered. The aim of this was to better understand how microbial cells respond when exposed to xanthate collectors and what effect the presence of microbial cells has on collector concentrations. These studies were conducted as microbial growth studies in the presence of increasing xanthate concentrations. Moreover, xanthate (at a fixed concentration) was contacted with microbes representing increasing microbial loading in waters with collector concentrations similar to those used during flotation. In both instances, residual xanthate present in solution was quantified temporally. In order to gain insights regarding collector adsorption from solution, xanthate was exposed to both microbes and ore particles and the removal of xanthate from solution monitored temporally. Microbial cell hydrophobicity before and after exposure to xanthate was also measured to assess the characteristics of the microbial consortium under flotation conditions.

In presenting the study, an introduction to the study is provided in Chapter 1. Chapter 2 provides a comprehensive literature review with particular emphasis on mineralogical composition of the ore used in the study, flotation fundamentals, flotation reagents, effects of water constituents on flotation performance and biodegradation of xanthate. This chapter is concluded by providing the objectives, hypotheses and key questions of the study. The aims and research questions are addressed in three research chapters, Chapters 4 to 6. Chapter 3 describes the methods used in these research chapters. The results gathered in the study are presented and discussed in Chapter 4, 5 and 6. Chapter 4 details the development of a method for the monitoring of microbial activity in an ore slurry and experimental determined cultivation conditions required for maintaining of the microbial community used in this study.The growth of these microbes in the presence of SEX and SIBX and their xanthate removal potential is presented in Chapter 5. The effect of the presence of this microbial community on flotation of a Cu-Ni bearing PGM ore is presented in Chapter 6. The study is concluded in Chapter 7 with includes recommendations for future research.

CHAPTER 2

2 LITERATURE REVIEW

This chapter provides a detailed review of the current literature pertinent to this study and the motivation behind the study. Section 2.1 briefly highlights the mineralogical composition of the Platreef ore from the Bushveld Complex in South Africa which was used in the present study. The preparation of the ore sample is presented in Section 2.2. Section 2.3 focuses on flotation fundamentals while Section 2.3.2 focuses on the factors which affect flotation performance. The most widely used reagents in flotation operations are presented in Section 2.4. The effects of the recycling and reuse of process water on water quality and flotation response are presented in Section 2.5. An overview of microbes in mineral beneficiation is presented in Section 2.6. The mechanisms by which microbial cells are introduced into flotation circuits are discussed in Section 2.6.2. Section 2.6.6 highlights bacterial attachment onto solid surfaces while Section 2.6.7 presents an overview of collector degradation with an emphasis on biodegradation. The chapter is concluded in Section 2.7, 2.8, 2.9 and 2.10 by presenting the problem statement, objectives, key questions and hypotheses, respectively addressed in this study.

2.1 Platreef ore

South Africa's Bushveld igneous complex (BIC) hosts the largest reserve of Platinum Group Minerals (PGMs) in the world. The BIC comprises of three distinct ore bodies namely the Merensky reef, the Upper Ground 2 (UG2) reef and the Platreef (Cawthorn, 1999; Jones, 2005; Becker et al., 2008). Typically, the proportion of the most abundant PGEs (Pt and Pd) in all deposits are as follows; Pt and Pd comprise around 55% and 32% in Merensky reef; 44% and 46% in Platreef; and 46% and 30% in UG2 reef, respectively with the other metals constituting around 15% (Cawthorn, 1999).

The BIC spans around 250 km x 450 km covering three different provinces in South Africa (North West, Limpopo and Mpumalanga) and is largely divided into three limbs: Western; Eastern and Northern limb (Figure 2-1). Platreef, located in the Northern limb, is of particular interest due to the presence of disseminated mineral reserves and commonly some fairly largeblebs of composite Base Metal Sulphides (BMS) (Viljoen and Schurmann, 1998).

CHAPTER 2

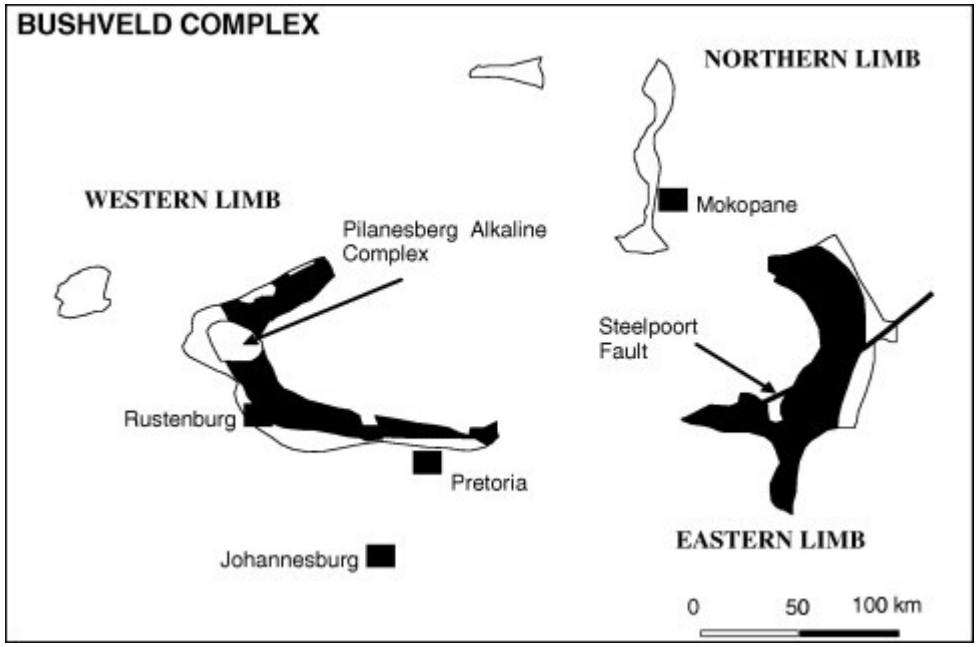

Figure 2-1: *A map of the Bushveld Igneous complex outcrops of South Africa showing the Western, Northern and Eastern limbs (Shamaila and O'Connor, 2008).*

As per Naldrettt et al. (2011), Platreef is a magmatic Ni-Cu (PGE) deposit. Further, it has been documented that Platreef ranks third globally as the largest PGE deposit, just behind the western and eastern limbs. Pyrrhotite, pentlandite and chalcopyrite, in descending abundance, are the most common base metal-hosting-minerals in the Platreef. PGMs tend to be erratically distributed within this reef. As pyrrhotite, pentlandite and chalcopyrite are sulphide minerals, the Merensky, UG2 and Platreef ores are commonly known as sulphide ores. Pt/Pd tellurides are the most important class constituting about 30% of PGMs, with arsenides constituting 21% of PGMs and 26%being alloys (Shamaila and Connor, 2008; Viljoen and Schurrmann, 1998). The PGMs as well as the base metals found in sulphide ores are recovered using flotation.

2.2 Ore processing for froth flotation

Preceding ore dressing, mining is carried out to extract ore bodies from the earth's crust. Within an ore sample, valuable mineral particles are disseminated and intimately associated with gangue material. The valuable minerals need to be liberated from the worthless gangue material - a process typically known as comminution. In the mineral processing plant, comminution is a sequential process of crushing and grinding. Crushing is a dry process and involves reduction of run of mine (ROM) ore to a particle size suitable for grinding. A simplified schematic representation of ore preparation and ore dressing is shown in Figure 2-2. Grinding is often carried out under wet conditions and achieves the liberation suitable for the ore. The degree of liberation can be defined as the percentage of the minerals that are occurring as free

particles in relation to the total ore sample. Thus, for successful downstream processing, sufficient liberation is of utmost importance (Wills and Napier-Munn, 2006).

The grinding conditions that are employed during the grinding process can influence the flotation response of the ground minerals. These include: the interaction between the minerals and the grinding media, the presence or generation of oxyhydroxide species in the pulp, the effects of chemicals or reagents used during grinding, the presence of gaseous atmosphere and most importantly the type of grinding media used (Bruckard et al., 2011).

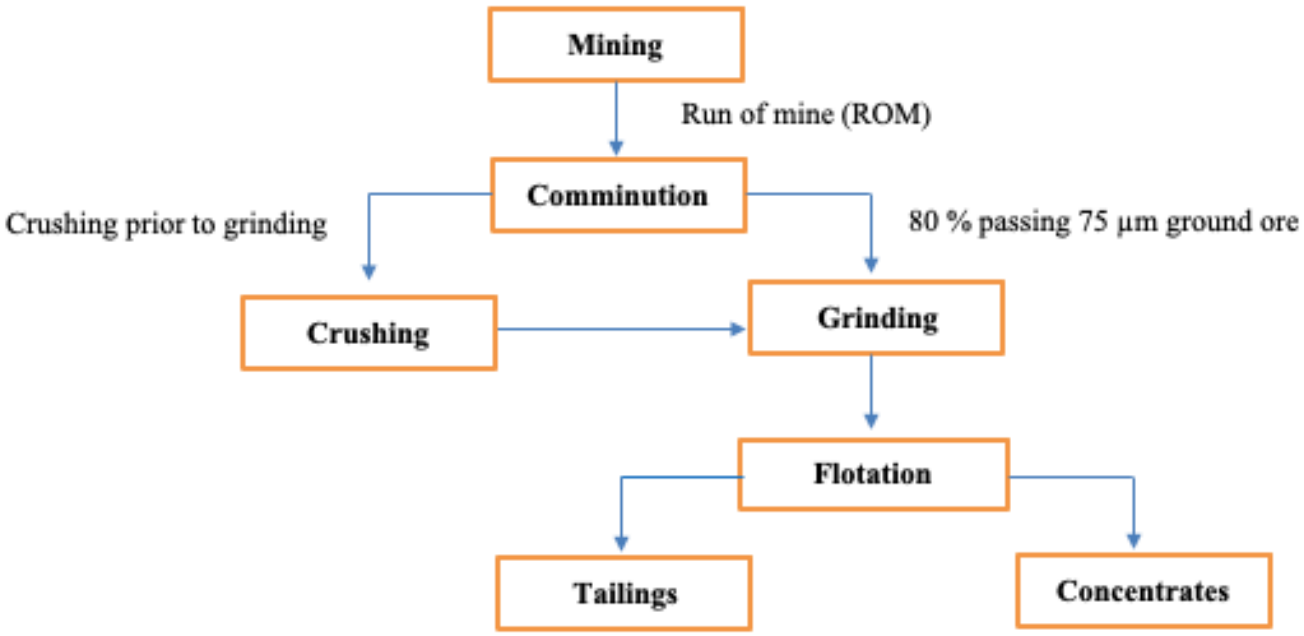

Figure 2-2: *Schematic representation of ore preparation and mineral processing.*

2.3 Froth Flotation

2.3.1 Fundamentals of flotation

Froth flotation is a highly versatile separation process which selectively separates target mineral particles from the non-valuable gangue material by exploiting their different surface properties (Bradshaw et al., 2005; Wiese et al., 2011). According to Moolman et al. (1995) and Wills and Napier-Munn (2006) the theory of froth flotation is multifaceted and involves three phases viz solids, water and froth with many subprocesses and intricate interactions, which are not completely understood. Typically, the mineral of interest is hydrophobic (water-hating) while the non-valuable particles (gangue material) are hydrophilic (water-loving). When air is bubbled through the ore slurry, typically known as the pulp phase, the hydrophobic minerals will attach to these air bubbles and float to the froth phase. At the same time, the hydrophilic particles will show no affinity for the air bubbles, hence they remain in suspension and drain off into the tailings stream (Kawatra, 2009). The mineralized froth is then skimmed off to form a target

mineral-rich concentrate relative to the original ore (Okibe and Johnson, 2002). Thus, froth flotation utilizes the differences in wettability between the valuable mineral and the non-valuable gangue material to achieve successful separation between particles in the pulp phase. Wettability is measured by measuring the contact angle (Section 2.3.4.1). Lower contact angle (≤ 90°) signifies greater wettability, whereas higher contact angle (≥ 90°) infers lower wettability. Thus, the greater the wettability, the higher the hydrophobicity and vice versa.

Some minerals, such as many coals, are generally hydrophobic in nature Fuerstenau et al. (1983) and will therefore be attracted to air bubbles; however, some valuable minerals are not ideally hydrophobic and chemical treatments (such as the addition of flotation reagents) are generally carried out to induce adequate hydrophobicity in order to achieve separation of the target mineral from the gangue material (Wills and Napier-Munn, 2006 and Kawatra, 2009). A schematic of a simplified flotation cell is shown in Figure 2-3.

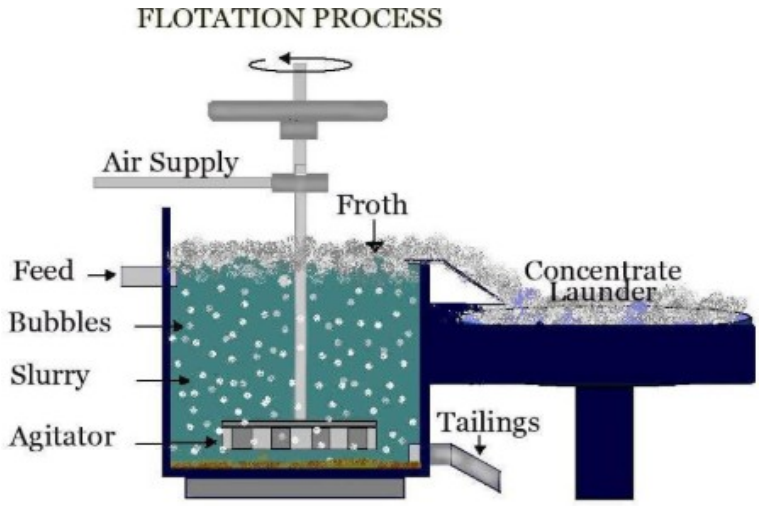

Figure 2-3: *Schematic representation of a flotation cell (Shumba, 2014).*

2.3.2 Factors affecting flotation

Flotation is a multifaceted process and can be affected by three main components: equipment,chemistry and operation (Klimpel et al., 1984). Within each of these components a number of subcomponents can be defined as per Figure 2-4. The present study considers the effects of the use of microbial laden water for flotation operations and the microbial response in the presence of flotation reagents with particular emphasis on xanthate collectors. The components under study are highlighted in red in Figure 2-4.

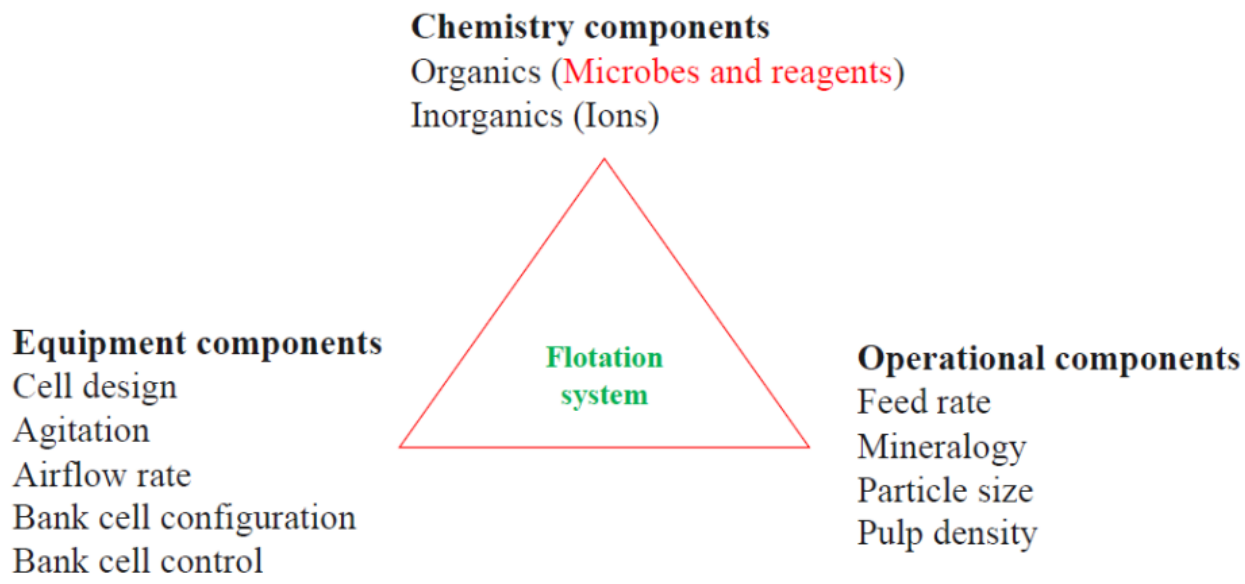

Figure 2-4: *Parameters which affect flotation (Adapted from Klimpel et al., 1994).*

Flotation comprises two major phases, namely the pulp phase and the froth phase. The former serves as the hub for the separation of the mineral of interest from the bulk and the latter serves as the zone for mineral recovery (Wills and Napier-Munn, 2006).

2.3.3 Pulp phase

The pulp phase serves to create an environment for particle–bubble attachment through physical and chemical means. Particles in the pulp phase report to the froth phase by true flotation, entrapment or entrainment (Smith and Warren, 1989). Hydrophobic particles which are meant to be floated attach to air bubbles and are lifted to the froth phase, this is termed true flotation. Entrainment is a process in which particles are suspended in the water which is trapped in the interstitial spaces between bubbles and subsequently report to the froth phase (Smith and Warren, 1989; George et al., 2004; Wills and Napier-munn, 2006; Kawatra, 2009), while entrapment is a process in which particles are physically entrapped between particles attached to air bubbles, sometimes referred to as aggregation (Savassi et al., 1998). True flotation is desired as it is selective, and entrainment/entrapment is undesired as they are unselective resulting in the flotation of gangue material which ultimately dilutes the concentrate (Smith and Warren, 1989; Wills and Napier-munn, 2006; Biçak et al., 2012).

Different water types such as borehole, tailings and concentrate thickener overflow waters are employed for flotation operations and contain different types of ions which can affect flotation. Besides these ion sources, it has been established that dissolution of ions from the milled material can introduce ions into flotation circuits and affect flotation (Coetzer et al., 2003). The mechanism by which some ions affect flotation are discussed in more detail in Sections 2.5.5 and 2.5.6. It is also worth mentioning that the

dissolution of ions from the grinding media itself (Rabieh et al., 2016) such as iron hydroxide species (Greet et al., 2004), for example $Fe(OH)_2$ and $Fe(OH)_3$ (Bruckard et al., 2011), can invariably precipitate and coat sulphide mineral surfaces (Greet et al., 2004 and Bruckard et al., 2011) and consequently affect their floatability (Johnson, 2002).

2.3.3.1 Electrical double layer

Grahame (1947) defined the electrical double layer as an array of charged particles and oriented dipoles which are thought to exist at every interface. The electrical double layer at the mineral interface is of primary importance in flotation. Some of the most important surface-physico chemical effects encountered in flotation include the following: (1) The sign and magnitude of the surface charge governs the adsorption of the physically adsorbing flotation reagents.

(2) Slime coatings are largely controlled by the electrical double layer. (3) Flotation kinetics relate directly to the effect of double layers on the rate of film thinning during bubble–particle contact. Thus, when a solid surface is immersed into an aqueous surface, it produces a region of inhomogeneity at the solid-solution interface. In many cases, the adsorption of collector at the mineral water interface is controlled by the electrical double layer and as a result the factors responsible for the surface charge on the solid surface are of great concern (Fuerstenau and Pradip, 2005).

Organics and inorganics can affect the electrical double layer on the mineral particles. In general, some organics and microbial cell surfaces carry a negative charge under physiological conditions with a few exceptions. As a result, bacteria generally experience electrical double layer repulsion when approaching negatively charged surfaces. Depending on the properties of the solid surface encountered by the bacterial cells, bacteria can either experience repulsion or attraction towards the electrical double layer. Studies have also suggested that the presence of inorganics such as Na^+ and Mg^{2+} can result in the compression of the electrical double layer (Yang et a1. 1998; Wang and Peng 2013; Manono et al, 2013).

2.3.4 Effects of particle size on flotation

Mineral particle size plays a crucial role in determining the performance output of the process. Sufficiently coarse particles settle rapidly and are not carried into the froth hence they are less prone to entrainment. On the other, too coarse particles favours low mineral recovery due to particle detachment from bubbles in high turbulence conditions owing to gravity or poor mineral liberation. As per Smith and Warren (1989), particles which are between 50 and 300 μm in size are generally less prone to entrainment. Fine particles, < 38 μm tend to settle slowly and hence are more easily caught in the bubble swarm in the direction of the froth. For example, if the fine particles are a few μm in size, the rate of their recovery in the froth is likely to be equal to that of water recovery and thus more prone to entrainment. In contrast, grinding fine improves mineral liberation and thus greater mineral recovery,

among others. As such, both fine and coarse grinding have shortcomings on flotation efficiency (Kawatra, 2009). Jameson, (2010) reported that generally, flotation works very well for particles that are typically in the range 20 to 150 µm in diameter, for sulphide ores. It should be noted that the PGM ore studied here, and described in Section 2.1, is a sulphide ore

2.3.4.1 Bubble-particle attachment

Flotation efficiency is dependent on the bubble size. Kawatra (2009) found that large bubbles relative to the particle size reduce particle–bubble interaction due to water surrounding the bubble. Conversely, smaller bubbles tend to lose their load back into the pulp phase due to insufficient buoyancy. The bubble size and the particle size must be comparable/compatible in order to ensure good contact between the bubble and particle (Kawatra, 2009). Therefore, there is a trade-off between particle size and bubble size.

Only minerals with a certain degree of hydrophobicity, either naturally or by the addition and absorption of flotation reagents, can attach to air bubbles (Liu et al., 2013a). It has been established that the efficiency of this attachment is determined by the contact angle between the air bubbles and hydrophobic particles to be floated (Chau et al., 2009). Contact angle measurement is usually regarded as a quick and simple method for quantifying surface wetting (Chao et al., 2009). The contact angle can be evaluated by Young's equation (Equation 2-1) (Chau et al., 2009) which is based on the surface tension, as shown in Figure 2-5.

$$YSA = YSW + YWACos\ \theta \qquad \text{Equation 2-1}$$

Where YSG denotes solid-air interfacial tension, YSL denotes solid-water interfacial tension, YLG denotes water-air interfacial tension and θ denotes contact angle.

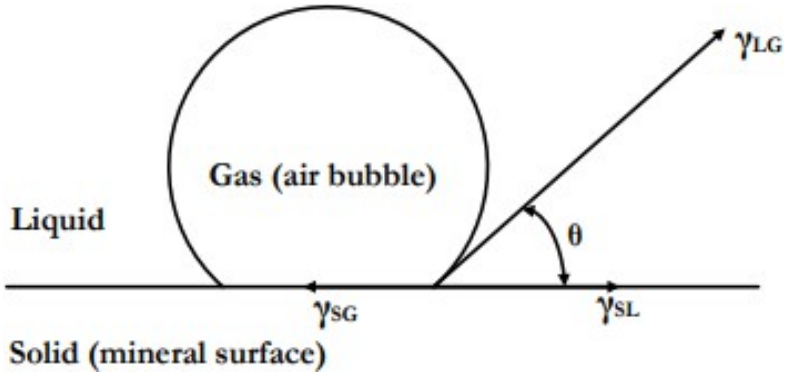

Figure 2-5: *Contact angle between a mineral particle surface and a bubble in an aqueous environment (Chau et al., 2009).*

Generally, contact angle measurements are used to determine attachment stability between particles and air bubbles. As a rule, the strength of attachment is directly proportional to the degree of the contact angle. The contact angle is usually 0^o when bubbles do not displace the aqueous phase. In contrast, a contact angle of around 180^o resembles complete displacement. As such, contact angle values between the particles and bubbles define a scale at which hydrophobicity can be estimated or described. For effective froth flotation, a contact angle of 90^o is deemed sufficient with 70^o being the optimum (Rao, 2004).

Some studies have also indicated that the attachment of microbes onto the mineral surfaces can affect the contact angle of bubble attachment. While investigating the effect of bacterial treatment on contact angle, Deo and Natarajan, (1998c) reported that following the contact of microbes to mineral specimens in an aqueous medium of pH 8–9 bubble attachment contact angles to quartz were increased from 55^0 (prior to treatment) to 76^0 (post treatment). The tests were performed with a *Paenibacillus polymyxa* culture, and the authors suggested that the change was due to enhanced hydrophobicity of the quartz surface following microbial contact. Contrarily, corundum and hematite contact angles significantly decreased from their initial values of 40^0 after a similar bio-pretreatment. This suggests that when microbes are present within flotation systems, they may affect key flotation parameters in unpredictable ways. This strengthens the value of research towards furthering our understanding of the effect microbes may have on flotation. Some of these aspects are addressed through fundamental research in this study.

2.3.5 Froth phase

Minerals are concentrated and upgraded in the froth zone; this serves as the final step in the flotation system and is hence crucial in determining the overall metallurgical performance. For optimum mineral recovery, froth stability is pivotal. Farrokhpay, (2011) defined froth stability as the ability of bubbles in the froth to resist bubble rupture/bursting and bubble coalescence. And Subrahmanyam and Forssberg (1988) simply described froth stability as the time of froth persistence. Froth stability is predominantly dependent on the type and concentration of the frother Farrokhpay, 2011).

Harris, (1982) defined two types of froth: unstable or metastable. The former refers to the froth which continues to break due to liquid drainage from between the bubbles (Harris, 1982). An unstable froth usually results in minerals dropping back into the pulp phase owing to bubbles rupturing before minerals are collected (Kawatra, 2009). In contrast, a metastable froth refers to a froth which can persist for a long time in the absence of disturbances (Harris, 1982). Additionally, according to Farrokhpay, (2011), a more stable froth is characterized by fewer bubble ruptures and coalescence events (small bubble size). Therefore, a froth of correct stability is of utmost importance. Although a more stable froth results in a marked increase in mineral recovery, it also results in loss of flotation selectivity and promotes

entrainment, which ultimately leads to gangue recovery, thereby contaminating the concentrate and lowering the grade (Moolman et al., 1995; Kawatra, 2009; Slatter, 2009). Contrastingly, an unstable froth results in a decrease in mineral recovery hence less often desired (Moolman et al., 1995). From a standpoint of managing both mineral recovery and grade, there is an optimum froth stability for a given flotation cell and the operating conditions (Farrokhpay, 2011).

It extremely important to note that the nature and concentration of the particles play a significant role in froth stabilisation (Farrokhpay, 2011). Generally, large hydrophobic particles (typically ≥ 50 μm) will tend to destabilase froth, while small hydrophobic particles (typically ≤ 5 μm) will tend to stabilize froth. In addition, hydrophilic particles generally do not alter froth stability (Johansson and Pugh, 1992) and, according to Lovell, (1976) lower solids concentration promotes froth destabilization, while higher solids concentration promotes froth stabilization. The mechanisms by which microbes and their metabolic products may affect froth flotation are discussed in Section 2.6

2.4 Flotation reagents

2.4.1 Collectors

Bulatovic (2007) referred to reagents as the most important part of the flotation process. Flotation reagents are added to the slurry to manipulate the surface properties of minerals to optimize the recovery of the valuable mineral and to depress flotation of the non-valuable material. Literature has suggested that several intricate interactions occur between flotation reagents during flotation operations (Bradshaw et al., 1998). To further compound these complex interactions, minerals which could be present in ores react in different ways to these interactions. Therefore, it becomes exceedingly difficult to isolate and quantify how each reagent behaves during these interactions (Bradshaw et al, 1998). Reagent suites usually include collectors, frothers and modifiers (Bradshaw et al., 1998; Wills and Napier-Munn, 2006).

Collectors are defined as organic species which are surface active and selectively adsorb onto target mineral surfaces giving them hydrophobic character and enabling their attachment to airbubbles (Wills and Napier-Munn, 2006 and Bulatovic, 2007). Generally, the collector type is matched in terms of nature or surface charge of the mineral (Bradshaw et al., 1998). Collectors adsorb onto mineral surfaces in two different ways - namely chemisorption and physisorption. Chemisorption, which can be defined as the formation of covalent bonds between the metals present in the mineral and the collector, is an irreversible and selective process. By contrast, physisorption, which is largely a result of electrostatic/Vander Waals forces, is reversible, non-selective and can largely be affected by changes in pH (Kawatra, 2009). A simplified schematic representation of collector adsorption onto mineral surface is illustrated in Figure 2-6.

CHAPTER 2

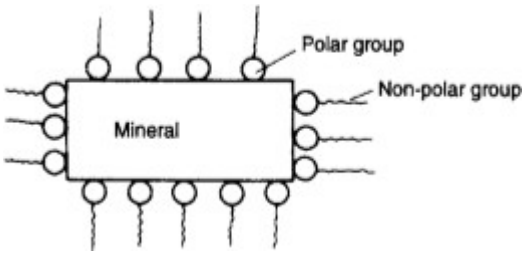

Figure 2-6: *Schematic diagram showing collector adsorption to mineral surface (Wills and Napier-Munn, 2006).*

Collectors can be broadly classified into two distinct groups: ionizing and non-ionizing (Figure 2-7) (Wills and Napier-Munn, 2006). Non-ionizing collectors are insoluble in water and are often used in coal and graphite flotation: non-ionizing collectors have an affinity for surfaces that are already partially hydrophobic such as coal (Kawatra, 2009). Conversely, ionizing collectors are heteropolar and dissociate in water. Ionizing collectors are often used in metal sulphide and metal oxide flotation. The underlying principle that governs this resides in the fact that sulphide minerals are semi-conductors and, as such, they can electrochemically react with thiol collectors (Bradshaw et al., 1998). Of particular importance to the present study is xanthate, which highlighted in red (Figure 2-7).

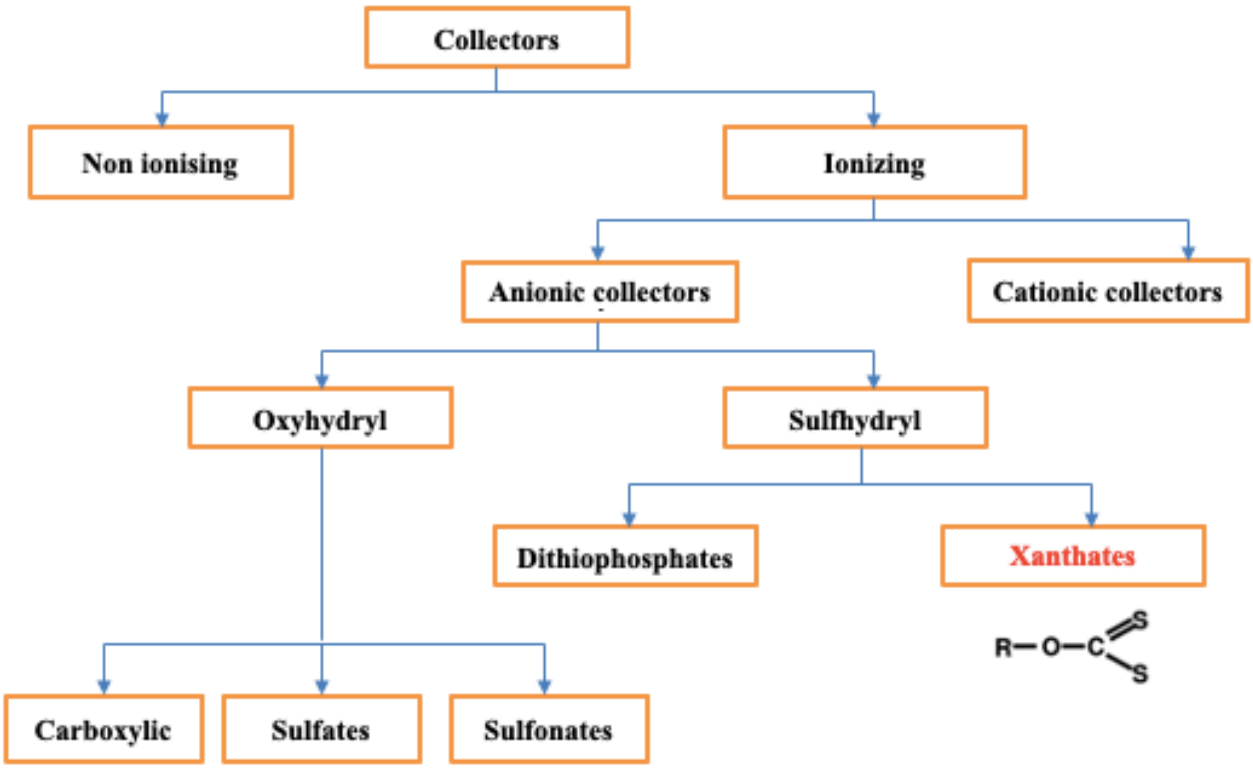

Figure 2-7: *Flotation collector classification with particular emphasis on anionic collector family. Xanthate which is the collector under study is grouped under anionic collectors (Adapted from Kawatra, 2009).*

When dissolved in water, the collector dissociates into cations or anions depending on the natureof the collector; thus, a collector is termed “cationic” or “anionic” based on the charge of the dissociated ions (Bulatovic, 2007). Figure 2-8 shows a schematic representation of an ionizing anionic collector.

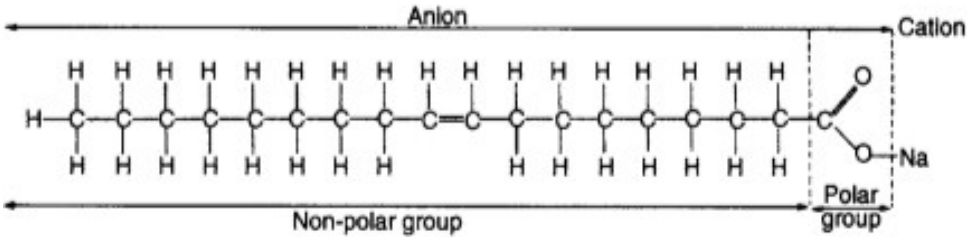

Figure 2-8: *A typical anionic collector structure (Wills and Napier Munn 2006).*

Typically, cationic collectors are amine salts and usually find application in silicate and sulphide bearing ore flotation under alkaline conditions. Anionic collectors, such as xanthates and sulfonates are usually used to float sulphides and metal oxides, under alkaline conditions. Anionic collectors can be further grouped into two distinct groups viz. oxyhydryl and sulphydryl. Collectors that belong to the oxyhydryl subgroup are primarily employed in the flotation of silicate, oxide and carbonate bearing ores. Contrastingly, collectors that fall under the sulphydryl subgroup, also referred to as thiol collectors, are most widely used in the flotation of sulphide minerals (Bulatovic, 2007). Xanthates and dithiophosphates are the most commonly used industrial collectors (Bradshaw et al, 1998; Bulatovic, 2007; Pearse, 2005).

Conceptually, the polar group (Figure 2-8) is thought to be responsible for reacting/interacting with the mineral of interest, while the non-polar hydrocarbon group gives the mineral its overall hydrophobic characteristic, as shown in Figure 2-8 (Bradshaw et al., 1998; Wills and Napier-Munn, 2006).

Sodium Ethyl Xanthate (SEX) is a widely employed xanthate collector for sulphide mineral beneficiation and was employed for flotation experiments conducted in this study. Therefore, particular emphasis has been placed on xanthate collectors. .

2.4.1.1 Xanthate collectors

Several studies have reported that xanthates are the most commonly used collectors in sulphide mineral flotation owing to their high selectivity (they have high affinity for sulphide minerals and little affinity for non-sulphide gangue minerals), efficiency and low cost (Hadler et al., 2005;Wiese et al., 2005). Xanthates are prepared by reacting an alcohol, carbon disulphide and alkali hydroxide as per Equation 2-2.

$$ROH + CS2 + KOH = ROCS2K + H2O \qquad \text{Equation 2-2}$$

where R denotes the hydrocarbon group usually between C2 and C6. A typical anionic sulphide collector, sodium ethyl xanthate (SEX), which is used in sulphide mineral flotation is shown in Figure 2-9.

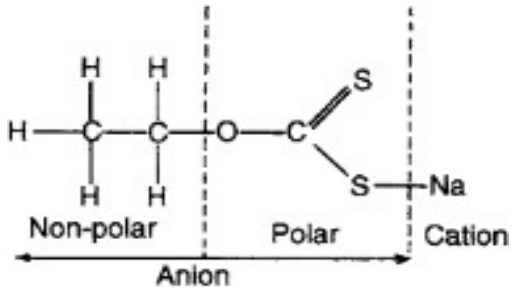

Figure 2-9: *Diagrammatic representation of Sodium ethyl xanthate (Wills and Napier-Munn, 2006).*

Selectivity, solubility, strength and reactivity of xanthate collectors are heavily affected by the difference in chain length. As per Nagaraj (1988), xanthates with longer chain lengths tend to be stronger and less selective while, xanthates with shorter chains are weaker but more selective. Also, the solubility of xanthates decreases with an increase in hydrocarbon chain length, and vice versa (Özün and Ergen, 2019).

2.4.1.2 Biocollectors

Several studies have highlighted the usage of microbial derived flotation reagents in place of the traditional flotation reagents, a process usually termed bioflotation. In bioflotation, microorganisms and their products can be used as collectors, depressants or frothers. In these cases, microbial cells are found to have positive impacts on flotation. In this case, microorganisms either living or their metabolites are employed for flotation operations in place of chemical flotation reagents and this is discussed in Section 2.6.4.

2.4.1.3 Collector degradation

Due to their high specificity for sulphide minerals, xanthates are usually added in relatively low concentrations during the flotation process. In general, 50-300 g of xanthate are required per ton of ore (Fu et al., 2015). Lam, (1999) reported that half of the collector added to the circuit is consumed with the residual reporting to the tailings discharge. As a result, excess unconsumed concentrations of xanthate collectors end up in plant effluents and are considered an environmental risk owing to their toxicity towards water and human life (Chockalingam et al., 2003). Most flotation plants recycle their water recovered following the tailings dewatering process ensuring that no effluent is discharged into the environment. The reuse of flotation process water may allow concentrators to employ less xanthate owing to residual/unconsumed xanthate which could be present in flotation waters which is recirculated within the flotation system. However, this is only possible for flotation plants where the pH of the water remains alkaline (approx. 8), given that the xanthate has not been degraded by acidic conditions. Studies by Oliviera et al. (2011) and Cifuentes et al. (2013) showed that there is a cascade of degradation

reactions which are associated with xanthate ions under acidic conditions usually as pH values below 6. Xanthate (ROCS2) hydrolysis which yields xanthic acid (ROCS2H) is shown in; Equation 2-3, followed by decomposition of xanthic acid as shown in Equation 2-4.

$$ROCS2^{-}+H2O \Leftrightarrow OH- + ROCS2H \quad \text{Equation 2-3}$$

$$ROCS2H \Rightarrow ROH+CS2 \quad \text{Equation 2-4}$$

From Reactions 2-3 and 2-4 it is evident why xanthates are inefficient as collectors under acidic conditions. Further, these degradation reactions proceed fast and are considered irreversible (Cifuentes et al., 2013)

In contrast, under alkaline conditions, hydrolysis of xanthates occurs slowly as per Equation 2-5. Xanthates have a pH stability of pH range 8-13 and are thus used in neutral or slightly alkaline aqueous environments. Flotation of most sulfidic ores are performed around pH 9 and it is a strongly buffered system due to the natural presence and dissolution silicate gangue material (Ekmekçi et al., 2005).

$$ROCS2^{-}+3H2O \Rightarrow 6ROH + CO3^{2-} + 3CS2 + 2CS3^{2-} \quad \text{Equation 2-5}$$

Trithiocarbonate ion ($CS3^{2-}$) undergoes further decomposition to produce CS2 and S^{2-} as per Equation 2-6

$$CS3^{2-} \Rightarrow CS2 \text{ and } S^{2-} \quad \text{Equation 2-6}$$

It is worth noting that xanthates are susceptible to atmospheric oxidation as demonstrated in Equations 2-7 and 2-8:

$$2ROCS2^{-} \Rightarrow (ROCS2)2+2e^{-} \quad \text{Equation 2-7}$$

$$2ROCS2 + 1/2\ O2+H2O \Rightarrow ROCS2)2 + 2OH^{-} \quad \text{Equation 2-8}$$

Since some of the products, for example dixanthogen $(ROCS_2)_2$, exhibit collecting characteristics, they adsorb non-selectively on sulphide mineral surfaces. As such, residual xanthate products remaining in recycled water may adversely affect flotation selectivity (Rao and Finch, 1989).

2.4.2 Frothers

Khoshdast and Sam, (2011) defined frothers as heteropolar surface-active compounds which comprise a polar group and a non-polar hydrocarbon radical. Frothers adsorb at the air-water interface, with the polar group attaching to water while the non-polar group attaches to the air bubble, as illustrated by Figure 2-10. Wills and Napier-Munn, (2006) described frothers as chemically similar to ionic collectors. Frothers unarguably play a pivotal role during froth flotation particularly on bubble size, stability and mobility of

the froth phase (Wills and Napier-Munn, 2006; Khoshdast and Sam, 2011). Frothers tend to reduce surface tension at the air-water interface, minimizing bubble coalescence, bubble rupture and preserving small bubbles, resulting in air bubble stabilization (Cho and Laskowski, 2002; Wills and Napier- Munn, 2006; Khoshdast and Sam, 2011).

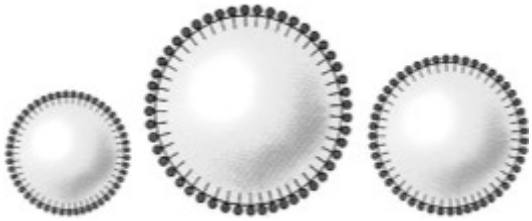

Figure 2-10*: Schematic diagram of frother orientation on bubble surfaces (Khoshdast and Sam, 2011).*

For frothers to be evenly distributed in an aqueous solution, they should be, at least to some extent, water-soluble (Wills and Napier-Munn, 2006). The most commonly used frothers are polyglycol ethers. The performance of polyglycol ethers can usually be related to their molecular weight and hydrocarbon chain length. Frothers with higher molecular weights produce more persistent froth compared with frothers having lower molecular weights. However, frothers with higher molecular weights tend to be less selective than frothers with lower molecular weights (Bulatovic, 2007).

There is some literature on the use of microorganism as frothing agents. A variety of microorganisms such as bacteria, yeasts and fungi are perceived to be able to produce biosurfactants. It has been suggested that these biosurfactants have a higher surface-activity, lower toxicity, higher biodegradability and better environmental compatibility compared to the synthetic/traditional surfactants (Wei et al., 2007). While investigating the frothing characteristics and applicability of rhamnolipid-type biosurfactants as frother, Fazaelipoor et al. (2010) experimentally determined that these biosurfactants have better surface-activity and static frothability, i.e. frother height and half-life, compared to Methyl Isobutyl Carbinol (MIBC), a synthetic surfactant.

Frother strength can also be characterized by their critical coalescence concentration (CCC) values. Based on empirical findings, Cho and Laskowski (2002) showed that the degree of bubble coalescence decreases with increasing frother concentration until a particular concentration, typically known as the CCC, is reached- as shown in Figure 2-11. At this point bubble coalescence is totally inhibited/prevented. Thus, frother strength can be characterized by CCC, with stronger frothers reaching CCC at lower concentrations.

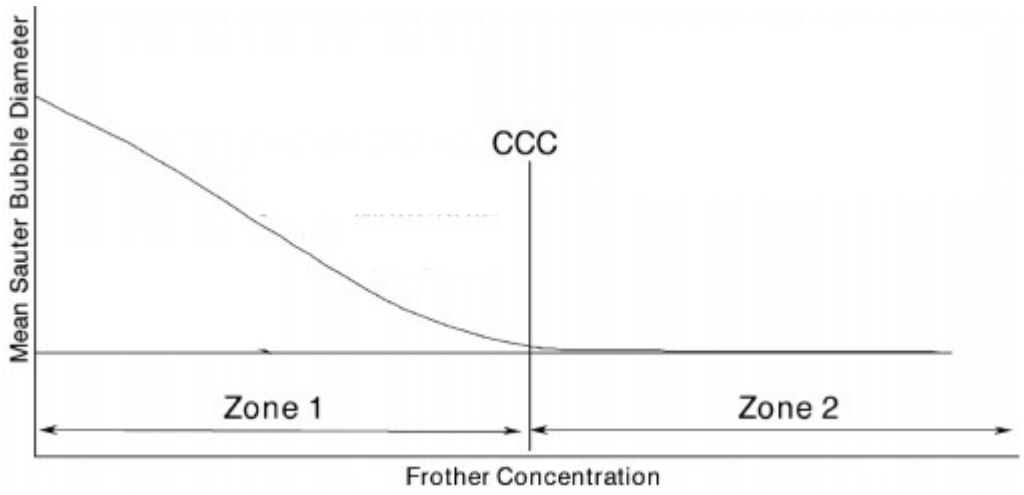

Figure 2-11: *The effect of frother concentration on bubble size (Cho and Laskowski, 2002).*

2.4.3 Modifiers

Depressants, activators and pH regulators, often referred to as modifiers or regulators, are employed in flotation processes to enhance collector function and govern the selectivity of theprocess (Bradshaw et al, 1998 and Bulatovic, 2007).

