2021

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Rogers, D. (2021) 'An investigation into the determination of ammonium in mine bioleaching solutions utilising an ammonia gas electrode', The Plymouth Student Scientist, 14(1), pp. 78-107.
http://hdl.handle.net/10026.1/17336

The Plymouth Student Scientist
University of Plymouth

*The Plymouth Student Scientist, 2021, **14,** (1), 78-107*

An investigation into the determination of ammonium in mine bioleaching solutions utilising an ammonia gas electrode

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Abstract

Fixed nitrogen is an essential nutrient for bacteria used in the mining industry to solubilise metals from their associated ores in a process known as bioleaching. Most bacteria are able to fix atmospheric nitrogen, but those which are not require a feed solution of aqueous ammonium to meet their nitrogen requirements. Bioleaching operations are large scale and therefore the cost associated with providing aqueous nitrogen is considerable. It is therefore important that only the minimum amount of aqueous nitrogen is added during the process to minimise costs. Camborne School of Mines were investigating the potential loss of aqueous nitrogen from bioleaching solutions through the precipitation of ammonium jarosite. In order to further their studies, they required development of a method for the determination of ammonium in a typical bioleaching solution so that they could research optimum bioleaching conditions to minimise loss of aqueous nitrogen. Various methods were researched and determination of ammonium using an ammonia gas selective electrode was investigated in detail. The method yielded promising results for determinations of ammonium between 0 and 50 mg/L in a typical bioleaching solution. Additions of base much in excess of ammonia electrode literature were required to liberate ammonia gas from bioleaching solutions, most likely due to the consumption of hydroxide during the precipitation of metal-hydroxide species. The performance characteristics of the method developed were assessed and the method and standard operating procedure developed were fit for purpose and adopted by Camborne School of Mines.

Keywords: mine bioleaching, ammonia gas electrode, fixed nitrogen, ammonium, ammonium jarosite, aqueous nitrogen, analysis.

Introduction

An Introduction to Bioleaching

The objective of this research was to develop an analytical method to determine the concentration of ammonium in a typical bioleaching solution. Bioleaching is a subbranch of hydrometallurgy, an approach to the recovery of metals from their associated ores through use of aqueous chemistry and bacteria, which is currently used to produce around 20 % of the world's copper (Davenport et al., 2011a).

The method for ammonium determination needed to be developed as it is thought that, under typical bioleaching conditions, in presence of Fe(III) at concentrations of 5-10 g/L, NH_4^+ would precipitate in the form of ammonium jarosite (Watling, 2006). The loss of ammonium from bioleaching solutions presented a problem as it removed nitrogen, a key bacterial nutrient, from bioleaching solutions. The method developed will be used in further research to determine the optimum bioleaching conditions and the impact, if any, of Fe(III) concentration upon the chemical equilibrium between aqueous ammonium and precipitated ammonium jarosite.

The bacterial catalysis for the oxidation of Fe and S was first demonstrated in the 1940s by Colmer and Hinkle (1947) and as the role of bacteria has become better understood, it has been utilised as a method to leach precious metals from ores which have already been primarily processed, resulting in a low-grade ore where the metal was previously thought to be uneconomical to recover. The benefits of bioleaching over traditional metal recovery approaches, such as the smelting of copper, are mostly environmental at this point in time. With smelting, ores must first be concentrated and then heated to temperatures of around 1250 ^oC to oxidise their copper content and to generate a copper-rich matte (Davenport et al., 2011b). Smelting is an efficient process and very near to 100 % of copper in the concentrated matte is recovered (Rawlings and Johnson, 2010a).

The bioleaching process works by bacterially catalysing a series of reactions which result in insoluble sulfide mineral compounds being converted to soluble sulfate compounds, which are then collected and processed to recover the leached metal. This conversion between insoluble and soluble compounds is achieved primarily by bacterial production of the ferric ion which oxidises the mineral surface. Bacteria of interest include *Acidithiobacillus ferrooxidans*, which generates the energy required for cell respiration via oxidation of ferrous iron and reduced sulfur compounds, *Leptospirillum ferrooxidans*, which also oxidises iron, and *Acidithiobacillus thiooxidans*, which oxidises sulfur. All three of these bacteria are mesophiles meaning they grow best in temperate conditions, usually between 20 – 45 ^oC (Jain et al., 2010a). Thermophilic bacteria, such as *Sulfobacillus sulfidooxidans*, are also of interest, especially with regard to leaching chalcopyrite ($CuFeS_2$), which does not leach well at temperatures below 50 ^oC (Dew et al., 2011; Dufresne et al., 1996).

An example of a bacterially catalysed leaching process is one used in the recovery of Cu from chalcocite (Cu_2S). Chalcocite's ionic lattice needs to be broken to release Cu^{2+} into solution and for this to occur, a strong oxidising agent and acidification is required, which is provided in the form of the ferric ion via bacterial oxidation of Fe(II) and production of sulfuric acid via oxidation of mineral sulfur (Davenport et al., 2011c). The process proceeds in two steps as follows:

Step 1: The ferric ion oxidises the mineral surface and in the process is itself reduced (see Equation 1). The rate of this step is fast but limited by absence of bacteria (Rawlings, 1997).

Step 2: Resultant Fe(II) is reoxidised by bacteria in the presence of oxygen and acid, which restarts the cycle (see Equation 2). The bacteria's sole role is regeneration of the ferric ion for continuous oxidative attack on the mineral's surface (Fowler et al., 1999).

$$Cu_2S_{(s)} + 2Fe^{3+}_{(aq)} \longrightarrow CuS_{(s)} + Cu^{2+}_{(aq)} + 2Fe^{2+}_{(aq)} \quad (1)$$

$$2Fe^{2+}_{(aq)} + 0.5O_{2\,(g)} + 2H^{+}_{(aq)} \xrightarrow{\text{Bacterial catalyst}} 2Fe^{3+}_{(aq)} + H_2O_{(l)} \quad (2)$$

The bacteria's interaction with the mineral surface via the biofilm layer can be visualised as follows; the standard reduction potentials driving the process also shown.

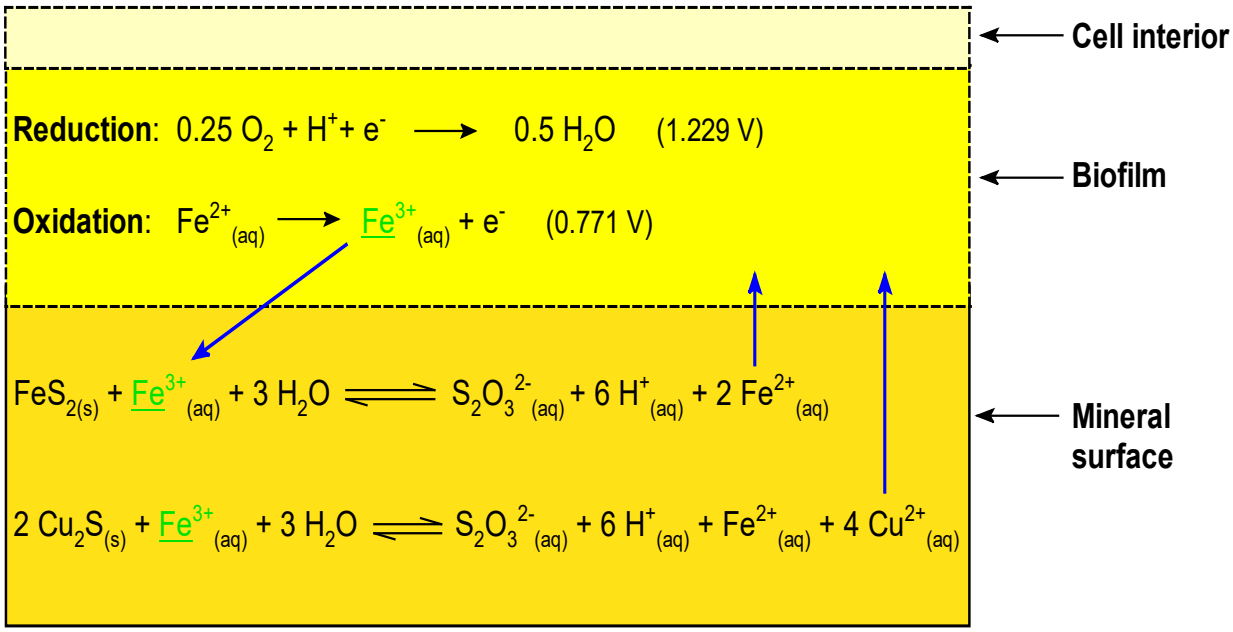

Figure 1: Diagram of *A. Ferrooxidans' interface with mineral surface.*

As shown, Fe^{2+} is oxidised in the bacteria's biofilm layer to Fe^{3+} in a redox reaction with O_2 being reduced to water. Fe^{3+} acts as an oxidising agent, oxidising Fe, S, and Cu, and dissolving the mineral surface layer. This process leads to insoluble FeS_2 and Cu_2S being converted to soluble Fe^{2+}, Cu^{2+}, and $S_2O_3^{2-}$. Thiosulfate is further oxidised to sulfate via an enzymatic process involving thiosulfate dehydrogenase (Kikumoto et al., 2012). The overall result is that metals in the sulfide mineral are

solubilised and so will impregnate the leach solution and drain to the bottom of the bioleaching heap pile.

Bio heap leaching is the process by which sulfuric acid is trickled through a heap of low-grade (around 2% copper, typically), homogenous ore with a particle sizes typically between 12-50 mm (Davenport et al., 2011d) containing the leaching bacteria to act as the oxidising agent. The leach solution is passed through the heap leach pile multiple times to ensure maximum extraction before the pregnant solution is sent for metal separation via a process such as electrowinning in the recovery of copper from aqueous solution (Kordosky, 2002). A typical chalcocite heap leach pile could yield an estimated 75-80% copper extraction within around 14 months (Rawlings and Johnson, 2010b). Heap leaching is widely used in the recovery process for non-sulfide ores, such as tenorite, which, as it does not require oxidation can be leached directly by sulfuric acid.

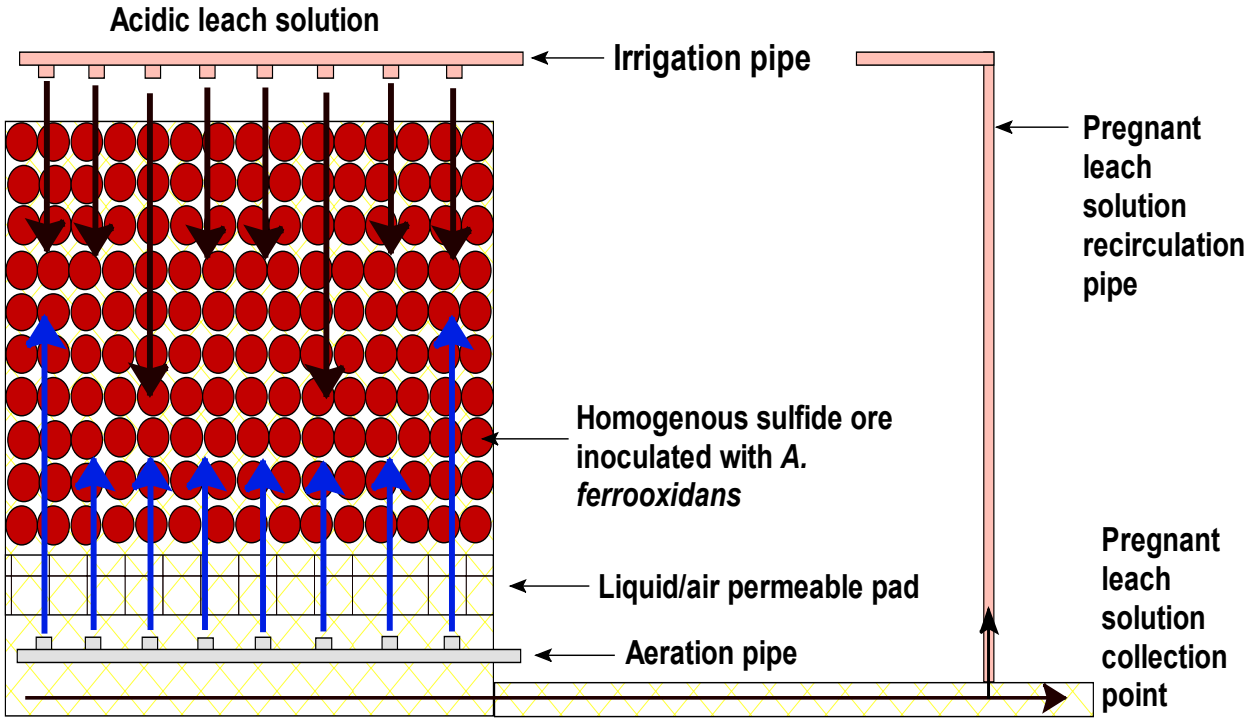

Figure 2: Diagram of bioleaching heap leach pile.

Jarosite Formation and Precipitation

Jarosite are insoluble iron-hydroxysulfate minerals with the general formula [$MFe_3^{3+}(SO_4)_2(OH)_6$] where 'M' can be substituted for any monovalent cation, such as Na+, K^+, H_3O^+ or NH_4^+. Jarosite formation occurs in acidic (approximately between pH 1.5 and 3), sulfate-rich environments, like that of sediment affected by acid mine drainage or in bioleaching systems (Jones et al., 2018).

Camborne School of Mines had a primary research interest in the ammonium determination method was in relation to the precipitation of NH_4^+ jarosite from bioleaching solutions, which results in loss of nitrogen, a key bacterial nutrient

(Willey et al., 2014a). CSM's research interest was in Cu leaching from chalcopyrite, $CuFeS_2$, which does not leach well at mesophilic temperatures and therefore the leaching bacteria of interest is the moderate thermophile Sulfobacillus sulfidooxidans. This bacterium is not able to fix nitrogen from the air (Zhang et al., 2017), unlike A. ferrooxidans (Mackintosh, 1978), and fixes nitrogen from NH_4^+ directly in solution in a process called ammonia assimilation (Bashkin, 2003; Willey et al., 2014b). It is for that reason that 100% of bacterium's nitrogen requirement has to be added to the bioleaching solution. The financial cost of the precipitation of ammonium as jarosite to mining companies is therefore significant as any ammonium precipitated is money wasted. Other nutrients added to the bioleaching solution include phosphate, potassium, and magnesium. The ammonium determination method was used to investigate the optimal bioleaching conditions required to keep ammonium dissolved in solution and therefore minimise formation and precipitation of ammonium jarosite and the subsequent loss of nitrogen from bioleaching solutions.

CSM's experimental setup includes the artificial bioleaching columns shown in the figure below. Their aim is that no ammonium will be detected in their leach solutions after passing through the columns as that will mean that the bacteria present have utilised it. If ammonium is detected in the solutions after passing through the columns that would mean that the detected amount is surplus to the bacteria's requirements and therefore the amount of ammonium in the feed solution is too high. The experimental question they were ultimately asking is "How much ammonium needed to be added to the bioleaching feed solution to nourish the bacteria?" The metal content of the leach solution is also monitored to determine the effectiveness of different leaching parameters upon metal extraction.

Jarosite precipitation is a well understood phenomena and is widely exploited in the zinc industry where it is used to remove dissolved iron from solution (Dutrizac, 1996).

Figure 3: Camborne School of Mines bioleaching columns at the Environmental Sustainability Institute.

Potential Ammonium Determination Methods

Many techniques have been developed for the determination of ammonium in sea or river water where metal concentrations tend to be relatively low in comparison to a typical bioleaching solution (Diallo et al., 2015). These techniques are often hindered by interfering species and therefore require a very 'clean' ammonium sample with few impurities. Due to the chemical nature of bioleaching solutions they are not clean and contain relatively high concentrations of interfering species, particularly Fe which interferes in many colourimetric determination methods such as with the Nessler's Reagent method (Crosby, 1968), and therefore may present difficulty in obtaining quality measurements or may require many sample preparation steps so as to make the method impractical.

It may be possible to control for interfering species by diluting the bioleaching solutions and utilising bioleaching solution free from ammonium as a blank. Further investigation would be required to determine whether the dilution factor applied would be sufficient to null the interference effects without resulting in an ammonium concentration in the sample which is below the limit of detection for the method. Typical ammonium concentrations of ammonium in the bioleaching solutions will range from 1 to 50 mg/L so there is room for a hundredfold dilution to remain above the typical limit of detection of 0.01 mg/L-N for methods such as the salicylate method (Verdouw et al., 1978) utilising a meter such as the HACH DR/890 Colorimeter.

Another potential method of controlling for interferences would be to extract the ammonium from the bioleaching solutions into a new solution and to then to measure its concentration free from the interferences in the new solution using a method such as the Nessler method or the salicylate method discussed previously. A typical extraction method is Kjeldahl digestion, which was originally developed for determination of organic nitrogen in samples such as plant tissue (Pepkowitz and Shive, 1942), however for determination of ammonium in the bioleaching solution, the method could potentially be modified. The digestion is effectively a distillation which relies upon the insolubility of ammonia in solutions with high pH. If bioleaching solutions were placed into a distillation apparatus and base added to raise the pH, the ammonia gas liberated could be condensed and captured in a solution of acid, of known concentration and volume. This would produce a 'clean' solution of ammonium for determination. Determination could then be completed via a titration with addition of a base and in the presence of an indicator dye. A back-titration calculation could then be used to determine the ammonium concentration or an analysis of the clean ammonium solution using a colourimetric method such as the Nessler method could be completed.