2.4.3.1 Activators

Activators serve to create a conducive environment for collector adsorption onto mineral surfaces. For example, copper sulphate (CuSO4) reacts with sphalerite (ZnS) and provides conditions necessary for collector-target mineral interaction. In the absence of a pre-treatment with CuSO4, xanthate collector cannot attach to ZnS surfaces owing to the formation of a soluble Zn-Xanthate compound which quickly dissolves. To counteract this phenomenon, a metal ion such as Cu^{2+}, in the form of $CuSO_4$, is reacted first with ZnS to form an insoluble Cu-xanthate precipitate. This results in the formation of a thin film of copper sulphide on the ZnS surface and thereby promoting xanthate attachment and rendering the ZnS particles hydrophobic and floatable (Kawatra, 2009).

2.4.3.2 pH Regulators

Since the surface chemistry of most minerals is affected by pH, pH regulators serve to regulate ionic composition of the pulp by changing hydroxyl concentrations. Accordingly, this results in improving collector interaction with the target mineral and reduces collector adsorption/interaction with the undesirable mineral (Bulatovic, 2007).

2.4.3.3 Potential Modifiers

Potential (Eh) modifiers can be used for redox adjustment in the system as these modifiers influence mineral surface properties to allow electrochemical flotation of sulphide minerals. Some examples of Eh modifiers are NaClO and H_2O_2 (Bulatovic, 2007).

2.4.3.4 Depressants

Bradshaw et al. (2005) suggested that the role of a depressant is opposite to that of a collector as it is designed to render the gangue material hydrophilic, thereby suppressing its flotation. This is achieved in two different ways; either by preventing collector adsorption on the hydrophilic species or by rendering partially or hydrophobic minerals hydrophilic. For example, depressants are used to reduce the floatability of hydrophobic talcaceous minerals by inducing hydrophilicity on their surfaces. The most widely used depressants are long chain polysaccharides such as carboxymethylcellulose (CMC) and guar gum (guar) (Bradshaw,et al., 1998), with the latter being susceptible to biodegradation (Qui et al., 2018). Figure 2-12 shows the typical structure of guar gum.

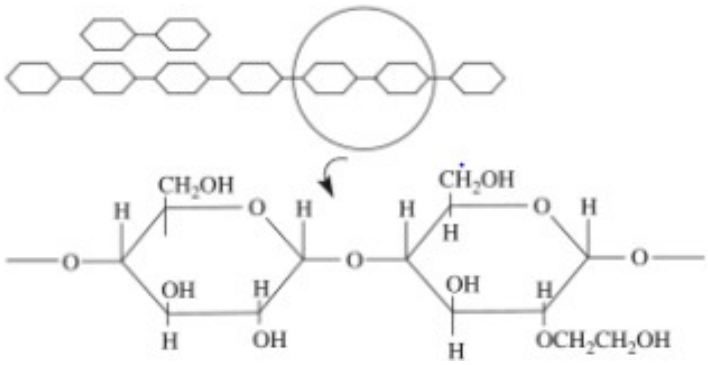

Figure 2-12: *A typical structure of guar gum (Bulatovic, 2007).*

2.5 Water quality in flotation

2.5.1 Water within flotation operations

Mining and mineral processing plants utilize water from the onset to the end of their operations,both as a transport agent and as a reagent. As such, water plays a pivotal role in such operations (Bailey, 1970 and Muzinda & Schreithofer, 2018). Bailey (1970) emphasized that during mineral processing operations, much of the water is utilized during flotation and, to a lesser extent, for processes such as heap leaching. With the ever-increasing pressure on mining companies to restrict freshwater use and limit mine water discharge, the minerals industry is increasingly putting practices in place to reduce consumption of fresh water, especially in water-stressed countries like South Africa. As a result, many concentrators have implemented on site water recycling and reuse, starting as early as the 1970's (Bailey, 1970). When

recycling water, it is assumed that water has been subjected to some form of treatment process whereas reuse refers to the direct reuse of water without any further treatment prior to use as water source for flotation operations. Water reuse results in the deterioration of process water which may negatively affect flotation performance. Recycling with the inclusion of the treatment step prior to flotation operations is preferred, however this is costly and time consuming.

Flotation is a water-intensive operation which employs water both as a reagent and transport medium. Studies by Levay et al. (2001) and Muzenda (2010) have shown that water accounts for about 80-85% by volume of the pulp in flotation circuits. For this reason, monitoring and understanding the effect of water chemistry and quality on flotation performance is of paramount importance (Rao & Finch, 1989; Liu et al., 2013; Muzinda and Schreithofer, 2018). It is well documented in literature (Muzenda, 2010; Rao and Finch, 1989, 1989; Muzinda and Schreithofer, 2018; Slatter et al., 2009) that, with continued reuse, the quality of process water tends to deteriorate over time, owing to the accumulation of organics and inorganics which can alter the water chemistry. The impacts of inorganics, ions in particular, are discussed in Section 2.5.4 and 2.5.5 while the impacts of organic material, particularly microbes, are discussed in Section 2.6.4 and 2.6.5.

Besides the adverse effects posed by changes in water quality owing to water reuse in flotation operations, it is in minimizing freshwater intake and retaining unconsumed reagents,which ultimately lowers costs regards water usage (Muzenda, 2010; Muzinda and Schreithofer, 2018). Moreover, water reuse tends to be a long-lasting and more sustainable option as it allows processing plants in water short areas to operate; mining companies are continuously looking for more ways in which to decrease their water footprint and water reuse is one such way. Discharge water typically contains toxic contaminants, such as metals and residual flotation reagents, which may pose a threat to human life and surrounding environments (in the case of tailings dams failure or leakage) (Slatter et al ., 2009; Lin et al., 2019) and therefore requires appropriate treatment before discharge to the environment.

2.5.2 Causes of water quality variations

Water quality variations have substantial and complex impacts on flotation owing to changing solid surface properties, which may result in compromised flotation performance (Levay et al., 2001; Liu et al., 2013a). According to a published framework by Liu et al. (2013a), concentrator water quality variations can be brought about by two broad categories of factors namely, internal and external factors.

Internal factors refer to those internal to the concentrator and comprise ore, reagent addition and water reuse within the concentrator as shown in Figure 2-13.

External factors can be divided into two distinct groups: external to the concentrator and external to the site. In terms of factors that are external to the concentrator (Figure 2-13) these may be within the

concentrator's proximity e.g., raw water streams and water external reuse in which water is recovered from multiple sites, including tailings storage facilities etc. In terms of factors external to the site, these are factors (Figure 2-13) not within the site's borders and may include raw and partially remediated waters. Since these waters are exposed to open systems, this results in interaction with the surrounding environments and local climate. Thus, mass and energy transfer can occur across the site boundary and these processes can be termed external to site factors, which are shown as inputs and outputs. For example, inputs (Figure 2-13) may include seawater, surface water, rainfall, groundwater, etc. while outputs may include seepage,evaporation, discharge of process water, etc. (Liu et al 2013a).

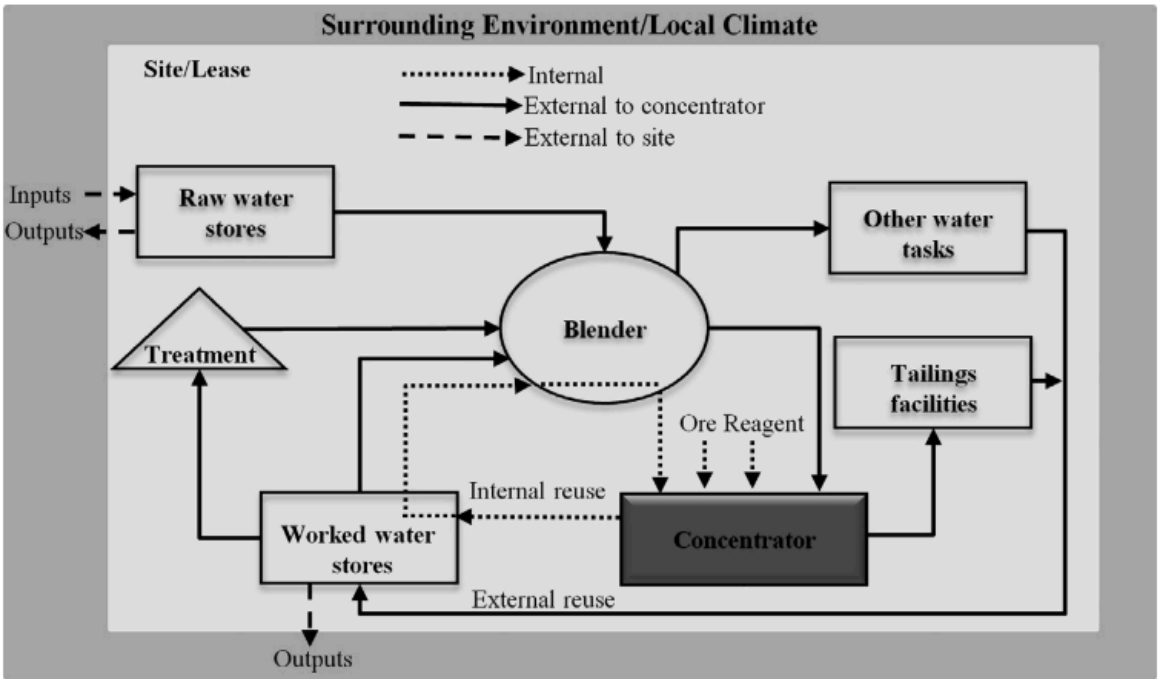

Figure 2-13: *Water sources on a mineral concentrator which may cause water quality variation (Liu et al., 2013a).*

The blender (Figure 2-13) simply represents site infrastructure, where different water streams can be combined prior to use on a site. Cote and Moran, (2009) defined raw water as water which has not been previously subjected to any use on a site. Worked water is water that has been subjected to use on a site and reclaimed for potential re-use.

2.5.3 Remediation of recycled flotation waters

Water treatment allows for the safe use of recycled water and avoids the need of adding fresh water to compensate for quality deficiencies. It is imperative to emphasize that the main goal of water treatment is to obtain water of sufficient quality to perform successful flotation. A myriad of water treatment techniques is available. The selection of these water treatment techniques is mostly dependent on several factors which includes suspended particle size, density, and concentration of water components to be

remediated. Electrochemical Water Treatment (EWT), polishing filtration and Dissolved Air Filtration (DAF) exemplify some of the water treatment techniques employed.

2.5.3.1 Electrochemical Water Treatment

EWT encompasses a group of physical-chemical methods that utilizes the potential difference from redox reactions generated between two electrodes in the electrolysis process (Sillanpää and Shestakova, 2017). These electrolytic processes/ reactions in solution results in the removal of contaminants in three different ways.

Firstly, conversion methods- conversion of the impurities to modify their properties in wastewater and these may include electrooxidation, electrocoagulation and electroreduction conversion methods. Secondly, separation without considerable changes in physical-chemical properties such as electroflotation or electrodialysis, where electric fields attract charged particles present in the wastewater The last method is simply the combination of the aforementioned methods (conversion and separation). Generally, it has been established that EWT methods results in the generation of well remediated water with little residual impurities (Feng et al., 2016).

2.5.3.2 Water polishing

Water polishing refers to the removal of suspended solids/fine particles present in low concentrations such as remaining solids from primary and secondary water treatments There are various types of polishing available and some of these includes sand filtration (Hamoda et al., 2004) and the Outotec Larox® LSF filter (Outotec, 2019), which uses adsorptive filtration to remove solids from process waters. Polishing filters offers high filtrate qualities at low costs.

2.5.3.3 Dissolve Air Filtration

DAF is defined as a particle separation process whereby microbubbles generated by a pressurized air stream is introduced into the water and attach to the particulate matter in suspension. The particles together with the bubbles float to the top of the flotation tank where they are withdrawn (Haarhoff and Edzwald, 2012). Rodrigues and Rubio (2007) reported that DAF can employed for the primary, secondary or tertiary treatment of waters containing colloids, fine and ultrafine particles, microorganisms, metal ions, or even oils and greases. Additionally, among others, this method can be used to treat larger volumes per unit of time compared to other treatment techniques such as filtration (Ferguson, et al., 1995).

2.5.4 Effects of water constituents on flotation performance

As reasoned by Liu et al. (2013a), the water constituents in recycled water can be broadly grouped into two major groups namely, inorganics/abiotic and organics/biotic components. The former can be defined

as the non-living component for example, metal ions, and the latter as the living component or components derived from living material, for example, microorganisms and their products. Each entity has a different impact on flotation performance and this is largely determined by the type of mineral and reagent suite present.

2.5.5 Positive impacts of inorganic components

2.5.5.1 Compression of the Electrical Double Layer

The presence of electrolytes in recycled waters results in the compression of the electrical double layer, described in Section 2.3.3.1, which improves particle-bubble attachment efficiencies. Therefore, electrolytes act as particle-bubble stabilizers (reduce electrostatic repulsion). A number of authors have reported that flotation recovery is markedly increased in the presence of increased levels of ions (Yang et a1. 1998; Wang and Peng 2013; Manono et al, 2013).

2.5.5.2 Formation of smaller bubbles

Due to the influence of electrolytes on gas solubility and surface tension, electrolytes facilitate the formation of much smaller and more stable bubbles. Smaller bubbles tend to improve particle-bubble collision probability and particle-bubble attachment efficiency (Pugh et al., 1997).

2.5.6 Negative impact of inorganic components

2.5.6.1 Activation of gangue material

There is evidence that some metal cations present in process water, such as Mg^{2+} and Ca^{2+}, activate gangue material/increase gangue material floatability, thereby adversely affecting flotation performance through a dilution of the concentrate and a decrease in grade (Rao and Finch, 1989., Liu et al.,2013; Seke & Pistorius, 2006; Muzinda & Schreithofer, 2018). Manono et al. (2016) showed that Na^{+} resulted in gangue activation during the flotation of a Cu-Ni containing ore within the flotation system. After adding bicarbonate ions to flotation water, Lutandula and Mwana (2014) reported that $HCO3^{-}$ ions boosted the flotation of the gangue material and depressed the mineral of interest. Most recently, while spiking Ca^{2+} and Mg^{2+} into different synthetic plant waters, Dzingai et al. (2020) noted that these ions lowered concentrate grade, indicating that gangue material is activated as proposed by Rao and Finch, 1989; Liu et al., 2013; Seke & Pistorius, 2006; Muzinda & Schreithofer, 2018.

2.5.6.2 Change in particle surface charge in the presence of metal ions

Metal ions such as Cu^{2+}, Fe^{2+} and Ca^{2+} present in flotation waters can alter particle surfaces and induce complications with respect to the interactions between particles and collector (Zhang et al., 1997). This

could adversely affect the formation of a particle-bubble interaction which could, in turn, affect the formation of a stable particle-bubble aggregate (Ata, 2012).

2.5.6.3 Precipitation of metal hydroxides on mineral surfaces

It has been established that the presence of some metal ions e.g. (Mg^{2+}, Ca^{2+}, Zn^{2+}), derived from the dissolution of minerals, are deleterious to flotation performance as, under the right conditions, they precipitate as hydrophilic hydroxides at the mineral surface. This forms a barrier for collector adsorption (Muzenda, 2010; Liu et al., 2013a).

2.6 Microorganisms

Microorganisms are broadly defined as living organisms which can only be seen under magnification using a microscope and are represented in all environments regardless of how extreme. They typically consist of a single cell (unicellular) and include bacteria, archaea, protozoa, algae and fungi. In some cases, multicell structures form, typically without cell differentiation. When considering microorganisms in mineral processing often only the beneficial microorganisms, used for mineral beneficiation through biooxidation, bioleaching or bioflotation are considered. However, the presence of high concentrations of microorganisms within otherwise non-microbial processes are currently being recognised as influencing process performance and are considered a topic worthy of including in the study of processes typically thought of as abiotic. The presence of microorganisms may be due to the increasing recycling of water sources and the use of unconventional fit-for-purpose water streams, resulting in enrichment of the prevailing microbial communities. The successful growth of microorganisms in flotation waters has been attributed to a number of factors including appropriate temperatures, nutrition from flotation reagents and abundance of oxygen amongst others. Currently we lack information on the impact the presence of significant microbial cell numbers in flotation waters may have on process performance.

2.6.1 Microorganisms in mineral beneficiation

Besides gaining attention in hydrometallurgical leaching operations, microorganisms have also shown promise for use in mineral beneficiation processes such as flotation and flocculation. Several investigations have revealed the potential use of microorganisms in place of the traditional flotation reagents, a method of operation known as bioflotation, which is believed to have potential to be more economical and environmentally friendly. However, although exploitation of these biotechnological processes is perceived to be environmentally friendly and cost effective, the practical implementation has not been commercially realized, particularly in the case of bioflotation. In contrast, bioleaching has been notably employed at a commercial scale. Microorganisms including autotrophic (utilizing carbon from CO_2), and heterotrophic (utilizing inorganic carbon) bacteria, fungi and algae, are among the

microorganisms implicated in mineral biomodification processes (Rao et al., 2010). However, microorganisms which could be naturally present in recycled flotation water, originating from poorly remediated water sources, and the ore employed for flotation operations, have been thought to be different from those employed in bioflotation or bioflocculation. The naturally prevailing microorganisms present in these poorly remediated waters have been linked to poor performance within mineral concentrators (Evdokimova. et al., 2012; Liu et al, 2013b; Bomberg et al, 2020).

Of particular interest to the present study is the effect of the microbial load present in poorly remediated flotation waters on flotation performance. As such, the effects of microbial load present in flotation waters originating from poorly remediated waste-water on flotation performance largely remains an unexplored subject.

2.6.2 Introduction of microorganisms in flotation circuits

The use of multiple water sources to produce a consistent process water for flotation, results in the introduction of organics and inorganics. Microbial cells and associated organics may be introduced into the flotation circuits via external water sources (Liu et al., 2013a), as make-up to process water with poor quality water sources has become prevalent across the world, especially in arid mining countries like South Africa. These alternative water sources, such as treated domestic water, to supplement freshwater may carry considerable microbial concentrations which are then introduced into flotation circuits (Liu et al., 2013a and Liu et al., 2013b). These waters comprise different bacterial communities at different concentrations, depending on their sources (Liu et al., 2016; Schumann et al., 2003). Another source which could result in the introduction of microbes is the ore itself (Smith and Miettinen, 2006). Further, it has been established that flotation circuits provide conditions which are conducive to microbial growth (Liu et al., 2013a).

Microbial growth in flotation circuits has been attributed to nutrients that are thought to be provided from some flotation reagents, adequate oxygen levels from aeration and suitable temperatures (Levay et al., 2001; Liu et al., 2013b). Carr et al. (2009) estimated that the microbial concentrations of typical raw sewage can be around 10^5 cells/ml, while Levay et al. (2001) reported that the total bacterial count can reach as high as 10^9 CFU/ml in flotation circuits. It has been postulated that microbial-ladened water facilitates complicated interactions among the geological component (mineral component), biological component (microbial community) and the chemical component (flotation reagents) of the flotation process (Liu et al., 2013b). These interactions have been understudied and as such are currently still poorly understood.

2.6.3 Bacterial lysis within the concentrator

Since bacterial cells are very sensitive to chemical, physical and biological factors in their surrounding environments, variations in these factors can result in bacterial lysis, a process in which bacterial cells undergo cell disruption. Bacterial lysis may occur within a concentrator due to various reasons. Amongst these reasons are mechanical forces, UV radiation and exposure to harsh conditions such as rapid changes in or extreme pH levels and the presence of various flotation chemicals. Bacterial lysis due to mechanical forces is usually due to forces exerted on bacterial cells by grinding media or possibly through shear within the float cell. Depending on the wastewater quality, UV can be employed to disinfect wastewater. While bacterial lysis may prevent the bacteria from acting, cell disruption may also prove problematic to flotation as cell disruption usually results in the release of organic components such as polysaccharides, proteins and DNA. These substances may passivate mineral surfaces, change surface properties and prove detrimental to flotation performance (Liu et al., 2016).

To date, however, the vast majority of studies have been devoted to elucidating the effects of inorganic components, particularly ions, in recycled water on flotation performance (Corin et al., 2011; Biçak et al., 2012; Ikumapayi et al., 2012; Manono et al., 2013; Lutandula and Mwana, 2014). The effects of organic components, particularly microorganisms, are far less studied and understood.

2.6.4 Positive effects of biotic components

In some cases, biotic components can have positive effects on flotation performance. The two major biotic constituents, namely residual reagents and bacteria, exemplify these positive effects. Retention of some residual reagents in process water results in a lowering of reagent consumption in flotation circuits, therefore lowering costs. Further, bacteria could be useful in selective flotation, such as bioflotation, where it is used to reduce or enhance the recovery of one mineral over another. Studies by Zheng et al. (2001) and El-midany and Abdel-khalek, (2014) showed that bacteria can selectively adsorb onto mineral surfaces and suppress mineral flotation. *Bacillus subtilis* and *Paenibacillus polymyxa* were used as depressants for the removal of impurities such as ash and sulphur in coal flotation. A study by Msipa (2018) showed that *P. polymyxa* (the organism itself) can be employed as a biocollector for the removal of pyritic sulphur in the second stage of the two-stage desulphurisation froth flotation process. Based on experimental results, Zheng et al. (2001) showed that *B. subtilis* can be used as a depressant for dolomite over apatite, using oleate as a collector by blocking oleate adsorption onto the dolomite. And *Mycobacterium phlei (M. phlei*) can be used as a depressant for apatite over *B.subtilis* owing to its higher affinity for apatite surfaces (Zheng et al., 2001). Merma et al. (2013) indicated that *Rhodococcus opacus* can be employed as both a biocollector and biofrother. From their findings, it was shown that *Rhodococcus opacus* facilitated the separation of apatite from quartz during phosphate flotation. In

addition, a study by Ramos-Escobedo et al. (2016) indicated that *Staphylococcus carnosus* can be employed as a biocollector in fine coal tailings flotation. Complementarily, Chiodza et al. (2020) demonstrated that raw algal lipids (RALs) and their derivatives (fatty acid methyl esters; FAMEs) can be used as biocollectors for the recovery of coal from ash-rich fine coal waste in froth flotation. These biocollectors performed similar or better (in terms of coal yield and recovery) than oleic acid commonly used as collector for coal flotation.

2.6.5 Negative effects of biotic components

Recycled flotation waters may contain biotic constituents such as organics and microorganisms which may act as flocculants, dispersants or surfactants thereby interfering with the flotation process performance (Rao & Finch, 1989; Levay et al., 2001; Liu et al, 2013). These negative effects are especially evident where the residing microbial communities represent many species enriched within the process from the water sources and the ore, compared to bioflotation where a specific species is utilised for its specific function within mineral recovery.

The presence of microorganisms and their metabolic products in flotation waters have been found to have detrimental effects on Cu and Au flotation (Schumann et al., 2003; Liu et al., 2013a). Further, some bacterial strains have been found to adversely affect flotation by interacting with mineral surfaces through bacterial passivation, which alters mineral surfaces and subsequent vital flotation subprocesses (Evdokimova et al., 2012; Liu et al., 2013a, 2013b, 2016).

As reported by Liu et al. (2013b), chalcopyrite flotation in three different mineral systems (highly pure chalcopyrite, synthetic mineralogy (known or controlled mineralogy) and Cu-Au bearing ore) was negatively affected by the presence of intact *E. coli* cells due to their adsorption on chalcopyrite surfaces reducing its surface hydrophobicity. Similarly, using high purity chalcopyrite as a model mineral, Liu et al. (2016) further performed batch flotation tests in the presence of disrupted *E. coli* cells and reported that disrupted *E. coli* cells negatively affected chalcopyrite flotation as evidenced by the decrease in mineral recovery owing to adsorption of organic material onto the mineral surfaces hindering collector adsorption. In their findings for both intact and disrupted cells, the magnitude of the negative impact for *E. coli* cells was dependent on cell concentrations, with increasing concentrations resulting in lower mineral recovery. Moreover, research by Attia (1990) showed that microbial attachment onto pyrite mineral surfaces resulted in the pyrite switching from a hydrophobic to a hydrophilic mineral.

Moreover, organics present in recycled waters, such as residual reagents, particularly xanthates and their oxidation products, dixanthogens, that contribute to process water, adsorb to most sulphide minerals, non-selectively reducing flotation efficiency in selective flotation processes (Rao andFinch, 1989; Seke and Pistorius, 2006).

It is also important to note that in most cases, for well understood microbial aided operations such as bioleaching and bioflotation, microbes are found to be beneficial. By contrast, in most cases for operations which are microbial independent, such as flotation, microbes and their products tend to inadvertently interact with minerals which, in most cases, has been reported to be detrimental to flotation. Thus, it is of paramount importance to assess and monitor the biotic water constitutes in microbial laden-water and their subsequent effects on flotation performance as recycled, or poorly remediated, water is increasingly employed as make up water within concentrators. Moreover, it is imperative to understand the impacts of microorganisms and their ultimate effects on flotation, particularly bacteria, as recycled waters from sewage, tailing dams, etc. are reported as a source of microorganisms (Slatter et al., 2009; Liu et al., 2013b).

2.6.6 Bacterial adhesion on mineral surfaces

Despite the fact that microbe-mineral interactions are not well understood, Vasanthakumar et al. (2013) demonstrated that some microbes attach to mineral surfaces through various interactions, for different reasons, which involve hydrophobic interactions, chemical attachment and physical adsorption. Microbial attachment onto solid surfaces has been highlighted and demonstrated in several studies (Watling, 2006). A study by van Loosdrecht et al. (1990) showed that the highest microbial activity was found on solid surfaces. The formation of a biofilm around the attached cells provides the microbes with an optimum and homogenous environment. As such, attached cells are able to function even in cases where the surrounding conditions are not optimal (Watnick and Kolter, 2000). In this context, microbial attachment onto solid surfaces is an imperative phenomenon as it allows optimum microbiological activity to occur.

Microbial adsorption onto mineral surfaces can bring about physicochemical changes which involve changes in the isoelectric point and the wettability of the mineral (Vasanthakumar et al., 2013). As per Rao et al. (2010), a microorganism's adhesion to a mineral surface is due to various reasons. For example, it has been found that *Shewanella oneidensis* utilize minerals as final electron acceptors in their respiratory cycle system. Bacteria which belong to the *Acidithiobacillus* genus recover energy from minerals through enzymatic oxidation processes of mostly the sulfidic components within the minerals. Additionally, attachment of microbial cells onto solid surfaces maybe initiated as a result of chemotactic response. A chemotaxis response refers to a microbial response owing to a particular stimulus. The stimulus may be an attractant or a repellent. In the case of the former, an attractant, such as a nutrient concentration gradient, is thought to mobilise bacteria towards higher stimulant concentrations. Contrarily, in the case of a repellent, the repellent will stimulate motile bacteria to move away from its higher concentrations. The processes make use of signal transduction proteins containing two functional domains; a sensory domain which interacts with the stimulant at the microbial cell surface and a

signalling domain which modulates effector activities within the cell causing movement toward or away from the stimulus (Rojas-Chapana et al., 1998).

Zobell (1943) was the first person to patent a model for microbe-mineral attachment. The proposed model encompassed two main stages viz: reversible attachment and irreversible adhesion. van Loosdrecht et al. (1990) refined this model and proposed a four-stage cascade of events, as depicted in Figure 2-14: transportation, initial adhesion (largely dependent on hydrophobic forces and isoelectric forces), firm attachment and colonization, resulting in biofilm formation. For microbes to attach to mineral surfaces, they should be transported to mineral surfaces. Amongst these transportation mechanisms are diffusive transport, active movement and connective transport. Regards diffusive transport, small microbes randomly contact mineral surfaces, which involves non-negligible Brownian motion. Active transport is associated with organism motility. Connective transport is due to liquid flow and is faster compared with diffusive transport. Of all the aforementioned transport processes, diffusive transport is the slowest.

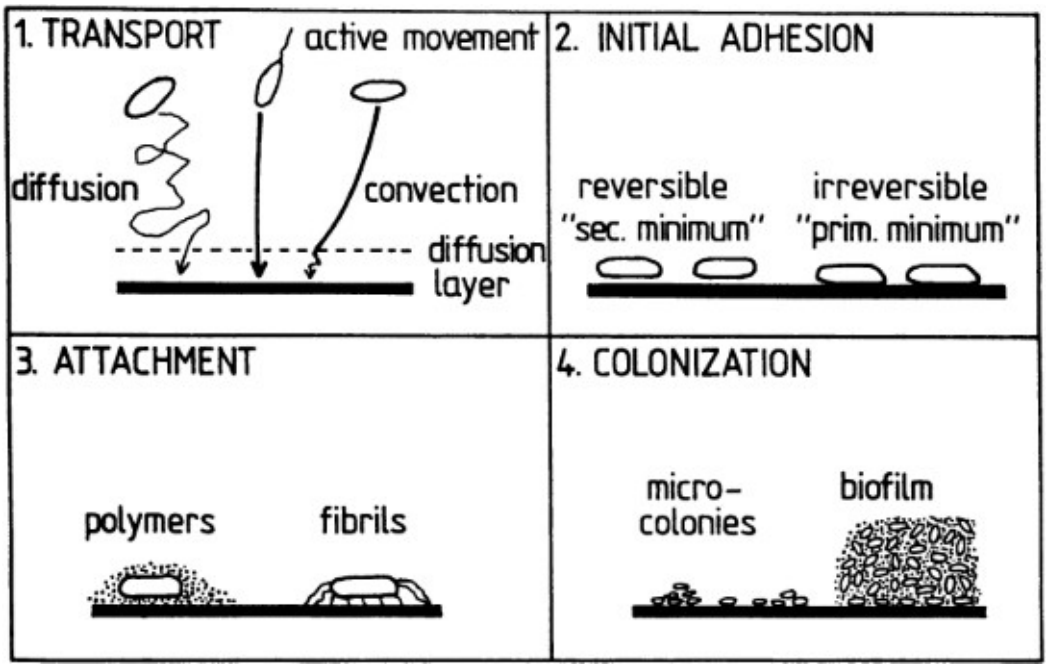

Figure 2-14: *Sequential attachment of microbes to solid surfaces (van Loosdrecht et al., 1990).*

Initial adhesion, which is mainly a physicochemical process (controlled primarily by hydrophobic and electrostatic forces), follows on from microbial transport to solid surfaces. It has been established that once the microorganism is in the vicinity of the solid surfaces, adhesion onto solid surfaces follows. During microbial adhesion onto solid surfaces, the cells become associated with the solid surfaces, either via a chemotactic response or by chance. The microbial-solid surface association is commonly termed "initial" or "primary adhesion" (van Loosdrecht et al., 1990). Primary or initial adhesion processes may

be either reversible or non- reversible. Reversible adhesion results in temporary bacterial adhesion to surfaces as it continues to exhibit Brownian motion. However, bacterial mobility and mild shear can remove them from surfaces. Irreversible adhesion is not accompanied by Brownian motion and the cells cannot be removed from the solid surfaces unless there is strong shear (Zobell, 1943;van Loosdrecht et al., 1990).

Following initial attachment, firm attachment occurs. Upon deposition of bacterial cells onto solid surfaces, strong links are formed between the solid surfaces and the cells, which is mainly facilitated by special structures such as fibrils and polymers. Further, polysaccharides have been shown to play a crucial role in surface film development. Colonization is the final step which results in formation of biofilm (van Loosdrecht et al., 1990).

Yee et al. (2000) demonstrated that the extent and rate of attachment are largely dependent on the surface of both the mineral and the microbe which, in turn, is affected by solution chemistry, mineral composition, and temperature, among others. High levels of microbial cell attachment onto pyrite and chalcopyrite surfaces have been observed, suggesting an affinity of the microorganism (*Metallosphaera hakonensis*) for attachment to sulphide minerals (Bromfield et al., 2011). Using particle-bubble attachment studies, Liu et al. (2013c) showed that chalcopyrite surface hydrophobicity decreased with an increase in concentration of *E. coli* cells present on the mineral surface. Interestingly, as per Liu et al. (2013c), *E. coli* cells compete with the collector for adsorption onto mineral surfaces. Consequently, this may result in *E. coli* cells acting as a barrier for collector adsorption. Furthermore, it has been established that, at elevated concentrations, microbes can desorb collector from mineral surfaces (Liu et al., 2013c). It should be therefore kept in mind that the microbes can act as either collector or depressant, depending on whether they induce hydrophilicity or hydrophobicity on the mineral surfaces (Rao et al., 2010). The microbial properties that induce hydrophobicity, is species specific and, in the case of a mixed microbial community, the most abundant species would elicit the greatest effect.

2.6.7 Collector biodegradation

Different microbial communities inhabit waters flowing from the concentrator to the return water dams (via the tailings storage facility) and the reuse waters within the concentrator and these waters contain residual toxic xanthate collectors as well as their decomposition products. The microorganisms within these waters are able to adapt and develop a natural tolerance towards xanthate toxicity. Based on empirical studies, Chen et al. (2011) and Natarajan and Praksan (2013) reported that microbes can degrade xanthate. Very little scientific evidence is available regarding the residual xanthate concentrations present in discharged effluents. According to Fuerstenau and Han (2003), approximately 0.12 g/L of residual xanthate have been reported to be destroyed in a biodegradable fashion. Some

microbes, for example *P. polymyxa,* have been reported to facilitate biodegradation of some organic collectors (Deo and Natarajan, 1998). Remarkably, biodegradation of some alkyl xanthates has been shown to be facilitated by activated sludge from wastewater treatment plants. Oxidation and dissociation of xanthates result in the production of CS_2 and alcohols which, in turn, are toxic to bacterial growth and activity at high concentrations. Accumulation of CS_2 results in an increase in permeability of the microbial lipid membrane (Dopson et al., 2006). At xanthate (isobutyl-, amyl- and ethyl) concentrations of around 10 mM, 90% inhibition of microbial growth (*A. ferrooxidans*) has been observed, while isopropyl xanthate did not affect the specific growth rate but resulted in a four-fold increase in the lag phase (Loon and Madgwick, 1995). Xanthate-treated microbial cells have been found to delay the attainment of stationary phase (Chockalingam et al., 2003; Natarajan and Prakasan, 2013).

While investigating the effects of collectors on microbes, Deo and Natarajan (1998) conducted bacterial (*P. polymyxa*) growth studies using Bromfield medium and replaced sucrose, a carbon source, with the organic collectors dodecyl ammonium acetate, isododecyl oxypropyl amine, sodium isopropyl xanthate and sodium oleate. It is of paramount importance to note that the normally employed Bromfield medium for microbial growth contains about 0.5 to 2% sucrose as a carbon source for efficient growth of *P. polymyxa* (Deo and Natarajan, 1998). Deo and Natarajan (1998) reasoned that, in the absence of sucrose bacterial growth will be limited. This is because the generation of exopolysaccharides and organic acids is directly related to the amount of sucrose present. However, based on experimental results, Deo and Natarajan (1998) reported that the bacterial cells seem able to utilize the collectors as a carbon source and grow efficiently. Therefore, from their findings, it became apparent that the bacterial cells could effectively grow whilst utilizing organic collectors as a carbon source in place of sucrose. In a similar study, Natarajan and Prakasan (2013) proposed a possible mechanism by which *P. polymyxa* could be grown using xanthate (SIPX) as a carbon source for metabolism. In their mechanism, they proposed that bacterial cells convert xanthate into an alcohol, which can safely enter the tricarboxylic acid cycle and satisfy the carbon requirements (Natarajan and Prakasan, 2013).

Interestingly, it has also been reported that single species bacterial isolates belonging to the *Pseudomonas* and *Methylobacterium* genera can utilize xanthates as a sole carbon source (Okibe and Johnson, 2002 and Natarajan and Prakasan, 2013). Using activated sludge (containing about 80% domestic sewage) as an inoculum, Chen et al. (2011) observed that sodium diethyldithiocarbonate and ammonium butyl dithiophosphate can be efficiently biodegraded, with ethylthiocarbonate being an exception. Apart from utilizing collectors as a carbon source, certain microbes have been found to adsorb collectors onto their cell surfaces. A study by Deo and Natarajan (1998c), in which they investigated the interaction between an amine collector and *P. polymyxa*, reported that the amine interacted bacterial

cells exhibited higher hydrophobicity compared to *P. polymyxa* cultivated in collector free medium, as shown by enhanced *P. polymyxa* floatability (interacted bacterial cells).

2.7 Problem statement

Froth flotation, a highly water consuming operation, is affected by the quality of the water utilised for operations. Mineral processing industries have started recycling and/or reusing water within their operations in a quest to reduce freshwater intake and mine water discharge. However, literature has highlighted that this results in the accumulation of organics and inorganics within the water, which may compromise water chemistry and thus flotation performance. Although the effects of microbes on microbial dependent operations such as bioflotation, bioleaching etc. are well researched and mostly understood, the effects of naturally occurring microbes, present in poorly remediated waters, which may be recirculated within flotation operations, are seldom reported and tend to be overlooked. Many studies have been devoted to elucidating the effects of the inorganics, particularly ions, on flotation; the effects of organics, particularly the microbial load, remains little explored. Studies to date have reported the detrimental effects of some microbial species on flotation performance, however these studies have used model bacterial species and, in most cases, pure mineral. Moreover, the mechanism by which the microbial cells could affect flotation is not understood. This is further compounded by the fact that the mechanism by which a particular entity affects flotation depends on the ore and reagent suite, often particular to a specific concentrator. Motivated by this gap in the literature, this study aims to tackle the underlying effects of organics, particularly microbes, in recycled water on the flotation performance of a Cu-Ni PGM bearing ore.

2.8 Objectives

The overarching aim of this work is to investigate the underlying effects of the microbial load in recycled flotation waters on the flotation performance of a PGM bearing ore.

Specific Objectives include:

- To establish methods for the measurement of microbial activity in an ore slurry.
- To investigate the attachment of microbes onto mineral surfaces.
- To investigate the effect of SIBX and SEX on microbial growth of a mixed consortium harvested from a platinum-bearing ore concentrator.
- To investigate the effects of microbes on flotation performance on overall metallurgical performance of a Cu-Ni PGM bearing ore.

2.9 Key questions

- Can microbial quantification be performed accurately in the presence of an ore slurry?

- Do microbes originating from a PGM concentrator circuit attach onto ore particles?
- Do microbes consume collectors as a source of energy (carbon source) and at what concentrations are collectors toxic to the microbial community studied?
- Do microbes affect water and solids recoveries, metal recovery and metal grade of a Cu-Ni PGM bearing ore concentrate?

2.10 Hypotheses

Based on the previously stated keys questions, the following was hypothesised:

- Collectors such as SEX and SIBX contain a carbon backbone structure and will act as a carbon source for microbes harvested from the concentrator circuit, resulting in an increase in microbial concentration and a decrease in collector concentration.
- Microbes adversely affect flotation performance (measured by metal recovery and grade) by attaching to mineral surfaces, resulting in a change to the mineral surface charge and/or hindering collector adsorption on mineral surfaces.

CHAPTER 3

3 EXPERIMENTAL DETAILS

In this chapter, the experimental details used to conduct the experiments applied, to investigate the key questions of this study, are provided. The general methods and analyses are presented in Section 3.1 while the experimental procedures are presented in Section 3.2.

3.1 General methods and analyses

3.1.1 Chemicals and solution preparation

The chemicals and reagents that were used for media and solution preparation were purchased from Merck chemicals, unless otherwise stated. For all the solutions, where applicable, autoclaving was carried out at 121^{o}C for 21 mins for sterilisation purposes.

To ensure sterility, microbial inoculation was performed in biosafety cabinets and the flasks were flamed and covered with cotton wool and foil paper. The microbial cultures were regularly checked using an Olympus BX-40 microscope

3.1.2 Media and solution preparation

3.1.2.1 Preparation of 1x Phosphate Buffered Saline (PBS)

A 1x PBS solution was prepared by dissolving 8 g of NaCl, 0.2 g of KCl, 1.44 g of $Na_2HPO_4.2H_2O$ and 0.24 g of KH_2PO_4 in 800 ml of deionized water. NaOH or HCl were used to adjust the pH to 7.4. Deionized water was used to achieve a final volume of 1 L.

3.1.2.2 Preparation of 1000x Trace elements solution

A 1000x trace elements solution was prepared by dissolving 10 g of $ZnSO_4.7H_2O$; 10 g of $FeSO_4.7H_2O$, 1 g of $CuSO_4.5H_2O$, 1 g of $MnSO_4.4H_2O$, 1 g of $CoSO_4.7H_2O$, 0.5 g of $Cr_2(SO_4)_3.15H_2O$, 0.6 g of H_3BO_3, 0.5 g of $Na_2MoO_4.2H_2O$, 1 g of $NiSO_4.6H_2O$, 1 g of $Na_2SeO_4.10H_2O$, and 0.1 g of $Na_2WO_4.2H_2O$ in 1 L deionized water. The solution was filter sterilized through a 0.22 µm syringe filter and stored at 4^{o}C.

3.1.2.3 Preparation of 1x Autotrophic Basal Salt (ABS) medium

1x ABS solution was prepared by dissolving 0.15 g of $(NH_4)_2SO_4$, 0.15 g of Na_2SO_4, 0.05 g of KCl, 0.5 g of $MgSO_4.7H_2O$, 0.05 g of KH_2PO_4, 0.014 g of $Ca(NO_3)_2.4H_2O$ in 800 mL of deionised water placed in a 1 L volumetric flask. The pH of the solution was adjusted to 7.4 with 96% H_2SO_4 or NaOH using a magnetic stirrer bar and plate to facilitate mixing. The salts were added one at a time to allow complete dissolution before the next salt was added.1 mL of trace element Solution (Section 3.1.2.2) and the solution was made up to 1000 mL.

3.1.2.4 Solid media for bacterial enumeration by spread plating

Two different agar plates were used in this study, commercial nutrient agar and ABS agar plates. Nutrient agar plates were prepared by adding 1.5% (w/v) bacteriological agar to the nutrient broth medium preceding autoclaving. For the preparation of ABS plates, 1.5% bacteriological agar was added to 1x ABS medium (Section 3.1.2.3) before autoclaving. After autoclaving, the agar solutions were poured onto plates in a biosafety cabinet to avoid the introduction of contaminants before solidifying. The resultant plates were left to set and dry before being covered and incubated at 30°C. Post the solidification process, the plates were spread-plated with the samples after appropriate serial dilutions using a spreader. The plates were incubated at 30°C following spread plating.

3.1.2.5 Growth media for flotation water microbes

Growth media was prepared by adding autoclaved 1x PBS solution (Section 3.1.2.1) and 0.1% yeast extract solution together with a sterilized 1x trace elements solution (Section 3.1.2.2), in a biosafety cabinet, flaming the neck of the Erlenmeyer flask to avoid contamination. The flasks were covered with cotton wool and foil paper to avoid contamination.

3.1.2.6 Preparation of synthetic plant water

Synthetic plant water (SPW) was prepared by adding different concentrations of inorganic salts to deionized water to a working volume of 40 L as per Wiese et al. (2005). The final concentrations of the inorganic ions present in 1 SPW are shown in Table 3-1. Wiese et al (2005) designed synthetic plant water at UCT with a total dissolved solids of 1023 mg/L, often referred to as 1 SPW, in order to mimic typical water quality at a PGM concentrator in South Africa. All the salts used were of analytical grade (> 95%) and acquired from Merck in powered form.

Table 3-1: *Final concentrations of each ion present in 1 SPW.*

Ion	Ca^{2+}	Mg^{2+}	Na^{+}	Cl^{-}	SO_4^{2-}	NO_3^{-}	NO_3^{-}	CO_3^{2-}
Concentration (ppm)	80	70	153	287	240	135	176	17

The ionic strength of the 1x PBS and 1 SPW was determined using Equation 3-1.

$$I = \frac{1}{2} \sum_{i}^{n} Z^2 iCi \qquad \text{Equation 3-1}$$

Where: I – is the ionic strength

Z – charge of the ion *i*

C – molarity of ion *i*

i – specific ion at which the ionic strength is evaluatedn – number of ions present in a system

3.1.3 Flotation reagent preparation

3.1.3.1 Collector

Sodium Ethyl Xanthate (SEX) was used as collector for sulphide mineral flotation. 1% (w/v) of SEX solution was prepared by dissolving 1 g of SEX in 100 ml of deionized water prior to flotation experiments. 2 ml of a 1% solution was added to the slurry to achieve a concentration of 100 g/t. Due to its proneness for decomposition, fresh solution was prepared every time prior to flotation experiments. The remaining SEX solution was discarded as per the laboratory safety rules. The collector was supplied in powder form by AECI Mining Chemicals at a purity of 99%. Sodium Isobutyl Xanthate (SIBX) (AECI mining chemicals at 97% purity) was also prepared in the same way as SEX for xanthate analysis in the presence of microbes. Different (SEX and SIBX) concentrations were prepared from 1% stock solutions for xanthate analysis in the presence of microbes (Section 3.2.4 - 3.2.7).