In contrast to a typical Kjeldahl digestion, it would not be required to bring the assay solution to a high temperature for a long amount of time. This is due to the nitrogen in the bioleaching samples being present only as volatile ammonia upon addition of base and therefore not needing to be released by the breaking down of larger organic molecules, such as with typical Kjeldahl digestions. Kjeldahl digestion is a process widely used in industry and the process has been mostly automated over the years though less costly setups can be assembled (Campins-Falco et al., 2008).

Due to the reasons aforementioned, a method that can selectively determine ammonium in the highly speciated bioleaching solutions was the most desirable, as it would remove the concerns of interference and lengthy sample preparation or extraction for ammonium assays and could potentially offer a relatively quick determination method.

Methods that can selectively determine ammonium in a speciated solution without the need for ammonium extraction are limited. Two potential options are ion chromatography (IC) and potentiometric determination via an ammonium ion selective electrode or an ammonia gas selective electrode (AGSE). Literature suggests that IC can offer selective determination of ammonium in solutions with metal concentrations multiple orders of magnitude higher than the ammonium concentration. Thomas et al (2002) found > 86 % recovery for all metals and 109 % recovery for ammonium in a solution of Li (2 mg/L), Na (100 mg/L), NH_4^+ (2 mg/L), K (20 mg/L), Mg (3 mg/L) and Ca (2 mg/L). They found that by using a modern high capacity column, environmental samples with a wide range of ionic strengths were able to be analysed without interference from the sample matrix. The main disadvantage of IC over the potentiometric approaches is cost. Whilst IC instruments are affordable to professional laboratories, the financial outlay is still in the order of tens of thousands of pounds. In contrast, electrode setups are available for less than a thousand pounds (GBP).

Of the two potentiometric approaches, the AGSE was deemed superior, due to potential interference effects from Na and K when using ammonium ion selective electrodes (Deyhimi, 1999). The interfering species for AGSEs are volatile amines, which lead to a false increase in the potential difference measurement of solutions. The magnitude of the interference effect is related directly to the interferent's basicity and therefore, the higher the pKa value of the amine, the greater the interference it causes (Lopez and Rechnitz, 1982). It is vital that interfering amines such as methylamine, dimethylamine, diethylamine and triethylamine either not be present in bioleaching samples for assay entirely or be able to be easily removed from the solutions before assay and this has been communicated to CSM. After comparison of the considered analytical methods, it was decided that the analytical method this research project would investigate would be potentiometric determination of ammonium in bioleaching solutions using an ammonia gas selective electrode.

Ammonia Gas Selective Electrodes

Ammonia gas selective electrodes work differently to traditional ion selective electrodes in that they utilise a gas-permeable hydrophobic membrane, which allows for the selective passage of ammonia gas liberated from assay solutions. Diffusion of ammonia occurs across the membrane until the partial pressure of ammonia is equal on both sides and an equilibrium is reached. The pressure at which the equilibrium is reached is proportional to the concentration of ammonia in the assay solution. The solution temperature and total ionic strength also impact the equilibrium (Meyerhoff, 1980). The precise composition of the membranes is proprietary; however, research has been conducted into membranes composed of poly(vinylammonium thiocyanate) for selective separation of ammonia from solution (Bhown and Cussler, 1991).

Once ammonia has diffused through the ammonia gas permeable membrane into the electrode's filling solution, the pH of the filling solution is increased, as the increase in ammonia concentration results in consumption of H_3O^+ from the filling solution. This decrease in H_3O^+ concentration pushes the equilibrium of Equation I to the left, which results in consumption of H^+ to produce more H_3O^+, thus lowering the concentration of H^+ in the filling solution and increasing its pH. The increase in pH is measured as a decrease in the potential across the hydrogen permeable membrane, as the potential is dependent upon the concentration of H^+ in the electrode filling solution. The overall process results in an inversely proportional relationship between the measured potential difference of an assay solution and its ammonium concentration.

The workings of the electrode and relevant chemical equilibria can be visualised as shown in the following figure:

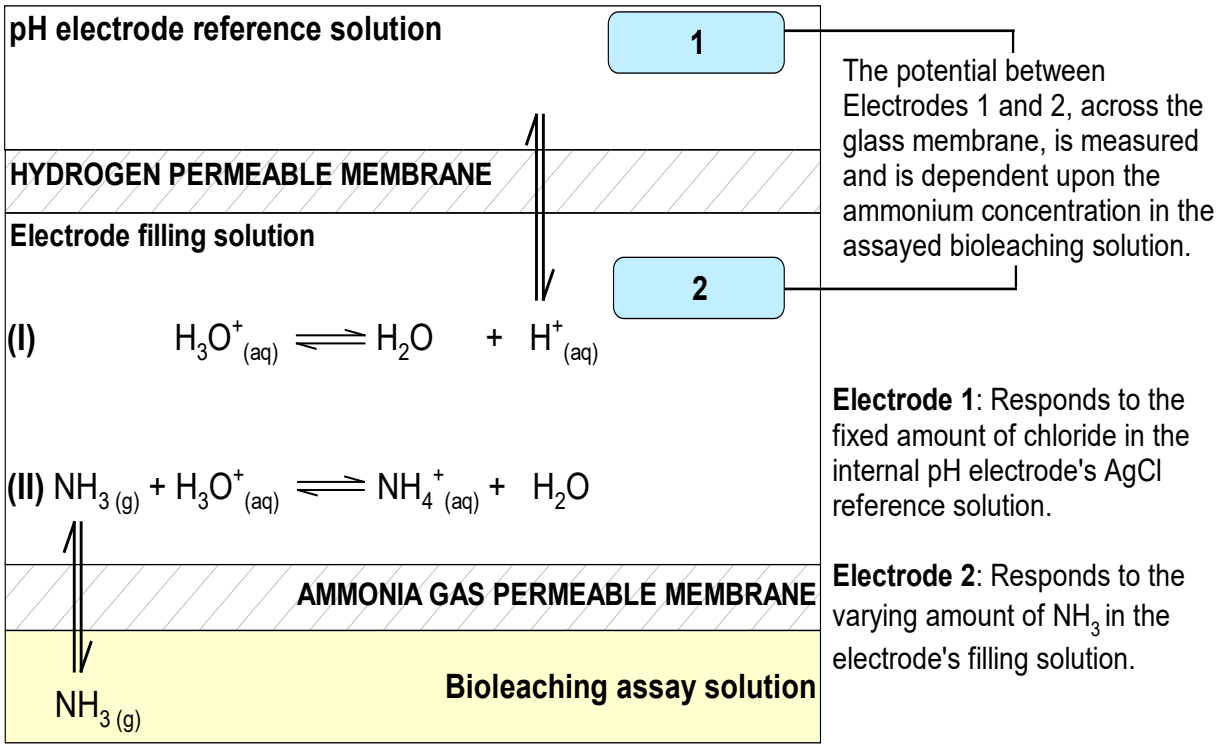

Figure 4: Diagram of ammonia gas selective electrode internal workings.

The relationship between the partial pressure of ammonia and its concentration (which is also equivalent to ammonia's solubility) in a given solution is described by Henry's Law, which states that, at a constant temperature, the amount of a given gas that is dissolved in a given type and volume of liquid is directly proportional to the partial pressure of that gas in equilibrium with that liquid. This relationship can be described mathematically as follows:

$$K_H = \frac{[NH_{3(aq)}]}{P_{NH_3}}$$

Where "K_H" is equal to the Henry's Law constant, a proportionality factor determined empirically (M/atm), "$[NH_3]$" is equal to the concentration of dissolved ammonia in solution, at constant temperature (M), and "P_{NH3}" is equal to the partial pressure of the gas at equilibrium (atm).

To liberate ammonia from acidic solutions requires an understanding of the ammonia/ammonium equilibrium, which can be described as follows:

$$NH_{3\ (g)} + H_2O \rightleftharpoons NH_4^{+}{}_{(aq)} + OH^{-}{}_{(aq)}$$

The pKa value for ammonia is 9.25 due to the formation of hydroxide ions upon dissolution and this value is equivalent to the pH at which the concentration of ammonia and ammonium in a given solution is equal at equilibrium. As shown, ammonia in an acidic solution with a concomitant low concentration of OH ions, is present as the protonated species ammonium. As the provided bioleaching samples are acidified to approximately pH 1.4, all ammonia is present in the aqueous protonated form ammonium.

It is shown that upon addition of OH ions to a solution of ammonium, notwithstanding absorbance of OH by other species present the equilibrium is pushed to the left side, in accordance with Le Chatelier's principle, resulting in an increase in the concentration (and therefore liberation) of insoluble gaseous ammonia. It was for this reason that assayed bioleaching solutions needed to be basified for potential difference measurements to be made by the electrode.