3.1.3.2 Frother

DOW 200 was used for all flotation experiments. The solution was stored in the refrigerator at approximately 5^{o}C. 8 uL of DOW 200 was added to the cell to achieve a final dosage of 40 g/t. The frother was supplied in a liquid form by Betachem (Pty) Ltd South Africa.

Table 3-2: *The flotation reagents type and its chemical composition.*

Reagent	Type of flotation reagent	Chemical formula	Molecular weight (g.mol-1)	Purity (%)	Dosages (g/t)
Collector	SEX	$C_3H_5NaOS_2$	144	99.9	100
Frother	DOW 200	$C_{10}H_{21}O_3$	264	99.9	40

3.1.4 Enrichment of microbes from concentrator for laboratory experiments.

Mine water from a South African platinum concentrator was collected. The mine water was decanted to remove the liquid phase and the resultant slurries were weighed. 0.4% (v/v) Tween 20 solution (a surfactant) (double the slurry weight), which was prepared in 1x PBS to detach attached microbes, was added to the slurry and the resultant slurry was agitated. The suspension was allowed to settle for approximately 30 mins. After 30 mins, the resultant supernatant (Tween wash) was combined with the decanted liquid fraction, removed prior to centrifugation using a Beckman Avanti-J-25 centrifuge at 2 831 x*g* using a JA-10 rotor for 10 mins to pellet the cells. The pellet was washed with 1x PBS to remove Tween 20. The resultant suspension was further centrifuged as outlined above to pellet the cells.

Subsequently, the resultant pellet was suspended in an autoclaved 1x PBS solution with 0.1%yeast extract, as a nitrogen and carbon source, and 1x trace element solution to a working volume of 100 ml in a 500 ml Erlenmeyer flask. The cultures were kept at 37^{o}C whilst being agitated at 140 rpm on an orbital shaker. Sub-culturing was conducted fortnightly by inoculating the growth media prepared, as described in Section 3.1.2.5**,** to maintain an active microbial stock culture.

3.1.5 Microbial cell growth measurements

3.1.5.1 Optical density for microbial growth measurements

For each experiment carried out, an overnight culture (Section 3.1.2.5) was prepared from the microbial stock culture (Section 3.1.4). To a 500 ml Erlenmeyer flask, 10% of inoculum was added to autoclaved media consisting of 1x PBS, 0.1% yeast extract and 1x trace elements solution, in a biological safety cabinet/ biosafety cabinet to avoid contamination. The microbial cell culture was cultivated at 37^{o}C at 140 rpm for 12-14 hours (until stationary phase was reached) in a rotary shaker. Microbial growth was monitored by measuring optical density (OD) at 600 nm using a Genesys 10s UV-Vis spectrophotometer, Thermo Scientific. Microbial growth was monitored hourly until the stationary phase of the growth was reached.

For all the tests performed, the OD values were correlated to cell dry weight (g\L) and cell concentrations (cells/ml). Different microbial dilutions were prepared in 1x PBS and their absorbances were determined at 600 nm using a Genesys 10s UV-Vis spectrophotometer, Thermo Scientific. Microbial enumeration was then carried out using microscopy by direct counting of each concentration prepared together with cell dry weight. Cell dry weight was determined by filtering each resultant solution through dry reweighed 0.2 µm filter papers. The filter papers with wet biomass were then oven dried in an 80^{o}C oven for over 24 hours. Cell biomass by definition, is the mass of living organisms (microorganisms) (Rosillo-Calle, 2006), After 24 hours, the filter papers were weighed, and the cell dry weight was determined upon subtracting the mass of filter paper alone. A standard curve (see Figure A-1) of cell dry weight vs OD at 600 nm vs cell concentration was generated, from which cell dry weight and cell concentration were read off from OD values for the tests conducted.

3.1.5.2 Fluorescein Diacetate (FDA) assay

A Fluorescein Diacetate (FDA) assay was used to determine microbial cell activity. 2 mg/ml of FDA was prepared by dissolving 20 mg of FDA in 10 ml of analytical grade acetone and this served as a stock solution. Owing to the fact that FDA is light and temperature sensitive, the stock solution was wrapped with foil paper and stored at -20^{o}C to prevent degradation. 62.5 µL of the stock solution was dissolved in 437.5 µL of PBS at pH 7.4 to a final volume of 500 µl with a concentration of 0.25 mg/ml. 12 µL of the solution was then added to 200 µL sample to achieve a final FDA concentration of 0.015 mg/ml

or15 µg/ml. The sample was mixed by vortexing and then incubated at 37°C for 5 mins. Following the five min incubation, the reaction was stopped with the addition of 300 µL chloroform. The sample was vortexed to effectively stop the reaction and centrifuged at 16 000 x*g* for 10 mins using a Heraeus biofuge Pico centrifuge. Subsequently, 100 µL of the supernatant was added to 100 µL of 1x PBS in 0.5 ml tubes and read at raw blue (excitation filters at 495 nm and emission filters at 510-580 nm) using a Quantus fluorometer. It should be noted that in some instances FDA was conducted on liquid fractions where possible and on slurries, however the solid fractions were subjected to centrifugation to obtain liquid fractions as the fluorometer only allows the measurement of liquid in the absence of particles.

3.1.5.3 Cell enumeration using a microscopy

Microbial enumeration was conducted microscopically using a Thoma counting chamber and an Olympus BX-40 epifluorescent microscope. Cells were observed at 1000x magnification using oil immersion and phase contrast. The cell concentration (in cells/ml) was then determined using the following equation.

$$\text{Volume of small square} = \text{depth x area} = 0.02 \text{ x } (0.05\text{x } 0.05)/1000 = 5\text{x}10^{-8} \text{ cm}^3 \quad \text{Equation 3-2}$$

$$\text{Cell conc} = \text{dilution factor x } \frac{\text{Cell count x N/n}}{\text{Volume of small square x total number of aquares}} \quad \text{Equation 3-3}$$

Where N = number of big squares (16)

n = number of squares counted (4)

3.1.5.4 Viable cell enumeration by serial dilution and spread plating

The samples were serially diluted using ten-fold dilutions until the samples were highly diluted. This was done to ensure that the colony forming units (CFU), which would grow on the plates, were in countable range. 100 µL was of the sample was serially diluted ten-fold five to six times. The dilutions were chosen depending on the expected microbial concentrations, based on the initial microbial concentration. 100 µL of the resultant samples were spread-plated onto the two different plates (Nutrient agar and ABS plates). Subsequently, the inoculated plates were incubated in the 30°C temperature-controlled room. Within 24 hours post inoculation, CFUs were determined by physically counting all the visible colonies present on the plates. It should be noted that only plates that had colonies in the range of 30-300 used. The microbial population during the recycling tests was evaluated using Equation 3-4.

$$\text{CFU/ml} = \frac{\text{Number of colonies x dilution factor}}{\text{Volume of culture plate}} \quad \text{Equation 3-4}$$

3.1.6 Phenol-sulphuric acid assay for carbohydrate analysis

The phenol-sulphuric acid method measures total carbohydrates in a variety of samples, including whole cells. The protocol followed causes complex carbohydrates to be digested into sugar monomers, which are then detected by the creation of an orange-yellow colour (DuBois et al. 1956). A standard curve was generated using set pure glucose concentrations, prepared froma stock solution of 1 g/L prepared in 1x PBS; the concentrations prepared were 50, 100, 150, 200 and 250 mg/L in the presence of 1x PBS. Absorbance values were then correlated to their respective concentrations to generate a standard curve, from which the glucose test assays were read off (see Figure A-2). Glucose is the most at widely used monomeric sugar in instances where the specific monomer sugar present in the cells is not known. For the test assay, the phenol-sulphuric acid assay was carried out in the presence and absence of a fixed collector at concentrations of 60 ppm and a working volume of 50 ml. 200 µL of 5% phenol was added to 200 µL of the sample and vortex.mixed for one min. This was followed by an addition of 1 ml of 99.9% analytical grade sulphuric acid. The resultant suspension was vortex mixed and 200 ml of the resultant samplewas added to a 96 microplate and read at 490 nm. It should be noted that the phenol-sulphuric acid assay was only carried out in the presence of two microbial concentrations: 10^8 and 10^9 cells/ml. In the presence of 10^6 and 10^7 cells/ml, the sulphuric assay was not considered owing to the fact that there was no response detected at high microbial concentrations (10^8 and10^9 cells/ml.

3.1.7 Techniques for flotation experiments

3.1.7.1 Ore preparation

The Cu-Ni PGE Platreef ore, which originated from the Bushveld Igneous Complex (BIC), was procured from a Platinum mineral concentrator in South Africa. The ore was reduced to -1 mm particle size using a TM Engineering Terminator jaw crusher (a particle size suitable for grinding). The ore was then homogenised and split using the rotary splitter to equal 1.0 kg portions prior to milling and sealed in plastic bags.

3.1.7.2 Laboratory scale milling

For all experiments conducted, 500 ml of 1 SPW was added to 1 kg of Platreef ore to achieve a 66 weight % solids during milling. A UCT-CMR stainless-steel laboratory rod mill was charged with 20 stainless steel rods of varying diameters (6 x 16 mm, 8 x 20 mm and 6 x25 mm). Prior to grinding, a milling curve was constructed (Figure 3-1). Based on the results from the milling curve, milling was carried out for 23 mins to achieve a particle size distribution of 80% passing 75 µm. The mill drive was maintained at 47 rpm for all experiments. The slurry was pressure filtered and the resultant “cake” was divided into six equal slices of approximately 200 g wet weight, that equates to a dry weight of 184 g. To ensure

homogeneity within the ore used, opposite slices of the resultant wet ore were combined and weighed (200 g wet weight).

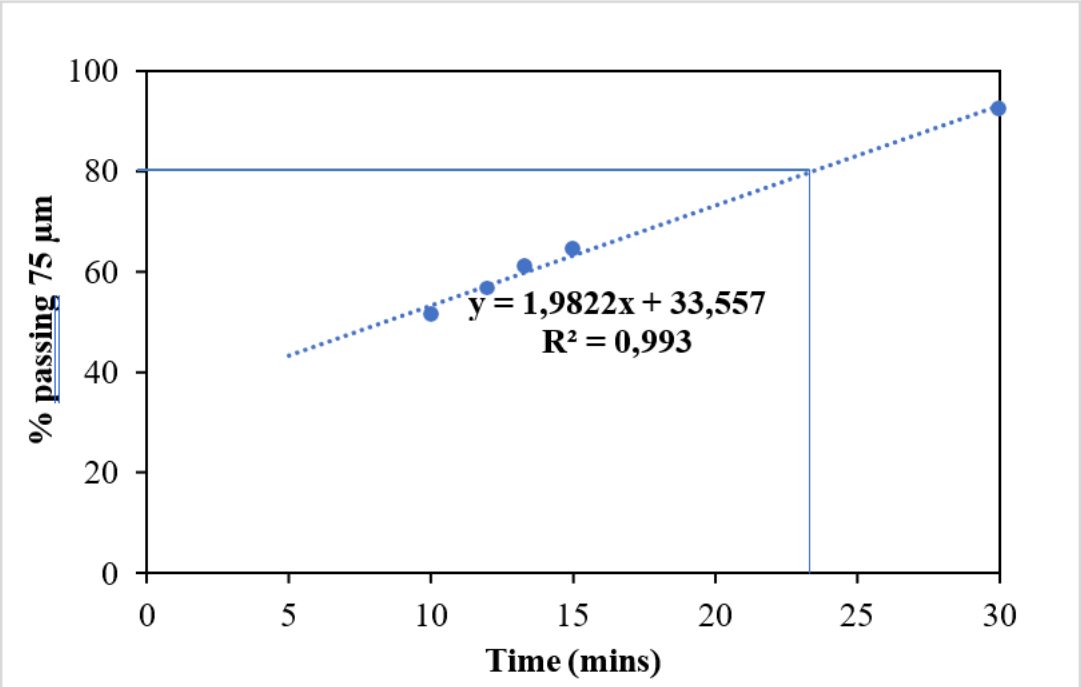

Figure 3-1: *Milling curve for the Platreef ore at different time intervals using a UCT rod mill*

3.1.7.3 Batch flotation

A 500 ml Chamic baby flotation cell (shown in Figure 3-2) was used for all the flotation experiments. Approximately 200 g of wet weight sliced ore was used for each of the flotation experiments carried out to achieve a solids loading of approximately 35% in the 500 ml flotation cell.

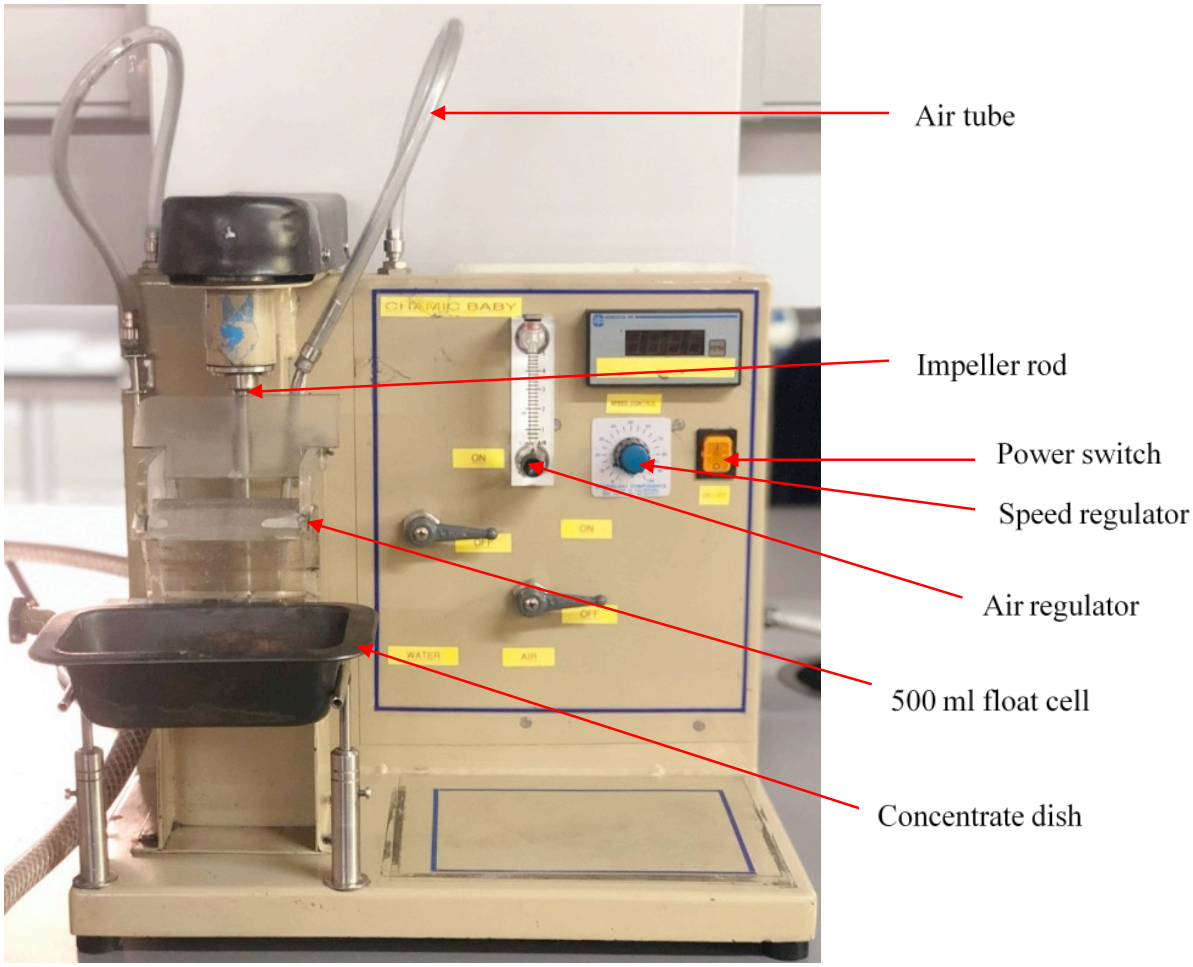

Figure 3-2: *Chamic baby laboratory flotation cell.*

3.2 Experimental procedures

Unless stated otherwise all biochemical experiments were performed in triplicate. As such, errors bars in the results and discussion sections, show the standard error between the triplicate or biological repeats and standard deviation for replicate tests.

3.2.1 Microbial cell harvesting for experiments performed with harvested cells

Microbial cells were harvested 12-14 hours post inoculation (after reaching stationary phase), by centrifugation in 500 ml (80% full) centrifuge bottles at 10 000 rpm using a JA-10 rotor for. 10 mins at 25^{o}C, using a Beckman Avanti-J-25 centrifuge. The resultant harvested cells were washed once with 1x PBS and resuspended in 1x PBS for subsequent experiments.

3.2.2 Shake flask adsorption studies

Adsorption studies were carried out to investigate the adhesion of microbial surfaces to ore particle surfaces. The adsorption studies were carried in 250 ml Erlenmeyer flask. 5 g of finely ground ore (80% passing 75 µm) was added to 100 ml of 1x PBS to achieve 5% solids loading. Microbial cells were harvested as per Section 3.2.1. A pre-determined microbial cell count of 1.36 x 10^{8} cells/ml was added

to the suspension. The samples were incubated at 37°C on a rotary shaker at 140 rpm. Sampling was performed every 30 mins for 2 hours, by removing 2 ml from the flask. The samples taken from the designated times were centrifuged using an Eppendorf Mini Spin Plus centrifuge at 800 xg for 30 secs to sediment ore particles. Centrifugation was done at the lowest speed in order to avoid pelleting microbial cells. The microbial cells in the supernatant were counted using a Thoma counting chamber after appropriate dilutions. The concentrations of adsorbed cells were calculated as the difference between initial and residual microbial concentrations as described by Africa, (2009). The percentage attachment was inferred using Equation 3.5. Direct cell counts were carried out in parallel with FDA assays as described in Section 3.1.5.2.

$$\text{Percentage attachment} = \frac{Co - C}{Co} \qquad \text{Equation 3-5}$$

Where:

Co = inoculum total cell number

C = total residual microbial cells

3.2.3 Microbial growth in the presence and absence of finely ground ore

To quantify the effect of ore particles on microbial cell growth, microbial cells were cultured in the presence and absence of ore. For experiments in the absence of ore, microbial growth was carried out in 500 ml Erlenmeyer flask to a working volume of 100 ml, as outlined in Section 3.1.5. 5 g of finely ground ore was added to 500 ml flasks with a total volume of 100 ml, to achieve 5% solids. 10% of inoculum was added together with 1x trace element solution. The microbial growth was then monitored by OD at 600 nm and FDA assay. Sampling was conducted hourly until stationary phase was reached (12-14 hours). It should be noted that for the experiment in the presence of finely ground ore, microbial growth was only monitored by FDA assay since mineral particles interfere with OD.

3.2.4 Microbial growth in the presence of xanthate collectors

The effects of the xanthate collectors, sodium ethyl xanthate (SEX) and sodium isobutyl xanthate (SIBX), the most commonly used xanthates in sulphide mineral beneficiation, on microbial growth were investigated.

In all the experiments, four different collector concentrations were added to the culture: 60 ppm, 120 ppm, 240 ppm and 480 ppm, to a working volume of 50 ml in 250 ml Erlenmeyer flask. A baseline experiment (the control) was carried out without the addition of collectors (in the presence of microbes alone). As xanthate decomposes over time, another control experiment was conducted in the absence of

microbes (in the presence of xanthate alone). For all the microbial experiments conducted, 10% of an active inoculum from the enrichment culture described in Section 3.1.4 was added to autoclaved media and collector solutions. The flasks were cultivated on a rotary shaker agitated at 140 rpm at 37°C. Samples (2 ml) were taken for analysis every one or two hours in the biosafety cabinet to avoid contamination. The microbial cell growth was monitored by reading absorbance at 600 nm, using plastic cuvettes in a Genesys 10s UV-Vis Spectrophotometer. For xanthates analysis, a dilution of 20x was performed in a 2 ml Eppendorf tube to a working volume of 1 ml. The xanthate analysis was conducted to measure residual xanthate in solution over time. The samples were vortex mixed using a Vortex-Genie, Lasec for 10 secs and centrifugedat 16 000 x*g* using a Heraeus biofuge Pico centrifuge for two mins to remove cells which maybe present in solution. 200 µL of each of the samples was pipetted out into a 96 well plate and read at 301 nm using a BMG omega UV-Vis Spectrophotometer. A wavelength of 301 nm is normally used to measure xanthates concentrations as xanthate ions exhibit maximum absorption at this wavelength (Jones and Woodcock, 1973). To determine the concentrations in ppm for all xanthate experiments, different xanthate concentrations were prepared before the test assays: 60, 120, 240, 480, 960 ppm (see Appendix A.3 and A.4) and their respective absorbances were determined. A standard curve was then generated. The absorbances for all xanthate analysis experiments were then read off from the standard curve in ppm. This was completed for all xanthate analysis experiments (Section 3.2.5, 3.2.6 and 3.2.7) carried out in this study. The K values (gradient) and volumetric removal rate were also determined for Section 3.2.5, 3.2.6 and 3.2.7. The gradient was determined by linearising xanthate concentration (ppm) and plotting a graph of ln concentration vs time. Consequently, the gradient (K) was graphically determined. It should be noted that only the linear portion was considered. The volumetric removal rate was inferred using Equation 3-6:

$$\text{Volumetric removal rate} = \frac{C1 - C0}{T1 - T0} \qquad \text{Equation 3-6}$$

Where C1 is xanthate final concentration and C0 is the initial xanthate concentration. T1 and T0 corresponds to the time at C1 and C2, respectively.

Xanthate percentage removal was inferred upon; Equation 3-7.

$$\text{Xanthate removal} = \frac{Co - C}{Co} \text{x } 100 \qquad \text{Equation 3-7}$$

Where Co is the initial xanthate concentration in parts per million (ppm) and C is the residual xanthate concentration in ppm at any given time.

3.2.5 Analysis of harvested microbial cells in presence of xanthate

Microbial cells were prepared as described in Section 3.1.5. To a 5000 ml Erlenmeyer flask, 100 ml of an active microbial cell community was cultured as described in Section in 3.1.5 to a working volume of 1000 ml. The microbial cell culture was incubated for 12-14 hours at 37^{o}C on a rotary shaker at 140 rpm. 12-14 hours post inoculation, microbial cells were harvested in a Beckman centrifuge Avanti-J-25 centrifuge at 10 000 rpm using a JA-10 rotor for 10 mins followed by 1x PBS washing. Subsequently, the microbial cells were then re- suspended in 1x PBS for subsequent experiments. Different microbial concentrations (10^6; 10^7;10^8 and 10^9 cells/ml) were prepared to a working final volume of 50 ml in 250 ml Erlenmeyer flasks in the absence and presence of xanthate. The experiment, which was carried in the absence of xanthate (presence of microbes alone), served as a control for the phenol sulphuric acid assay and was compared to that in the presence of both xanthate and microbes. Since xanthate is likely to decompose over time, another control experiment was prepared at a xanthate concentration of 60 ppm (in the absence of microbial cells). All the flasks were incubated at 140 rpm at 37^{o}C on a rotary shaker. The samples were taken at designated times for analysis. Residual xanthate was checked by a multi well plate using a BMG omega Spectrophotometer at 301 nm. For the microbial supplemented studies, the samples were pelleted in 2 ml Eppendorf tubes at 16 000 x*g* using a Heraeus biofuge Pico centrifuge for two mins to remove microbial cells. Xanthate analysis was performed in parallel with OD to monitor bacterial growth using Genesys 10s UV-Vis spectrophotometer at 600 nm. Concurrently, polysaccharides produced by the microbial cells were monitored by carbohydrate assay using the phenol-sulphuric acid assay.

3.2.6 Analysis of SIBX in the presence of finely ground ore

This experiment was conducted to determine the effect of microbes on collector adsorption in a system where both the mineral of interest and microbes coexist. The microbial response in the presence of xanthate was conducted in the presence of the previously stated collector concentration of 60 ppm. The microbial cells were cultured as per Section 3.1.5 and centrifuged as previously stated in Section 3.2.1. To a 50 mL 1x PBS working solution, approximately 17.8 g of finely ground ore was added to achieve a solids density of 30%. Four different tests were set up. The experiments were carried out in such a way that the ore particles and microbes were added to SIBX separately and to a combination of both microbes and ore particles. Also, due to SIBX decomposition over time, a baseline experiment in the presence of SIBX alone was conducted. In all the microbe added systems, a microbial concentration of 10^8 cells/ml was used. This concentration was used as it closely resembles typical microbial concentrations in waters employed for flotation operations on site. Levay et al. (2001) reported that microbial counts in flotation waters can reach as high as 10^9 CFU/ml while Liu et al (2013b) further that the concentration of microbial cells in river water employed for flotation could be as high as 10^8 cells/ml. A concentration of

60 ppm SIBX was used, equating approximately to 60 g/t, the normal collector dosage used on site (this was mathematically determined in this study).

3.2.7 Xanthate analysis in the presence of live and dead microbes

To determine whether dead microbial cells/solid surfaces would still cause removal of xanthate from solution, experiments were conducted in the presence of live and dead microbes (10^9 cells/ml) at a fixed xanthate (SEX and SIBX) concentration (60 ppm). A control was added in which no microbes were contained. The dead microbes were heat killed by autoclaving as per Section 3.1.1. Sampling was performed as previously discussed in other xanthate analysis experiment carried out with harvested microbial cells (Section 3.2.5).

3.2.8 Cell surface characterization

Cell attachment onto mineral surfaces is a physio-chemical process. Thus, this necessitates a full understanding of cell surface properties, which mainly drives the interaction between the solid surfaces and the microbial cells.

3.2.8.1 Hydrophobicity test (Microbial Adhesion To Hydrocarbons - MATH)

The microbial cells were harvested as previously stated (Section 3.2.1) and diluted with1x PBS. Dilutions were done in such a way that OD at 600 nm was between 0.3 and 0.4. 3 mL of the cell suspension was placed in a test tube together with 0.8 ml of hexadecane. The suspension was vortex mixed for one min followed by a 30 min incubation. After 30 mins, the aqueous solution was measured at 600 nm. Adapted from Natarajan and Das, (2003), the formula shown in Equation 3.8 was used to evaluate the microbial cell hydrophobicity. In order to assess reproducibility, the experiments were carried out in triplicate.

$$\text{Hydrophobicity} = \left[1 - \frac{OD\ final}{OD\ initial}\right] \times 100 \qquad \text{Equation 3-8}$$

3.2.9 Microbial load quantification during flotation recycling experiments

Flotation recycling experiments (water and ore reuse) were conducted in the absence and presence of added microbes. Prior to flotation experiments, a contact time of 2-hours between water components and ore particles was allowed. Samples were collected at designated times during the 2-hour incubation and flotation period for microbial load quantification. The microbial load quantification within the mineral slurry was determined using two different microbial quantification methods: namely FDA assay (Section 3.2.9.1) and spread plating (Section 3.2.9.2).

3.2.9.1 FDA assay for microbial quantification during recycling tests

The microbial load was quantified in three different phases: slurry, supernatant and mineral. For the slurry phase, 200 µL of the slurry phase was withdrawn from the pulp in the float cell at designated points (during 2-hour incubation and flotation time). FDA assay was then carried out as stated earlier in Section 3.1.5.2. To analyse the planktonic phase, the slurry was first subjected to centrifugation using an Eppendorf Mini Spin Plus centrifuge for one min at 800 xg (lowest speed on the centrifuge) to remove the mineral particles from the solution. It should be noted that care was taken when centrifugation was conducted in the presence of both ore particles and microbes in order to avoid pelleting the microbial cells. 200 µL of the resultant supernatant was then subjected to FDA assay as described in Section 3.1.5.2. The microbial load which could be present/attached onto the ore particles phase was conducted in such a way that the supernatant was removed, and 100 µL of 1x PBS was added to a 200 mg of the ore particles portion was added. 200 µL ml of the resultant sample was then subjected to FDA as per Section 3.1.5.2.

3.2.9.2 Plating for microbial quantification during the flotation recycling tests

Plating, as previously outlined in Section 3.1.5.3, was carried out in parallel with FDA analysis. The microbial load was determined by plating the supernatant (planktonic phase) onto agar plates. Since a mixed microbial community was used in this study, maybe comprising different microbial communities (autotrophs and heterotrophs), it was necessary to prepare two different agar plates, namely, nutrient agar plates and ABS agar plates.

3.2.10 Batch Flotation procedures

The milled slurry (Section 3.1.7.2) was transferred to the 500 ml Chamic baby cell (Figure 3-2). 1 SPW was added to the float cell to achieve a pulp density of approximately 35%. Air flow rate and impeller speed were kept at 4 L/min and 170 rpm, respectively for all batch flotation work. SEX was added to the slurry and conditioned for two min followed by DOW 200 which was conditioned for one min. The collector and frother dosages were kept constant throughout the study. After conditioning, air was introduced into the cell, and the froth was skimmed off at 15 seconds intervals for 20 mins, generating four concentrates at 2, 6, 12, and 20 mins. A constant froth height of two cm was sustained throughout with the addition of 1 SPW. 20 ml of feed was drawn from the cell using a 20 mL syringe. At the end of the float two 20 ml samples of tails were drawn from the slurry. Water recoveries were recorded using mass balance. Feed, concentrates and tails were filtered and dried in an oven at 80^{o}C for 24 hours. The dry flotation products were then sent for analysis to determine the amount of copper and nickel in the samples. Copper and nickel content analysis was done using a Bruker S4 Explorer X-ray Fluorescence (XRF) Spectrophotometer.

Table 3-3: *Actions involved during flotation at designated time intervals.*

Action	**Time (mins)**
Milling (80% passing 75 mm)	23 mins
Transfer to float cell	
Impeller turned on	
Feed sample (20 ml)	
Collector (SEX 100 g/t)	0
Frother (DOW200 40 g/t)	2
Air turned on	3
C1	5
C2	9
C3	15
C4	23
Air turned off	
Tails samples for assay (2 x 20 ml)	
Impeller turned off	
Empty float cell to final tails	

3.2.11 Flotation recycling experiments in the absence and presence of added microbes

To quantify the effect of microbial load and water recycling on water and solids recoveries, metal recovery and grade, flotation tests were conducted. Two sets of flotation tests were conducted. One set was conducted in the presence of microbes and the other was conducted in their absence. Each set comprised three batches of flotation tests, with only the first batch of tests using finely ground fresh ore (80% -75 microns) with 1 SPW (Section 3.2.10) The first batch of flotation tests was performed in triplicate with the filtrate collected and used in the second batch of flotation tests. The concentrates and tailings from the first flotation test were combined, after filtration, and used as feed for the second batch of flotation tests. Due to limited water and feed, the second batch of flotation tests could only be performed in duplicate. Again, the concentrates and tailings from the second flotation tests were filtered and the filtrates collected and used as water for the third batch of flotation tests, with the concentrates and tailings again being used as feed to the third batch of tests. The limited water and feed meant the batch of flotation tests only comprised a single test. For all the experiments conducted, there was a contact time of two hours between water components and ore, prior to flotation. It should be noted that during laboratory float tests (also known as bench floats, representing rougher flotation on a

concentrator), flotation takes place over a set time - 20 minutes in these experiments. However, on a flotation circuit the contact time between the water components and ore may extend to hours and this is the reason why a two hour contact time was given prior to flotation. The flotation tests were designed in such a way that the first set of tests was performed on.

Two sets of recycling tests were carried out, the first (control) was conducted using only ore and 1 SPW water, while the second was conducted with ore, 1 SPW water and the deliberate addition of microbial cells. Only the first batch of flotation tests were dosed with microbial cells; after that the microbes were assumed to be recycled in the second and third batches of flotation tests. Samples (two ml) for each of the flotation tests were collected every 30 mins for the entire 2-hour incubation period for plating and FDA assay. A simplified schematic representation for all these recycling experiments is depicted in Figure 3-3. The concentrates produced for the flotation experiments were collected at 2, 6, 12 and 20 min respectively, by collecting the froth into a collecting pan every 15 seconds. The cell was drained to collect the tails at the end of each test. Representative samples for the first and second batches of flotation tests, were drawn out using a syringe from each of the concentrates and tails and filtered on filter papers of known mass to determine their wet mass. The dry mass was determined by calculating moisture content of the samples (which was 8.2%). For the third batch of flotation tests (a single test) (absence and presence of microbes) the feed sample, concentrates and tails were filtered on filter papers of known mass and dried in an oven at 80°C for 24 hours. The dried feed, concentrates and tails was weighed for yield determination for all the flotation experiments. For all batches of tests, collector was added prior to the 2-hour incubation period.

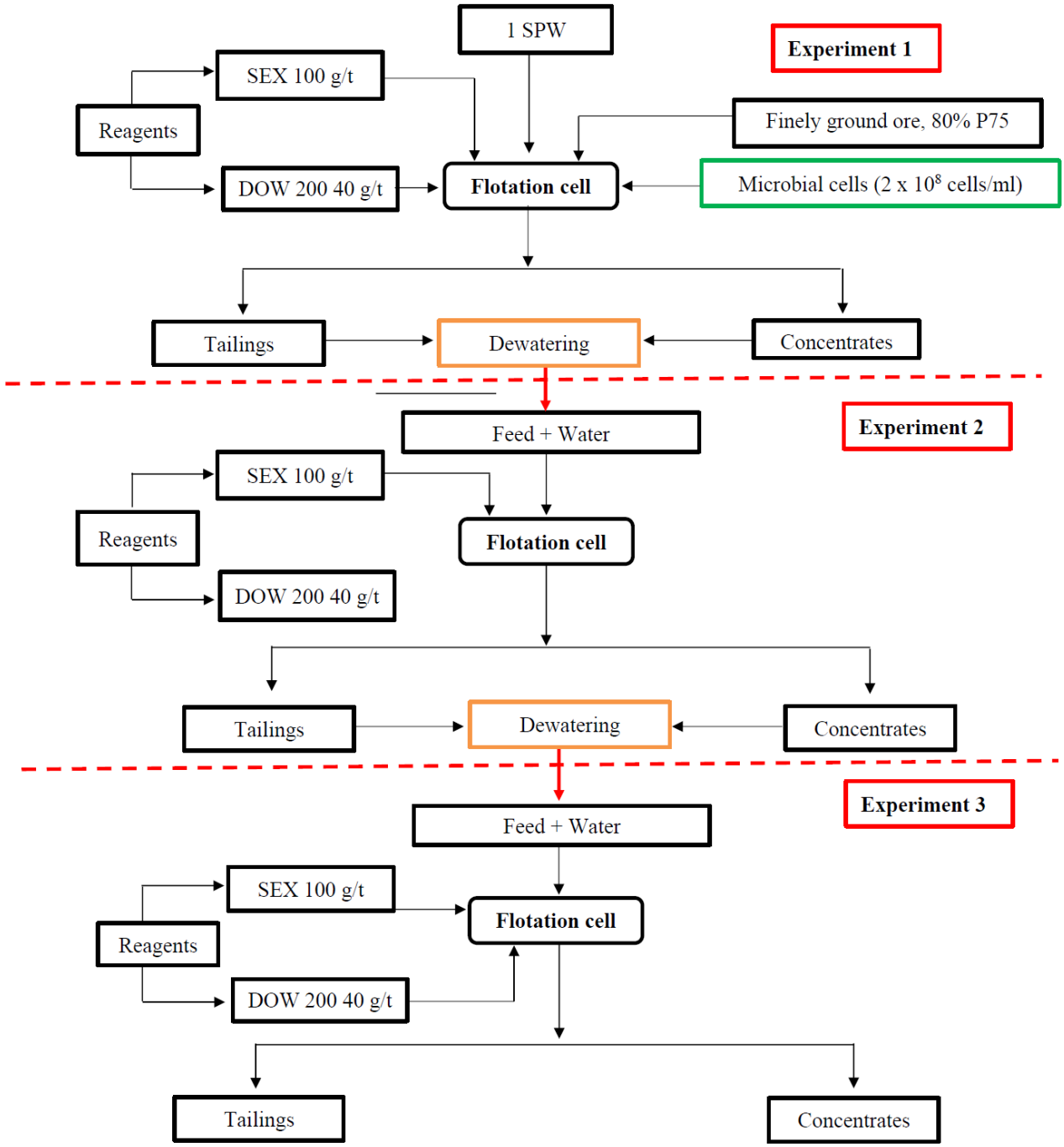

Figure 3-3: *Flow diagram of the recycling experiments in absence and presence of added microbes*

CHAPTER 4

4 DEVELOPMENT AND OPTIMIZATION OF METHODS FOR THE MEASUREMENT OF MICROBIAL LOAD AND ACTIVITY IN AN ORE SLURRY

This chapter presents results for the development of methods and the optimization of experiments conducted prior to their implementation for use in studying the effect of microbes on ore particle flotation, in Chapters 5 and 6.

4.1 Introduction

The usage of microbial laden water, such as treated domestic water, for flotation operations, has become common in the minerals processing space. While this serves to reduce the use of high-quality fresh water, this practice may result in the introduction of organics and microbial cells to flotation circuits, a situation that has been postulated to be detrimental to flotation performance (Slatter et al., 2009; Evdokimova et al., 2012; Liu et al., 2013b, 2013a). The work presented here was performed to better understand the effects of microbes in flotation systems. However, very few techniques have been developed and validated for the quantification of microbial cells in an ore slurry, such as those from flotation circuits. In cases where the effects of microbial load on flotation have been reported, attachment of microbes to ore particles has not been considered. This gap in literature is addressed in the current study.

The fluorescein diacetate (FDA) assay, a simple assay which can be employed to measure metabolically active microbial cell activity and thus provide a representation of active microbial cell concentration, was optimized and used for microbial quantification, in the absence and presence of fine ore particles. FDA assays were complemented with standard microbial enumeration techniques where possible. Since the microbial community originated from mine waters and no culturing conditions existed for this microbial community, it was deemed necessary to investigate a suitable media for cultivation of these microbes at a laboratory scale. Further, the attachment of these microbial cells onto ore particle surfaces was also evaluated.

4.2 Experimental approach

Water and slurry samples were collected from a South African platinum concentrator and microbes were harvested according to the procedure given in Section 3.2.1. The microbial community was cultured in the presence of 1x Phosphate Buffered Saline (PBS) or 1 Synthetic Plant Water (SPW) supplemented with yeast extract (0.1%), as a carbon and nitrogen source, with the addition of a trace element solution, as described in Section 3.1.4. Growth studies were performed in the presence of the two different media (1x PBS and1 SPW), both with and without ore particles (80% passing 75 μm) at 5% solids loading. The attachment of microbes present within the microbial consortium used in this study to the ore surfaces,

was evaluated in shake flasks at a 5% (w/v) solids loading over a two-hour incubation period. The experiments were carried out in 500 ml Erlenmeyer flasks agitated at 140 rpm. Samples were taken at designed time intervals and the residual microbial concentration in solution was evaluated by direct cell counting using microscopy, as described in Section 3.1.5.3. The % attachment was calculated by subtracting residual microbial cells in suspension from the initial inoculum cell number and dividing the resultant answer by the inoculum cell number. The attachment is presented as percentage attachment see Equation 3.5 (Chapter 3). It should be kept in mind that no apparent microbial growth was observed in the presence of 1x PBS alone. Thus, the attachment studies were conducted in the presence of 1x PBS as the background medium without any carbon or nitrogen source supplementation. Hence, it was assumed that no growth would be expected in the 2-hour attachment study.

4.3 Results and Discussion

4.3.1 Microbial biomass measurement in the presence of fine ore particles

In the present study, two methods were used to quantify microbial cells during microbial growth, namely, optical density (OD) measured at 600 nm and fluorescein diacetate (FDA) assay. The former is a spectrophotometric measurement of scattered light facilitated by the presence of particles such as microbial cells and thus measures cell turbidity in suspension. From an OD value, biomass concentration can be inferred by preparing an OD to cell number or biomass concentration standard curve, by using either direct cell counts under light microscopy or by determining the cell dry weight of microbial suspensions at specific OD values.

The FDA assay is a simple and rapid technique that measures microbial cell activity, which is indicative of the metabolically active microbial cell concentration present. FDA hydrolysis is facilitated by several different enzymes, such as proteases, lipases, and esterases, present in all microbial cells. Following FDA hydrolysis by enzymatic reaction, fluorescein, the product of FDA hydrolysis, is released and can be easily measured spectrophotometrically (Lundgren, 1981 and Green et al., 2006) or by using a fluorometer. Fluorescence is more sensitive than absorbance (OD) as it can used to detect lower microbial concentration through detection of fluorescein liberation, allowing the kinetic measurement of enzyme activity. This allows a quantitative measure of metabolically active cells which can be used to infer microbial concentration. For some experiments in this study it was necessary to quantify microbial growth in the presence of an ore slurry. Optical density measurements could not be employed as ore particles increase the scattering of light. Direct cell counting by microscopy is also challenging in the presence of fine ore particles, which may result in increased errors when using this method. The FDA assay, which can measure microbial activity in the presence and absence of an ore slurry, was

therefore optimized and used for microbial cell quantification during microbial cell growth in the presence of ore particles.

A standard curve was drawn up to measure microbial activity, in the presence of ore particles, using the FDA assay, using the relationship between microbial cell concentration and microbial activity. To achieve different microbial cell concentrations, dilutions of an overnight microbial culture were prepared with 1x PBS used as dilutant. Figure 4-1 shows the linear relationship that exists between Relative Fluorescence Units (RFU) and cell concentration. This graph suggests that microbial activity measured by FDA assay is indicative of the metabolically active microbial cell concentration present within a sample. This finding confirms that the FDA assay can be used to determine microbial cell concentration in slurries. To validate the use of the FDA assay to monitor the increase in microbial biomass in the presence of fine ore particles, microbial growth curves were conducted in the presence and absence of ore (Figure 4-2).

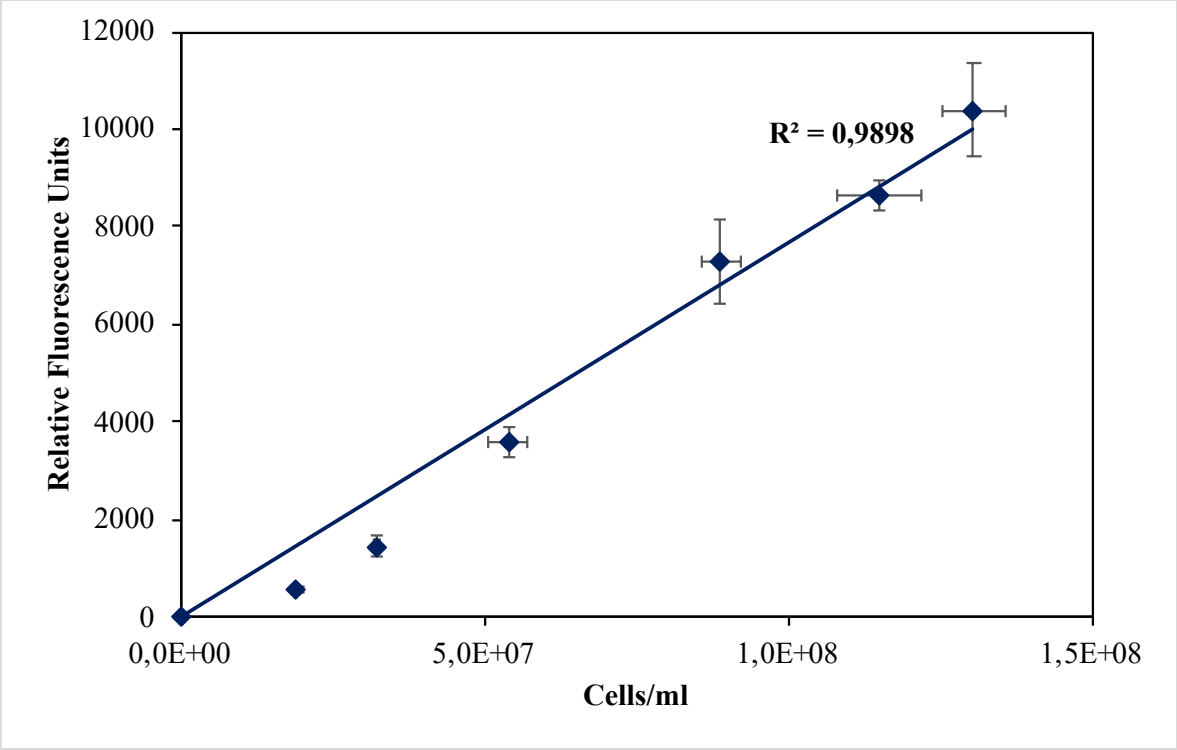

Figure 4-1: *Relative Fluorescence Units (RFU) at different microbial cell concentrations achieved by using 1x PBS as a diluent after a five min incubation time with fluorescein diacetate substrate. The data points represent the average of three technical repeats and error bars show the standard deviation of these.*

Microbial cell growth, in the absence of ore particles, was monitored using OD at 600 nm; for growth, the FDA assay was conducted in parallel on the same samples. The effect of two different media types, PBS and SPW, both supplemented with 0.1% yeast extract and 1x trace elements solution, on the growth

of the microbial consortium, was also tested. Owing to the fact that waters ages/deteriorates over time and as a result the water quality cannot be controlled, it is not common practice/impractical to conduct experiments using process water, and as such synthetic water is generally employed for laboratory flotation tests. The UCT 1 SPW was designed by Wiese et al. (2005) and contains inorganic ions which typically mimic the water quality in PGM concentrators across the Bushveld Igneous Complex in South Africa, while PBS is often used as a physiological buffer to maintain an isotonic environment for microbial cells. For these reasons, the two media were separately employed to culture the microbial cells at a laboratory scale and microbial response towards the studied microbial cells was recorded. The RFU achieved by FDA assay and microbial cell concentration over time is shown in Figure 4-2. OD and cell concentration as a function of time is shown in Figure 4-3.