The equilibrium constant for the ammonia/ammonium relationship in the electrode's filling solution can be expressed as follows:

$$K_{eq} = \frac{[NH_4^+][OH^-]}{[NH_3]}$$

As gaseous ammonia is liberated from assay solutions and enters the electrode's filling solution, the concentration of NH_4^+ increases, in line with Le Chatelier's principle. However, as ammonium in the reference solution is highly concentrated, any small change to its concentration will not impact its overall value by a significant amount and therefore the ammonium concentration in the filling solution can be treated as constant. The equation can therefore be expressed with ammonium concentration omitted (Thomas and Booth, 1973):

$$K_{eq} = \frac{[OH^-]}{[NH_3]}$$

The relative amount of ammonia to ammonium in the filling solution directly affects the pH as addition of ammonia to the solutions results in production of OH ions. As previously discussed, it is this change in pH that the electrode measures as a change in the potential difference between its working electrode in the filling solution and its reference electrode, which is held at a fixed potential as it responds to a fixed amount of chloride in the electrode's reference solution (Evans and Foulkes, 2019). Fundamentally, the ammonia gas selective electrode is a pH electrode with an

ammonia-selective polymer membrane placed in front of the pH electrode's glass hydrogen membrane (Crow, 1974). The change in the measured potential between the two electrodes across the glass hydrogen-permeable membrane therefore corresponds directly to the ammonia concentration in the sample (Bier, 2018). The relationship between the measured potential difference between the working and reference electrodes and the ammonia concentration is described by the Nernst equation:

$$\mathrm{E} = \mathrm{E}^0 - 2.303\ \frac{\mathrm{RT}}{\mathrm{nF}}\ \log\left[\mathrm{NH}_4^+\right]$$

Where "E" is the Measured potential difference (conventionally in mV), "E^0" is the reference potential (also conventionally in mV), "R" is the gas constant, equal to 8.314 J mol^{-1} K^{-1}, "T" is the temperature in Kelvin, "n" is the number of electrons transferred, equivalent to the ionic valency, in this case 1, "F" is the Faraday constant, equivalent to 96,485 C mol^{-1} and $[NH_3]$ is the concentration of ammonia in the solution, in mol/dm.

As the above equation is in the form of a linear function, $y = mx + c$, it can be rearranged as follows, where *y* and *x* are the axes values and *m* and *c* correspond to the gradient and y axis intercept value of the plotted straight line:

$$\mathrm{y} = \quad \mathrm{m} \quad \mathrm{x} \quad + \mathrm{c}$$

$$E = -2.303\ \frac{RT}{nF}\ \log[NH_4^+] + E^0$$

Once plotted, the gradient of the graph is dependent only on solution temperature as all other terms in the *m* portion of the equation are constants.

Therefore, if the logged ammonium concentration of a prepared series of ammonium standards are plotted against their corresponding potential difference values, and a calibration graph is plotted, the ammonium concentration in an unknown sample can be determined by solving the linear equation for *x* and anti-logging, as follows:

$$\left[\mathrm{NH}_4^+\right] = 10^{\frac{\text{Electrode response} + |\text{y intercept value}|}{\text{Gradient of calibration graph}}}$$

Methodology

Throughout all laboratory work conducted as part of this research project, glassware was prewashed and soaked using 2% nitric acid for a minimum of 30 minutes and, unless otherwise stated, deionised water was used in making volumetric flasks up to the mark. Calibrated analytical balances, analytical and bulb pipettes were used for weighing of reagents and preparation of calibration standards. The ammonium salts used were reagent grade and provided by Sigma Aldrich. Risk assessments and COSHH forms were completed for all reagents used and also for the bioleaching solutions provided.

This section of the paper discusses the laboratory work completed related to the use of an ammonia gas selective electrode (AGSE). The first section covers the "proof of

concept" laboratory work, carried out with a faulty AGSE belonging to the University of Plymouth which was modified to perform bioleaching solution analyses; the second part covers the method development laboratory work, completed with the AGSE purchased and provided by Camborne School of Mines (CSM) upon their review of the proof of concept laboratory work.

Proof of Concept Laboratory Work

The ThermoFisher Orion 9512 AGSE provided by the University was disassembled and placed into pH 4.0 and pH 7.0 pH electrode calibration solutions. Repeat attempts to calibrate the electrode were made but no response was recorded when checking for pH or change in potential difference using the mV mode of the meter, a model 3510 manufactured by Jenway. The internal electrode of the ThermoFisher Orion 9515 AGSE was therefore deemed to be faulty and was therefore not able to be used.

As only the internal electrode of the AGSE was faulty, the electrode casing, which serves as a holder for the ammonia-selective membrane and as a container for the electrode filling solution were still able to be used. A slim pH electrode which could fit inside of the casing and replace the faulty electrode was therefore sourced and 2.5 mL of electrode filling solution was added to the case so a usable electrode could be fashioned. A slim pH electrode manufactured by Mettler Toledo was first sourced but produced poor sensitivity so another more modern FB68801 slim pH electrode manufactured by Fisherbrand was sourced, which performed acceptably in both simulated and diluted bioleaching solutions. Results were reported to CSM, which led to their purchase of a new AGSE, manufactured by ThermoFisher, which was used in method development part of the laboratory work.

To preserve the limited amount of bioleaching solution provided, tenfold dilutions were applied to all bioleaching solution used in the proof of concept laboratory work, as the primary objective was to validate the concept of measuring ammonium as ammonia in the bioleaching solutions, rather than to generate actual measurements or to develop a repeatable method. The experimental setup for the proof of concept laboratory work was as shown in Figure 5.

Care was taken to ensure that the electrode apparatus was set up in the same way each time before any measurements were taken so as to not introduce experimental error between measurements. Of particular note were the electrode depth in the solution and the solution temperature. A 100 mL lab beaker was used to provide a good solution depth to volume ratio whilst still allowing room for the magnetic stir bar.

The analysis procedure was adapted from EPA Method #350.3 (Environmental Protection Agency, 1974). Three sets of calibration standards were prepared at concentrations of 0.0, 0.1, 1.0, 10, 100 and 1000 mg/L-NH3 using three separate matrices: a synthetic bioleaching solution using a 0.5 M NaCl in deionised water solution to simulate the ionic strength of the real bioleaching solution diluted tenfold, the Fe(II) bioleaching solution provided by CSM and the Fe(III) bioleaching solution provided by CSM, both diluted tenfold.

Figure 5: Experimental setup for proof of concept laboratory work. The figure shows the Mettler Toledo electrode (1), the ThermoFisher Orion 9512 electrode casing with ammonia selective membrane and filling solution (2), the thermometer (3), the electrode stand (4), the magnetic stir bar (5) and the Jenway 3510 pH meter (6).

The purpose of the 0.05 M NaCl matrix was to determine whether electrode response was impacted by the specific ionic species present in solution or by the total ionic strength of the solution. This was an important distinction to make because if electrode response is not dependent upon the speciation then CSM would be able to produce a series of blank solutions or varying ionic strengths using a non-ammonium salt which could then be used to 'zero' their electrode. This would allow for quick ammonium determinations as the electrode could be zeroed in the relevant premade blank solution of an ionic strength approximately equal to that of the assay matrix and then could be placed into the assay solution for approximate ammonium determination without the need for construction of a new calibration curve for every new assay matrix investigated.

Comparison of calibration curves in solutions of approximately equal ionic strength (the 0.05 M NaCl matrix vs. the tenfold diluted bioleaching solution) allowed for this determination to be made. Theoretically there should be no difference between the

curves as, as previously discussed, the solubility of ammonia is dependent on the total ionic strength of the solution, as opposed to the specific species present.

The total ionic strength of the bioleaching solutions was calculated using the composition data provided by CSM and the formula devised by Debye and Hückel as part of their research on non-ideal solutions (Atkins, 1998) and found to be approximately 5 M for the Fe(II) solution and 5.6 M for the Fe(III) solution.

Calibration standards were then prepared using analytical grade $(NH_4)_2SO_4$ as the ammonium source and were prepared using the serial dilution method, starting with a 1000 mg/L-NH_3 solution, prepared by addition of 0.970 g of $(NH_4)_2SO_4$ to a 250 mL volumetric flask made up to the mark, and diluting downward to a lowest concentration of 0.1 mg/L-NH_3 in increments of tenfold dilutions using 100 mL volumetric flasks made up to the mark with the desired matrix.

The inner body of the electrode was soaked in the supplied electrode filling solution for a period of 12 hours beforehand (this solution is typically a 0.1 M solution of NH_4Cl with a small amount of $AgNO_3$). The electrode was then placed into a 10 mg/L ammonium solution for 15 minutes, per the manufacturer's recommendation, before any measurements were performed.

The electrode was placed into 100 mL of the blank solution so that its tip was 3 cm from the base of the beaker and the stir bar was turned on to provide a small amount of mixing but not enough to form a vortex, which could lead to excessive loss of ammonia from the solution, potentially impacting the amount of ammonia diffusing into the electrode. 5 mL of 1 M NaOH was then added and the metre was set to millivolt mode and the reading was allowed to stabilise. Once stable, after approximately five minutes, the "relative" button on the meter was pressed so that the mV reading of the blank solution would be set to zero and all further measurements would be relative to that of the potential difference generated by the blank solution and 5 mL of 1 M base.

Next, 100 mL of the lowest concentration calibration standard was transferred from its volumetric flask into a 100 mL beaker for analysis. The stir bar was again turned on to provide a light amount of mixing. Next, 5 mL of 1 M NaOH was added to the beaker to raise the solution's pH and liberate ammonia. Once added, the mV reading decreased gradually over a period of approximately 30 minutes before settling. The same amount of base was added to each assay in order to raise the pH equally in all samples in a calibration series. Addition of an equal volume of base to each standard is important as ammonia's solubility is proportional to the pH of the solution and error would be introduced by variance of pH between the standards.

The instrument reading was allowed to stabilise until the meter's 'stable reading' symbol appeared constantly for over a minute, and the millivolt value was then recorded. Stabilisation took an average of around 30 minutes for ammonia measurements taken in 0.5 M NaCl and tenfold diluted bioleaching solution matrices. Between measurements the electrode was rinsed with deionised water and dabbed dry with a paper towel, per the manufacturer's instructions.

The procedure was repeated for all calibration standards in order from lowest to highest concentration and four calibration graphs were then plotted, as follows:

1. Mettler Toledo internal electrode in 0.5 M NaCl matrix
2. Fisherbrand FB68801 internal electrode in 0.5 M NaCl matrix
3. Fisherbrand FB68801 internal electrode in Fe(II) matrix
4. Fisherbrand FB68801 internal electrode in Fe(III) matrix

Spiked samples using both Fe(II) and Fe(III) solutions (diluted tenfold) with known additions of ammonium were analysed and plotted on the simulated bioleaching solution calibration graphs to assess whether the simulated solution provided a good estimation of the real solution and to determine if assay matrix impacted the electrode performance. Repeat measurements of the blank solution were also taken between calibration standard measurements in the tenfold diluted Fe(III) bioleaching solution to assess the instrument drift and determine the limit of quantification.

Method Development with Electrode Provided by Camborne School of Mines

The proof of concept laboratory work validated the concept of determining ammonium in the bioleaching solutions as ammonia using an ammonia gas selective electrode. As a result of this CSM purchased their own AGSE, an Orion 9512HPBNWP electrode manufactured by ThermoFisher Scientific. The second part of the laboratory work focused on verifying the newly purchased electrode operated within manufacturer's specifications and assessing its performance characteristics, namely the precision, as measurements in this section were performed in triplicate, the linear range, and investigating how the electrode responded in undiluted bioleaching solutions, with higher concentrations of metals, which affected the amount of base required, than in the proof of concept work. The other focus of the method development laboratory work was to develop a repeatable method for use by CSM in their research.

To verify correct operation of the electrode, a series of ammonium calibration standards were again prepared in two matrices: 0.05 M H_2SO_4 and undiluted Fe(III) bioleaching solutions. This concentration of H_2SO_4 was chosen as it was readily available in the laboratory and approximated the pH of CSM's research bioleaching solutions (around 1.5) well. The pH of the 0.05 M H_2SO_4 solution was determined as follows:

$$\mathrm{pH} = -\log_{10}[\mathrm{H}^+] = -\log_{10}(0.05) = 1.30$$

First, a 10,000 mg/L-NH_4^+ stock solution was prepared by addition of 3.64 grams of reagent grade $(NH_4)_2SO_4$ to a 100 mL volumetric flask, made up to the mark with 0.05 M H_2SO_4. Acting upon feedback from CSM, standards were prepared as ammonium as opposed to ammonia as in the proof of concept laboratory work Tenfold dilutions of the 10,000 mg/L-NH_4^+ stock solution was made down to 1 mg/L-NH_4^+, each time making up to the mark with either 0.05 M H_2SO_4 or Fe(III) bioleaching in 100 mL volumetric flasks. Blank solutions of 0.05 M H_2SO_4 and Fe(III) bioleaching solution with no ammonium addition were also prepared.
The electrode apparatus was set up as shown previously with the exception that a 60 mL beaker was used for analyses to preserve bioleaching solution, acting upon feedback from CSM that using smaller volumes of bioleaching solution in their assays was preferable. Using a slim beaker allowed for an electrode depth of 2 cm in

30 mL of assay solution. The measurement procedure was otherwise the same as for the proof of concept work with the exception being that the procedure was repeated a total of three times to provide triplicate measurements so instrument precision could be determined. The same calibration standards were used as there was insufficient bioleaching solution to prepare three separate sets of standards.

Particular attention to the volume of base added to the calibration standards when making ammonium measurements was made. This was due to the two matrices being assessed being more acidic than those in the proof of concept laboratory work.

The theoretically appropriate amount of base needed to be added to a metal-free assay solution was determined to be 1.25 mL, assuming a solution pH of 1.5 and a target ammonia liberation pH of 12 though experimental trial and error with this amount of base proved poor for obtaining potential difference measurements. No instrument response was recorded in 30 mL assay solutions with under 5 mL of 1 M base addition and slow instrument response (greater than 25 minutes for the reading to first reach the stabilisation value) was observed when using between 5 mL and 15 mL. Larger amounts of base produced the best measurements with additions of 30 to 40 mL producing sharp instrument response and stabilisation within 10 minutes of addition and was therefore the amount of base used for the method development calibration standard measurements.

After the three series of potential difference measurements for the two series of calibration standards were made, calibration graphs were produced using the average measurement value with error bars added so that the sensitivity, linear range, and precision of the electrode could be assessed.

To assess the accuracy of the electrode, CSM provided two new bioleaching solution samples containing 50 mg/L-NH_4^+ and two corresponding blanks, which possessed the same matrix but no ammonium. The electrode was zeroed to the relevant blank solution and 40 mL of 1 M NaOH was added to liberate ammonia from the solutions. As only a small amount of the relevant blank was provided, it was not possible to produce a calibration graph for this new matrix. Due to this, an electrode curve typical of the solution temperature was used. This introduced error and uncertainty into the accuracy assessment but still provided a good assessment of the electrode's performance in line with the objective of the laboratory working using CSM's electrode by allowing for calculation of an approximate recovery factor. The recovery factors were determined as follows:

$$\text{Recovery Factor} = \frac{\text{Determined concentration value (mg/L)}}{\text{Maximum theorectical concentration value (mg/L)}}$$

Results

Proof of Concept Laboratory Work

The instrument responses for the two internal pH electrodes in the simulated tenfold diluted bioleaching solution calibration standards using 0.5 M NaCl were as shown in Table 1.

Table 1: Calibration standard concentrations and electrode response in simulated tenfold diluted bioleaching solutions.

Ammonia Conc. (mg/L)	**Ammonia Conc. (M)**	**$(NH_4)_2SO_4$ conc. (mg/L)**	**Instrument Response (mV)**	
			Mettler Toledo electrode	Fisherbrand Electrode
0.00	0.00	0.00	0	0
0.10	5.87×10^{-6}	0.388	-2.7*	-5.8*
1.00	5.87×10^{-5}	3.88	-29.4	-62.3
10.00	5.87×10^{-4}	38.8	-42.9	-119.1
100.00	5.87×10^{-3}	388	-77.7	-175.0
1000.00	5.87×10^{-2}	3880	-122.0	-230.7

The instrument responses for the Fisherbrand internal pH electrode in the bioleaching solution matrices were as shown below.

Table 2: Calibration standard concentration and electrode response in bioleaching solution matrices with Fisherbrand electrode.

Ammonia Conc. (mg/L)	**Ammonia Conc. (M)**	**$(NH_4)_2SO_4$ conc. (mg/L)**	**Instrument Response in Fe(II) Solution (mV)**	**Instrument Response in Fe(III) Solution (mV)**
0.00	0.00	0.00	0	0
0.10	5.87×10^{-6}	0.388	-5.0*	-5.1*
1.00	5.87×10^{-5}	3.88	-60.9	-61.3
10.0	5.87×10^{-4}	38.8	-121.5	-120.4
100	5.87×10^{-3}	388	-179.8	-180.6
1000	5.87×10^{-2}	3880	-234.2	-236.5

*These measurements were later discounted.

The instrument responses for the Fisherbrand electrode in the spiked tenfold diluted bioleaching solution assays were as shown below. The spiked sample measurements are only shown on the calibrations graphs for the Fe(II) bioleaching solution as the Fe(III) values were very similar. The values are shown as green squares on the calibration graphs.

Table 3: Spiked samples predicted vs actual electrode response in tenfold diluted bioleaching solution.

Electrode and Matrix Used	**Ammonia Conc. (mg/L)**	**Expected Instrument Response (mV)**	**Actual Instrument Response (mV)**	**% Diff**	**Measured Ammonia Conc. (mg/L)**	**Recovery Factor**
Mettler Toledo Electrode - Fe(II)	5	-42.96	-36.0	-16.2	3.00	0.60
Mettler Toledo Electrode - Fe(III)	5	-42.96	-37.1	-12.9	3.25	0.65
Fisherbrand Electrode - Fe(II)	5	-101.83	-98.5	-3.3	4.36	0.87
Fisherbrand Electrode - Fe(III)	5	-101.83	-100.1	-1.7	4.66	0.93

The instrument responses for the Fisherbrand electrode in the spiked bioleaching solution assays were as shown below.