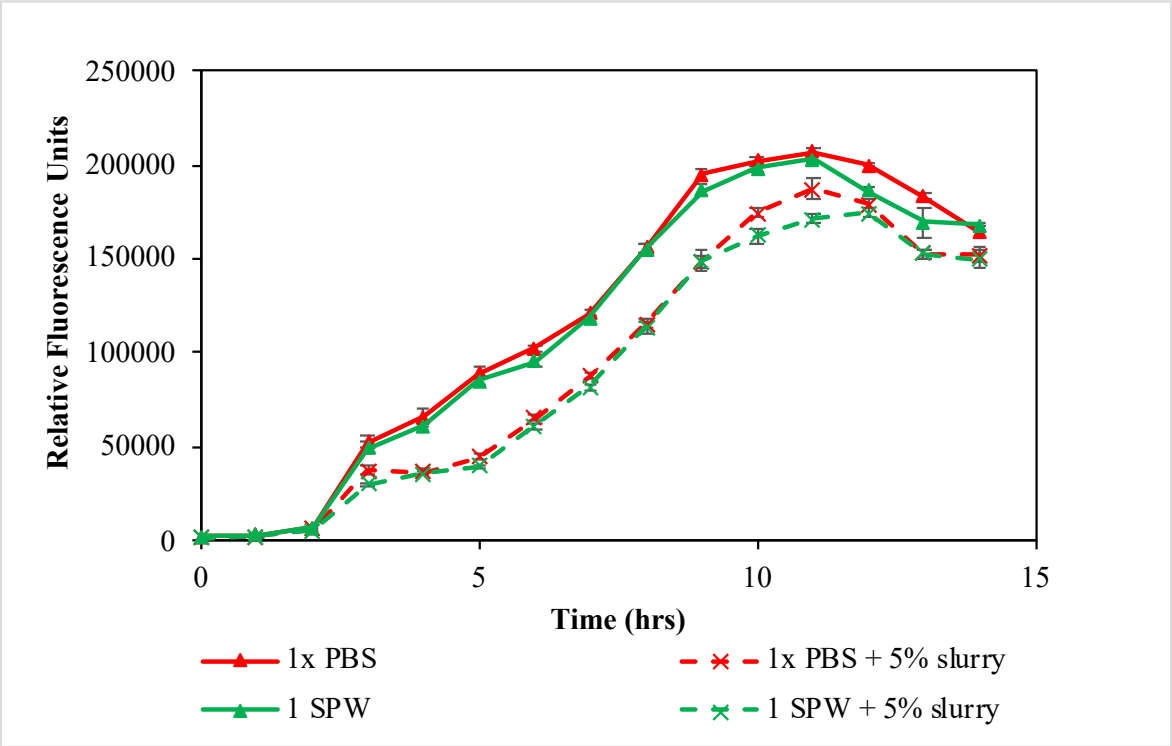

Figure 4-2: *Relative Fluorescence Units (RFU) over time in different media (1x PBS and 1 SPW) supplemented with 0.1% yeast extract and 1% trace elements solution in the absence and presence of finely ground ore (5% solids loading). The growth curves were initiated by the addition of an active inoculum (10% [v/v]). Data points represent the average of triplicate tests. Error bars indicate the standard error between triplicate tests. Where error bars are not visible, they are smaller than the data marker.*

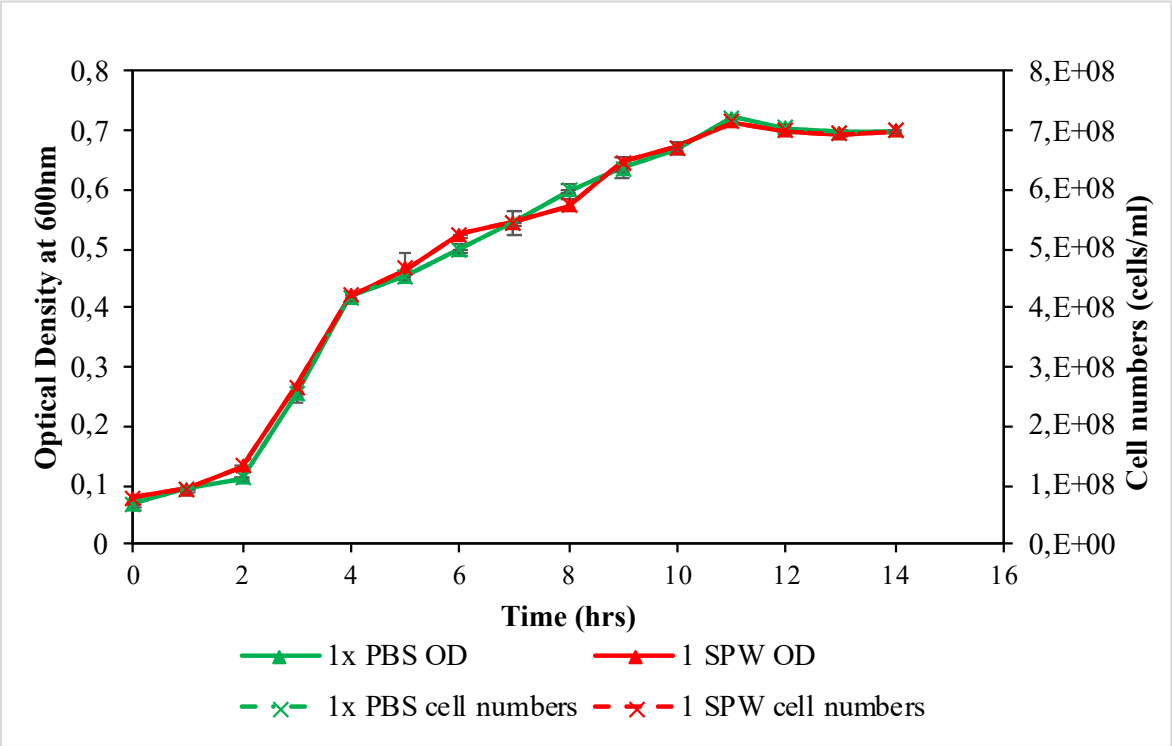

Figure 4-3: *Optical Density (OD) at 600 nm and cell concentration over time in two different media (1 PBS and 1 SPW) supplemented with 0.1% yeast extract, 1% trace elements solution and 10% active inoculum for the microbial consortium harvested from a South African Platinum concentrator. The error bars represent standard error between triplicate tests.*

It is apparent from Figure 4-2 that RFU increased over time in the absence and presence of finely ground ore. Complementary to this, cell numbers increased with time (Figure 4-3). The OD recorded for this experiment was correlated to microbial cell numbers (Figure 4-3) using microbial cell numbers to OD conversion factors determined by preparing a standard curve (Figure A- 1). It should be noted that the curves, in Figure 4-3, are overlapping suggesting that OD and cell number gave similar results. The increase in RFU and cell numbers, in both curves in Figure 4-3, is indicative of microbial cell growth and thus increased metabolic activity measured in the case of the FDA assay. This finding is in accordance with Swisher and Carroll (1980) who demonstrated that the amount of fluorescein produced (given as RFU in the present study) by the hydrolysis of FDA was directly proportional to the microbial population growth. Thus, growth was observed in both media types which simulate the ionic strength of concentrator water.

It can also be seen from Figure 4-2 that no apparent difference was observed between the two media types, 1x PBS and 1 SPW, in the presence and absence of finely ground ore. This suggests that the microbial consortium studied here responded in the same way to the two media used. The FDA results were validated by performing cell biomass measurements in parallel for the cultures in the absence of fine ore particles. Figure 4-3 illustrates OD and cell biomass as a function of time using two different

growth media, 1x PBS and 1 SPW. Similar to the RFU values, OD and microbial cell numbers increased over time as evidenced in Figure 4-3 which indicates microbial growth over time. From Figure 4-3**,** it is apparent that the microbial cell community responded in the same way in both media types. From Table 4-1 it can be seen that the two background media have very different compositions and ionic strengths. Ionic strength measures the concentration of electrolytes dissolved in a solution and was calculated using; Equation 3.2 (Chapter 3). The ionic strength of 1x PBS was found to be 0.17 M while that of 1 SPW was found to be 0.025 M. Despite the fact that the ionic strength of 1x PBS was markedly higher than that of 1 SPW, the microbial cells responded similarly to both media. The presence of small quantities of additional nutrients such as nitrate in SPW and phosphate in PBS appear to have had no effect on the microbial growth observed in those media. It is possible that the growth was supported by the yeast extract as carbon and nitrogen source. Additionally, some of the microbial consortium may be capable of autotrophic growth and fix carbon for growth by utilising carbon dioxide. The autotrophic behaviour of acidophilic mineral associated microbial populations has been well documented (Nancucheo and Johnson, 2020). A microbial abundance study of flotation waters from the Kevitsa Cu-Ni concentrator in Finland highlighted the presence of abundance of autotrophic metabolic pathways for this community (Bomberg et al., 2020).

Microbial activity was lower in the presence of finely ground ore as compared with growth media containing no ore (Figure 4-2). These findings suggest that the presence of ore particles affected the microbial activity. This mirrors findings by Schnurer and Rosswall (1982) as well as those by Adam and Duncan (2001), in which they experimentally determined that the presence of soil particles negatively affected FDA by adsorbing fluorescein. However, Schnurer and Rosswall (1982) reported that the adsorption of fluorescein to soil particles did not exceed 7% and was mostly lower than 5% and could therefore be regarded as minimal. Other possible reasons for lower microbial activity in the presence of ore particles could include the ore sample causing the adsorption of the FDA to be less accessible to the microbial cells, or the microbial cells may attach to the surfaces of the ore particles thus reducing the diffusion of the fluorescein product from the cells. It is also possible that the presence of ore particles in the shake flasks induced microbial shear as the culture serving as inoculum for this study was cultivated in the absence of ore. The initial shear could have reduced the initial RFUs measured by FDA until the cells had adjusted to the presence of suspended ore particles.

The above results suggest that FDA can be employed to quantify microbial cell concentration in a slurry through the measurement of microbial activity. This leads to the conclusion that the microbial load, which may be present in ore slurries in flotation circuits during flotation operations, can be effectively quantified using the FDA assay, especially for microbial communities well adapted to slurry conditions.

Table 4-1: Ionic composition in mmols present per litre in 1x PBS and 1 SPW growth media

Ions	1 SPW	1x PBS
Cations		
Na^+	6.66	157.17
K^+		4.45
Mg^{2+}	2.91	
Ca^{2+}	2.32	
Anions		
Cl^-	8.74	136.89
NO_3^-	2.83	
$H_2PO_4^-$		1.76
HPO_4^{2-}		10.14
CO_3^{2-}	0.28	
SO_4^{2-}	2.50	
Ionic strength	0.025 M	0.17 M

4.4 Microbial attachment to finely ground ore

An investigation into the attachment of microbial cells from the consortium used in this study, onto ore particles was initiated. The study was conducted by monitoring the microbial cell concentration present in solution through direct cell counting using microscopy, following addition of a known microbial concentration to a 5% (w/v) ore in suspension in 1x PBS. Figure 4-4 depicts residual microbial cells in suspension in the presence of finely ground ore over a two-hour incubation period.

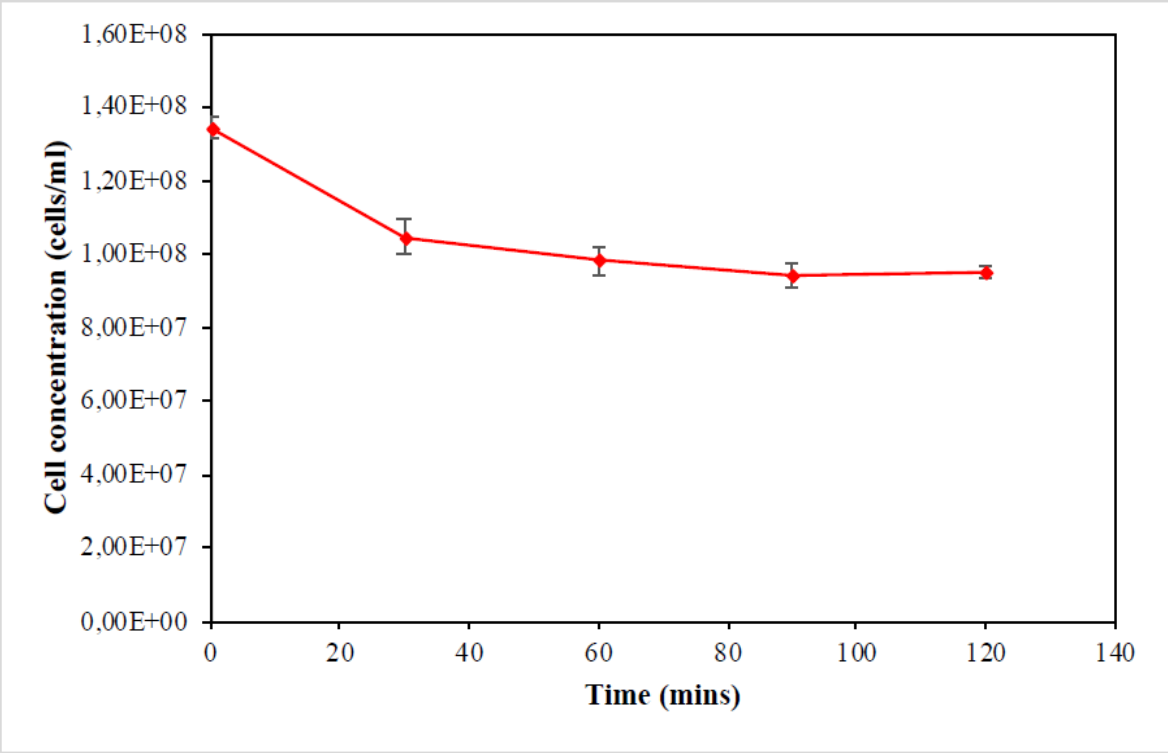

Figure 4-4: *Residual microbial cells in solution in the presence of finely ground ore at 5% solids loading over time at an initial microbial concentration of 1.36 x10^{8} cells/ml. Error bars represent standard deviation between triplicate tests.*

This study was initiated based on the assumption that the cells may attach to mineral particles in the ore. The microbial cells present in the microbial-ore suspension decreased with time as shown in Figure 4-4. This suggests the attachment of microbial cells onto ore particles.

Attachment of microbes onto mineral surfaces is well established (Van Loosdrechtet al., 1990; Africa, 2009; Rao et al., 2010; Bromfield et al., 2011; Liu et al., 2013b) and is largely governed by the surface characteristics of the specific microorganisms and the mineral. Despite the fact that this study did not consider the preferential attachment of the microbial consortium onto the mineral surfaces present in the ore, Mhonde et al. (2020) showed that the same microbial consortium resulted in changes in electrochemical properties of sulphide minerals, which may suggest microbial passivation of the sulphide mineral surfaces present in the ore sample. The rate, or extent of attachment, onto ore particles was more pronounced during the first 30 min of the incubation period, and decreased between 30 and 60 min, while it remained relatively constant for the final hour of the incubation.

The FDA results obtained in the presence of finely ground ore suggest that the presence of the fine ore particles may inhibit microbial growth. However, it is not thought that the presence of ore particles resulted in the death of microbes, as the FDA results over the first hour of the growth curve are similar in the absence and presence of ore particles (Figure 4-2).

During this attachment study, no yeast extract was added to facilitate microbial growth. It is important to note that the study was carried out for a short period of time and as a result, there was insufficient time for biofilm formation. Therefore, the adhesion of the microbial cells can be only explained by electrostatic and hydrophobic interactions which govern the initial adhesion of cells onto mineral surfaces (Zobell, 1943; Van Loosdrecht et al., 1990). It is worth pointing out that the presence of ore particles did not induce microbial death as shown in Figure 4-2 as no apparent RFU difference was observed in the presence and absence ore particles in the first hour. Therefore, the decrease of microbial cells in the liquid fraction was believed to be only through microbial attachment onto ore particles. Studies to date have mostly focused on the attachment of acidophilic microorganisms to low grade ores suitable for bioleaching or biooxidation. Some research has been done on the attachment of neutrophilic single bacterial isolates and type strains to ore particles. In this study a mixed neutrophilic microbial community originating from a South African platinum concentrator was used.

The % attachment was inferred from Equation 3.5 (Chapter 3). After two hours, approximately 29% of the microbes were removed from the liquid fraction and believed to be attached to the ore particles. Africa (2009) reported a similar level of attachment (32% after two hours) for the acidophile, *Acidithiobacillus ferrooxidans,* onto a low-grade chalcopyrite containing ore at a solids loading of 2% (w/v). Furthermore, Africa (2009) recorded a high level of attachment of *At. ferrooxidans* to pyrite and chalcopyrite concentrates, which suggests that the microorganism has an affinity for attachment to sulphide minerals. Bromfield (2011) conducted attachment studies using the thermophilic acidophile *M. hakonensis*, adapted to elemental sulphur, in the presence of a low-grade chalcopyrite-containing ore at 2% w/v solids loading, and reported an attachment percentage of 28% to sulphide mineral; this attachment is comparable with the 29% reported in the present study.

Furthermore, Liu et al. (2013b), while investigating the adsorption of *Escherichia coli* onto chalcopyrite surfaces, reported that *E. coli* cells attached to chalcopyrite minerals and the attachment levels increased with an increase in *E. coli* cell concentration. Also, *E.coli* cells decreased the surface hydrophobicity of chalcopyrite. Thus, despite the fact that the microorganisms used were different to those used in this study, the present microbial consortia show affinity for sulphide minerals present in the real ore sample used.

According to Schouwstra et al. (2013), geochemical characterization of three different pits originating from the Platreef ore, similar to that used in the present study, indicated that Cu and Ni feed grade averaged in the range of 0.6 - 0.7 and 0.11 - 0.14 g/t, respectively. Cu primarily represents the target mineral chalcopyrite while Ni primarily represents the mineral pentlandite. As such, the low-grade sulphide constituents offer only a minor portion of the bulk ore to which the microbes would likely attach.

Attachment of the microbes present in the flotation waters, onto mineral surfaces, can lead to the formation of a microbial layer at the mineral surfaces which may subsequentially act as a barrier for collector adsorption onto mineral surfaces. This could result in changes in mineral hydrophobicity, compromising particle-bubble attachment, a vital secondary sub-process of flotation. This may in turn affect the formation of particle-bubble aggregates, a third vital sub-process (Liu et al., 2013b), ultimately limiting valuable mineral recoveries.

Additionally, it has been widely acknowledged that the adhesion of microbial cells alters mineral hydrophobicity. Surface treatment of sulphide minerals with microbial solution has been shown to influence sulphide superficial chemical properties, which in turn alter their responses in processes such as flotation (Seifelnassr and Abouzeid, 2013). Thus, in light of this, it is evident that the formation of the microbial layer on the mineral surfaces can adversely affect flotation.

4.5 Conclusions

The present study shows that FDA can be used to monitor microbial concentration in the presence and absence of an ore slurry. Despite the fact that the presence of ore particles affected the RFUs achieved by FDA assay, the effect was marginal and can therefore regarded as minimal. Thus, FDA is suitable for microbial quantification in an ore slurry.

From the growth studies in the presence of two media (1x PBS and 1 SPW), it was concluded that the microbial consortium responded in the same way to both media. Also, shake flask studies revealed that the microbial community attached onto the ore particles surfaces. This observation supports the work of Van Loosdrecht et al. (1990); Africa, (2009); Rao et al.(2010); Bromfield et al. (2011); Liu et al. (2013b), who all reported that microbial cells, both acidophiles and neutrophiles, attach to mineral surfaces. The degree of attachment of the microbial cells to low grade ore, was found to be similar to that reported by Africa (2009) and Bromfield et al., (2011). High levels of attachment were attained in the first 30 mins and decreased for the remaining 1.5-hour period tested. Thus, the microbial community exhibited adsorption onto the ore surfaces, which could result in microbial passivation onto target mineral surfaces. Microbial attachment onto mineral surfaces can compromise vital sorption processes in flotation and subsequently affect flotation performance.

CHAPTER 5

5 MICROBIAL CELL RESPONSE IN THE PRESENCE OFXANTHATE

This chapter provides experimental data on the response of a microbial community, harvested from a South African platinum concentrator, to the presence of two primary xanthate collectors, widely employed in sulphide mineral beneficiation, namely: Sodium Ethyl Xanthate (SEX) and Sodium Isobutyl Xanthate (SIBX). The study investigates microbial growth in the presence of increasing xanthate concentrations, and the effect of the microbes on xanthate concentration.

5.1 Introduction

Several studies have indicated that residual/unconsumed xanthate collectors, present in poorly remediated wastewater (Alto et al., 1977 and Deo and Natarajan, 1998) that is recirculated for flotation operations, are toxic to microbial and aquatic life (Natarajan and Prakasan, 2013). A study by Webb et al. (1975), in which they investigated the toxicity of xanthate on rainbow trout, revealed that SEX was more toxic than SIBX to this fish species. Moreover, similar findings were attained by Tuovinen (1978) who found that SIBX was less toxic to the acidophilic bacterial strain *Acidithiobacillus ferrooxidans*, compared with SEX, which was found to be highly toxic. Interestingly, it has been established that some microbial strains can develop a natural tolerance towards xanthate by causing the xanthate to be degraded, a phenomenon known as bioremediation (Chen et al., 2011a). This raised the question that if the microorganisms in the recycle water accelerate the degradation of flotation reagents, flotation would be affected. Owing to the cost effectiveness of bioremediation, compared to the traditional methods for the removal of organics in waste waters, some mineral concentrators have implemented bioremediation prior to wastewater discharge to the tailings storage facilities. In light of this, studies on xanthate in the presence of microbial cells have largely focused on bioremediation (Chockalingam et al., 2003; Chen et al., 2011a; Cheng et al., 2012; Natarajan and Prakasan, 2013). The current study aimed to elucidate the impact of microbes within flotation waters on the performance of the flotation process. Thus, the response of the microbial community to the presence of xanthate collectors was investigated.

5.2 Experimental approach

The effects of xanthate (SEX and SIBX) at various concentrations on the microbial growth of the microbial community harvested from a South African platinum concentrator were evaluated. Microbial growth studies were conducted in the absence and presence of xanthate, using increasing xanthate concentrations to determine microbial tolerances, for over 30 hours. For all the growth studies, 10% (v/v) of an active overnight culture was used as inoculum as described in Section 3.1.4. 1x Phosphate Buffered Saline (PBS) was used as background media and supplemented with 0.1% yeast extract for carbon and nitrogen sources. Cultures were also supplemented with trace elements. Optical Density

(OD) was used to monitor the microbial growth at 600 nm in the absence and presence of xanthate, while the residual xanthate in solution was quantified spectrophotometrically by UV Vis at 301 nm as described in Section 3.2.4. Moreover, the effects of xanthate on microbial cells, at increasing cell concentrations, and the effects of microbes on xanthate in solution were elucidated. In this instance, 1x PBS was used as the background media without the addition of yeast extract. Different microbial concentrations were prepared and supplemented with 60 ppm xanthate. Residual xanthate in solution in the absence and presence of microbial cells was determined by UV Vis (Section 3.2.4). The carbohydrate content of the microbial cells was quantified using the phenol-sulphuric acid assay in the absence and presence of xanthate to determine whether xanthate may act as an additional carbon source for these microorganisms (Section 3.2.5). An experiment was performed to determine if the absorption or bioremoval of xanthate from solution by microbes would affect the adsorption of the collectort to the mineral surfaces contained within ore samples. For this experiment a 60 ppm SIBX solution was contacted with either 30% ore (w/v) or 10^8 cells/ml as detailed in Section 3.2.6. The findings from these experiments were compared with those obtained by the combined exposure of the 60 ppm SIBX solution to 30% ore and 10^8 cells/ml. A control of 60 ppm SIBX in the absence of ore and microbes was included. Further, microbial cell hydrophobicity was conducted using the Microbial Adhesion To Hydrocarbons (MATH) test using 1x PBS as a background media. To further shed light on whether the microbial cells were adsorbing collector on microbial surface, the microbial cells were contacted with collector prior to performing the MATH test.

The precision of the results obtained in this chapter was evaluated by standard error analysis, presented as error bars in the following graphs. In some cases, the error bars were small and are therefore not visible at the scale used. Unless stated otherwise, all biological studies in this chapter were conducted in triplicate, and non-biological studies (xanthate controls) in duplicate (Section 5.3), while xanthate degradation experiments, at a fixed xanthate dosage of 60 ppm, were carried out in triplicate, in both the biological and non-biological (xanthate controls) studies.

5.3 Results and discussion

5.3.1 Microbial response in the presence of SEX/SIBX during growth studies

The effect of xanthate collectors (SEX and SIBX) on the microbial cell response during microbial growth was considered. Growth studies were carried out in the presence of increasing concentrations of xanthate (60 ppm, 120 ppm, 240 ppm and 480 ppm). Microbial cell growth was monitored using OD at 600 nm. Concurrently, residual xanthate in solution was monitored spectrophotometrically by UV Vis at 301 nm. The OD recorded for all the experiments was correlated to microbial cell dry weight (Figure 5-1 and

Figure 5-2) using dry weight to OD conversion factors, determined by preparing standard curves for these (Figure A-1).

5.3.2 Microbial growth in the presence of xanthate

Microbial growth in the presence of increasing concentrations of xanthate collectors (SEX and SIBX) in solution was investigated and the results are shown in Figure 5-1 and Figure 5-2 below. Microbial growth was monitored by OD, which was then converted to cell dry weight.

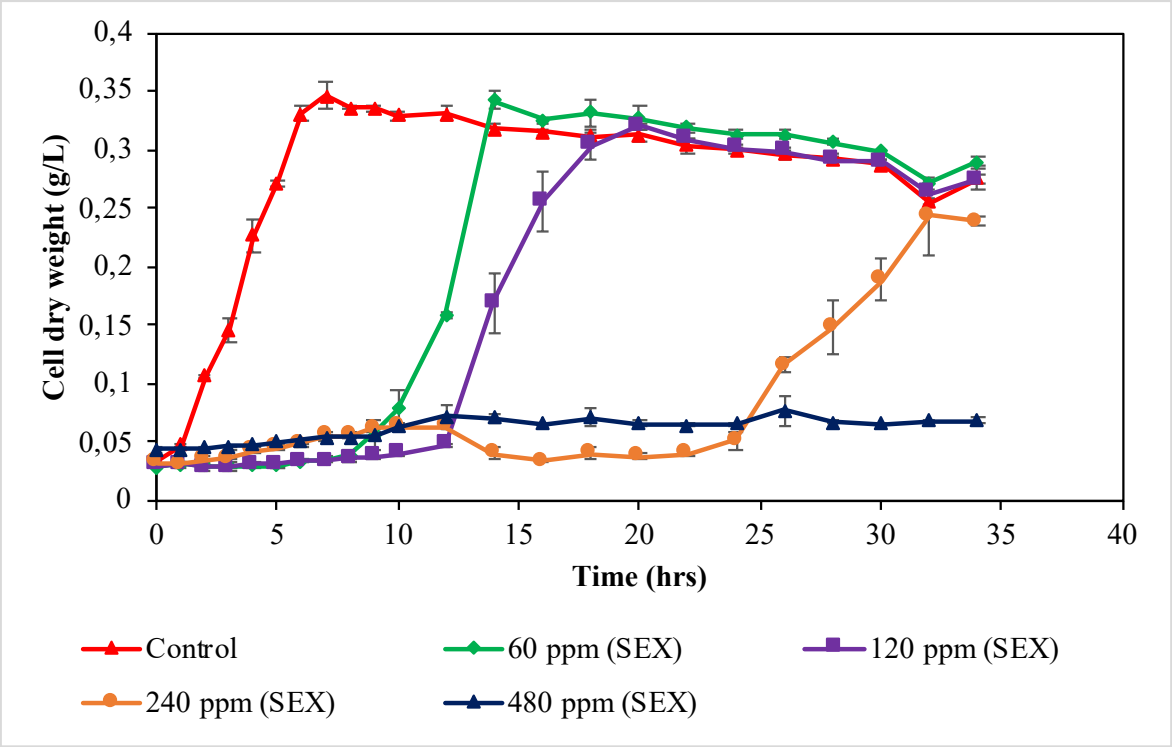

Figure 5-1: *Cell dry weight of a mixed consortium in the absence of SEX (control) and in different SEX treatments over time. Each data point represents an average of three repeats for SEX supplemented cultures and two repeats for the Control.*

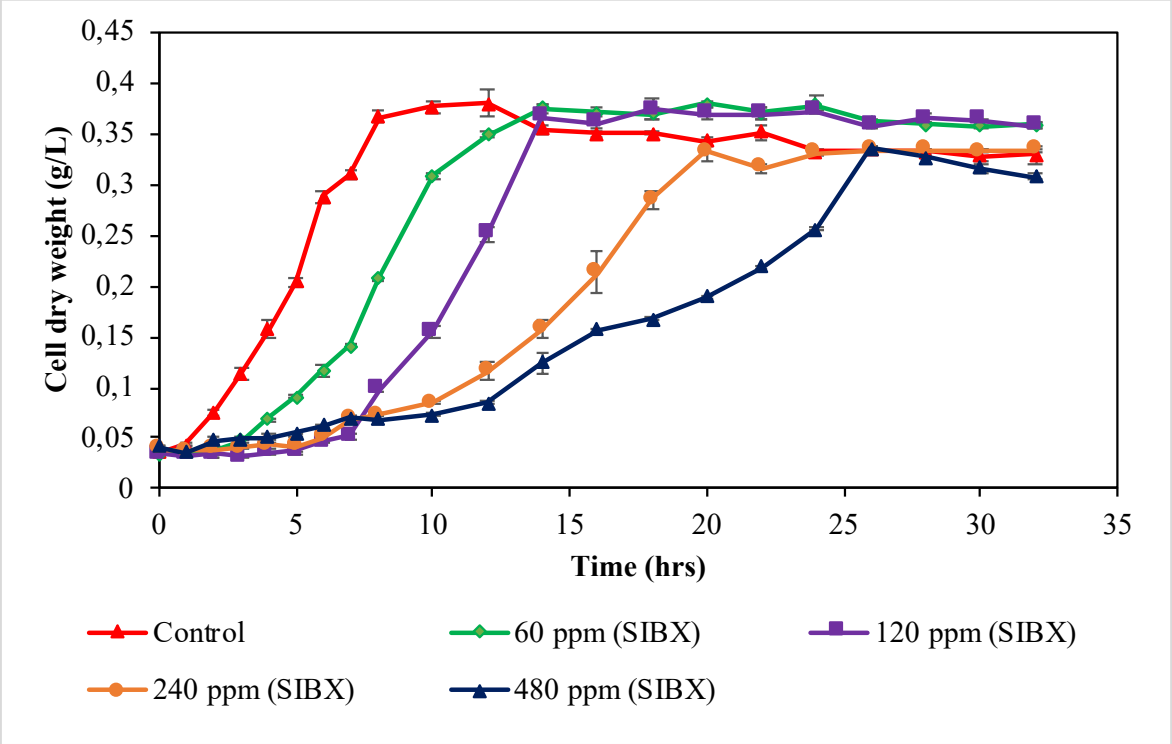

Figure 5-2: *Cell dry weight of a mixed community in the absence of SIBX (control) and in different SIBX concentrations over time. Each data point represents an average of two repeats and three repeats for the Control and SIBX supplemented cultures, respectively.*

An increase in cell biomass which, indicates microbial growth (Deo and Natarajan,1998) can be seen in Figure 5-1 and Figure 5-2. During microbial growth in solution, 3 phases occur, namely the lag phase, the exponential growth phase and the linear or declining growth phase. During the lag phase, it is assumed that the cells adapt to the new environment and hence do not replicate i.e., during this time the specific growth rate is zero. In the exponential growth phase there is an exponential increase in cells and the specific growth rate is assumed to be a constant. The exponential growth phase is often followed by linear, or limited and declining growth phases for many cultures. Once the stationary phase has been reached, there is no net increase in cell population and the specific growth rate returns to zero (Buchanan et al., 1997) .

From Figure 5-1 and Figure 5-2 it can be seen that the cell biomass of all samples increased over time, although with differing lag times for all concentrations, except at 480 ppm of SEX, where little cell growth occurs (Figure 5-1); the control samples have the shortest lag phases. Prolonged lag phases can be seen in the presence of higher xanthate concentrations, for example, in 240 SEX and 480 ppm SIBX, which is consistent with the toxicities of these collectors suggested in literature (Deo and Natarajan, 1998; Natarajan and Prakasan, 2013). Longer lag phases occur as a result of the delayed response by the microbial population to a sudden change of environment (Swinnen et al., 2004). It therefore follows that the longer lag phases, detected in the presence of xanthate, may be due to the microbial cells

adapting to presence of the collector in solution. Albeit not experimental proven in the present study, it is expected that if the consortium is serial sub-cultured in the presence of the xanthate, the microbial community would adapt and develop xanthate tolerant mechanisms. As a result, the microorganisms will better facilitate the removal of xanthate at higher concentrations. Similar observations were noted by Deo and Natarajan (1998a).

OD measurements were continued until the stationary phase was reached for all SIBX concentrations tested. Tests performed in the presence of all SEX concentrations were conducted for a similar period (over 30 hours) with SIBX concentrations however stationary phase was not attained for all SEX concentrations. Growth profiles for the different SIBX concentrations suggested that the microbial community exhibited differences in the growth rates achievable with increasing collector concentration. This effect was less pronounced in the presence of SEX for concentrations up to 120 ppm; however, a lower growth rate occurs at 240 ppm SEX and it should be noted that at the highest SEX concentration (480 ppm), no apparent growth was detected (Figure 5-1). These findings complement the studies of Loon and Madgwick, (1995); Deo and Natarajan, (1998a); Dopson et al. (2006); Chen et al. (2011a); Natarajan and Prakstan (2013) who reported that higher xanthate concentrations inhibit microbial growth. From this data it can be suggested that at an SEX concentration of 480 ppm, xanthate becomes toxic to the microbial community studied here.

The prolonged lag phases were not as pronounced in the presence of increasing SIBX concentrations (Figure 5-2). This indicates that SIBX was less inhibitory to microbial growth when compared to SEX.

Bararunyeretse et al. (2017) investigated the effect of sodium isopropyl xanthate (SIPX) and SIBX on soil microbes and found that the shorter propyl chain (SIPX) had a more pronounced toxicity on microbes than the longer butyl chain (SIBX) at the same concentrations. This may be explained by the fact that there are less functional anionic sulfhydryl ($OCSS^-$) groups, believed to be responsible for the toxicity of xanthates, in the longer chain length xanthate SIBX (Mr 172.2 g/mol) compared with the shorter length SEX (Mr 144.18 g/mol). However, at xanthate concentrations below toxicity levels to microbes, Deo and Natarajan, (1998b) proposed a possible mechanism whereby xanthate collectors could be used as metabolic substrates. In their mechanism, Deo and Natarajan (1998b) suggested that microorganisms can convert xanthate into an alcohol, which could safely enter the tricarboxylic acid (TCA) cycle and satisfy the carbon requirements. Other possible mechanisms which could aid microbial xanthate removal from solutions are that the microbial cells could consume the xanthate without utilizing it as an energy source, or that xanthate is adsorbed onto microbial surfaces. The mechanisms by which the microbes cause xanthate removal are further explored in the forthcoming sections.

The microbial cell growth was monitored in parallel with residual SEX and SIBX analysis by UV Vis at 301 nm. Owing to the decompositional nature of xanthate collectors, controls were included for each of

the xanthate concentrations tested; the controls contained no microbes, only xanthate (Figure 5-3 and Figure 5-4). Given that the microbial community originated from neutral environments and hence this microbial community consisted of neutrophiles, all experiments were performed at a pH of 7.4, thus the pH effect on xanthate degradation can be eliminated. Since it was not apparent which mechanism was responsible for xanthate bioremoval from solution, degradation or adsorption, the word "removal", in this study, is used to describe a decrease in xanthate concentration.

Residual xanthate in solution was measured concurrently with the monitoring of the microbial growth over time.

Figure 5-3 and Figure 5-4 report residual xanthate over time. Notations used in this section are given as, for example, 60 ppm Control and 60 ppm Exp, where SEX or SIBX concentration in parts per million are followed by whether the microbes are absent (Control) or present (Exp), respectively.

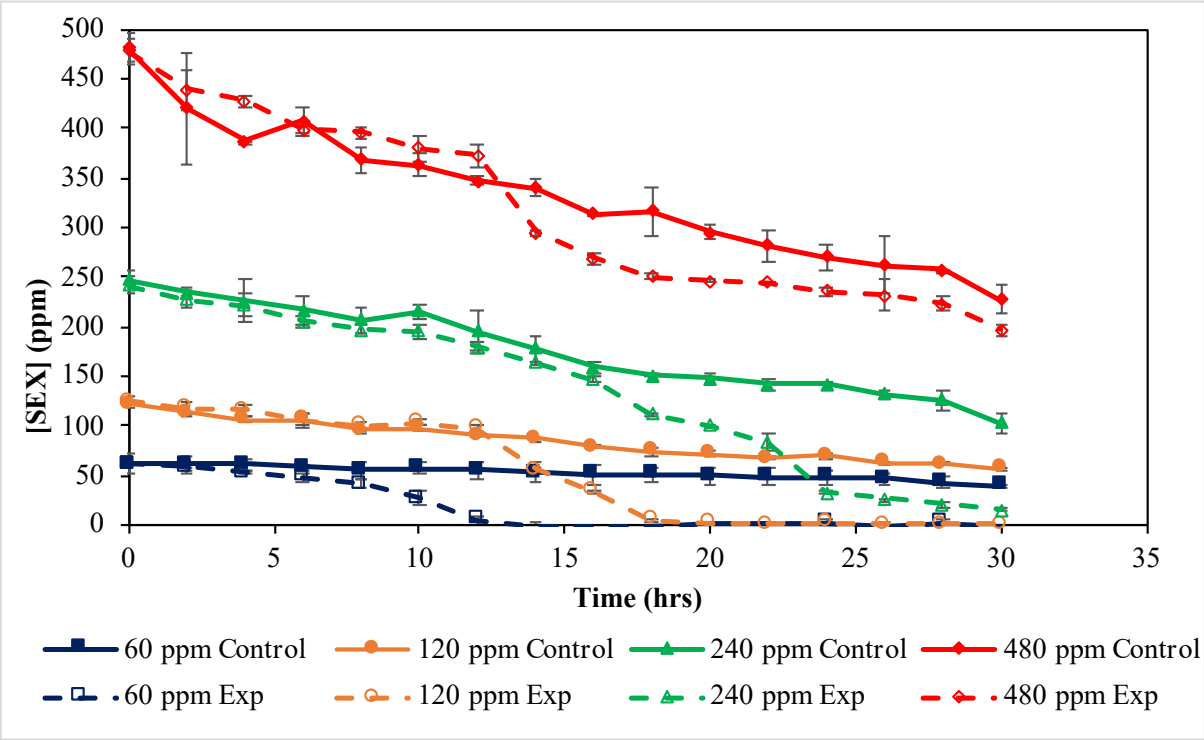

Figure 5-3: *SEX removal from solution at different initial SEX concentrations in the absence (Control) and presence (Exp) of the microbial cells as a function of time. Data points represent the average of two independent flasks for the controls and three flasks for the experiments performed in the presence of microbes. Error bars represent standard errors of the mean from the Exp flasks (triplicates) and standard deviation between duplicate for control flasks.*

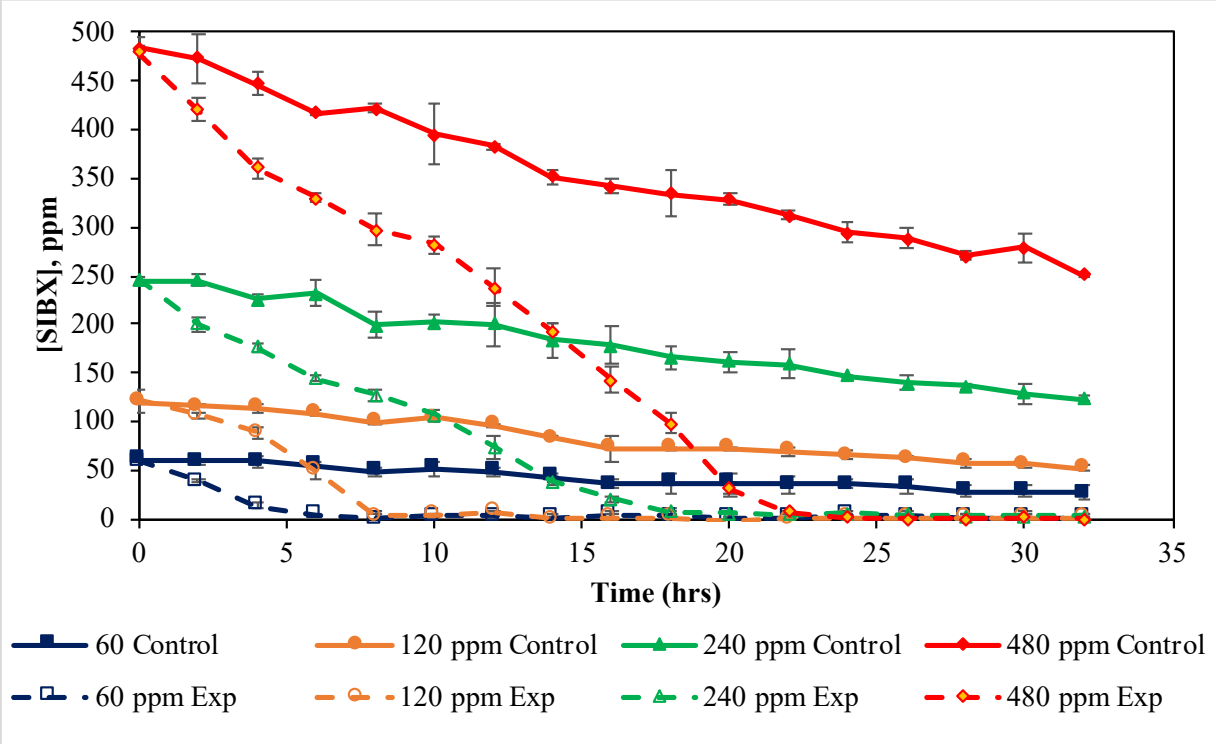

Figure 5-4: *SIBX removal from solution at different initial SIBX concentrations in the absence (Control) and presence (Exp) of microbial cells as a function of time. Each data point represents an average of two repeats and three repeats for the Control and SIBX supplemented cultures, respectively. Error bars represent standard errors of the mean from the Exp flasks (triplicates) and standard deviation between duplicate for control flasks.*

A marked decrease in xanthate concentration is noted in the presence of microbes relative to the controls. The decrease in xanthate concentration corresponded with the increase in microbial biomass reported in Figure 5-1 and Figure 5-2 and suggests that the microbial cells could effectively remove xanthate from aqueous solutions. Similar biodegradation has been reported for other microbial species and communities (Deo and Natarajan, 1998a; Okibe and Johnson, 2002 Chockalingam et al, 2003;Chen et al., 2011b; Natarajan and Prakasan, 2013; Bararunyeretse et al., 2017). Complete SEX removal was achieved at 12-14, 20 and 30 hours for 60, 120 and 240 ppm SEX in the presence of microorganisms, respectively. This approximately translates to a complete SEX percentage removal from solution in over 12-14, 20, 30 hours for 60 ppm Exp 120 ppm Exp and 240 ppm Exp, respectively - see Appendix B for percentage xanthate removal in non-xanthate and treated xanthate treated samples. Although a slight decrease in SEX was observed in the presence of microorganisms at 480 ppm SEX, as indicated in Figure 5-3, the decrease was not as apparent as at the lower SEX concentrations where microbial growth was observed. It is possible that the microbial community tolerated SEX to a SEX concentration of 240 ppm, beyond which microbial growth was adversely affected and, in turn, affected the bioremoval of SEX.