Table 4: Spiked samples predicted vs actual instrument response in bioleaching solution matrices.

Bioleaching Solution	Ammonia Conc. (mg/L)	Expected Instrument Response (mV)	Actual instrument Response (mV)	% Diff	Measured Ammonia Conc. (mg/L)	Recovery Factor
Fe(II)	5	-102.78	-97.6	-5.0	4.07	0.81
Fe(III)	5	-102.78	-99.2	-3.5	4.34	0.87

Four calibration graphs were plotted from the data obtained and linear regression trendlines were added.

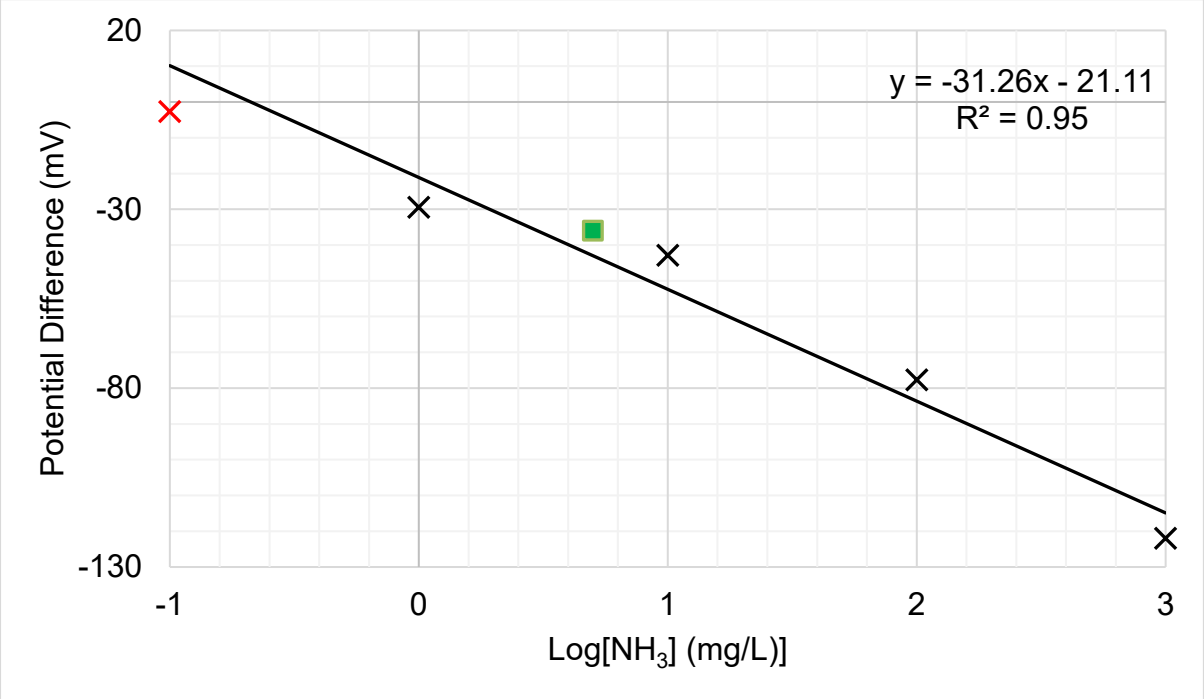

Figure 6: Mettler Toledo electrode calibration graph in simulated tenfold diluted bioleaching solution calibration standards. The red cross denotes the discounted average measurement for the 0.10 mg/L-NH_3 solutions. The green square denotes the spiked sample's logged concentration in relation to the "expected" instrument response per the linear regression line.

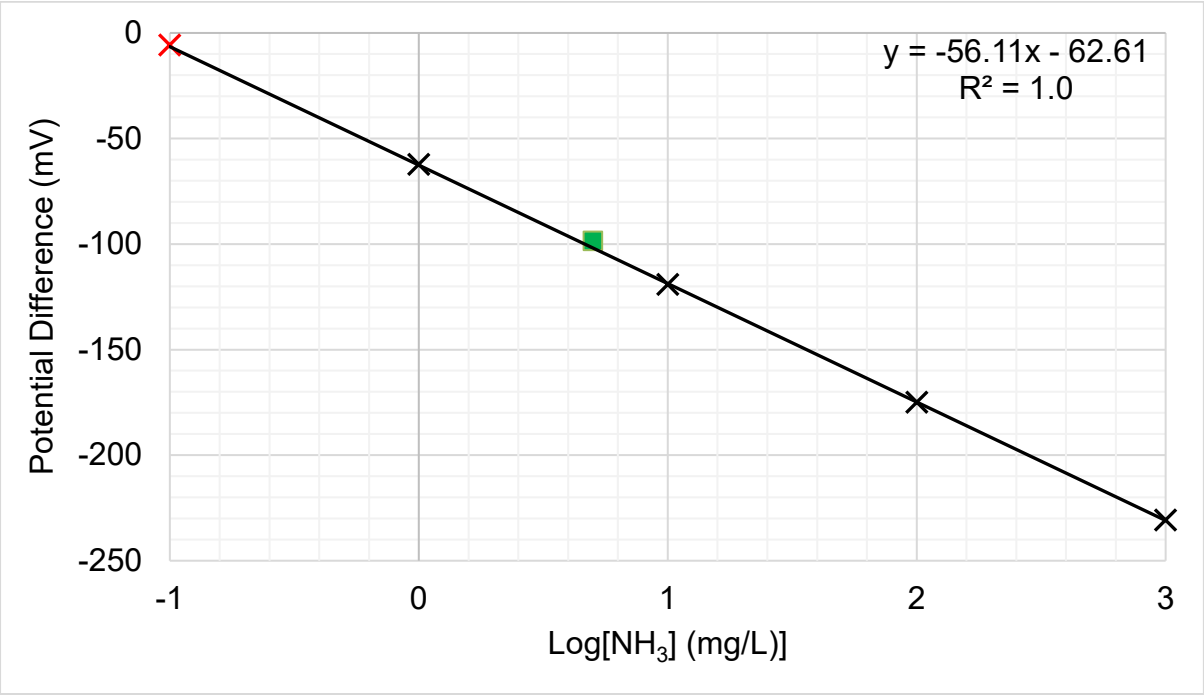

Figure 7: Fisherbrand electrode calibration graph in simulated tenfold diluted bioleaching solution calibration standards. The red cross denotes the discounted average measurement for the 0.10 mg/L-NH_3 solutions. The green square denotes the spiked sample's logged concentration in relation to the "expected" instrument response per the linear regression line.

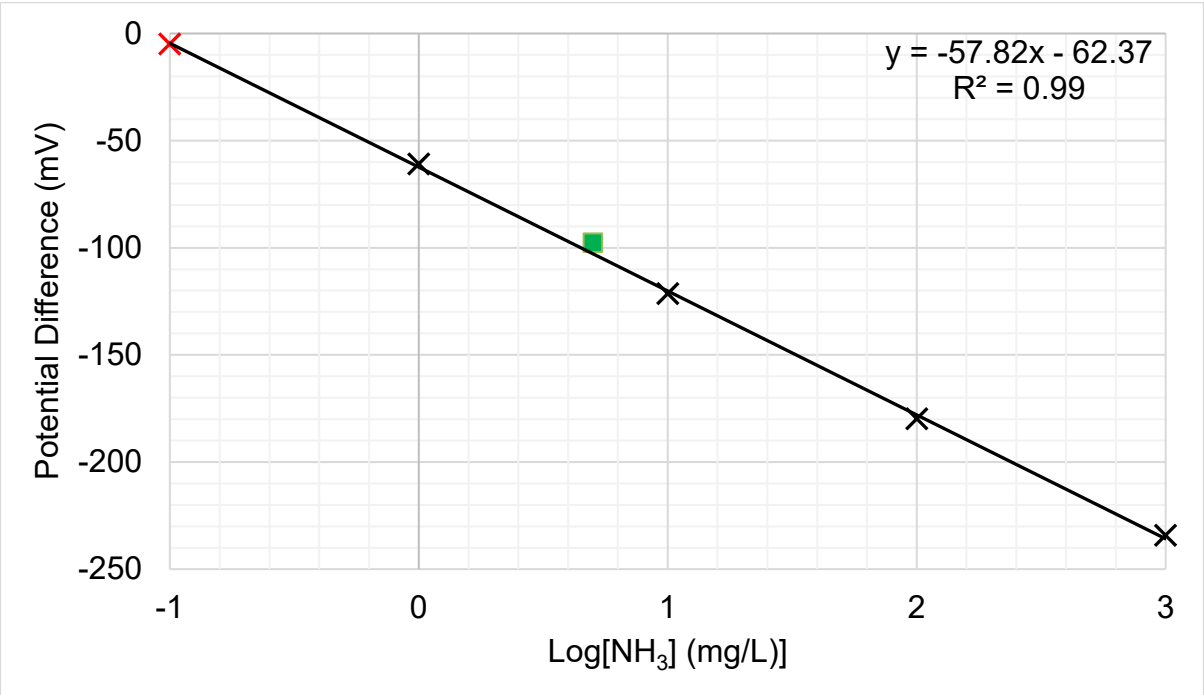

Figure 8: Fisherbrand electrode calibration graph in Fe(II) bioleaching solution calibration standards. The red cross denotes the discounted average measurement for the 0.10 mg/L-NH3 solutions. The green square denotes the spiked sample's logged concentration in relation to the "expected" instrument response per the linear regression line.

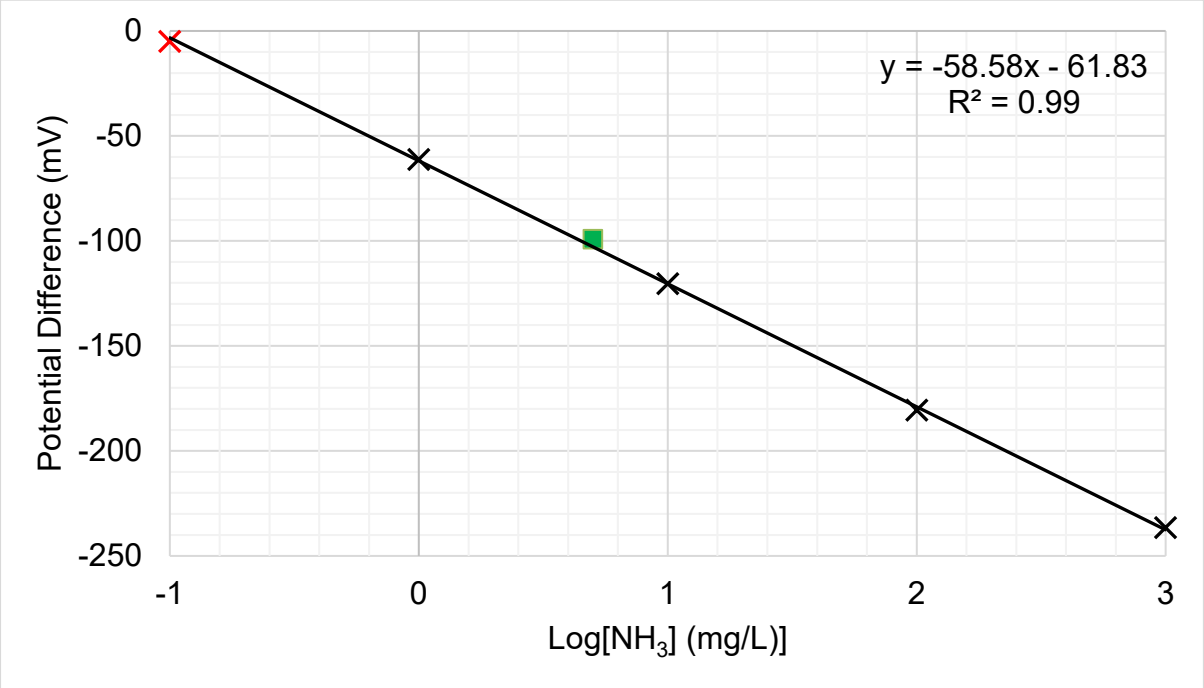

Figure 9: Fisherbrand electrode calibration graph in Fe(III) bioleaching solution calibration standards. The red cross denotes the discounted average measurement for the 0.10 mg/L-NH_3 solutions. The green square denotes the spiked sample's logged concentration in relation to the "expected" instrument response per the linear regression line.

Limit of Quantification Determination

After the instrument had been zeroed to the blank calibration standard's potential difference, further measurements of the blank sample were taken after analysis of each calibration standard. This provides valuable data about how much, if any, instrument drift is present and allowed for the method's limit of quantification (LOQ) to be determined. The LOQ is defined as the lowest concentration of analyte the method can determined from that of a blank sample with adequate precision for most practical purposes was determined as follows:

$$X_{LOQ} = \frac{10s_0}{b}$$

Where "s_0" is the sample standard deviation of the series of blank measurements taken, and "b" is the gradient.

The results of repeat measurements of the blank sample taken between calibration standard measurements were as follows:

Table 5: Repeat blank sample analysis measurements for LOQ determination.

Calibration standard NH_3 conc. (mg/L)	**Potential difference relative to blank (mV)**				
	1	**2**	**3**	**4**	**5**
0	-0.5	-0.8	-0.7	-1.3	-2.0

The LOQ was determined to be 0.8 mg/L-NH_3 using the previous Equation shown.

Method Development with Electrode Provided by Camborne School of Mines
Instrument responses for the CSM electrode in the 0.05 M H_2SO_4 and Fe(III) calibration standards were as shown in the tables below.

Table 6: Calibration standard concentrations and electrode response in 0.05 M H_2SO_4 matrix.

Ammonium concentration (mg/L)	Measurement (mV)			Average (mV)	Standard deviation (mV)	RSD %
	1	2	3			
0.0	0.0	0.0	0.0	0.0	0.00	0.0
1.0	-61.9	-60.8	-62.3	-61.7	0.78	1.3
10	-119.1	-120.6	-121.2	-120.3	1.08	0.9
100	-179.5	-181.6	-177.2	-179.4	2.20	1.2
1000	-237.3	-283.0*	-235.5			

***This measurement was deemed to be anomalous and therefore no average, standard deviation or rsd could be determined for the 1000 mg/L solution as three reliable measurements were not made.

Table 7: Calibration standard concentrations and electrode response in Fe(III) bioleaching solution matrix.

Ammonium concentration (mg/L)	Measurement (mV)			Average (mV)	Standard deviation (mV)	RSD %
	1	2	3			
0.0	0.0	0.0	0.0	0.0	0.00	0.0
1.0	-60.1	-59.2	-60.4	-59.9	0.62	1.0
10	-121.0	-121.3	-121.8	-121.4	0.40	0.3
100	-180.1	-179.7	-178.7	-179.5	0.72	0.4
1000	-241.6	-241.4	-240.7	-241.2	0.47	0.2

Two calibration graphs were plotted from the data obtained and linear regression trendlines were added (Figures 10 & 11). Error bars equivalent to three times the standard deviation in either direction was added to each potential difference value, which meant that the was a 99.73 % likelihood of the true value falling within the error range shown (Harvey, 2000). The error bars were not effectively shown at the half-page scale so are not shown in this section. The anomalous measurement highlighted in Table 6 is shown as a red circle on the corresponding calibration graph. The anomalous measurement as not included for purposes of calculating averages and standard deviations for the error bars nor for calculation of the linear regression line.

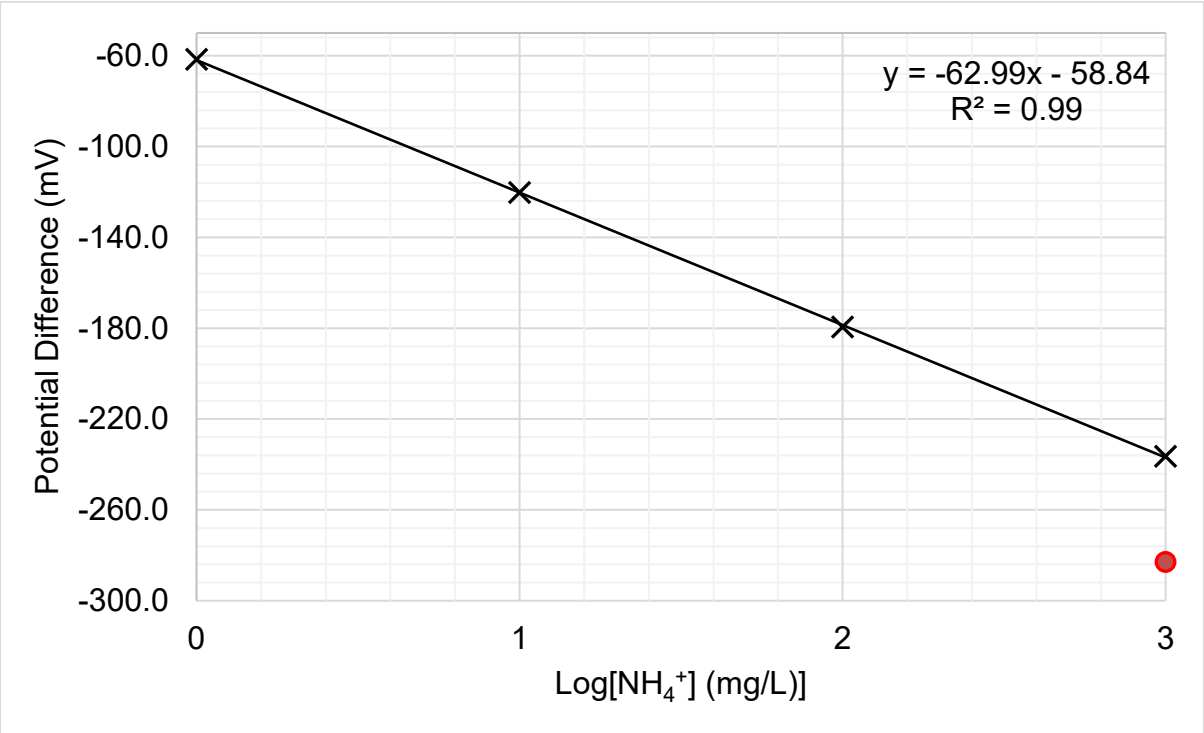

Figure 10: CSM electrode calibration graph in 0.05 M H_2SO_4 calibration standards. The red dot denotes the -283.0 mV anomalous measurement.