While this is the case for SEX, in the case of SIBX, Figure 5-4, its bioremoval from the solution was completed within 6-8, 8, 18 and 22 hours for 60, 120, 240 and 480 ppm SIBX, respectively after the commencement of the test. This approximately correlates to a complete SIBX removal from solution in over 6-8, 8, 18, 22 hoursfor 60 ppm Exp, 120 ppm Exp, 240 ppm Exp and 480 ppm Exp, respectively see Appendix B for percentage xanthate removal in non-xanthate and treated xanthate treated samples.

It is thus apparent from Figure 5-3 and Figure 5-4 that the extent of SEX and SIBX removal in the presence of microbial cells was predominantly dependent on the initial xanthate concentration, since the inoculum concentration was kept constant throughout the studies. When considering the degradation of xanthate in the absence of microorganisms, the extent of removal was markedly lower than in the presence of microbes. While at lower concentrations xanthate was removed from the microbial supplemented cultures over a shorter period, at higher concentrations xanthate was removed over a longer period. These observations are in accordance with studies by Deo and Natarajan (1998a,b) and Natarajan and Prakasan (2013) who showed that the extent of xanthate biodegradability/removal was largely dependent on the initial xanthate concentration in solution. They showed that at low concentrations xanthate was degraded over a shorter period compared with xanthate removal at higher concentrations. When comparing the two collectors, bioremoval of SIBX was higher compared with SEX for all tested concentrations. It therefore follows that xanthate with longer carbon chains tends to be bioremoved more easily from solution, compared to xanthate with shorter carbon chains (Okibe and Johnson, 2002; Bararunyeretse et al., 2017).

5.4 Xanthate analysis in the presence of increasing microbial cell concentration

From Section 5.3.1 it remained unclear as to which mechanism (adsorption or biodegradation) was responsible for xanthate removal from solution. Therefore, the removal of xanthate at different microbial concentrations; 10^6, 10^7, 10^8 and 10^9 cells/ml was assessed at a fixed starting xanthate concentration of 60 ppm. For all the tests conducted, 1x PBS was used as the background medium without supplementation of a carbon or nitrogen source to support microbial growth.

Figure 5-5 and Figure 5-6 shows the residual xanthate concentration in solution vs time, in the absence and the presence of different microbial cell concentrations. It should be noted that for lower microbial concentrations (10^6 and 10^7), the extent of xanthate removal was very low and as such sampling was performed every three hours and then left overnight. The controls, 60 ppmSEX or SIBX without the addition of microbes, only showed slight degradation during the course of the study for both xanthates tested (Figure 5-5 and Figure 5-6). From Figure 5-5, it can be seen that SEX concentration in the absence and presence of 10^6 and 10^7 cells/ml showed a minimal decrease after 21 hours and remained at similar concentrations as the control. The same trend was observed in the SIBX treated microbial cells at 10^6 cells/ml, while in the presence of 10^7 cells/ml an initial decrease of approx. 20 ppm SIBX was observed

over the first six hours of the test. These results could suggest that the microbial concentrations were too low to permit collector removal, or complete removal in the case of the 10^7 cells/ml with SIBX. Most notably, a relatively steep decrease in SEX and SIBX concentrations occurred in the presence of 10^8 cell/ml and 10^9 cells/ml (Figure 5-5 and Figure 5-6). This is attributed to the fact that at these concentrations, microbial cells can aid xanthate bioremoval, as reported for other microbial species and communities (Deo and Natarajan, 1998; Chockalingam, et al., 2003; Chen et al., 2011b; Natarajan and Prakasan, 2013). The success of bioremoval appears to be dependent on xanthate chain length and microbial concentration.

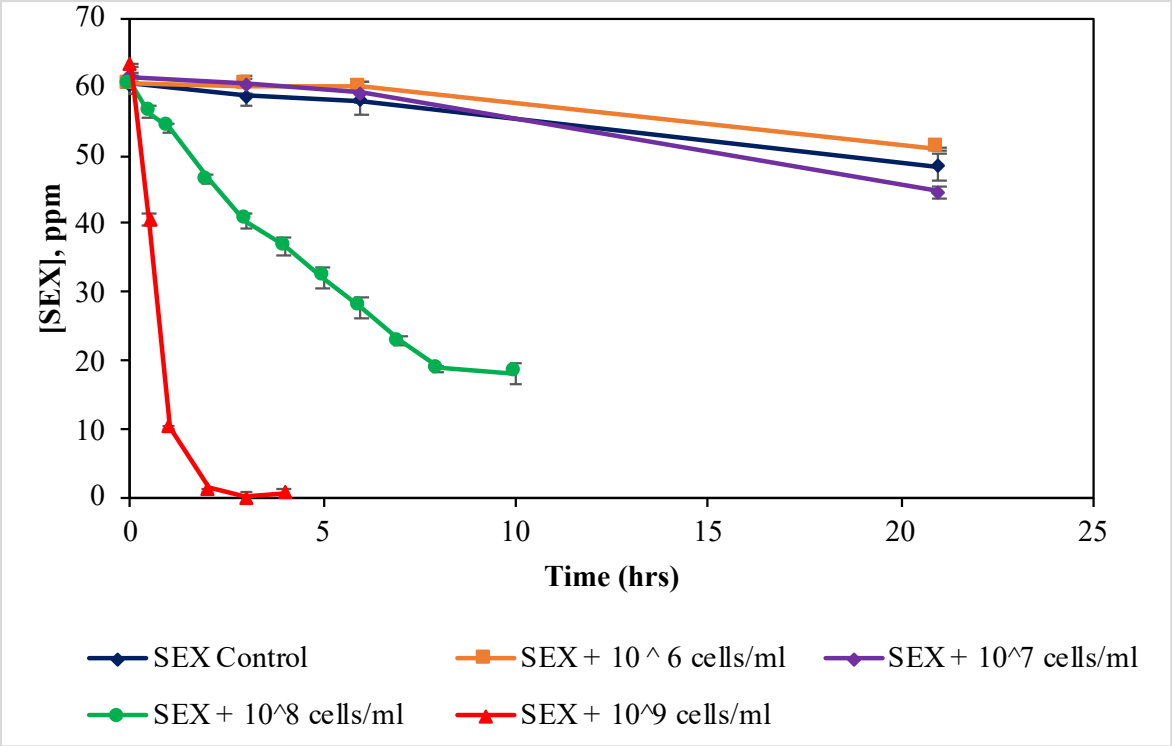

Figure 5-5: *Residual SEX concentration in the presence of different microbial concentrations over time at an initial starting SEX concentration of 60 ppm. Each data point represents an average of three repeats for all experiments. The error bars show standard error calculated from the mean for three repeats.*

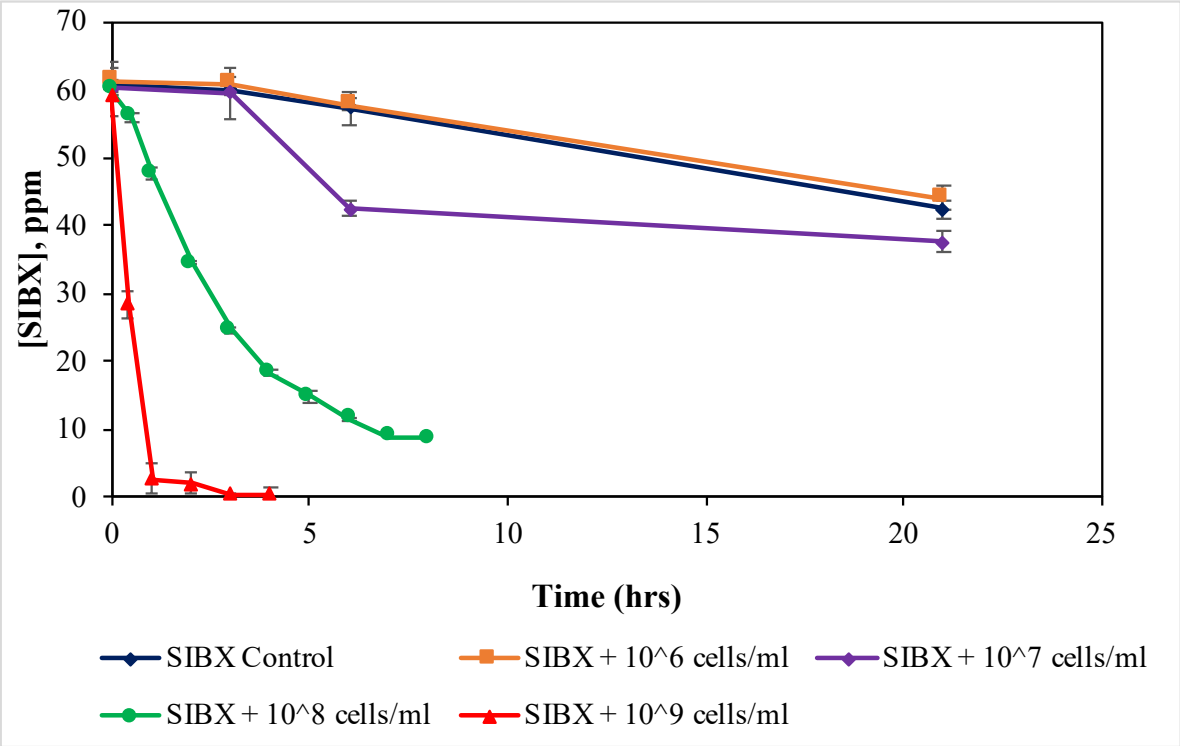

Figure 5-6: *Gives an account of SIBX removal over time in different microbial concentrations at an initial SIBX concentration of 60 ppm. Each data point represents an average of three repeats for all experiments. The error bars show standard error from the mean for three repeats. Error bars were notvisible at the scale used.*

When considering the extent of bioremoval of SEX, complete SEX removal occurred within two hours in the presence of 10^9 cells/ml. Complete removal of SEX at the other, lower, cell concentrations was not achieved within the time given for these experiments or, the microbial concentration was too low to achieve higher removal of xanthate. For SIBX, 100% bioremoval was achieved over an hour in the presence of 10^9 cells/ml whereas for the other microbial concentrations complete SIBX bioremoval was not attained within the given time ofthe experiments (Figure 5-6) (see Appendix- B for percentage bioremoval).

From the data presented in Figure 5-5 and Figure 5-6, the rate constants (K) and volumetric removal rates (ppm/hr) were extracted from the linear portion of the data following the conversion of the xanthate concentrations to their natural logarithmic (ln) values (presuming exponential). These results are given in Table 5-1 and Table 5-2. Higher volumetric removal rates are evident as the microbial concentration increases.

Table 5-1: *K values and volumetric removal rate in the presence of an initial concentration of 60 ppm SEX in the absence and presence of different microbial concentrations. There was no error evaluation.*

Treatment	Time period (Linear portion)	Gradient (K)	Volumetric removal rate, ppm/hr
SEX Control	0 - 21 hrs	0.01	0.58
SEX + Microbes (10^6 cells)	0 - 21 hrs	0.01	0.45
SEX + Microbes (10^7 cells)	0 - 21 hrs	0.02	0.80
SEX + Microbes (10^8 cells)	0 - 10 hrs	0.13	4.23
SEX + Microbes (10^9 cells)	0 - 3 hrs	1.99	20.99

Table 5-2: *Shows K values and volumetric removal rate in the presence of an initial concentration of 60 ppm SIBX in the absence and presence of different microbial concentration as a function of time.*

Treatment	Time period (Linear portion)	Gradient (K)	Volumetric removal rate, ppm/hr
SIBX Control	0 - 21 hrs	0.02	0.87
SIBX + Microbes (10^6 cells)	0 - 21 hrs	0.02	0.82
SIBX + Microbes (10^7 cells)	0 – 6 hrs	0.06	2.95
SIBX + Microbes (10^8 cells)	0 - 8 hrs	0.26	6.43
SIBX + Microbes (10^9 cells)	0 - 2 hrs	1.74	28.62

It is important to note that the K values and volumetric removal rates were higher for SIBX compared with SEX. These results suggest that a possible reason for the increased lag period and toxicity of SEX at 480 ppm may be due to the lower removal rates achieved by this microbial consortium for SEX.

These results shown in Figure 5-5 and Figure 5-6/ Table 5-1 and Table 5-2 above, suggest that SEX and SIBX can be removed from the solution, a phenomenon facilitated by microbial cells. Moreover, it can be inferred that a positive correlation exists between microbial cell concentration and the extent of xanthate removal from solution. The same can be said in the case of the microbial concentrations and the rates.

5.5 The effect of microbial carbohydrate content on bioremoval of xanthate

An investigation into the effect of carbohydrate content of the microbial cells was initiated to determine whether the microbial cells were consuming or adsorbing carbon from SEX and SIBX onto or into their cells. Xanthate analysis was conducted simultaneously with the phenol - sulphuric assay to quantify carbohydrate content of the cells in the presence and absence of SEX and SIBX. The carbohydrate assay was carried out concurrently with xanthate analysis in the presence and absence of xanthate at microbial concentrations of 10^8 and10^9 cells/ml.

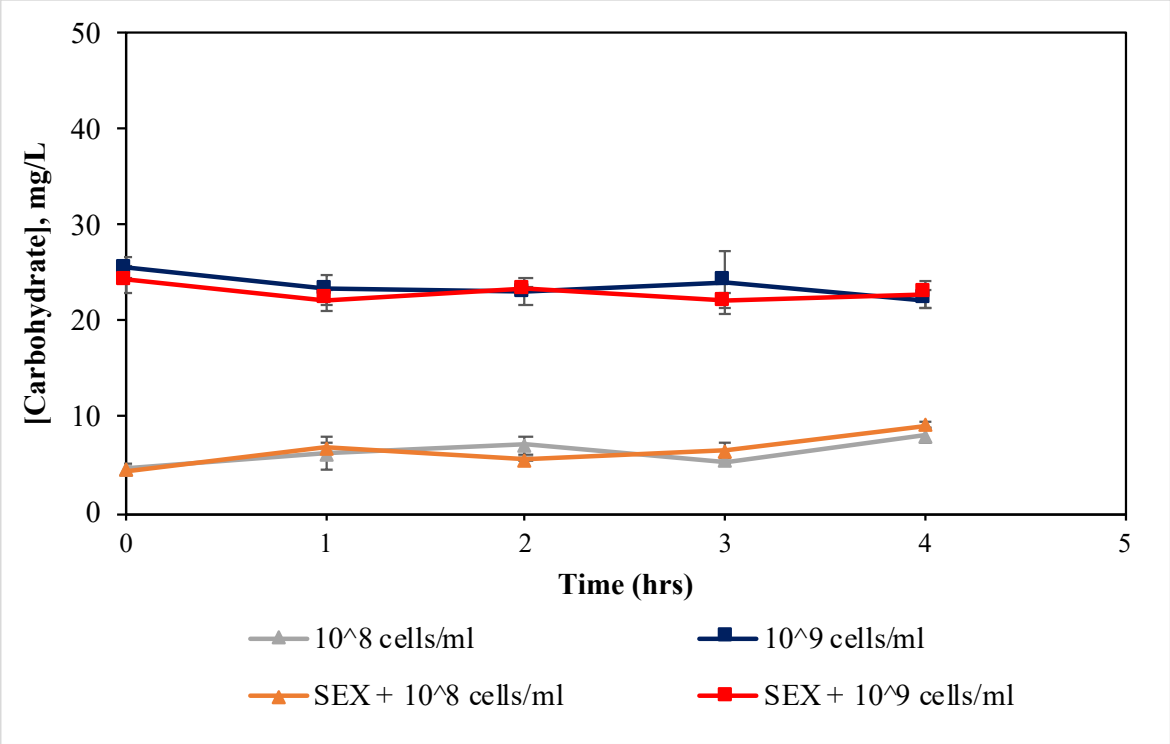

Figure 5-7: *Carbohydrate concentration of microbial cells in the absence and presence of 60 ppm SEX in microbial concentrations of 10^8 and 10^9 cells/ml. Each data point represents an average of three repeats. Error bars represent the standard error of the mean for these data points*

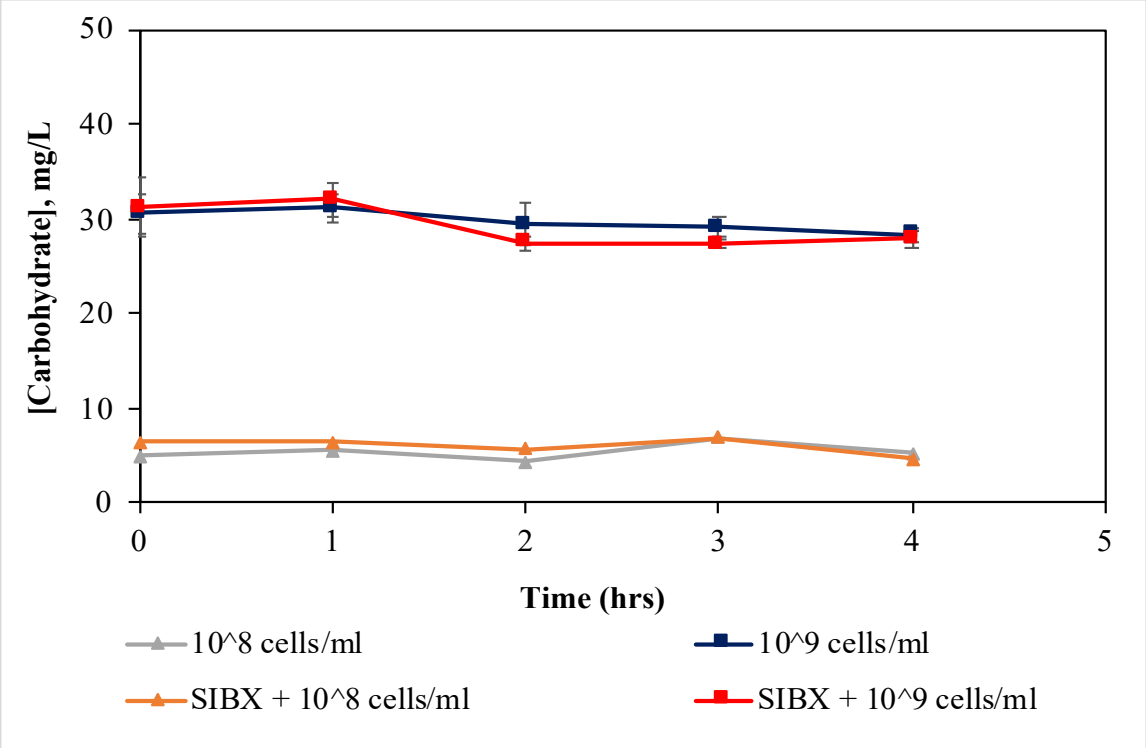

Figure 5-8: *Carbohydrate concentration vs time in the absence and presence of 60 ppm SIBX concentration of in different microbial concentrations (10^8 and 10^9 cells/ml). Each data point represents an average of three repeats. Error bars represent the standard error of the mean for these data points.*

Figure 5-7 and Figure 5-8 illustrate the carbohydrate concentration as a function of time in the presence and absence of xanthate at different microbial concentrations. As per Figure 5-7 and Figure 5-8, no apparent carbohydrate content difference was observed in the absence and presence of SEX and SIBX at either microbial concentration (10^8 or 10^9 cells). Therefore, these results suggest that the carbon backbone of the xanthate collectors is not adsorbed onto the cells, thereby contributing to the total carbohydrate content of the cells. However, it is noted that the assay may not be sensitive enough to measure small increases in the total carbohydrate concentration. The microbial consortia used in this study was found to compromise tiny cells compared with the cell sizes such as *E. coli*. As such, it may be possible that the studied microbial cell concentrations contain a relatively low carbohydrate content.

The xanthate removal measured in these tests was between 40 and 60 ppm, which corresponds to between 9 and 21 mg/L carbon which, if assimilated or absorbed, should result in a measurable increase in the carbohydrate content of the microbial cells. Other published findings on this subject suggest that *Bacillus polymyxa (B. polymyxa)* can utilize xanthate as a sole carbon source resulting in the production of polysaccharides (Deo and Natarajan, 1998a; Deo and Natarajan, 1998b; Chockalingam et al., 2003). However, it must be stated that this study considers the use of a microbial community, unlike the

aforementioned researchers who used an individual strain – *B. polymyxa* - which has been studied extensively.

No increase in microbial biomass was measured for the microbial community used during the tests, suggesting that the cells were not able to utilize the xanthate as carbon source. This data was validated with cell counts and no increase in cell concentration was noted. Despite this, it is clear that the microbial cells aided the removal of xanthate from solution.

5.6 Xanthate analysis in the presence of microbes and ore particles

From the above studies into microbial growth in the presence of xanthate collectors, and the bioremoval of xanthates from solution by various microbial concentrations, suggest that the microbial cells were able to facilitate the removal of SEX and SIBX from solution. From Section 5.5 above, it appears that xanthate is not adsorbed onto the microbial cells. This raised the question of whether, in a system containing both microbial cells and ore particles (such as a concentrator circuit), microbes could compete with the mineral for collector adsorption, or if microbes would remove xanthates from solution before they were able to adsorb to the mineral surface.

Figure 5-9 below shows the change in SIBX concentration as a function of time under different conditions of ore and microbes. The SIBX concentration remained fairly constant in the 60 ppm SIBX control during the study (Figure 5-9). A decrease in SIBX concentration in the presence of microbes alone was observed, although a greater decreased occurred in the presence of ore particles alone. The combined xanthate removal in the presence of both ore particles and microbes was not significantly different from the removal by ore alone (based on the overlap of the error bars for these data sets). Complete % SIBX was only witnessed in the presence of 30% ore alone and 30% ore in the presence10^8 cells/ml (see Appendix B - Figure B-5). These observations imply that absorption of SIBX onto the minerals in the ore sample (Wills and Finch, 2016) was more pronounced than the bioremoval achieved by the microbial cells.

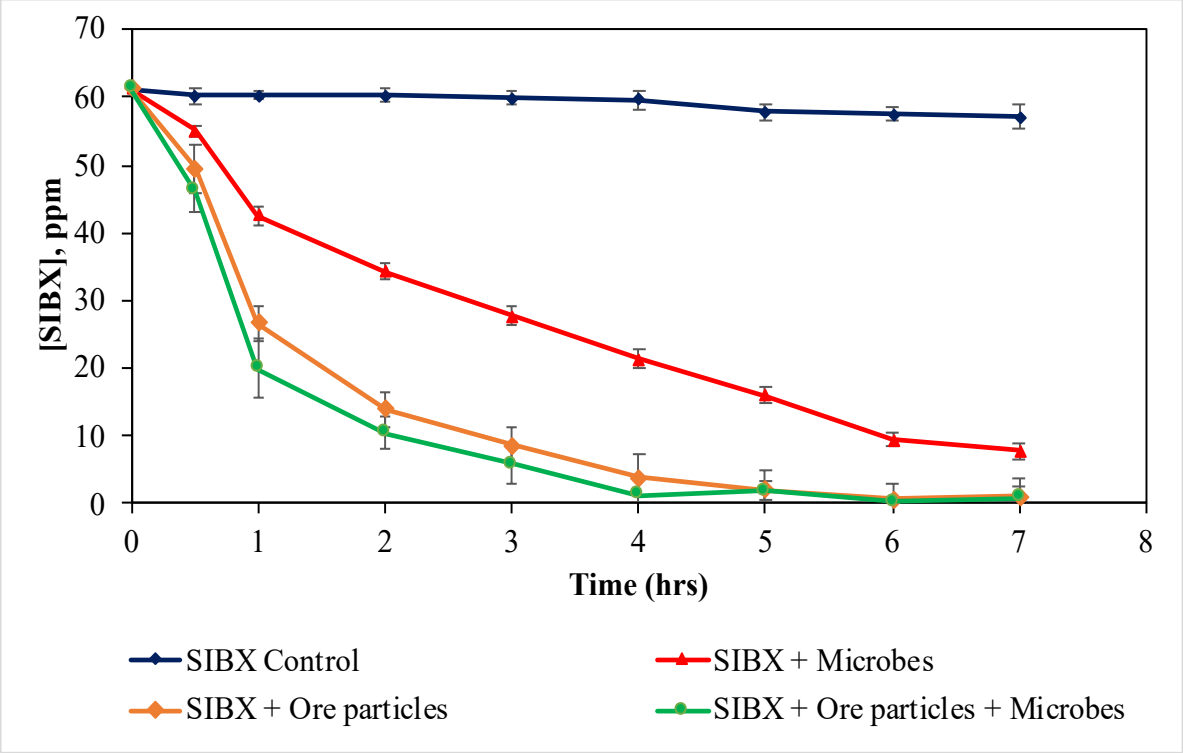

Figure 5-9: *SIBX concentration in the different microbial and ore systems at an initial concentration of 60 ppm and a microbial concentration of 10^8 cells/ml at a solids loading of 30 % over time. Each data point represents an average of three repeats.*

Although the volumetric rates for xanthate removal in the presence of ore alone and with ore and microbes appear to be similar, taken from the curves in Figure 5-9, the K values in Table 5-3 shows that a relatively higher rate was found in the presence of added microbes when compared to their absence in the ore particles system (between one and five hours). It is worth noting that the rates are additive, i.e., (SIBX + Ore particles + Microbes) = (SIBX + Microbes) + (SIBX + Ore particles) (Table 5-3). These findings could imply that in a system where both ore particles and microbes coexist, the rate of collector removal from solution would be higher than compared with that of a system containing ore particles only.

Table 5-3: *The gradient at linear portion and volumetric removal rate values (3 significant figures) in different SIBX systems at an initial SIBX concentration of 60 ppm as a function of time.*

Treatment	Time period (Linear portion)	Gradient (K)	Volumetric removal rate, ppm/hr
SIBX Control	0.5 - 4 hrs	0.003	0.17
SIBX + Microbes	0 - 7 hrs	0.29	7.63
SIBX + Ore particles	0 - 5 hrs	0.68	11.80
SIBX + Ore particles + Microbes	0 - 5 hrs	0.92	11.86

As previously reported (Section 5.4) higher microbial concentrations (10^9 cells/ml) resulted in greater collector removal (compared with) 10^8 cells/ml. From this finding it stands to reason that high microbial concentrations would mean that less xanthate is available to attach to mineral surfaces. In a study by Liu et al. (2013a), in which the effect of *E. coli* cells on chalcopyrite flotation was investigated, they suggested that when metallurgists encounter low mineral recoveries on site, when using microbial laden water for flotation operations, they usually need to increase collector dosages. From the present results, it can be suggested that in the presence of high microbial counts, the efficacy of collector adsorption onto the target mineral surface could be greatly decreased, due to bioremoval of xanthate. This could result in insufficient collector adsorption onto the target mineral, resulting in less hydrophobic particles/target mineral reporting to the concentrate, which in turn results in lower valuable mineral recovery. The findings from this study suggest that in the presence of both ore particles and microbial cells, xanthate removal is accelerated. As such, xanthate availability for mineral attachment would be limited due to bioremoval. The microbial community is able to facilitate the bioremoval of xanthate from solution, suggesting that an increase in collector dosage would circumvent/remedy the detrimental effect.

5.7 Xanthate analysis in the presence of live and dead microbes

From the previous findings, (see Section 5.5 where it is concluded that xanthate does not adsorb to the microbes) it is postulated that the dominant mechanism responsible for the removal of xanthate from the solution is via the active degradation of xanthate by microbial action. The following experiment, to prove this hypothesis, used dead microbial cells (to determine whether the dead microbial cells/solid surfaces would still permit the removal of xanthate from solution.

Figure 5-10 and Figure 5-11 shows the residual xanthate available in the presence of live and dead microbial cells - killed by autoclaving (Section 3.1.1). As can be seen from the figures, the concentrations of SEX and SIBX remained the same throughout the six hours in the absence of microbes (control test). Complete xanthate removal occurred in the presence of live microbes over a two hour period (see Appendix-B- Figures B-6 and B-7). In contrast Figure 5-10 and Figure 5-11 show a slow decrease in xanthate concentration in the presence of dead microbes. Thus dead microbial cells appear to facilitate slow xanthate removal, possibly via adsorption of xanthate onto the residual cell debris; complete xanthate removal was not observed within the time of the experiment.

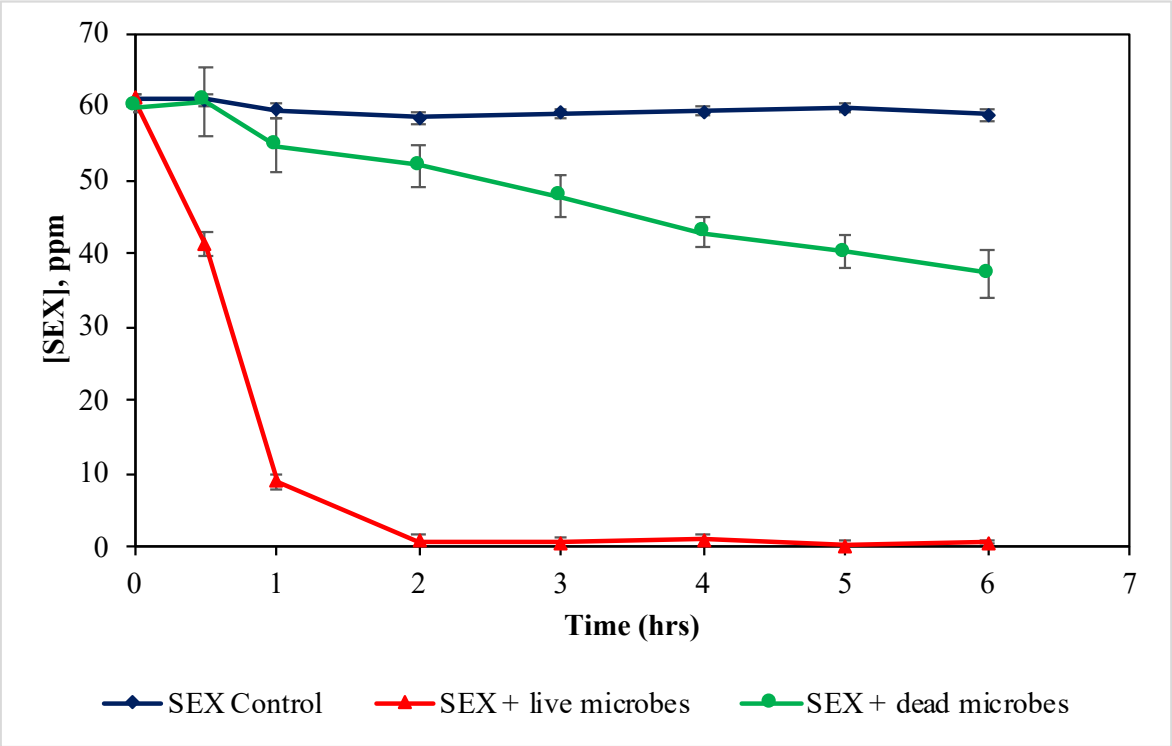

Figure 5-10: *SEX concentration at an initial concentration of 60 ppm in the absence and presence (live and dead) of microbes (10^9 cells/ml) over time. Each data point represents an average of three repeatsand the error bars depict standard error of the mean.*

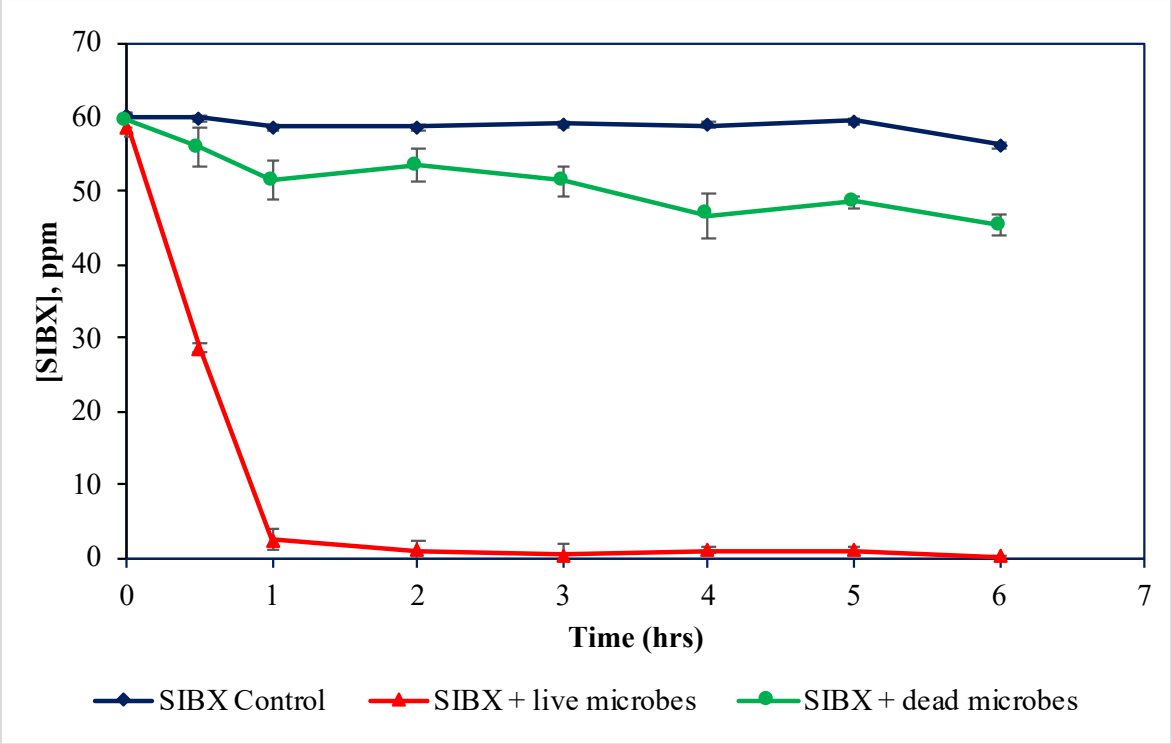

Figure 5-11: *SIBX concentration in absence and presence of live and dead microbial cells (10^9 cells) as a function of time. The starting concentration was 60 ppm SIBX. Each data point represents an average of three repeats. Error bars were not clearly visible at the scale used.*

Regarding the K values and volumetric removal rate depicted in Table 5-4, it is apparent that the K values and volumetric removal rates were higher in the presence of dead microbes compared to in their absence, suggesting bioremoval of xanthate is facilitated by dead microbes.This supports the findings in Figure 5-10 and Figure 5-11 that dead microbes facilitate the removal of xanthate from solution, or be it at a slow rate. The K values and volumetric removal rate was much higher in the presence of live cells compared with dead cells and their absence (Control) (Table 5-4) suggesting that there is some adsorption and natural degradation facilitated by the microbial cells with significant biodegradation in the presence of an active microbial culture. It is worth pointing out that autoclaving could have resulted in cell lysis and release of intracellular components which could have also facilitated xanthate bioremoval.

Table 5-4: *Gradient (K) and volumetric removal rate (3 significant figures) in the absence and presence of microbes at a microbial concentration of 10^9 cells/ml. The starting concentration was 60 ppm SIBX in both the absence and presence of microbes.*

Treatment	Timeperiod (Linear portion)	Gradient (K)	Volumetric removal rate, ppm/hr
SEX control	0 - 6 hrs	0.004	0.01
SEX live	0.5 - 3 hrs	1.69	16.22
SEX dead	0 - 6 hrs	0.083	3.79
SIBX control	0 - 6 hrs	0.003	0.38
SIBX live	0 - 3 hrs	1.55	14.42
SIBX dead	0 - 4 hrs	0.04	3.22

As would be expected, dead microbes do not consume the collector as a carbon source or actively biodegrade the collector. Heat killed microbial cells could only facilitate xanthate removal through adsorption onto microbial surfaces or onto microbial breakdown products of autoclaving.

5.8 Microbial cell hydrophobicity (Microbial Adhesion to Hydrocarbons – MATH)

The relative microbial cell hydrophobicity was determined using a two-phase partitioning protocol using the organic solvent hexadecane ($C_{16}H_{34}$) and 1x PBS as aqueous phase. The protocol developed by Natarajan and Das (2003) was followed and performed in triplicate. The microbial cells were subjected to hexadecane for 30 min. The portion partitioned to the aqueous phase (1x PBS) was quantified by UV-Vis spectroscopy at 600 nm and direct count using microscopy. The partitioning of microbial cells to the organic phase was evaluated by Equation 5-1.

$$\mathbf{A\ \%} = [(A_1 - A_2)/A_1] \times 100\ \% \qquad \text{Equation 5-1}$$

where **A%** is the percentage of microbial cells partitioning to the hexadecane (organic phase), A1 is the initial absorbance and A2 is the absorbance after 30 min interaction with hexadecane.

Table 5-5: *OD at 600 nm measuring cell concentration resuspended in 1x PBS before and after partitioning with hexadecane for 30 mins.*

	Absorbance (A)	1	2	3	Average
0 min	A1	0.371	0.371	0.370	0.371
30 min	A2	0.347	0.343	0.344	0.343

As previously stated in **Equation 1**, the percentage of the microbial cells adhering to the organic phase was evaluated as follows,

A% = [(A1-A2)/A1] x 100 % = **7.56%** (0.63)

The percentage value represents an average of three percentage repeats (with the standard error noted in brackets).

The calculated A% indicates that only **7.56%** of the microbial cells partitioned with the organic phase (hexadecane). This indicates that the remaining microbial concentration (**92.44%)** remained in the aqueous phase. Similar results were achieved when cell numbers from direct cell counting by microscopy was used to calculate A%. This suggests that the microbial cell community is largely hydrophilic. These results complement the recent findings of Mhonde et al. (2020) who carried out micro-flotation with the same microbial community and suggested that the microbial community may have adhered to mineral surfaces and induced hydrophilicity on sulphide mineral surfaces resulting in mineral depression. The MATH test was repeated after subjecting the microbial cells to SIBX for 30 mins, to determine if exposure to collector may increase their hydrophobicity. Table 5-6 shows absorbance at 600 nm in the hexadecane and aqueous phases following exposure to SIBX for 30 mins and allowing partitioning of hydrophobic cells to the hexadecane phases for 30 mins.

Table 5-6: *OD at 600 nm measuring cell concentration resuspended in 1x PBS before and after partitioning with hexadecane for 30 mins with an initial 30 min 60 ppm SIBX contact time.*

	Absorbance (A)	1	2	3	Average
0 min	A1	0.371	0.371	0.370	0.371
30 min	A2	0.326	0.340	0.340	0.335

A% = [(A1-A2)/A1] x 100 % = **9.53% (**1.30**)**

The percentage value represents an average of three percentage repeats (with the standard error noted in brackets).

As shown by A%, only a slight increase in hydrophobicity (**1.97%**), **9.53%** is reported in the presence of SIBX. The standard error calculated for this value suggests that no significant difference in the hydrophobicity of the cells was induced in the presence of SIBX. Further, a T-test was conducted between the resultant OD in the absence and presence of SIBX. The critical value (p value) was found to be greater than 0.05 (0.404) hence the null hypothesis is true i.e., strong evidence towards the null hypothesis which implies that the difference between the two treatments is statistically insignificant. Deo and Natarajan. (1998) subjected *B. polymyxa* to a pre-treatment with SIBX prior to flotation and observed a marked increase in *B. polymyxa* floatability, suggesting that SIBX (collector) interaction with the microbial cell surface increased the cell hydrophobicity. This does not appear to be true for the microbial community used in this study.

5.9 Conclusions

This study has shown that the microbial community is able to grow in the presence of xanthate collectors (SEX and SIBX) and simultaneously aid in xanthate removal from solution. Despite the fact that microbes can grow in the presence of xanthate, xanthate negatively impacted microbial growth by extending the lag phase; this effect was more pronounced in the presence of SEX when compared to SIBX. Moreover, higher xanthate concentrations could not support microbial growth as shown in the case of 480 ppm SEX.

In order to better understand the mechanism by which the microbial cells facilitate xanthate removal from solution, it was necessary to conduct xanthate analysis at a fixed xanthate concentration (60 ppm) in different harvested microbial cell concentrations. This study was carried out in parallel with a carbohydrate assay of the contacted microbial cells. Considering the effect of xanthate on the harvested microbial cells, it was found that microbialcells enabled the removal of xanthate from solution, a phenomenon which was predominately dependent on microbial cell concentration. No increase in the cell carbohydrate content or microbial biomass was reported, suggesting that these microbes could not utilize xanthate as a carbon source.

The findings presented in Chapter 5 strongly suggest that the main mechanism by which these microbial cells facilitated xanthate removal from solution was through active degradation.

Moreover, it was shown that in a system where ore and microbial cells coexist, collector absorbed onto the mineral surfaces at a faster rate than the microbially mediated bioremoval rate.

CHAPTER 6

6 FLOTATION RECYCLING EXPERIMENTS

This chapter investigates the effect of microbial load and water reuse on the flotation performance of a Cu-Ni PGM bearing ore. The effects of microbial load and water reuse were evaluated using four metallurgical performance indicators: water recovery, solids recovery, metal recovery and concentrate grade.

6.1 Introduction

There is limited scientific literature regarding the effect of microbes that naturally prevail in flotation process waters on flotation. Different microbial communities are introduced to the flotation process from various sources including the ore material (Smith and Miettinen, 2006), the reused water and the top up water, which could originate from poorly or partially treated water sources such as treated domestic water (Levay et al., 2001 and Slatter et al., 2009). These sources have been widely acknowledged to contribute microbial contamination to mineral processes (Bomberg et al., 2020) and it appears that the microbial cells occurring in flotation waters can significantly affect flotation (Levay et al., 2001; Slatter et al., 2009; Evdokimova et al., 2012; Liu et al., 2013a, 2013b, 2013c; Liu et al., 2016; Bomberg et al., 2020), although the effect of microbes on flotation is usually overlooked in most controlled flotation studies, where microbial load may be low or negligible due to the use of laboratory prepared process water (synthetic water).

Several studies have focused on the effects of inorganic constituents or chemical parameters present in flotation water, such as ions, and thus the effects of these are better studied and understood compared with organic components such as microbes (Rao and Finch, 1989; Muzenda, 2010; Corin et al., 2011; Ikumapayi et al., 2012; Manono et al., 2013, 2016; Lutandula and Mwana, 2014; Shengo et al., 2014; Linet al., 2019). In instances where the effects of organic constituents on flotation, such as microbial cells, have been reported, often single pure microbial strains have been utilized, which do not necessarily represent the microbes present in real mine waters (Evdokimova et al., 2012; Liu et al., 2013b, 2013c, 2016). Generally, these studies have used pure single minerals and not real ore, which is more representative of a flotation system (Mhonde etal., 2020). Motivated by this, the present study considers a more pragmatic approach in which the effects of a naturally occurring mixed microbial consortium, harvested from a platinum mine concentrator, is considered in the context of the flotation of a complex Cu-Ni PGM bearing ore

6.2 Experimental approach

Flotation recycling experiments were conducted to determine the impact of water reuse within flotation systems. To simulate a flotation circuit and allow greater contact time between water components and the ore, flotation recycling experiments were performed (Section 3.2.11).

During a general laboratory flotation test, the traditional contact time between the water and ore extends to approximately 20 mins. However, when considering the progression of ore and water through a flotation circuit, the contact time between flotation waters and ore may extend to hours. To extend the contact time 2 hours was allowed between flotation waters and ore prior to the flotation tests. A detailed flow chart of all the components added into the flotation cell during this experiment is given in Chapter 3 - Figure 3-3.

For Experiment 1 (Exp 1) fresh finely ground ore was milled and floated with 1 SPW (Table 3-1). The concentrates and tailings from Exp 1 were dewatered, combined and used as feed for Experiment 2 (Exp 2). The concentrates and tailings from Exp 2 were dewatered, combined and used as feed for Experiment 3 (Exp 3). In addition to the reuse of the ore, water recovered from Exp 1 was used for Exp 2 to simulate the reuse of water in a flotation circuit. During Exp 1, three floats were performed to allow the recovery of enough water and combined ore (concentrate and tailings) for Exp 2. Exp 2 was performed in duplicate, and Exp 3 was carried out as a single flotation experiment due to the loss of ore and water during the recycling.

To determine the effect of the presence of microbes on flotation and the reuse of microbial laden waters, recycling experiments were performed in the absence (Control) and presence of microbes (+ microbes). The control recycling test was conducted using 1 SPW, while the test in the presence of microbes was conducted with 1 SPW, supplemented with the microbial consortium harvested from a Cu-Ni PGM bearing ore concentrator circuit at a final concentration of $2x\ 10^8$ cells/ml (Section 3.2.1). (No additional microbes were introduced during the flotation study). The two base metals used to evaluate recovery and grade were Copper (Cu) and Nickel (Ni), which were derived from chalcopyrite and pentlandite, respectively, within the ore. Owing to the strong association of PGMs with base metals, it is widely acknowledged that the flotation behaviour of base metals serves as a proxy for the flotation behaviour of the PGMs (Wiese et al., 2005). Microbial quantification was conducted during the 2-hour incubation period and again following the recovery of the concentrate and tailings fractions, using two different methods: FDA for ore slurry samples and cell plating for water samples. Microbial concentration was inferred from microbial activity measured by FDA assay (Section 3.1.5.2) and colony forming units, determined by plating on both nutrient rich medium, Nutrient agar (NA) and minimal, Autotrophic Basal Salts (ABS) agar (Section 3.1.5.4).

All results obtained were evaluated using standard error analysis, which is presented as error bars in the graphs. In some cases, the error bars were too small and therefore not visible at the scale used. In the case of Experiments 2 and 3, the standard error could not be calculated due to the lack of biological replicates. Unless stated otherwise, all the experiments which were carried out in the absence of deliberate addition of microbial cells (referred to as "in the absence of microbes"), while those with deliberate addition of microbes are termed "in the presence of microbes" or "+Microbes". Besides the microbial interactions with the minerals and their subsequent effects on flotation, there are multifaceted interactions which occur between flotation reagents and minerals. It is for this reason that the tests were conducted with only collector and frother, no other flotation reagents were added. .

6.3 Results and discussion

6.3.1 Water and solids recoveries

The effect of the presence of microbial cells at an initial concentration of 2x 10^8 cells/ml on water and solids recovery was evaluated. In addition, the effect of water reuse on flotation performance in both the absence and presence of microbial cells was evaluated. The recorded water and solids recovery are presented as curves in Figure 6-1, Figure 6-2 and Figure 6-3 and as a comparative bar graph (Figure 6-4).

Figure 6-1 depicts water recovery with flotation time for all the experiments conducted, both in the absence and presence of microbes. A marked increase in water recovery was recorded across all tests with continuing water reuse. This may be attributed to a build-up of frother, as additional frother dosages were added to each recycling experiment. Tests performed in the presence of microbes recorded higher water recoveries relative to the control experiments, suggesting that the microbial cells may have affected frothability within the system.

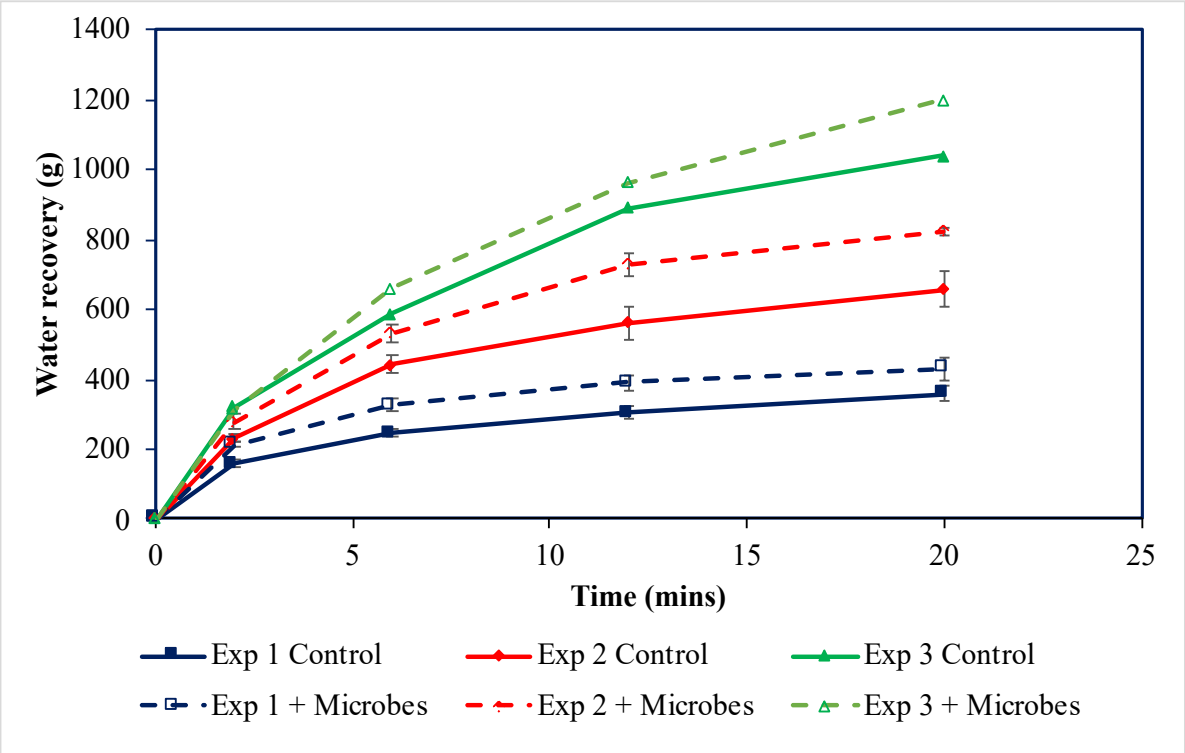

Figure 6-1: *Water recovery for all the recycling experiments in the absence and presence of microbial cells (2x 10^8 cells/ml) per flotation time of a Cu-Ni PGM bearing ore. Microbes were only dosed in "Exp 1 + Microbes" and subjected to recycling. Error bars represent standard error between triplicate tests for Exp 1 and standard deviation for duplicate tests for Exp 2, For the third experiment no duplicate or triplicate floats could be performed, and thus there was no evaluation of error in Exp 3. Apart from Exp 3, where error bars cannot be seen, they are smaller than the data marker.*

The effect of increased water recovery upon water reuse, assumed to be due to the accumulation of frother, was more pronounced in the presence of microbes when compared to the increased water recovery measured in the absence of microbial cells. The increase in water recovery from Exp 1 to Exp 3, is assumed to be as a result of frother accumulation, although microbes appeared to increase water recovery over and above the accumulated frother. A comparison between the effect of microbes only and reuse (residual frother) only on water recovery was performed. The difference in water recovery between the Exp 1 Control and the Exp 1 + Microbes, (microbe only effect) was compared to the difference in water recovery between the Exp 1 Control and the Exp 2 Control (reuse effect). The effect of reuse, attributed to residual frother accumulation, on water recovery was nearly fourfold (297.7 g) of that of the increase in water recovery measured in the presence of microbial cells (71.7 g). When comparing water recoveries in Exp 2 Control (297.7 g) and Exp 2 + Microbes (394.5 g), a synergistic effect of reuse and microbial presence on water recovery is suggested.

Figure 6-2 shows solids recovery against flotation time. Like the water recovery trend, the solids recovery trends show that with more water reuse, more solids are recovered and the microbes add to the solids recoveries.

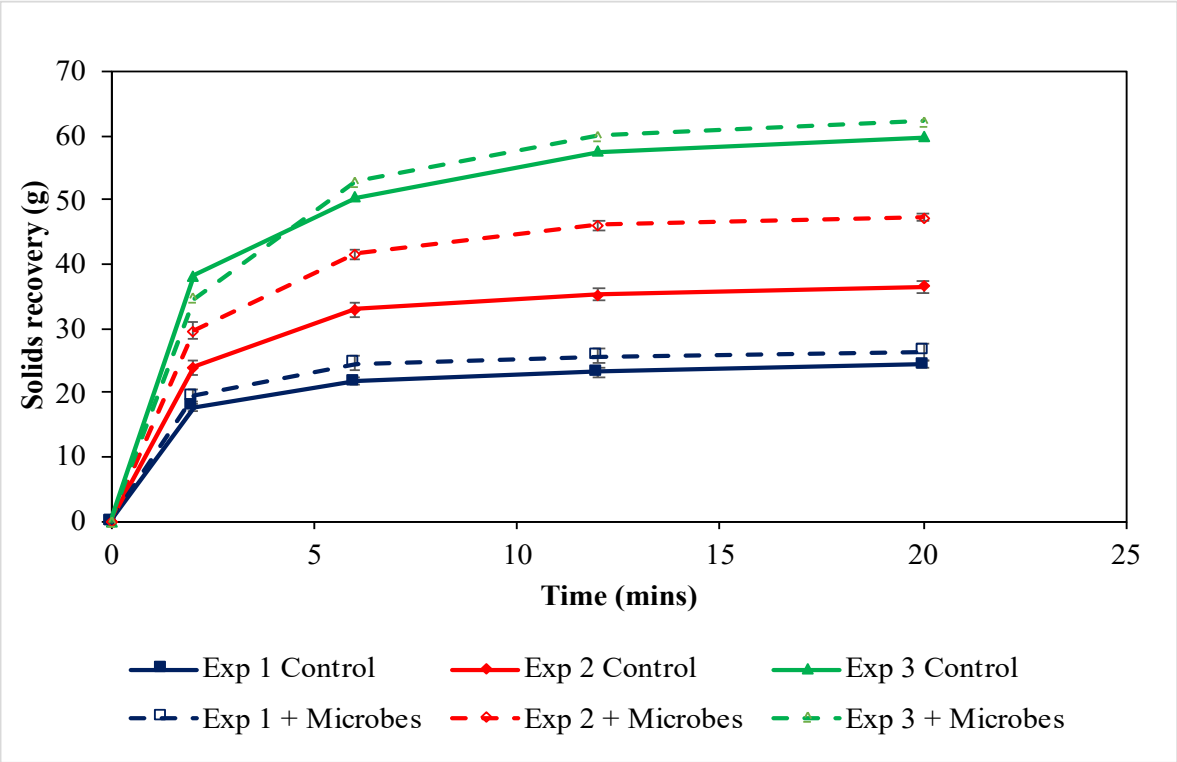

Figure 6-2: *Solids recovery vs flotation time of a Cu-Ni PGM bearing ore in the absence and presence of microbial cells (2x 10^8 cells/ml) in Exp 1 + Microbes only and thereafter subjected to recycling. Error bars represent standard error between triplicate tests for Exp 1 and standard deviation betweenduplicate tests for Exp 2. There was no evaluation of error in Exp 3. Apart from Exp 3, where error bars cannot be seen, they are smaller than the data marker.*

Figure 6-3 depicts cumulative solids recovery as a function of cumulative water recovery. The general trend observed is that high water and solids recoveries were obtained across all tests in the presence of added microbes when compared to the control experiments. It can be deduced that, higher water recoveries coincided with higher solids recoveries across all experiments, possibly due to an increase in total entrained material/solids as the water recoveries increased. Overall, the results show a marked increase in water and solids recoveries as the water was reused multiple times (in both the absence and presence of microbes).

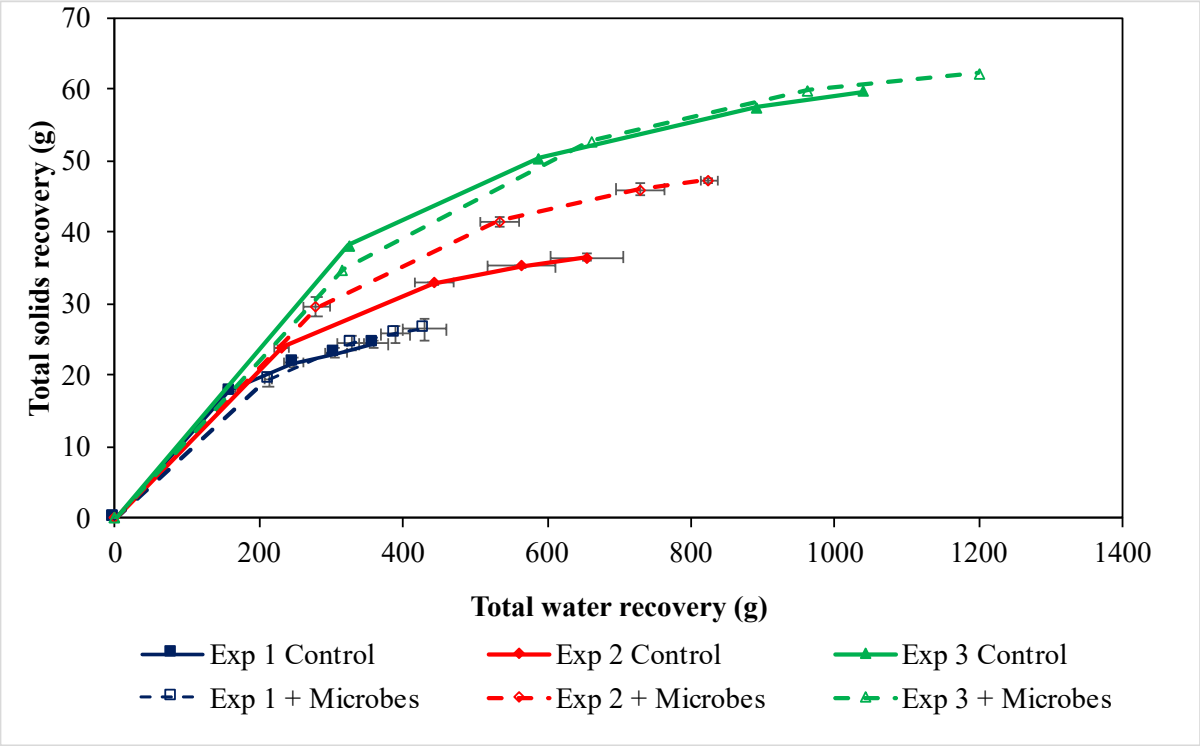

Figure 6-3: *Solids recovery vs water recovery in the absence and presence of deliberately added microbial cells during recycling tests. Error bars represent standard error between triplicate tests (Exp 3) and standard deviation between duplicate tests in Exp 2 and standard deviation between duplicate tests for Exp 2. There was no evaluation of error Exp 3.*

Figure 6-4 shows a summarized graph of the effect of added microbial cells on total water and solids recovery for all experiments. Generally, the observed trend is that cumulative water and solids results show that both were higher in the presence of microbes and both total water and solids recoveries increased during water reuse in both systems (in the absence and presence of microbes).

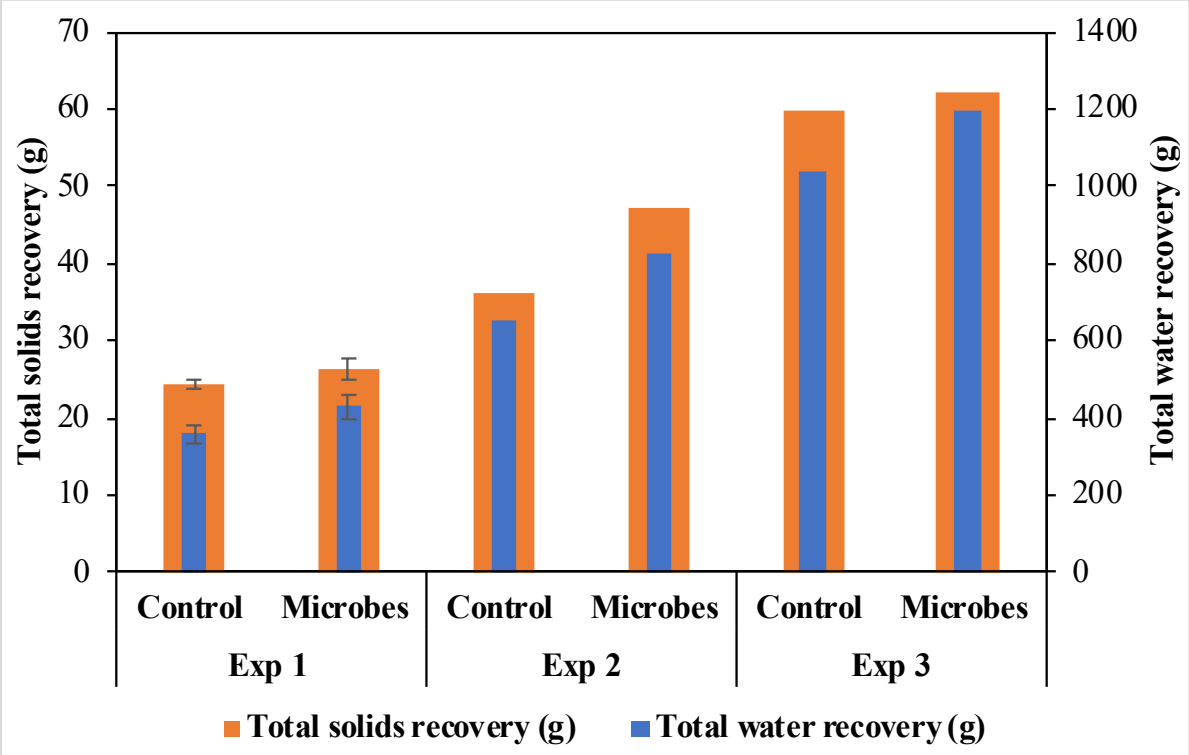

Figure 6-4: *Total solids recovery vs total water recovery in the absence and presence of microbial cells for the recycling experiments. Error bars represent standard error between triplicate tests (Exp 1). There was no evaluation of error in Exp 2 and 3.*

The results presented above suggest that with increased water reuse, increased water and solids are recovered. This effect is enhanced with the presence of microbial cells in the system. As reported by other authors, water recovery is a good indication of froth stability (Wiese, 2009, Wiese et al., 2011; Corin et al., 2011; Biçak et al., 2012). Since new frother was dosed at the beginning of each test, there was a possibility of residual frother accumulating in the system, which translates to an increase in frother concentration within the reused waters. Accordingly, the residual frother in the reused water could have resulted in pronounced frothability, resulting in an increase in water recoveries as the water reuse progressed.

Process water in flotation circuits always contains organic materials, such as frother, which affect froth stability (Farrokhpay and Zanin, 2012). It has been widely acknowledged that an increase in frother concentration results in finer bubbles, owing to reduced surface tension and inhibition of bubble coalescence, hence increasing froth stability (Cho and Laskowski, 2002). Similar findings were made by Aldrich and Feng. (2000); Corin and Wiese.(2014) who indicated that froth stability increased with increasing frother concentration.

Tests performed in the presence of microbes recorded higher water recoveries relative to the control experiments, suggesting that the microbial cells may also have an effect on frothability within the

system. It has been established that a number of microorganisms can produce biosurfactants (Khoshdast et al., 2012). Thus, the increased frothability could be attributed to the fact that microbial cells and their products may act as surface-active compounds and therefore reduce the surface tension of water. This is suggested by the results of Khoshdast et al. (2012) who investigated the effect of rhamnolipid, a biosurfactant (of microbial origin), on surface tension and found that rhamnolipid drastically lowered the surface tension of water from about 70 to 30 mN m^{-1}. Furthermore, while investigating chalcopyrite flotation in the presence of mineral adapted bacterial cells as bio-collectors, Sharma and Rao (1999) showed that excessive frothing occurred, confirming the impact of microbes on frothability. In a recent paper by Mhonde et al. (2020) in which the same microbial community was used as in this study, micro-flotation tests also suggested that the microbes, and/or their metabolic products, may have acted as bio-frothers.

Increased water reuse may have facilitated the dissolution of certain ions from the ore material, which could have influenced gangue activation, resulting in more solids being pulled to the concentrate launder. For example, while spiking feed water with $HCO3^-$, Lutandula and Mwana (2014) found that the $HCO3^-$ ions promoted entrainment of gangue minerals. Moreover, it has been illustrated that the presence of elevated amounts of certain ions, such as Ca^{2+}, Mg^{2+}, Cl^- and $SO4^{2-}$ in the flotation system, possibly due to water reuse, results in increased froth mobility, which increases the rate at which both the solids and water are recovered (Manono etal., 2016).

Even though the solids concentration increased with water reuse across the experiments, it is apparent that a greater number of solids were recovered in the presence of microbes when compared to their absence in the system. In addition to the increase in froth stability, which may be caused by microbes and their metabolic products as explained above, another possible explanation for this finding could be that the microbial cells activated the gangue minerals, similar to the action proposed for increased ions. This suggestion agrees with the observations made by Sharma and Rao (1999), in which they observed increased pyrite floatability, a gangue mineral, in the presence of microbial cells.

6.4 Base metal recoveries and grade

The base metal recoveries and grades are presented in the following sections. Chalcopyrite recovery and grade are represented by Cu while pentlandite recovery and grade are represented by Ni. To quantify the effect of water recovery on metal recovery, metal recovery vs water recovery plots were plotted in addition to metal recovery vs time plots.

6.4.1 Cu recovery and grade

The effects of microbial cells and water reuse on Cu recovery and grade were quantified. Figure 6-5, Figure 6-6; Figure 6-7 and Figure 6-8 presents Cu recovery vs flotation time, Cu recovery vs water recovery, grade vs recovery and recovery-grade cumulative bar graphs, respectively.

Figure 6-5 and Figure 6-6 show that most of the Cu is recovered in the first concentrates across all the tests, with little chalcopyrite being recovered in the subsequent concentrates. A higher percentage of Cu was recovered in the initial concentrate in Exp 1 Control compared with Exp 1 + Microbes or any of the water reuse tests performed subsequently (Figure 6-5). Cu recovery over time graphs for the reuse tests (Exp 2 and Exp 3), with and without the addition of microbes, showed similar trends (Figure 6-5).

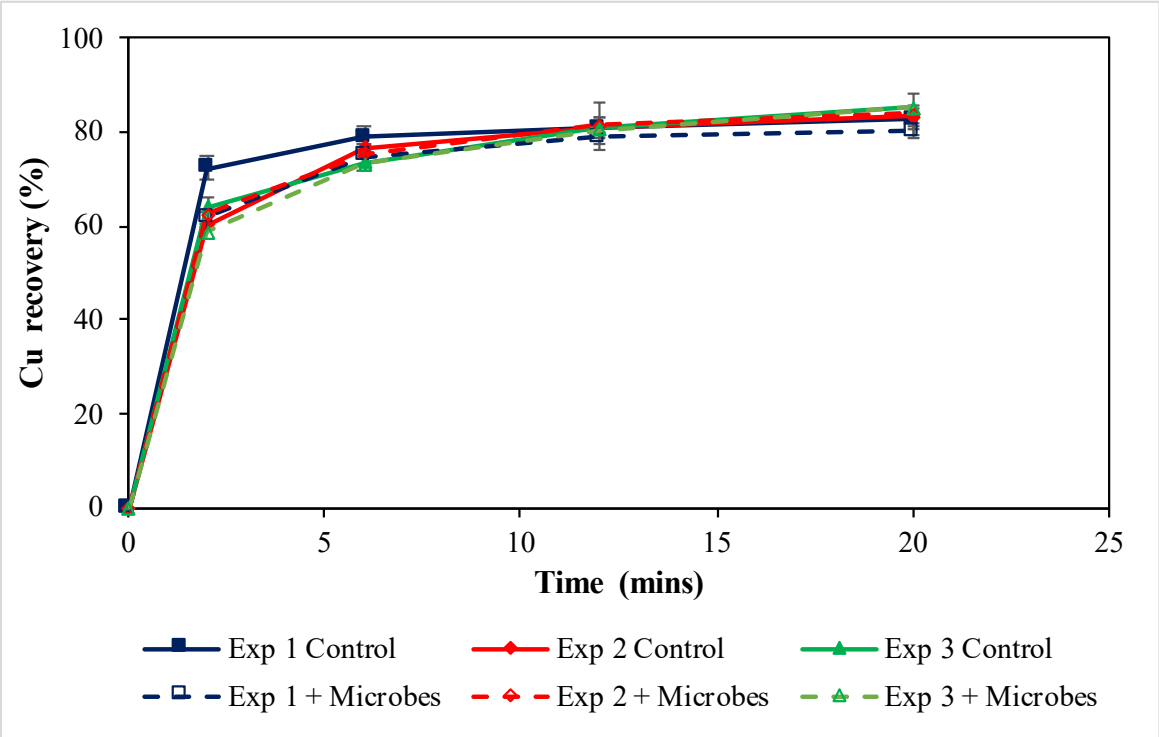

Figure 6-5: *Cu recovery vs flotation time in the presence and absence of microbial cells during flotation recycling tests. Error bars represent standard error between triplicate tests (Exp 1) and standard deviation between duplicate in Exp 2. There was no evaluation of error in Exp 3.*

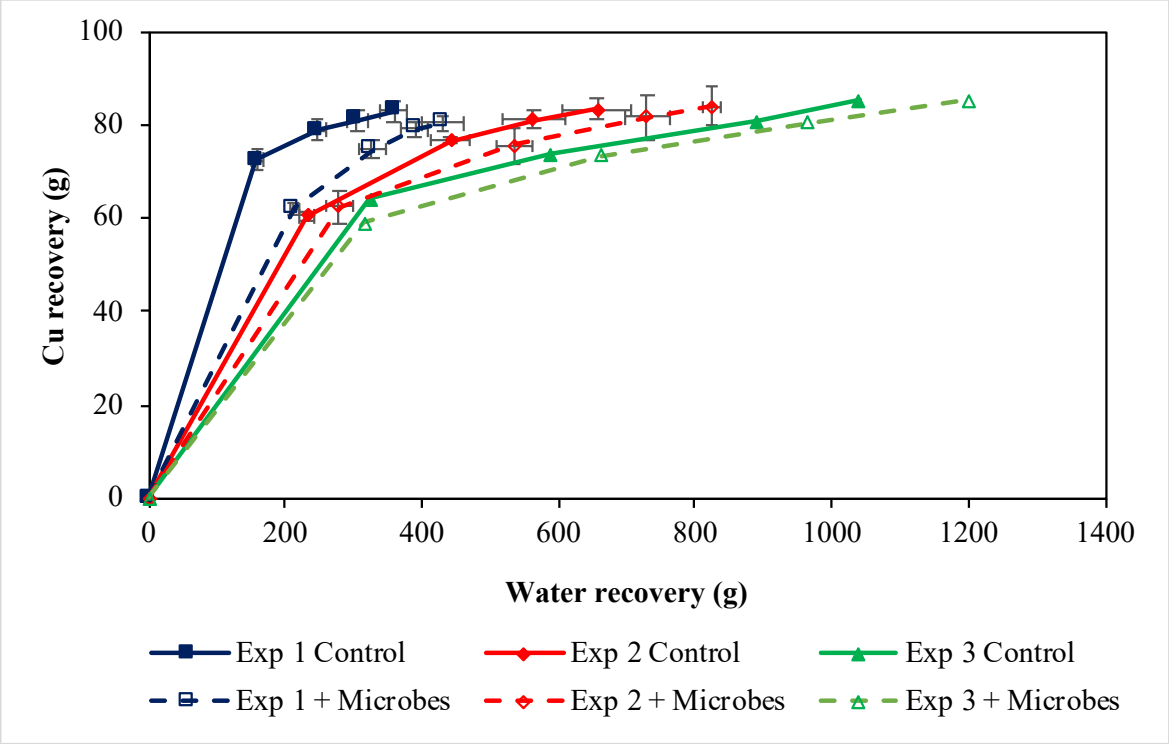

Figure 6-6: *Cu recovery as a function of water recovery in the absence and presence of microbial cells for the recycling experiments. Error bars represent standard error between triplicate tests (Exp 1) and standard deviation between duplicate in Exp 2. There was no evaluation of error in Exp 3.*

Tests performed in the presence of microbes corresponded to higher water recoveries (g) per % Cu recovered across all tests, with the lowest water to Cu recovery (g/%) reported for the Exp 1 Control (Figure 6-6). It is apparent that as the recycling test progressed, higher water recoveries were recorded at each final Cu recovery % in the absence and presence of microbes. For the test performed in the absence of microbes (Controls), final Cu recovery % was achieved at lower water recoveries compared to in the presence of microbes (+ Microbes).

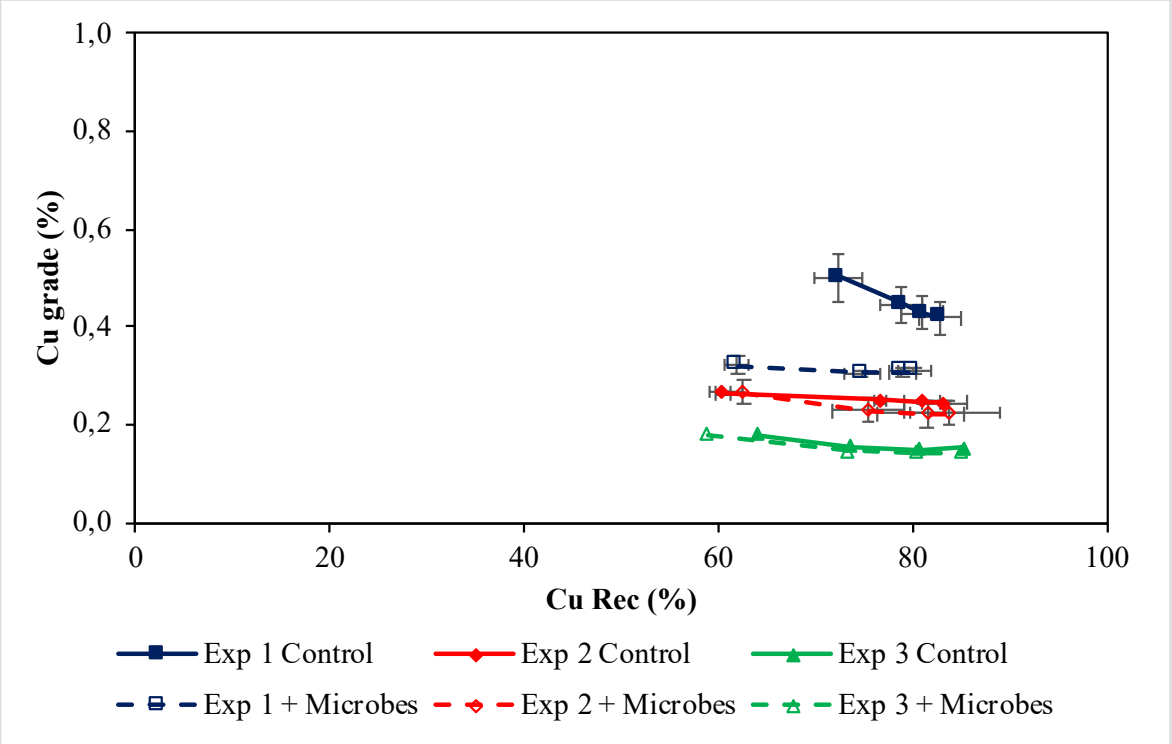

Figure 6-7: *Cu grade vs Cu recovery in the absence and presence of microbial cells for the recycling experiments. Error bars represent standard error between triplicate tests (Exp 1) and standard deviation between duplicate in Exp 2. There was no evaluation of error in Exp 3.*

Figure 6-7 shows Cu grade vs Cu recovery. It is evident that a relatively similar Cu recovery was yielded in the absence and presence of added microbes for the reuse experiments, Exp 2 and 3. However a much lower Cu grade was recovered in the presence of added microbes (Exp 1 + Microbes) relative to the control (Exp 1 Control) for all four concentrates recovered in Exp 1. A steeper slope can be seen for the Exp 1 Control which shows a decrease in grade with an increase in recovery compared to Exp 1 + Microbes. As illustrated in Figure 6-7, no real change in Cu grade and recovery was reported for Exp 2 and 3 (in the absence and presence of microbial cells). These results are also presented and further discussed in Figure 6-8 as cumulative total grade recovery bar graphs.

Figure 6-8 provides cumulative Cu recovery and recovery in the absence of microbial cells and their presence while Table 6-1 illustrates the numeric values for Cu recovery and grade.

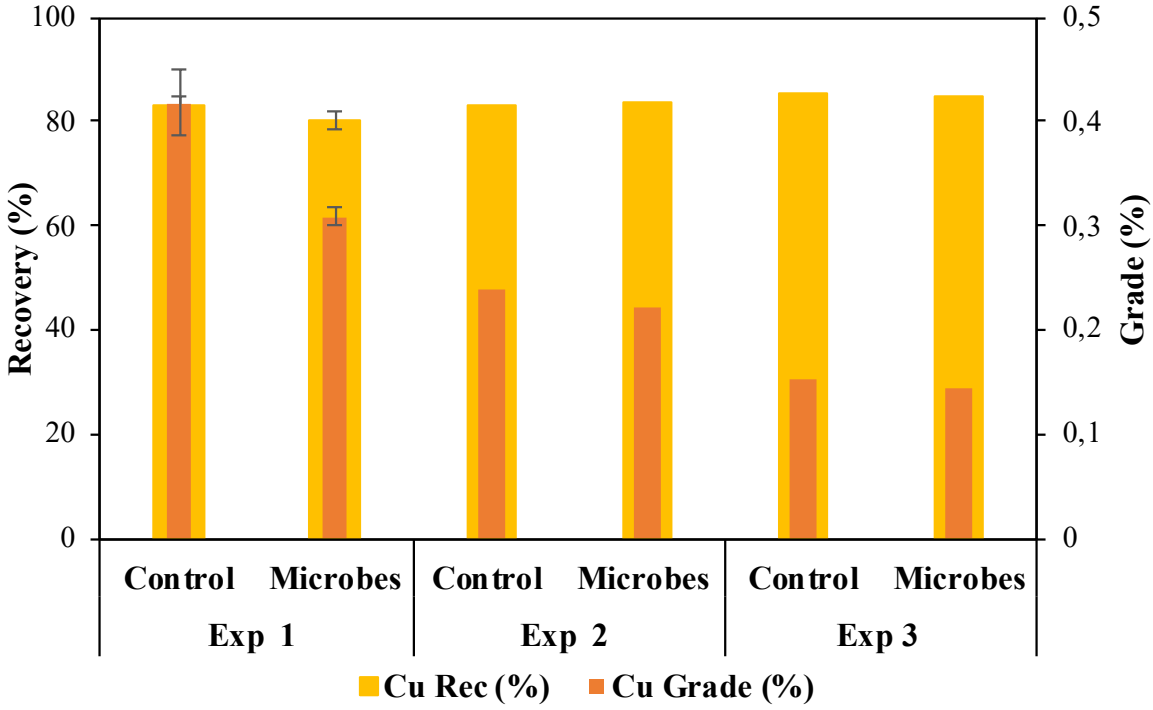

Figure 6-8: *Total Cu grade and total Cu recovery in the absence and presence of microbial cells for the recycling experiments. Error bars represent standard error between triplicate tests (Exp 1). There was no evaluation of error in Exp 2 and 3.*

Table 6-1: *Final percentage Cu recoveries and grades in the presence and absence of microbes for all the recycling tests.*

		Cu Rec (%)	Cu Grade (%)
Exp 1	Control	82.83 (2.08)	0.42 (0.03)
	Microbes	80.18 (1.58)	0.3 (0.01)
Exp 2	Control	83.21	0.24
	Microbes	83.78	0.22
Exp 3	Control	85.14	0.15
	Microbes	85.08	0.14

For Exp 1 the values represent an average of three repeats (with the standard error noted in brackets)

A higher final Cu grade was achieved in the absence of microbes relative to their presence for Exp 1, with this effect becoming less apparent as the ore and water was reused during the test (Figure 6-8 and Table 6-1). This suggests that the presence of microbes markedly affected Cu grade. The decrease in

metal grades on concentrator sites have been perceived to be the major detrimental effect posed by water reuse onsite.

The possible mechanism which may have accounted for the lower Cu grade in the presence of microbes could be that the microbial cells activated the gangue mineral which negatively affected Cu grade. This observation supports the work of Sharma and Rao (1999), in which they reported increased pyrite floatability in the presence of microbial cells; pyrite is a gangue mineral present in the ore used in the current study. As shown in Figure 6-3, high water recoveries coincided with high solids recoveries which may have decreased the Cu grade in the presence of microbes. Since the amount of water recovered is directly proportional to the amount of gangue mineral reporting to concentrate launder through entrainment (Subrahmanyam and Forssberg, 1988; Smith and Warren, 1989), it is expected that the enhanced frothability, induced by microbial cells, resulted in nonselective flotation which, in turn, resulted in the recovery of gangue minerals. Similar findings were attained by Evdokimova et al. (2012) who showed that microbial laden water negatively affected flotation of apatite, a non-sulphide mineral. The deterioration of floatability of apatite was ascribed to the bacterial cells interacting with the Ca containing mineral, thereby reducing the selectivity within the system.

Water reuse across all tests also affected Cu recovery. Cu recovery marginally increased in both the absence and presence of microbial cells as reuse progressed from Exp 1 to Exp 3 (Figure 6-8 and Table 6-1). This effect may have been a result of residual frother, which promoted water and mineral recoveries (Wiese et al, 2006). However, the marked increase in mineral recoveries (Figure 6-4) was accompanied by a notable decrease in Cu grade as the recycling continued (Figure 6-8 and Table 6-1). In general, it has been found that the higher the recovery of water, the higher the proportion of gangue and the lower the concentrate grade (Smith and Warren, 1989). A general conclusion which can be drawn from these findings is that Cu recovery remained in the range of 80% - 85% during water reuse i.e., Cu recovery did not appear to be negatively affected by water reuse. However, there was a substantial decrease in Cu grade with continued reuse due to the increased recovery of gangue material. The impact of microbial cells on the final Cu recovery and grade was most pronounced in Exp 1, where a reduction in Cu recovery and grade was reported (Figure 6-8 and Table 6-1). This finding shows that the microbial consortium in the water has a negative effect on flotation.

6.4.2 Ni recovery and grade

Flotation performance in the absence and presence of microbes was evaluated by quantifying pentlandite recovery and grade, which is represented by Ni.

Ni recovery as a function of time and water recovery was evaluated in the absence and presence of microbial cells as illustrated in Figure 6-9 and Figure 6-10.

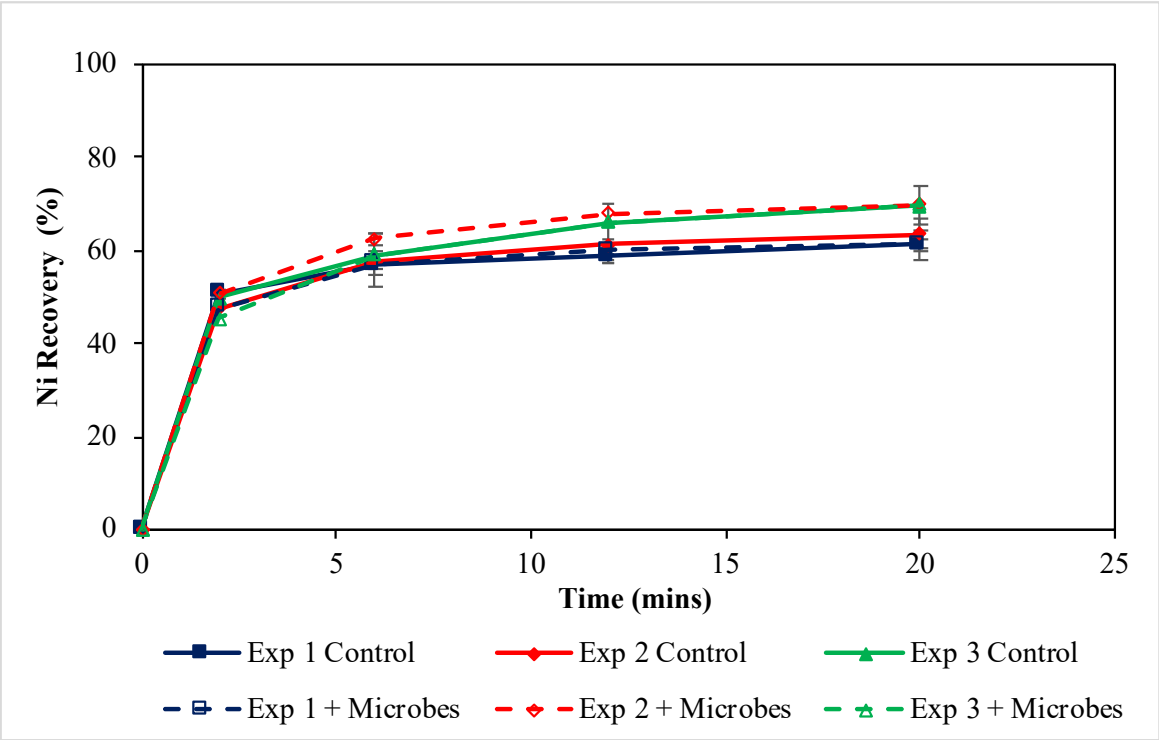

Figure 6-9: *Compares Ni grade as a function of time in the absence and presence of microbial cells for all the recycling tests. Error bars represent standard error between triplicate tests (Exp 1) and standard deviation between duplicate tests (Exp 2). There was no evaluation of error in Exp 3.*

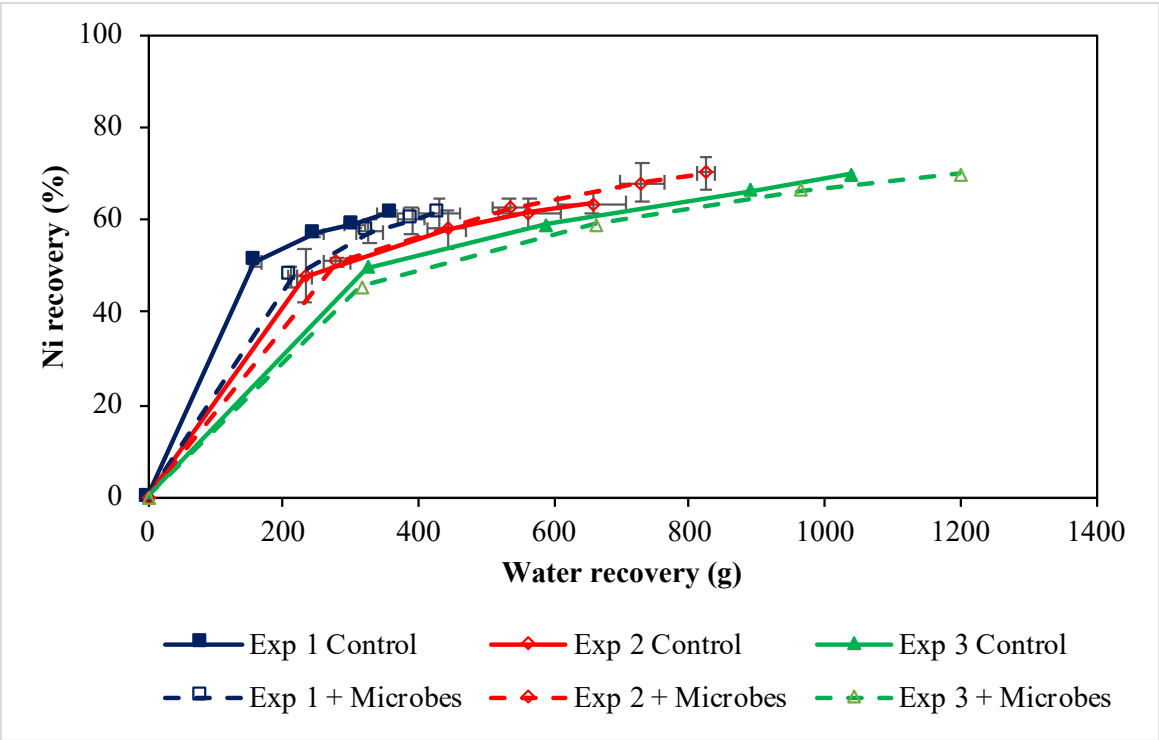

Figure 6-10: *Compares Ni recovery vs water recovery in microbial and non-microbial systems for the recycling tests. Error bars represent standard error between triplicate tests (Exp 1) and standard deviation between duplicate tests (Exp 2). There was no evaluation of error in Exp 3.*

Figure 6-9 illustrates Ni recovery as a function of time while Figure 6-10 depicts Ni recovery as a function of water recovery. In general, and as expected, Ni recovery increased with time across all experiments. The mineral recovery was highest in the first concentrate and then plateaus with a slight difference in Ni recovery for the rest of the flotation period. Like Cu, Ni recovery increased marginally with continuing water reuse for all tests (Figure 6-10). For the test conducted in the absence of microbes (Controls), final Ni recovery % was achieved at lower water recoveries compared to in the presence of microbes (+ Microbes) (Figure 6-10).

Figure 6-11 shows Ni grade as a function of Ni recovery. Ni recovery was relatively the same in the absence and presence of microbes for Exp 2 and Exp 3. In Exp 1 all four concentrates showed a lower Ni grade in the presence of microbes (Exp 1 + Microbes).

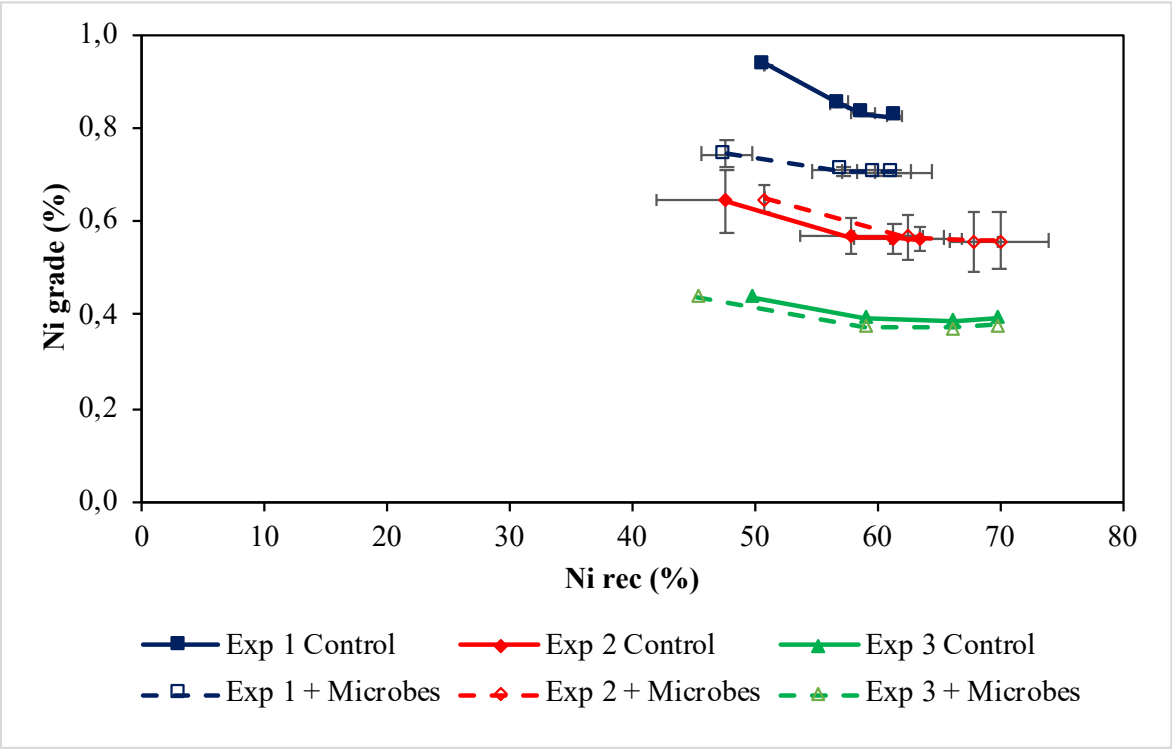

***Figure 6-11**: Ni grade vs Ni recovery in the absence and presence of microbial cells at an initial microbial concentration of 2x 10^8 cells/ml (Exp 1 + Microbes) for all recycling test tests. Error bars represent standard error between triplicate tests (Exp 1) and standard deviation between duplicate tests (Exp 2). There was no evaluation of error in Exp 3.*

Higher Ni recoveries were yielded in Exp 2 in the presence of microbes (Exp 2 + Microbes) in comparison to the control (Exp 2 control) across all four concentrates. No apparent difference in Ni recovery and grade was reported in the absence and presence of microbes in Exp 3 (Figure 6-11).

The effect of microbes on Ni flotation was evaluated by comparing Ni recovery and grade in the presence and absence of microbes as accumulative bar graphs in Figure 6-12. The Ni recoveries and grades are also presented numerically in Table 6-2.

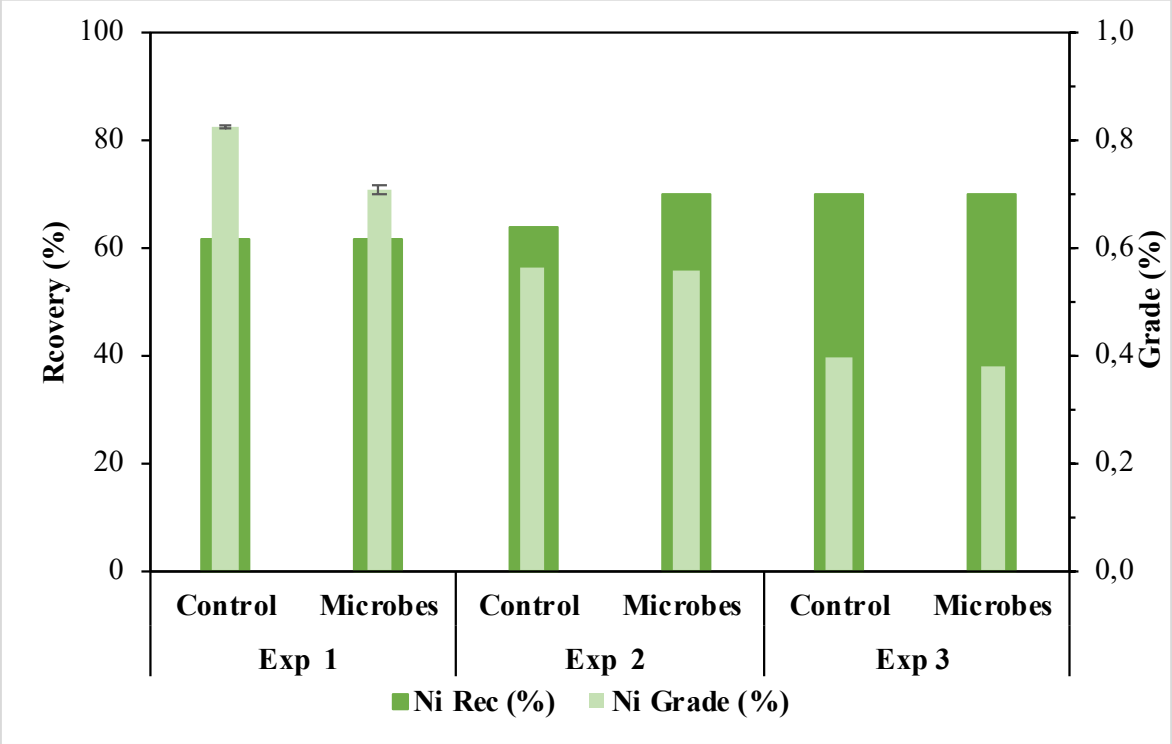

Figure 6-12: *Total Ni grade and total Ni recovery in the presence and absence of microbial cells for all the recycling tests. Error bars shows standard error between triplicate tests (Exp 1). There was no evaluation of error in Exp 2 and 3.*

Table 6-2: *Total Ni recoveries and grades in the presence and absence of microbes for all the tests. conducted.*

		Cu Rec (%)	Cu Grade (%)
Exp 1	Control	61.41 (0.75)	0.82 (0)
	Microbes	61.36 (3.08)	0.71 (0.01)
Exp 2	Control	63.52	0.56
	Microbes	69.97	0.56
Exp 3	Control	69.75	0.39
	Microbes	69.91	0.38

The values represent an average of three repeats for Exp 1 (with standard error noted in brackets).

In Exp 1 and 3, cumulative Ni recovery is relatively the same in both the absence and presence of added microbial cells. Pentlandite is slow floating (Chen et al., 1999; Bradshaw et al., 2005), and hence more susceptible to changes in the flotation chemistry (Wiese et al., 2005); however, its flotation was not

affected by the presence of microbial cells (Figure 6-12). Therefore, this shows that the presence of microbes had no apparent effect on the recovery of pentlandite.

Exp 2 reported slightly higher Ni recoveries in the presence of added microbial cells relative to the control, although this may be as a consequence of variation in the two replicate float experiments performed.

In considering cumulative Ni grade, higher cumulative Ni grade is seen in the absence of added microbial cells relative to the + Microbes in Exp 1 (Figure 6-12 and Table 6-2). It is therefore clear that pentlandite grade was negatively affected in the case of Exp 1 + Microbes. This agrees with observations by Levay et al. (2001) in which it was shown that Ni grade increased after the removal of organic species, such as microbes, from water. As in the case of Cu above, microbial facilitated gangue activation could have resulted in the lower Ni grade (Sharma and Rao 1999) in the concentrates recovered, due to the dilution of the concentrate grade. The detrimental effect of microbial laden water employed for flotation operations on the mineral grade recovered has also been reported by other authors (Evdokimova et al., 2012; Liu et al., 2013b).

Complementary to the effects of microbial cells on the flotation of pentlandite, Ni flotation was prone to changes posed by the water reuse effects as witnessed by changes in Ni recovery and grade with continuing reuse. The marginal difference (Table 6-2) which was observed in both instances (in the absence and presence of microbes) as the recycling progressed across all the tests was most possibly due to an increase in water and solids recovery (Figure 6-4). The mechanism by which water recovery may affect Ni mineral recovery and grade are comparable to those suggested for Cu (Section 6.4.1).

6.5 Microbial quantification during flotation water reuse experiments

For the water reuse tests, a 2-hour incubation period between water and ore was performed to simulate retention times in a flotation circuit, and to allow microbial cells to attach to the ore surfaces. Microbial quantification was monitored at designated intervals during the 2-hour incubation and the flotation period. To reduce experimental errors and allow the quantification of microbes within an ore slurry (Chapter 4), two microbial quantification protocols were employed: fluorescein diacetate (FDA) assay, to determine microbial activity within water and slurry fractions, and plating of the water fraction onto agar plates, to determine colony forming units (CFUs). The studies were conducted as previously explained in Section 3.2.9. For clarity, the following abbreviations have been used throughout this section:

t 0, t 30, t 60, t 90 and t 120 where t denotes time in minutes and the numeric value the number of minutes.

C1, C2, C3 and C4 denotes concentrate 1 through to 4 collected during the flotation test. T denotes the bulk tailings remaining in the flotation cell at the end of the test.

For the tests performed in the absence (Control) and presence of microbes (+ Microbes), microbial activity using FDA assay was determined in three different fractions: the ore slurry representing a representative sample of the solid and liquid phase, liquid phase representing unattached planktonic microbes and solid ore particle phase, as detailed in Section 3.2.9.1.

The microbial activity in the flotation experiments performed in the absence of additional microbial cells (Controls) was measured using the FDA assay. The results are given as Relative Fluorescence Units (RFU). Figure 6-13, Figure 6-14 and Figure 6-15 show the FDA results for Exp 1, Exp 2 and Exp 3 Control water reuse tests. FDA provided a representation of active microbial cell concentration and, since microbial activity was observed in the absence of added microbial cells, it became apparent that the ore/slurry used in this study contained a native population of microbial cells. It should be noted that the 1 SPW used was synthetically produced in the laboratory according to Table 3-1 using deionized water and only contained inorganic ions, and no microbes, before being contacted with the ore material.

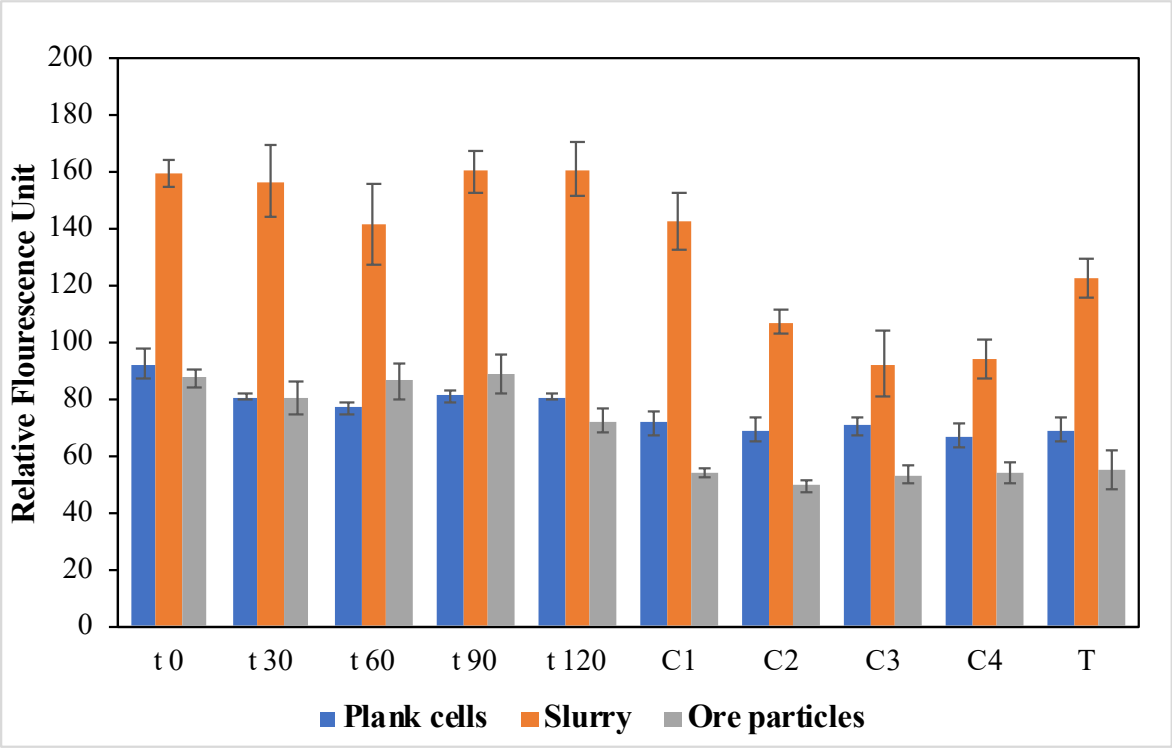

Figure 6-13: *Microbial activity given as RFU for Exp 1 (control) during the 2 -hour incubation and flotation times. Error bars show standard error from three repeats*

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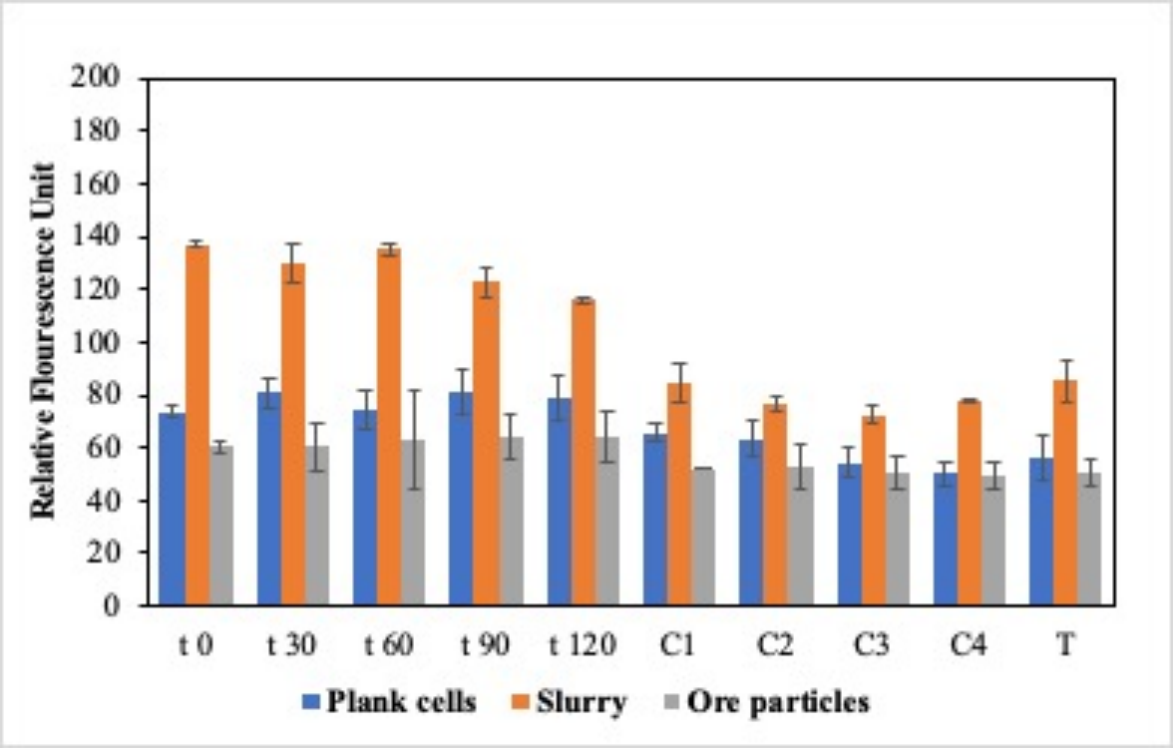

Figure 6-14: *Microbial activity given as RFU during 2-hour incubation and flotation time for Exp 2 (Control). Error bars show standard deviation from two repeats.*

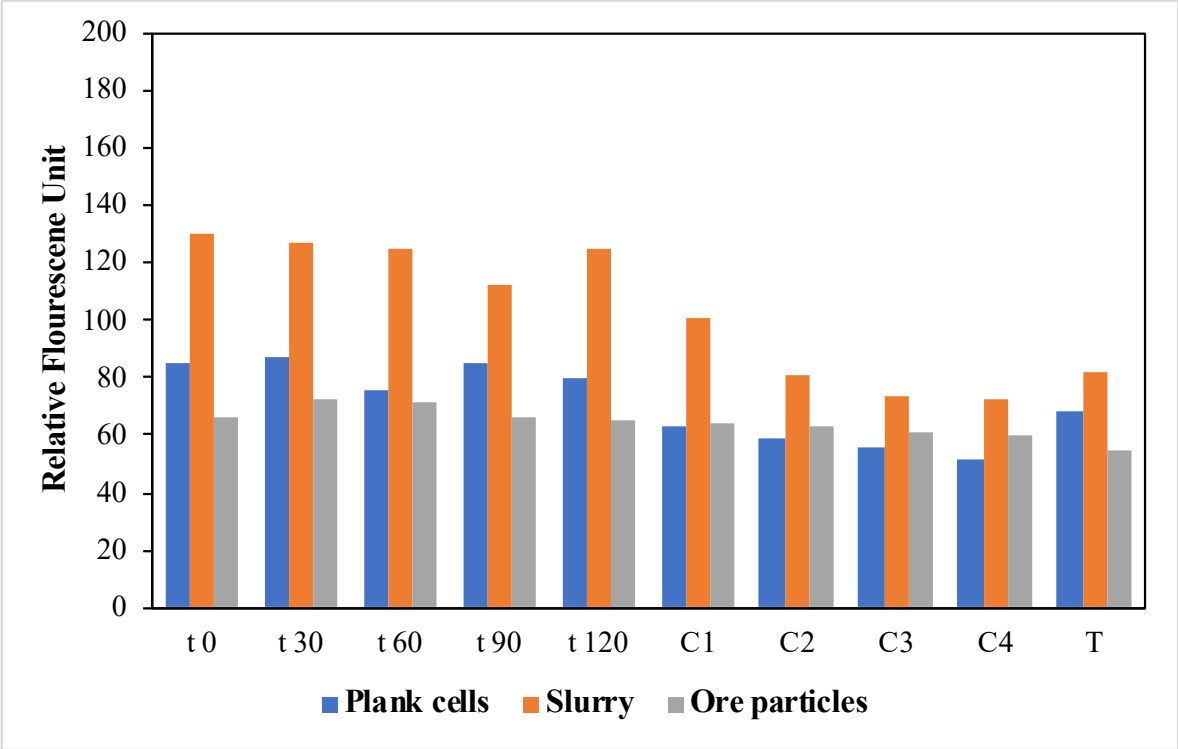

Figure 6-15: *Microbial activity measured as RFU under 2-hour incubation and flotation time for Exp 3 (Control). There was no evaluation of error in Exp 3.*

Generally, the microbial activity remained constant during the 2-hour incubation time in the three phases (slurry, planktonic and ore particles phase), and decreased in all three phases during the flotation period

across all three experiments. A decrease in microbial activity was expected in Exp 1 (Figure 6-13) since there was an addition of top-up water into the flotation system which ultimately diluted the microbial cells and decreased the microbial concentration. A negligible difference in microbial activity was detected in Exp 2 (Figure 6-14) during the incubation period. Top up water which was used in Exp 2 and 3 originated from Exp 1 and Exp 2, respectively and hence there was no addition of fresh water into the flotation system in Exp 2 and Exp 3.

Cell plating on two different agar media was conducted in parallel with FDA, to allow the quantitative reporting of CFU/ml in the liquid/water phase of the flotation samples. The nutrient agar plates were used to quantify heterotrophic microbes, while the ABS agar plates quantified autotrophic microbes. The growth of mixotrophic microbes is supported on both types of media. Figure 6-16 and Figure 6-17 provides CFU/ml using 2 different agar plate types in the flotation waters generated from experiments performed in the absence of added microbial cells.

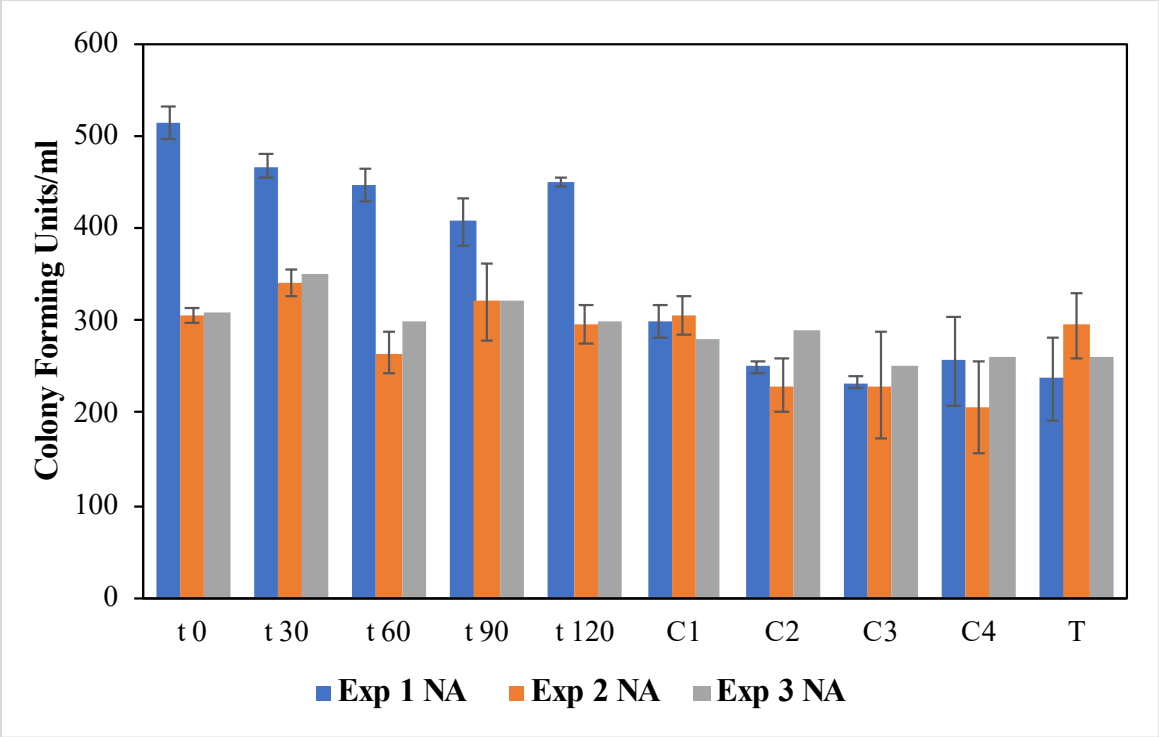

Figure 6-16: *CFU/ml on NA plates in the absence of added microbes at designated time intervals during the 2-hour incubation and flotation period. Error bars shows standard error between triplicate tests (Exp 1) and standard deviation in the case of duplicate tests (Exp 2). There was no evaluation of error in Exp 3.*

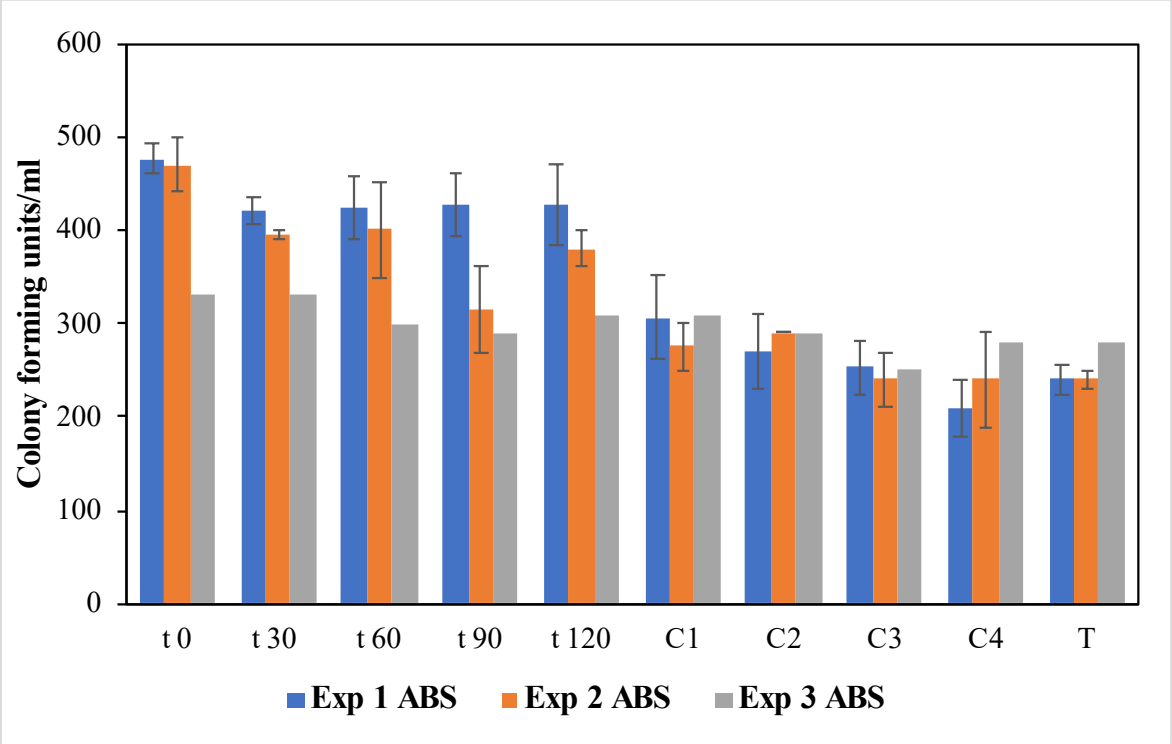

Figure 6-17: *CFU/ml at designated periods on ABS plates in the absence of added microbes during the 2-hour incubation and flotation period. Error bars represent standard error between triplicate tests (Exp 1) and between duplicate tests (Exp 2). There was no evaluation of error in Exp 3.*

The results in Figure 6-16 and Figure 6-17 suggest that the CFUs/ml were similar on the two medium plate types tested. The decrease in microbial concentration measured during the 2-hour incubation period and the flotation concentrates and tailings waters collection during bench flotation of Exp 1 is the result of dilution from the addition of top up water during the collection of the concentrates. In general, the drop in CFU/ml corresponded with high amount of top up water. This is most evident in CFU/ml from t120 to C1. This decrease in microbes is seen for both plate types used (Figure 6-16 and Figure 6-17). Exp 2 counts achieved on ABS plates during the 2-hour incubation period were similar to those measured during the incubation period for Exp 1 (Figure 6-17). The microbial concentrations reported were significantly higher than those measured in the concentrate and tailings waters. This may suggest that microbial growth was supported in the overnight time period between Exp 1 and Exp 2. This effect was not evident between Exp 2 and Exp 3 and may suggest that the water matrix may have become limited in nutrients capable of supporting microbial growth at this stage.

FDA assay results for recycling tests performed in the presence of 2x 10^8 microbial cells (+ Microbes) are presented in Figure 6-18, Figure 6-19 and Figure 6-20.

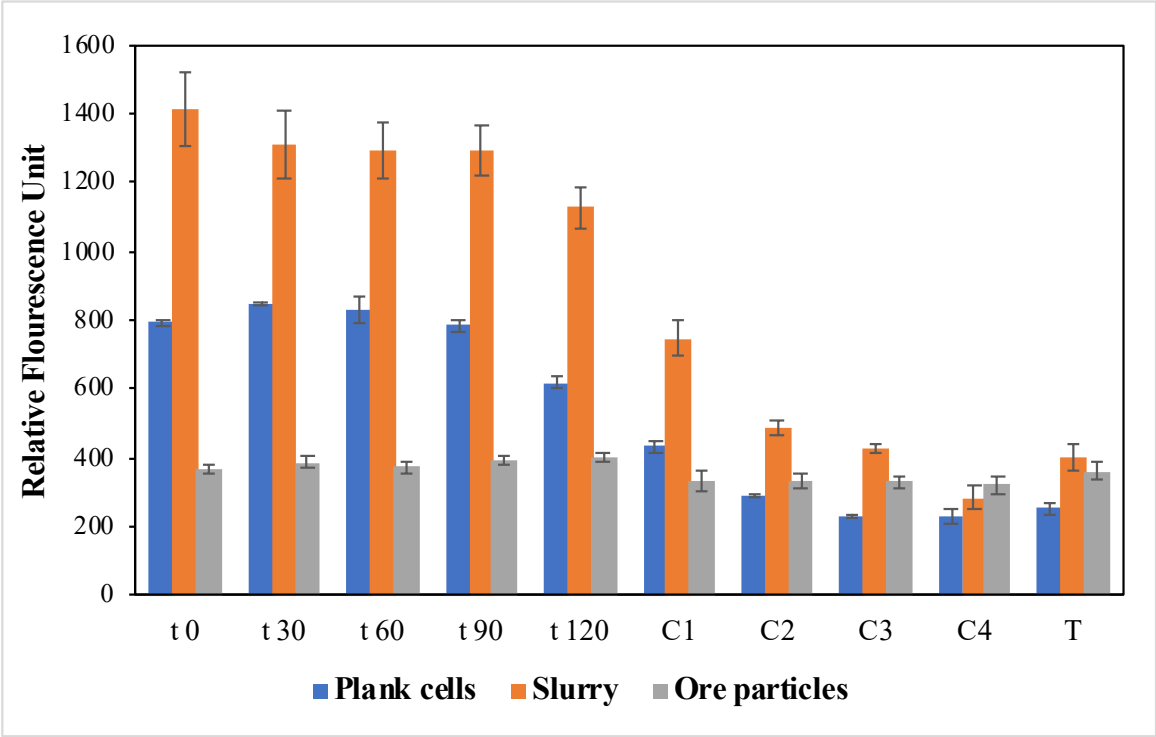

Figure 6-18: *Microbial activity given as RFU in the presence of predetermined microbial cell concentration of 2 x 10^8 cells/ml for Exp 1 during the 2-hour incubation and flotation period. Error bars represent standard error between triplicate tests (Exp 1).*

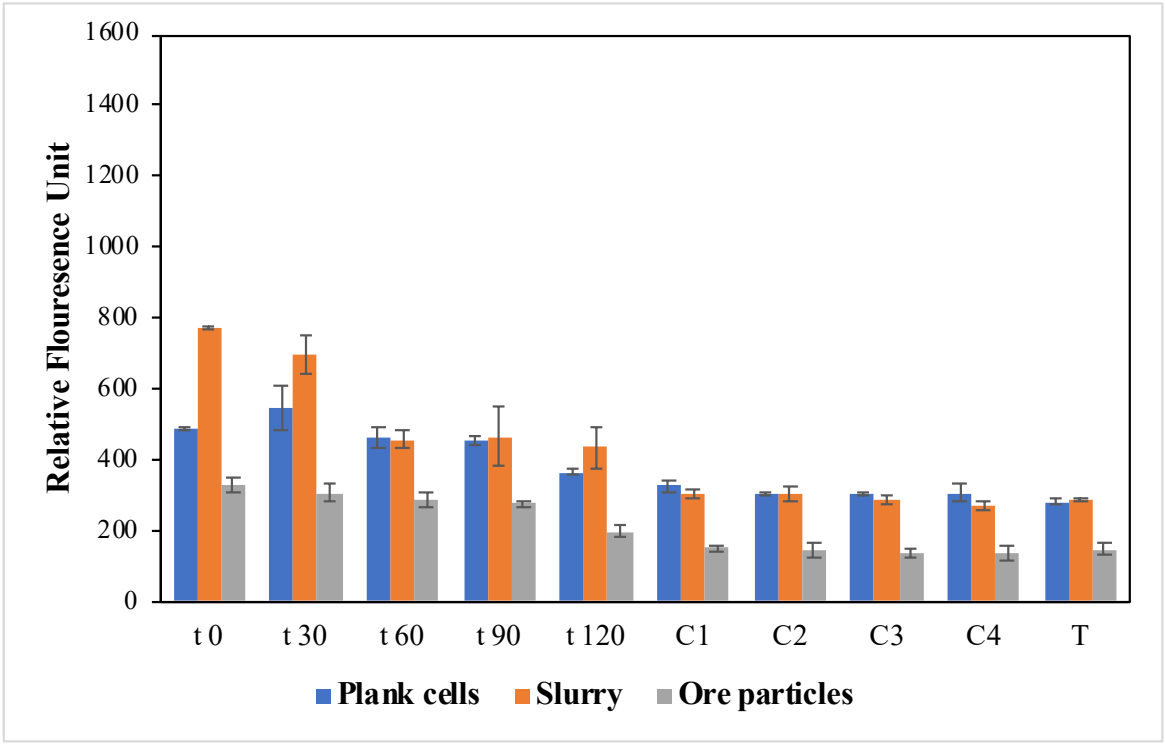

Figure 6-19: *Microbial cell activity given as RFU in the presence of microbial cells for Exp 2 + Microbes during the 2-hour incubation and flotation time. Error bars represent standard deviation between duplicate tests (Exp 2).*

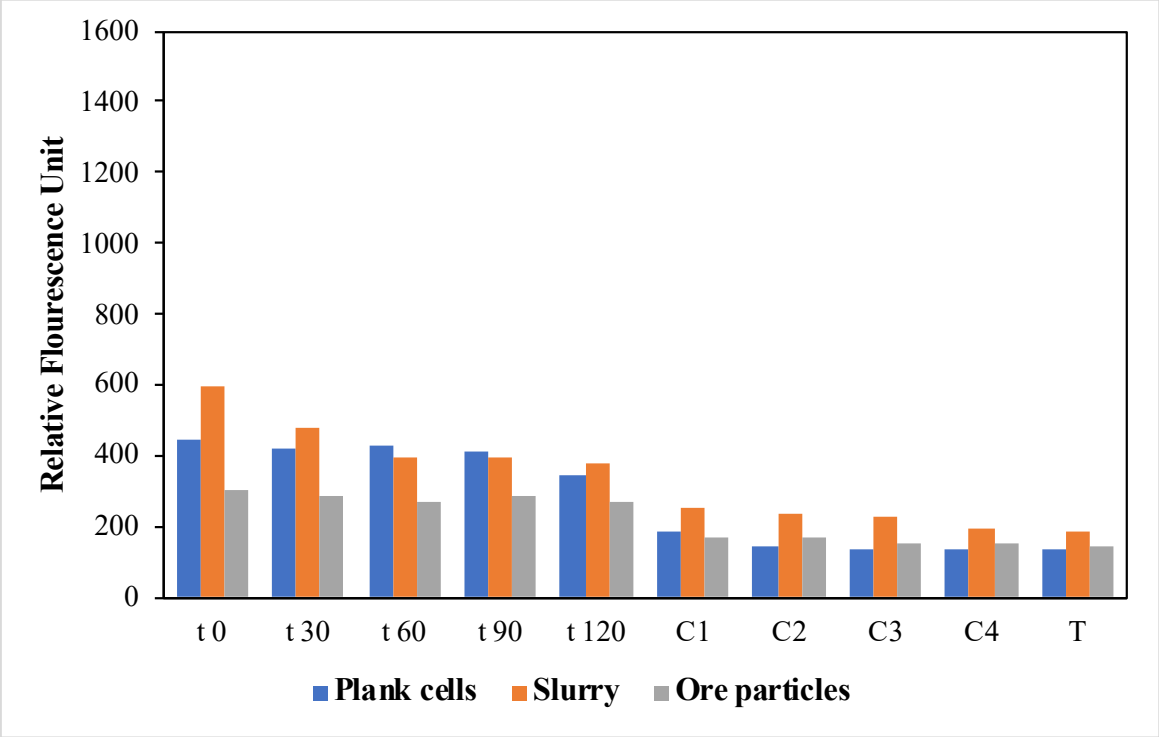

Figure 6-20: *Microbial cell activity given as RFU in the presence of microbial cells for Exp 3 + Microbes during 2-incubation and flotation period. There was no evaluation of error in Exp 3.*

From Figure 6-18 high microbial activity was noticed at t 0 which suggests a high microbial cell concentration of metabolically active cells. The microbial activity in the concentrates (C) and tailings (T) were significantly lower in Exp 1 (Figure 6-18), due to the addition of fresh top-up water during flotation, as also suggested in the Control experiment results. There was a decrease in microbial activity in the slurry overtime during the 2-hour incubation and flotation time across all the experiments (Figure 6-18, Figure 6-19 and Figure 6-20) in all the three phases (planktonic, slurry and the ore phase). In Exp 1, it is difficult to pinpoint whether the decrease in microbial activity was due to microbial attachment or shear given that there was an addition of fresh water which did not contain microbes. However, a decrease in the microbial activity, which translates to a decrease in microbial concentration, was also evidenced in Exp 2 and Exp 3 during the 2-hour incubation and flotation in the slurry phase where there was no addition of fresh water into the system.

Figure 6-21 and Figure 6-22 show CFU/ml on NA and ABS plates, respectively during the 2-hour incubation and flotation period for the +Microbes recycling experiments.

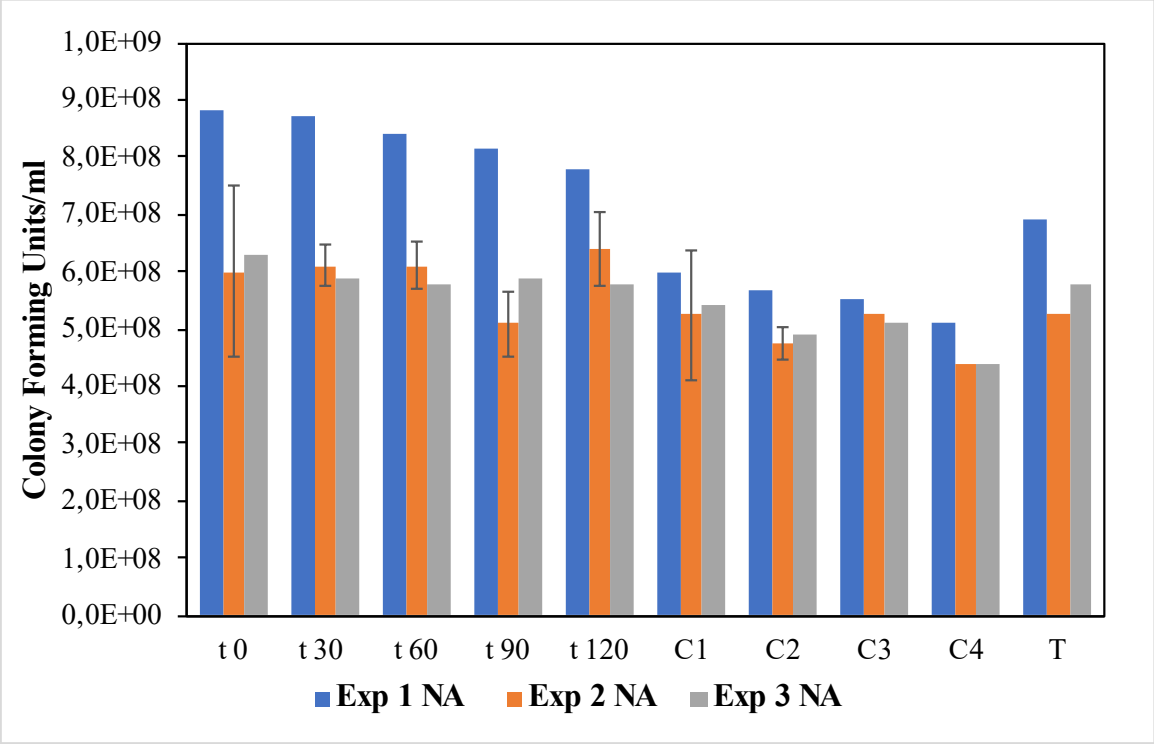

Figure 6-21: *CFU/ml on nutrient agar (NA) plates during the 2-hour incubation and flotation period. Error bars represent standard error between triplicate tests (Exp 1) and duplicate tests (Exp 2). There was no evaluation of error in Exp 3.*

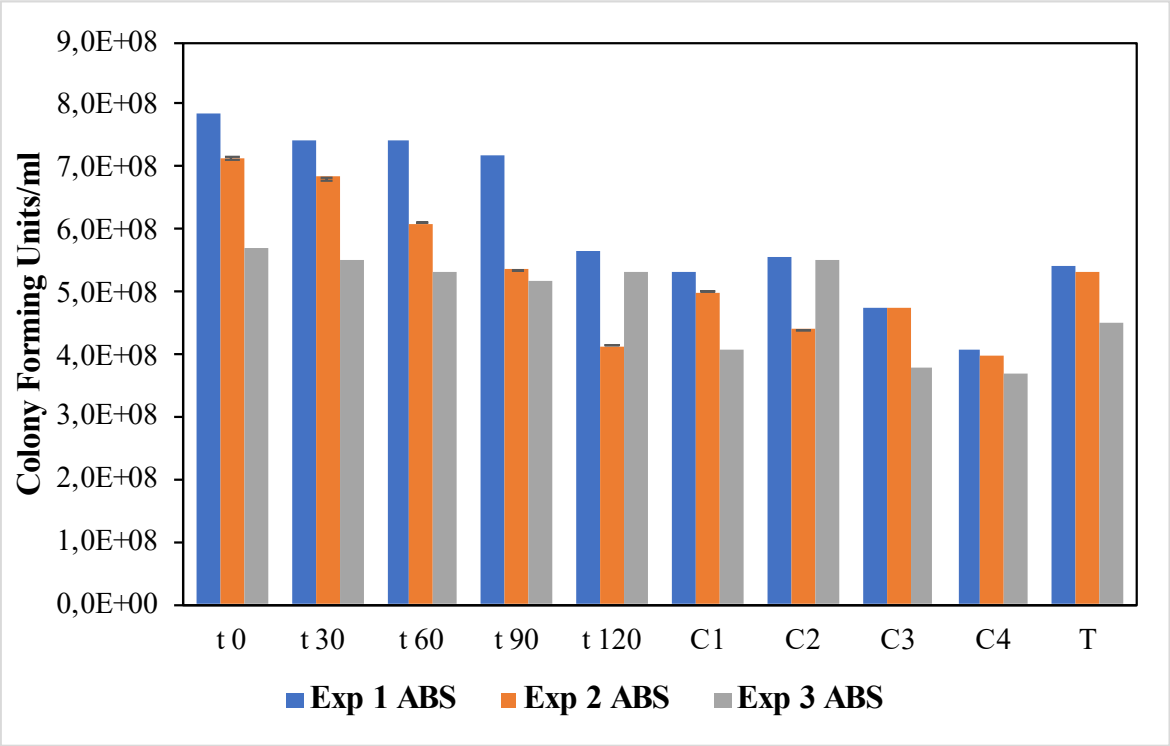

Figure 6-22: *CFU/ml on ABS agar plates during 2-hour incubation and flotation period. Error bars represent standard error between triplicate tests (Exp 1) and duplicate tests (Exp 2). There was no evaluation of error in Exp 3.*

As can be seen in Figure 6-21 and Figure 6-22 the CFU/ml decreased between Exp 1, Exp 2 and Exp 3 water reuse tests, with the most pronounced decrease observed between Exp 1 and Exp 2. These results are consistent with the FDA data. When comparing NA agar plates to ABS agar plate, similar CFU/ml was achieved on the two plate types. This may suggest that the majority of microorganisms represented in the community used for this study were mixotrophic in nature and could therefore utilize both organic and inorganic carbon sources for growth.

From the results above (Figure 6-13), the RFU was 160 in Exp 1 in the absence of microbial cells whereas in their presence, RFU was 1413 (Figure 6-18). This was expected given that there was a deliberate addition of microbial cells into the system. The microbial activity that was observed in the absence of a deliberate addition of microbes in this test, particularly in Exp 1 (Figure 6-13), confirmed that the ore sample contained microbial cells, since the deionized water used in this study did not contain microbes. This is consistent with other studies which have indicated that microbial cells could be introduced in flotation circuits through the ore sample (Bomberg et al., 2020).

The above findings (Figure 6-13 - Figure 6-15 and Figure 6-18 - Figure 6-20) show a decrease in microbial activity in the slurry phase in both tests from incubation time to flotation which may suggest microbial shear in the float cell owing to mechanical forces within the float cell. Microbial cell disruption, by any means – including shear, results in the release of organic molecules (Madigan and Martinko, 2006) and can be detrimental to flotation performance owing to the release of organics such as polysaccharides, proteins and DNA, which can readily passivate and alter mineral surfaces (Liu et al., 2016). The FDA assay, which measures microbial activity, can determine microbial shear as compromised cells would display lower metabolic activity. This effect would be less evident when microbial concentration is measured as CFU/ml using agar plates, as the lengthy incubation time could allow recovery of cells in a nutrient replete environment.

The significant RFU decrease in the planktonic phase and slurry phase, particularly in the presence of added microbes (Figure 6-18 - Figure 6-20), could suggest attachment of microbes onto the ore particle surfaces. This is in accord with the studies of Van Loosdrecht et al. (1990); Sharma and Rao (1999); Vilinsk et al. (2008); Africa, (2009); Bromfield, (2011); Liu et al. (2013c); Behera and Mulaba-Bafubiandi, (2017) who reported microbial attachment onto mineral surfaces. No significant increase in microbial activity was measured in the ore fraction throughout these tests, suggesting that the decrease in microbial activity and concentration in the planktonic and slurry fraction were not as a result of microbial attachment in this bench flotation study.

It is worthwhile noting that even though this study was not focused on bioremediation, the microbial consortia studied here is capable of causing xanthate removal from solution. It can thus be concluded that the consortia could be employed for xanthate bioremoval in xanthate polluted waste waters.

6.6 Conclusions

The outcomes of the water reuse tests revealed that water and solids recoveries generally increased with continuing water reuse across all the experiments. This could be attributed to the dissolution of certain ions which promote water and solids recovery. From the water and solids recoveries profiles, it appears that higher water and solid recoveries were yielded with the addition of microbes, compared with the controls, a phenomenon that can be attributed to microbial induced frothability, most probably from the release of microbial proteins and metabolites into solution, rather than microbial facilitated gangue activation. This finding is supported by the decrease in microbial activity (FDA assay) and cell concentration (plating) reported during this study. The addition of microbial cells did not affect Cu or Ni recoveries; which were relatively similar during the recycling test; however, Cu and Ni grades were affected by the presence of microbial cells due to the apparent activation of gangue minerals by the microbes.

It is important to note that this study was performed with a microbial community harvested from flotation waters and subsequently enriched in the laboratory to allow sufficient microbial cells to allow experimental study. The specific species abundances may therefore differ from those present within the actual concentrator waters. For this microbial consortium, the presence of microbes adversely affected the flotation performance of the base metal sulphide minerals chalcopyrite and pentlandite, strengthening the proposed hypothesis that the presence of microbes increase water and solids recovery, negatively affecting metal grade recovery. This implies that where the usage of microbial laden waters for flotation operations is prevalent, there is a need to monitor and identify the microbial content in these waters as they may prove to detrimentally affect flotation performance. Furthermore, water reuse, which is also widely practiced on mineral concentrators, may tend to result in reagent accumulation, which may inadvertently activate processes which could affect flotation.

CHAPTER 7

7 CONCLUSIONS AND RECOMMENDATIONS

The overarching aim of this study was to investigate the effects of microbial loads in poorly remediated point source water and any water reused by the base-metal sulphide concentrator, on the flotation performance of a Cu-Ni PGM bearing ore. In addition, the effect of xanthate on microbial cells, and vice versa, was considered. Since flotation is a water intensive process, water quality is of paramount importance as it largely determines the overall performance of the process and as a result water quality has attracted a great deal of interest in the research space. As such, there is a growing need to gain fundamental knowledge on the effects of microbial laden waters on PGM recovery. To address this, key questions and hypotheses were formulated. In this chapter, the conclusions of this study are presented from the findings from experiments designed to answer the proposed key questions. The findings from these studies are also discussed in light of the hypotheses proposed for the study.

7.1 Conclusions

7.1.1 Key questions

1. **Can microbial quantification be performed accurately in the presence of a mineral slurry?**

The present study has shown that the FDA assay can be effectively employed for microbial quantification in ore slurries (see Section 4.3.1). It should be noted that even though the FDA assay can be used for quantifying microbial activity, the metabolic activity measured using this technique cannot be directly related to a specific cell concentration or cell dry weight. This is due to changes in the specific metabolic activity of cells during the various phases of growth. However, the trends observed between different treatments and ore slurry phases (liquid or solid) can be accurately compared.

2. **Do microbial cells attach onto ore surfaces?**

The attachment studies have indicated that the microbial community can attach to the ore surfaces, a phenomenon largely driven by surface characteristics of both the microbes and the mineral fractions contained within the ore (see Section 4.4). The attachment of the microbes was found to be of a similar magnitude to other studies conducted using low grade ore (Africa, 2009 and Bromfield et al., 2011).

3. **Does the microbial community use xanthate as a carbon source and at what concentration does xanthate become toxic to microbes?**

This study provided a framework on the microbial response to the presence of xanthate. The study showed that the microbial community can grow in the presence of xanthate collectors (SEX and SIBX), with the addition of a carbon source to the growth medium. The microbes did not appear to use xanthate

as a carbon source, as no growth was observed in the absence of an additional carbon source, such as yeast extract. The microbes were shown to remove xanthate from solution i.e. there was bioremoval of xanthate.

It remained uncertain as to which mechanism was responsible for xanthate bioremoval. Xanthate analysis at a fixed collector concentration and increasing microbial cell concentrations (see Section 5.5) indicated that microbial cells could aid xanthate bioremoval, a process which was largely dependent on the microbial cell concentration. It was shown that higher microbial cell concentration could easily aid xanthate removal from solution compared to lower microbial cell concentrations. In other words, higher rates (rate constant (K) values and volumetric removal rates) were noted in the presence of higher microbial concentrations compared to lower microbial concentrations suggesting a positive correlation between volumetric rates and microbial concentration.

The microbial cells used in this study were found to tolerate xanthate to a certain xanthate concentration; beyond this concentration xanthate was thought to be toxic to the microbes. The tolerance level for SEX was shown to be less than 480 ppm, while a much higher tolerance was seen with SIBX. It is also worthwhile to mention that the presence of xanthate (for all the xanthate treated cultures) induced a prolonged lag phase of growth for the microbial community studied, in comparison to the non-xanthate treated cultures. When comparing the xanthate collectors, it was shown that SEX, with a shorter carbon chain length, exhibited higher toxicity compared with SIBX, which has a much longer carbon chain length (see Section 5.3.1).

Xanthate analysis indicated that, in the presence of ore particles, the microbes competed with xanthate for attachment to the mineral surface and the microbes also appeared to metabolise xanthate through active degradation as no substantial adsorption was witnessed in this study (see Section 5.7). The rate of xanthate removal from solution was much faster in the presence of ore than the rate achieved when only microbes and no ore were present. There was, however, an additive effect of xanthate removal in the presence of ore and microbes i.e., more xanthate was removed with ore and microbes present in solution and this amount was the same as that when only ore or microbes were present. This suggests that microbe laden water has a detrimental effect on flotation operations, indicating that higher concentrations of collector would be needed to achieve the mineral recoveries required.

4. **Do microbes and water reuse affect water and solids recoveries, metal recovery and metal grade of a Cu-Ni PGMs bearing ore concentrate?**

Generally, from the gathered data in the present study, the presence of the microbial cells added during experimentation, induced pronounced frothability as shown by the water recovery profiles. Water and

solids recoveries increased with water reuse and higher water and solids recoveries were detected in the presence of microbes relative to their absence.

The effect of residual and newly added frother, from the second and third water reuse experiments, was less pronounced than that of frother combined with microbes, suggesting that frothability may have been induced by microbial cells and, possibly, their by-products (see Section 6.3.1). Even though the residual frother was not measured, the increase in water recoveries with water reuse was most likely due to residual frother accumulation.

In considering the metal grades and recoveries, the Cu recovery remained relatively similar in the presence and absence of additional microbes. This could be due to the well-known fast floating nature of chalcopyrite. On the other hand, Cu recoveries marginally increased during as water was reused, possibly owing to the increase in solids and water recoveries. Ni recoveries marginally increased in both systems (in the absence and presence of microbes) during the course of water reuse. However, the presence of added microbes did not affect Ni recovery when compared to the Control across all tests.

Regarding the impact of microbes on metal grades, the presence of microbes negatively affected Cu and Ni grades. This was more pronounced in Exp 1 compared to the other experiments (see Section 6.4). The possible reasons for these findings may be due to the following: (1) microbes could have activated gangue mineral or (2) microbial induced frothability may have promoted gangue mineral flotation. The recovery of unwanted gangue minerals with the concentrate, lowered the metal grade. This highlights the detrimental effect microbial cells may have on metal grade recovery. It is also worth noting that water reuse, regardless of the presence (added microbes) or absence of microbes, resulted in a decrease in metal grades across all tests. This may have been due to residual frother being retained in the water leading to higher water and solids recoveries. Thus, going forward, tests to determine the optimal amount of frother to use when reusing water, need to be performed. Decreasing the frother addition will decrease reagent costs.

7.1.2 Review of Hypotheses

The first hypothesis stated that “Collectors such as SEX and SIBX contain a carbon backbone structure and will act as a carbon source for microbes harvested from the concentrator circuit, resulting in an increase in microbes and a decrease in collector concentration”. This appears not to be true for the microbial community used in this study. Results gathered from this study suggest that either the microbial cells adsorbed xanthate onto the microbial cell surfaces or actively degraded the xanthate without utilising it as a carbon source.

The second hypothesis stated that “Microbes that naturally prevail in flotation waters and waters reused in the concentrator, adversely affect flotation performance (measured by water recovery, solids recovery,

metal recovery and grade) by attaching to mineral surfaces, resulting in a change to the mineral surface charge and/or hindering collector adsorption on mineral surfaces". Results from this study strengthen the suggestion that flotation performance is negatively affected in the presence of high concentrations of microbes. The addition of microbial cells promoted the recovery of water and solids to the concentrates and, although metal recoveries were not affected, grade was decreased with the presence of microbes.

This study suggests that there might be potential risks to flotation efficiency on concentrator sites where microbial laden water is employed for flotation operations.

7.2 Recommendations

Based on the presented results, the following recommendations were made.

1. As it was necessary to understand the effects of microbes on flotation performance in a simple flotation system, such as in the absence of a depressant, it is recommended that studies be conducted in a flotation system in which depressant is added. .
2. Since microbial cells may respond differently to different ore types it is possible that the microbial cells used in this study may behave differently in the presence of a different ore. As a result, notwithstanding the negative effects of naturally prevailing microbial cells on flotation performance, generalizations cannot be made. Similarly, since the ore material is not sterile it may contain a natural endemic microbial consortium, and it is possible to find different consortia associated with different ores. Similarly, the water sources utilised for point source addition may also differ in the microbial consortia they introduce to the concentrator circuit. The literature has indicated that different micro-organisms impact bioflotation differently, so an impact of microbial type is also expected.
3. It would be interesting to carry out attachment, and possibly imaging, studies using specific pure mineral types and gangue minerals to determine whether the microbial cells show preferential attachment to the valuable minerals of interest or the gangue minerals.
4. The microbial community used in this study, enriched from a commercial concentrator, showed potential as a consortium for application in a bioremediation treatment for the removal of xanthate from concentrator waters. This technology may have application preceding the further bioremediation, using xanthate sensitive microbes, or through the supplementation of other microbial consortia utilised for bioremediation to allow the co-current xanthate removal and further bioremediation. The application of this consortium for this purpose requires further investigation.

5. It would be imperative to ascertain the microbial communities which could be present in the concentrator as this presents ways by which the microbial growth could be reduced or limited within the concentrator. It is also of great importance to understand the dominant microbial communities/species within the concentrator in order to eradicate the prevalent microbial species before reaching high microbial counts which could be detrimental to flotation operations. Moreover, where possible, the microbial growth should be limited.

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9 A Appendix A- Standard curves

A.1 Standard curves generated for microbial enumeration of Platinum concentrator microbes

Microbial dilutions were prepared and their OD was determined in parallel with cell concentration (cells/ml) by direct microscopic cell counting and cell dry weight (g/L) (FigureA-1) determination as described in Section 3.1.5.

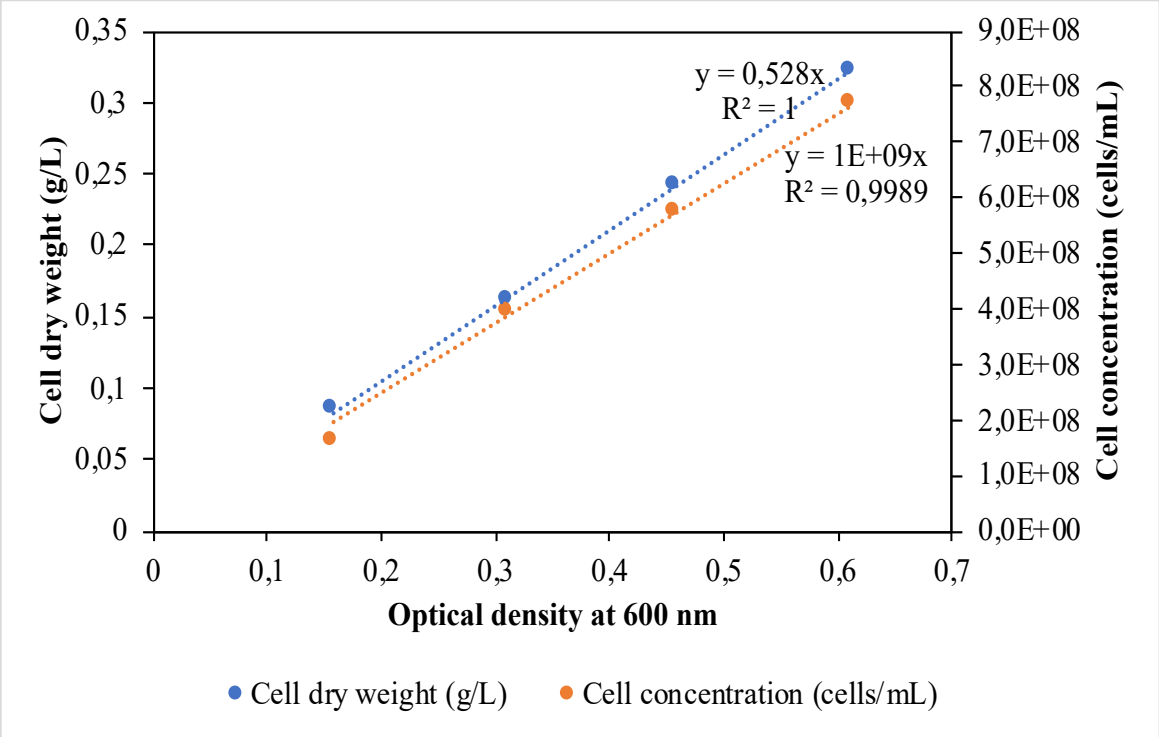

Figure A- 1: *Aa standard curve for the platinum concentrator microbes*

A.2 Carbohydrate standard curve generated for phenol sulphuric assay method

Different glucose concentrations, 0 to 250 mg/L, were prepared and their absorbances values were determined at 490 nm. Carbohydrate quantification in microbes in the absence and presence of xanthate was then calculated from the OD results achieved and the coefficient from the generated standard curve (Figure A-2).

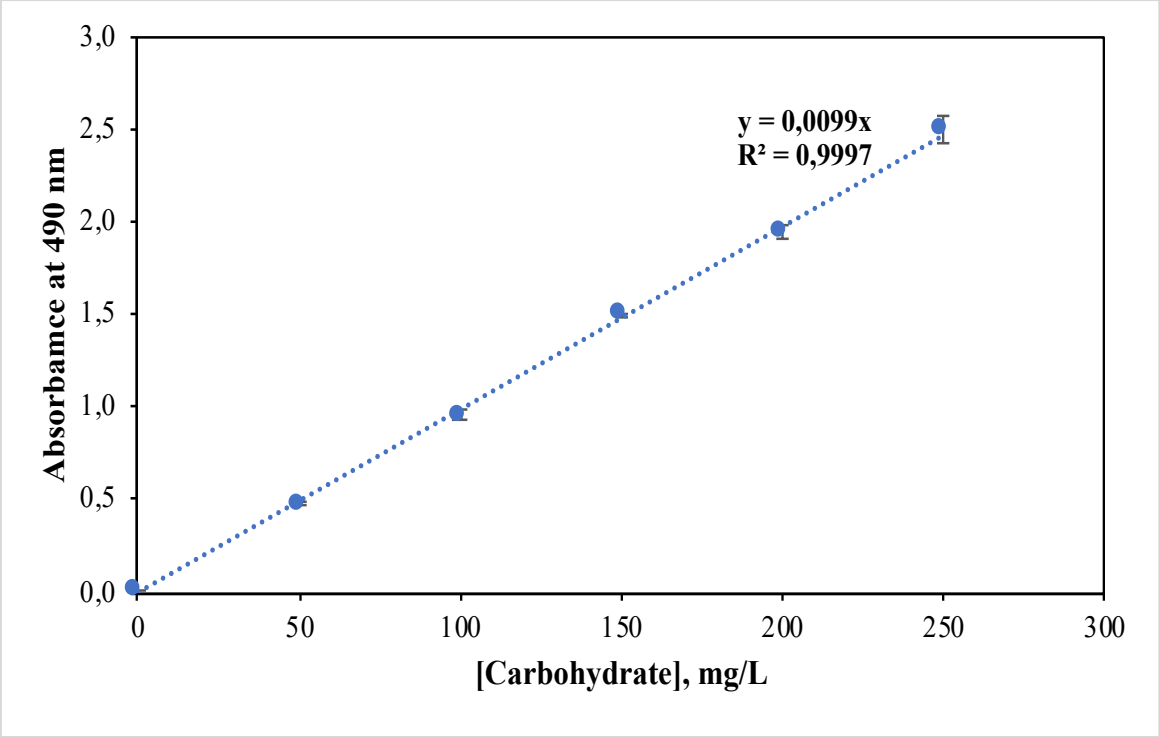

Figure A- 2: *Absorbance values measured at 490 nm for different glucose concentrations following application of the phenol sulphuric assay*

A.3 SEX standard curve

For all xanthate experiments conducted in this study, different xanthate concentrations were prepared, and their absorbance were determined at 301 nm. SEX standard curve was constructed by measuring the absorbance of standard solutions of the collector between 60 and 500 ppm from which the experimental data in ppm was determined (Figure A-3).

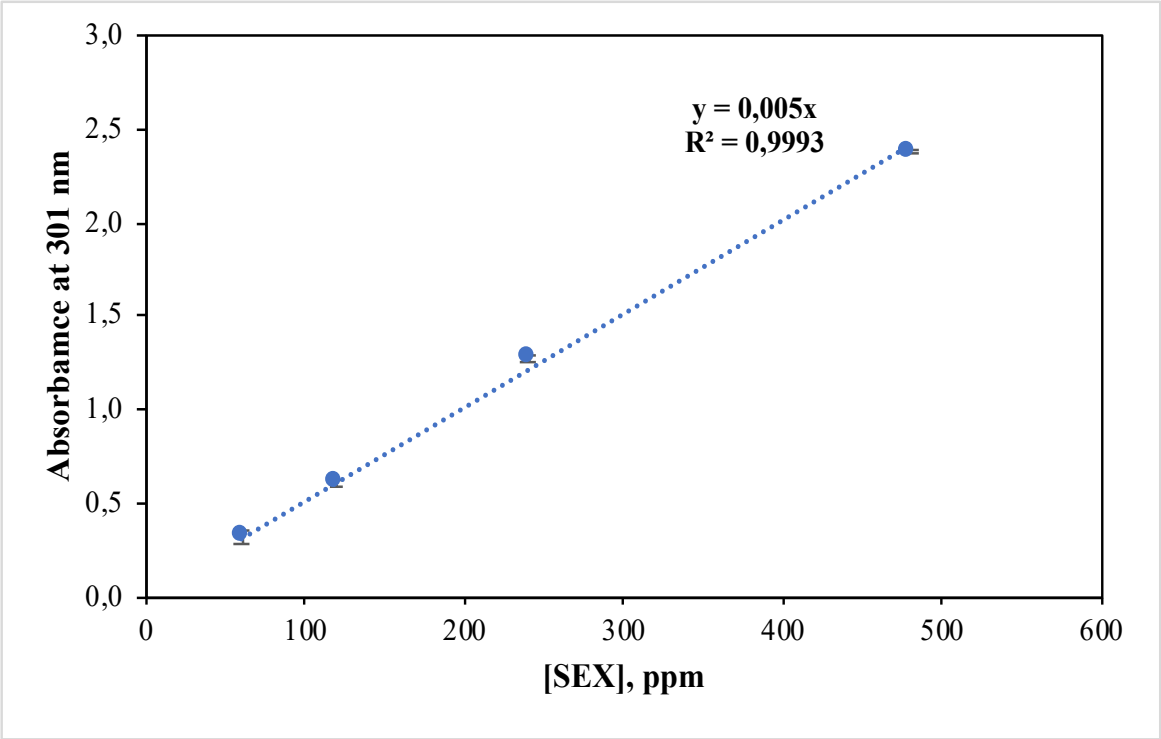

Figure A- 3: *SEX standard curve*

A.4 SIBX Standard curve

A SIBX standard curve was constructed from which the experimental data in ppm was determined by measuring the absorbance of standard SIBX solutions between 60 and 500 ppm at 301 nm as shown in Figure A-4

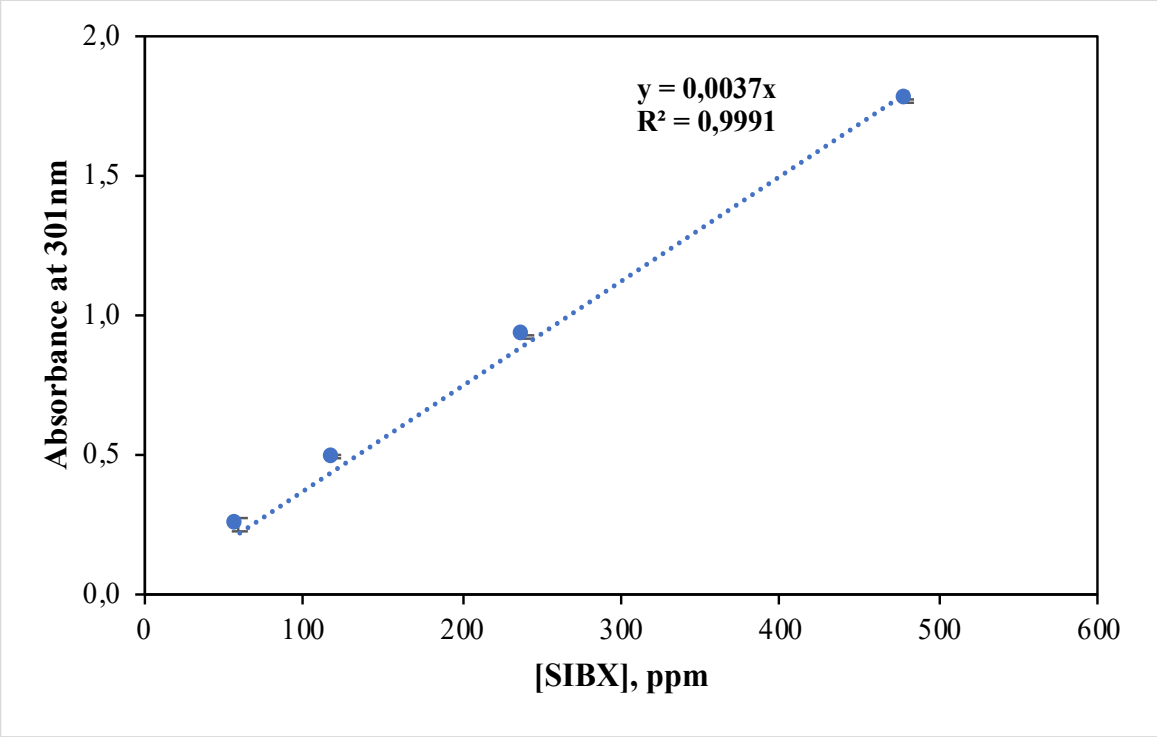

Figure A 4: *SIBX standard curve*

10 B Appendix B- Xanthate percentage removal

Xanthate percentage removal was calculated in the absence and presence of microbial cells (Section 3.2.4 - 3.2.7). The xanthate percentage removal was determined using Equation 3.7 (Section 3.2.4). Figure B-1; Figure B-2; Figure B-3; Figure B-4; Figure B-5; Figure B-6 andFigure B-7 shows xanthate (SEX and SIBX) percentage removal upon different xanthate treatments.

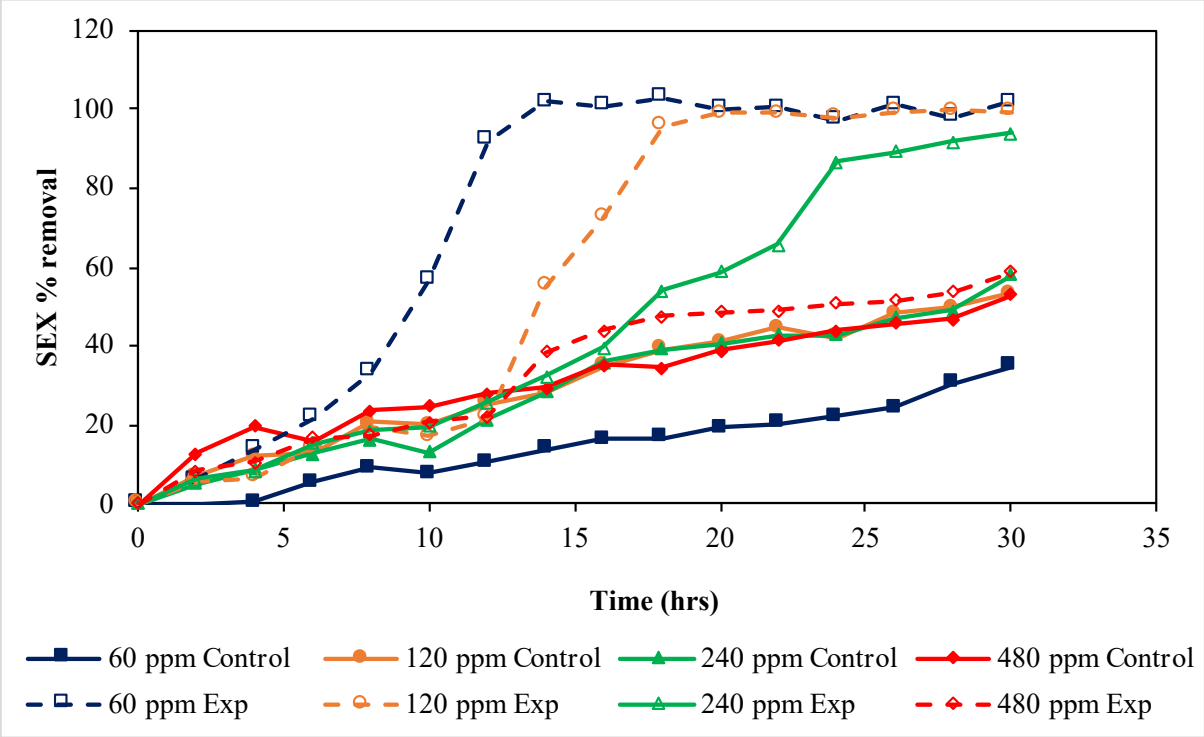

Figure B- 1: *SEX percentage removal in the absence (Control) and in the presence of microbes at increasing SEX concentration*

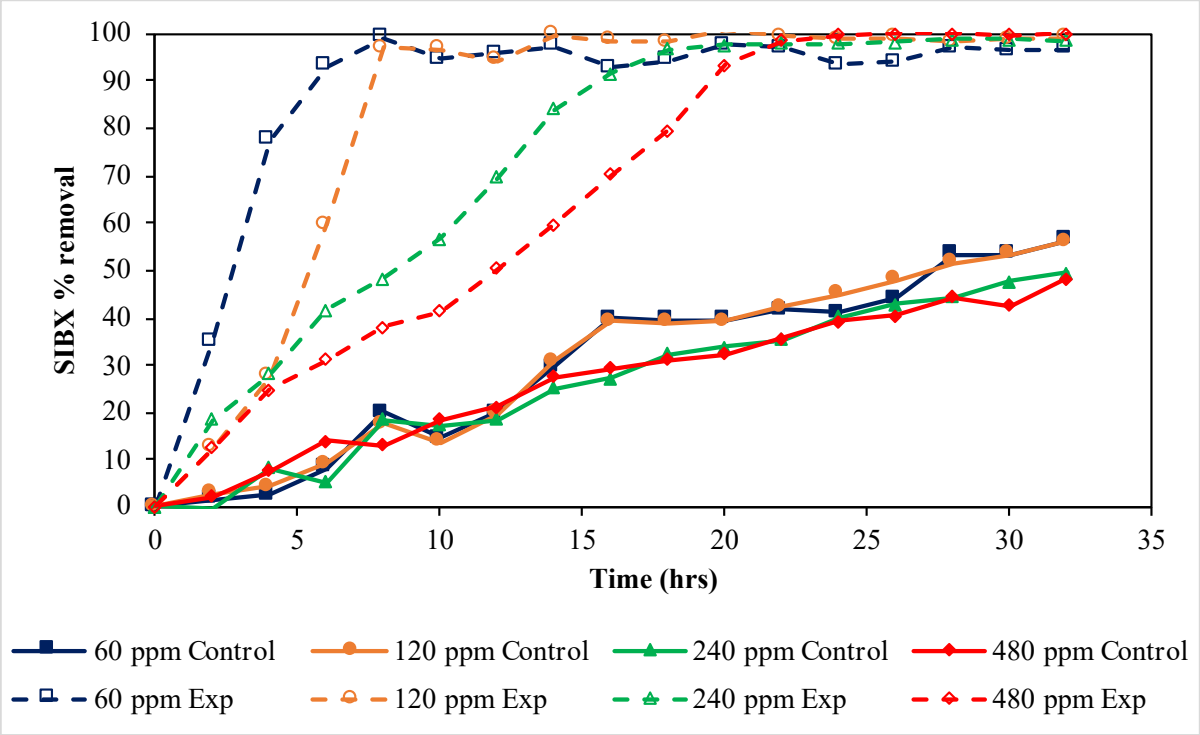

Figure B- 2: *SIBX percentage removal in the absence (Control) and in the presence of microbes (Exp) at increasing SIBX concentrations*

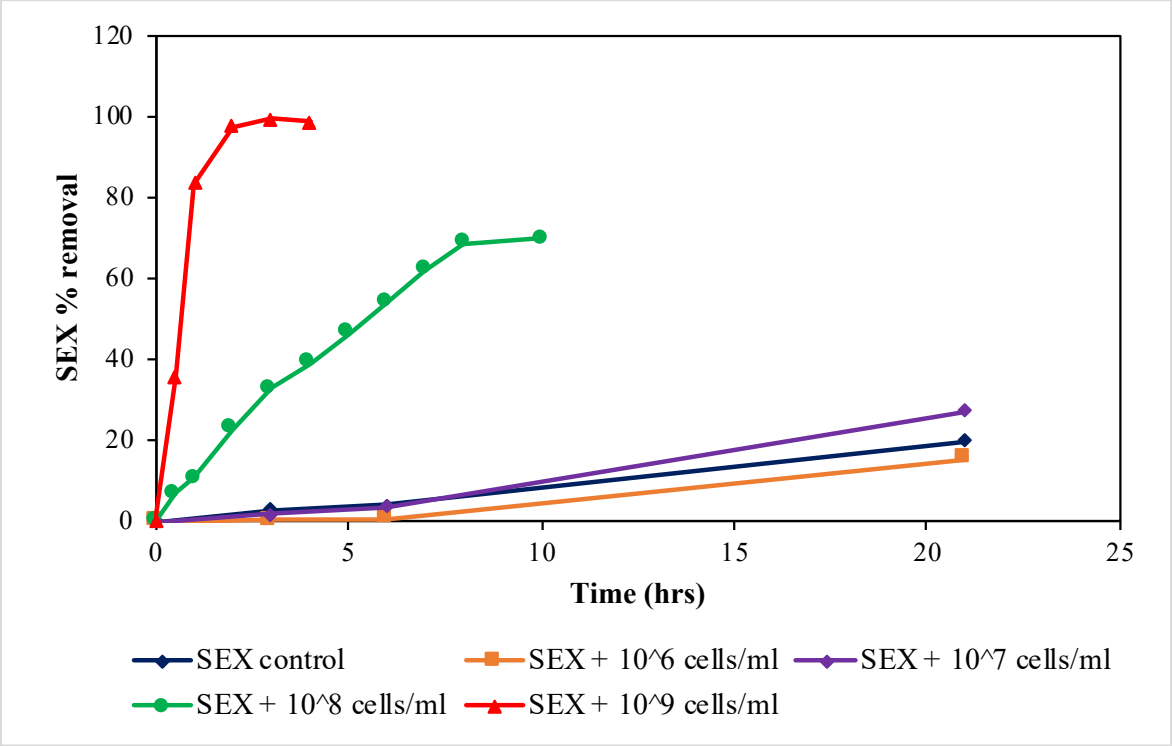

Figure B- 3: *SEX percentage removal in the presence of increasing microbial cell concentrations. The initial SEX concentration was maintained at 60 ppm across all experiments*

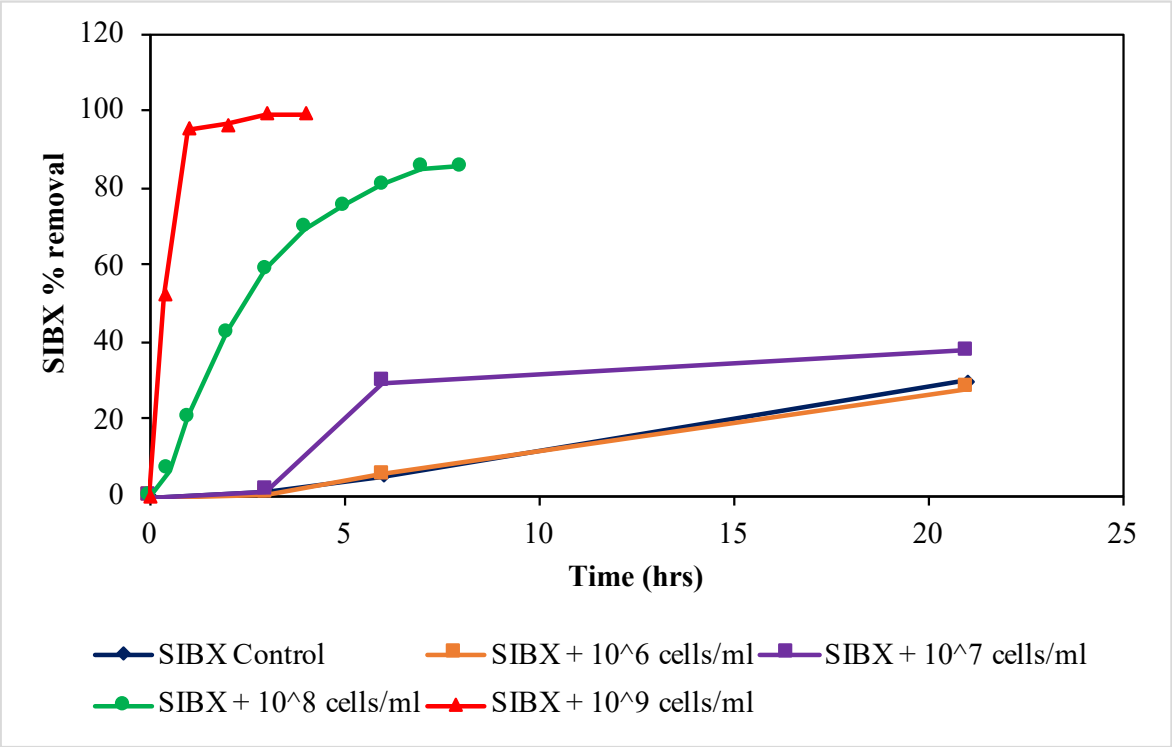

Figure B- 4: *SEX percentage removal in the presence of increasing microbial cell concentrations. The initial SEX concentration was maintained at 60 ppm across all experiments*

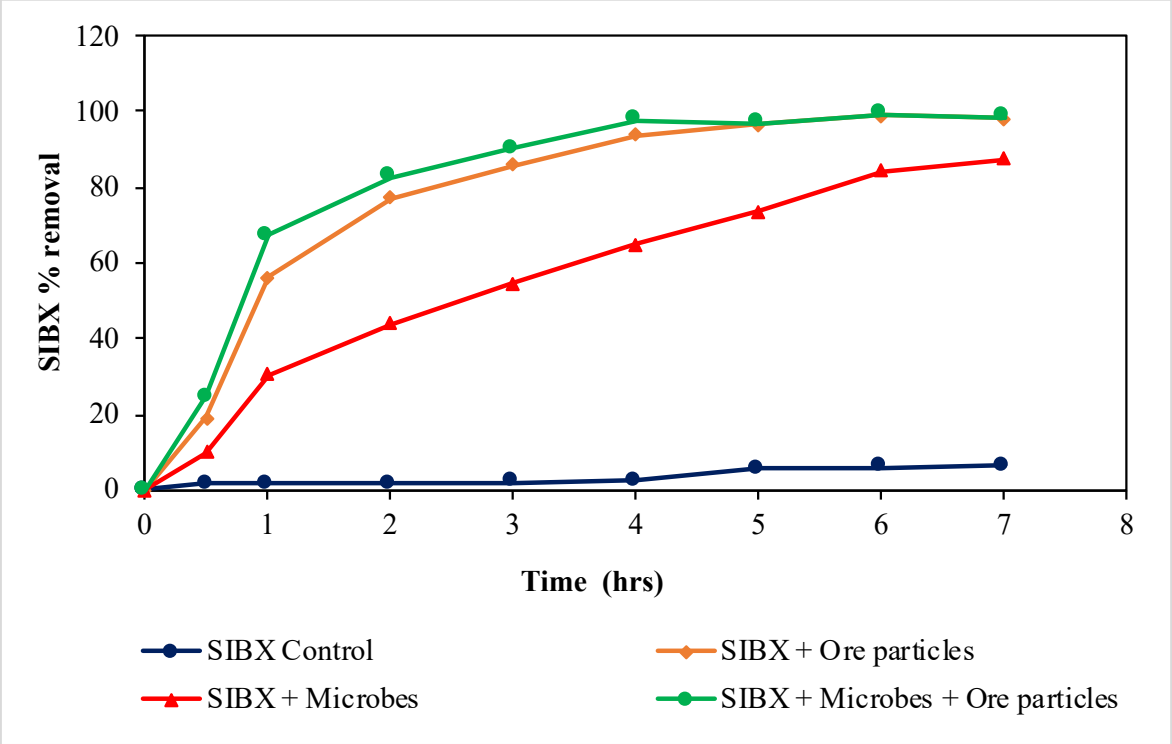

Figure B- 5: *Percentage removal of SIBX in different microbial and mineral systems. The initial SIBX concentration was maintained at 60 ppm across all tests*

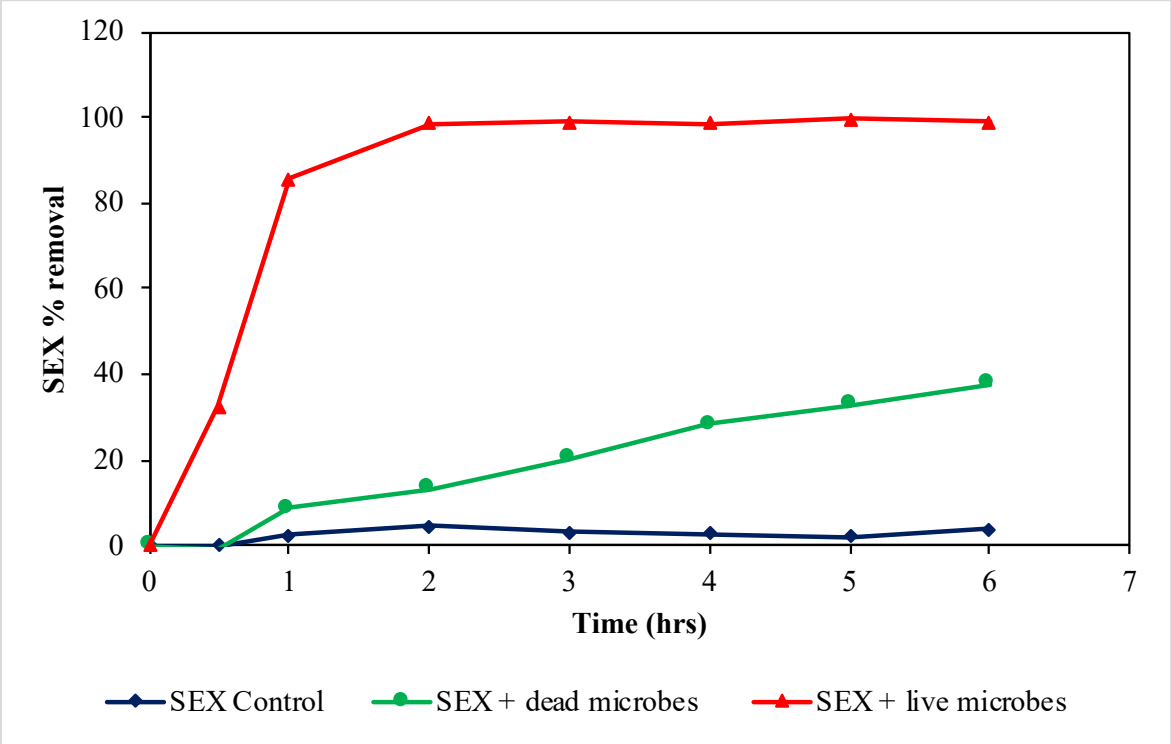

Figure B- 6: *Percentage removal of SEX in the presence of dead and live microbes. The microbial concentration was maintained at 10^9 cells/ml at an initial SEX concentration of 60 ppm.*

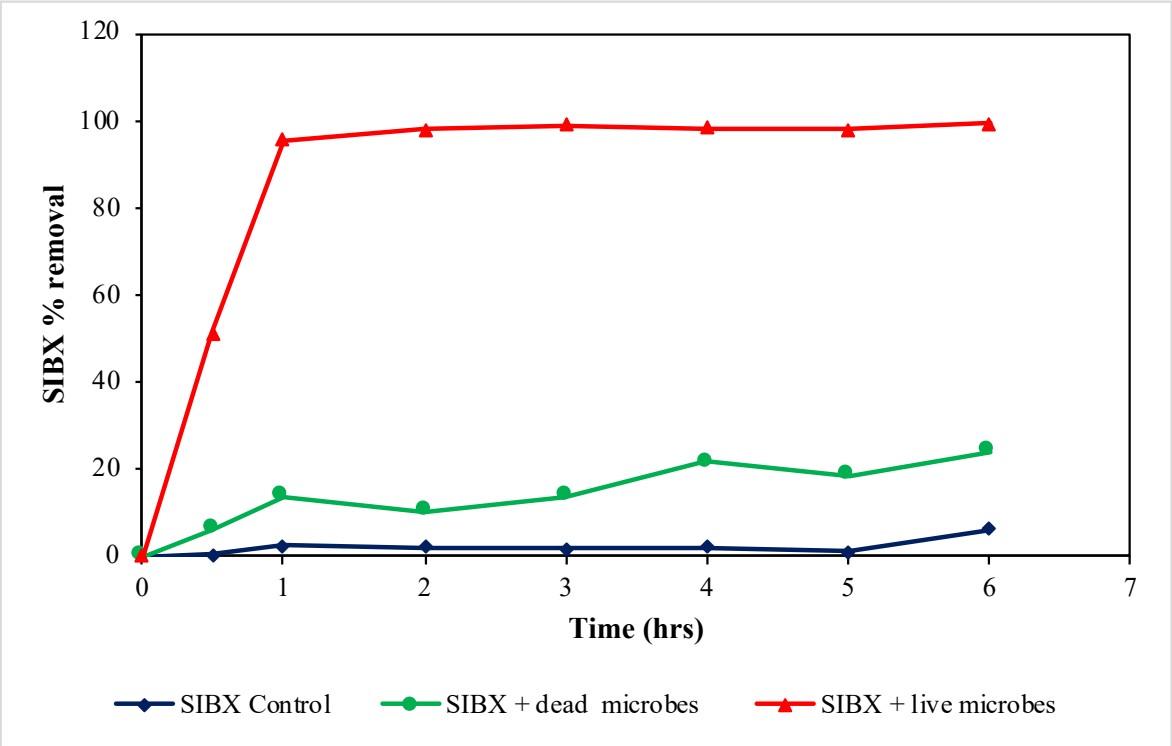

Figure B- 7: *Percentage removal of SIBX in the presence of dead and live microbes. The microbial concentration was maintained at 10^9 cells/ml at an initial SIBX concentration of 60 ppm.*