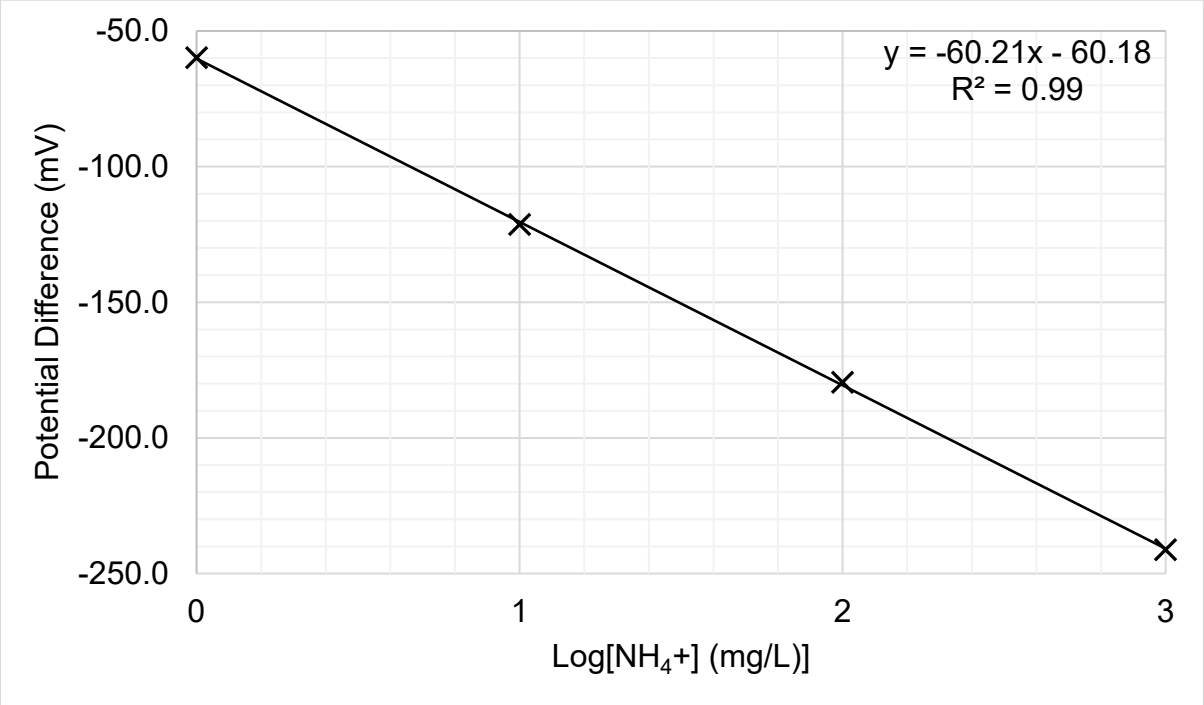

Figure 11: CSM electrode calibration graph in Fe(III) bioleaching solution calibration solutions.

The ammonium measurements for the two 50 mg/L-NH_4^+ solutions provided by CSM were as shown in the table below. As previously discussed, the assumed electrode curve used was y = -60.21x -60.18, which was the reported curve for the electrode in the Fe(III) bioleaching solution, was used to produce the "expected instrument response" column.

Table 8: Recovery factors for ammonium determination in further bioleaching solutions provided by CSM.

Solution	Ammonium Conc. (mg/L)	Expected Instrument Response (mV)	Actual instrument Response (mV)	% Diff	Measured Ammonia Conc. (mg/L)	Recovery Factor
1	50	-162.4	-160.4	-1.23	46.2	0.92
2	50	-162.4	-161.4	-0.62	48.0	0.96

Discussion

Proof of Concept Laboratory Work

The slope of the calibration graphs represents the sensitivity of the electrode used, the amount of electrode response per unit concentration. The Mettler Toledo electrode returned poor sensitivity of -31.26 mV/decade, which is around half of the literature sensitivity, and what the Nernst equation predicts for an electrode measuring pH, of between -58 to -64 mV/decade, depending on temperature. Degradation of the sensitivity was likely due to the electrode's age of approximately 20 years. No attempt to recondition the electrode was made as the Fisherbrand electrode was sourced. The Fisherbrand electrode returned an average sensitivity of -57.50 mV/decade, which was in line with literature and the manufacturer's documentation. The Fisherbrand electrode also returned an average R^2 value of 0.999, which indicated that the electrode response was linear over the four orders of magnitude of the calibration standards. The Fisherbrand electrode slopes were broadly equivalent in the Fe(II) and Fe(III) matrices, which confirmed that the level of Fe oxidation in the assay matrix did not impact electrode response, as expected for an ammonium-selective method.

The potential difference measurements for the 0.1 mg/L-NH_3 calibration standards using both electrodes were of concern. The measurement values were hypothesised to be approximately equal to the sensitivity of the electrode the ammonia concentration was being measured with (-31.26 mV for the Toledo electrode and -57.50 mV for the Fisherbrand electrode) however, both measurements were close to 0 mV, the relative potential difference value of the blank solution. It was determined that it may not be possible to determine this low an ammonia concentration with an AGSE without modification of the assay solutions and that these measurements likely responded to electrode drift rather than ammonia liberated from the solutions. The potential differences measured for the 1.00 mg/L-NH_3 lent credence to this theory as the values of -29.4 and -62.3 mV were in line with being equal to one decade of sensitivity and it was therefore inferred that around 1.00 mg/L-NH_3 is the lowest ammonia concentration the electrodes were able to measure under the assay conditions used.

Due to the unreliability of the 0.10 mg/L-NH_3 measurements, they were not included for purposes of determining the linear regression equations for the lines of best fit and are shown in red on the calibration graphs. The method development calibration standards were decided to therefore start from a concentration of 1.0 mg/L-NH_4^+.

The spiked sample recovery factors of 0.60 and 0.65 for the Mettler Toledo electrode were poor which was to be expected as the low sensitivity of the electrode meant that difference between expected and actual electrode response was magnified (e.g. 10 mV difference corresponds to greater difference in ammonia concentration at -30 mV/dec sensitivity vs. -60 mV/dec sensitivity, as the gradient of the electrode slope is lower). As expected, the recovery factors for the more sensitive *Fisherbrand* electrode were much better in all matrices investigated, ranging between 0.81 and 0.93.

The determined limit of quantification of 0.8 mg/L-NH_3 reflected the relatively low amount of instrument drift from the Fisherbrand electrode. The limit of quantification was acceptable for the intended use of the electrode by CSM to determine ammonium in bioleaching solutions at concentrations of under 50 mg/L. Concentrations determined at values of less than 0.8 mg/L-NH_3 will need to be reported as “<LOQ”.

Method Development Laboratory Work

The electrode purchased and provided by CSM after their review of the proof of concept laboratory work performed in line with literature returning a sensitivity of around -63 mV/dec in the H_2SO_4 calibration standards and around -60 mV/dec in the Fe(III) bioleaching solution calibration standards. The electrode response was linear in both sets of calibration standards. This again showed that linearity is independent of assay matrix and that instrument response is dependent upon only the ammonium concentration in the assay, as indicated by the observed R^2 values of 0.997 and 0.999 for the two assessed matrices.

The second measurement of -283.0 mV for the 1000 mg/L-NH_4^+ calibration standard in the H_2SO_4 matrix was of interest but was deemed to be anomalous due to all other measurements for the 0, 1.0, 10 and 100 mg/L-NH_4^+ in that matrix being in close proximity to one another, as shown by their standard deviation and relative standard deviation values. Therefore, as three reliable measurements were not available for the 1000 mg/L-NH_4^+ solution, an average, and therefore a standard deviation, could not be calculated and error bars were therefore not added to that data point.

The main experimental observation during the method development laboratory work was the large difference in the amount of hydroxide addition required to obtain measurements. The metals in the provided bioleaching solutions and their respective hydroxides species are shown in Table 9. The concentrations listed are based upon data provided by CSM. During precipitation, the metal-hydroxides removed dissolved OH from the bioleaching solutions, therefore lowering the pH and therefore increasing the solubility of ammonia. When analysing an assay with a corresponding calibration graph, it is important that the solubility of ammonia was equivalent for both the assay and the calibration standards used to produce the calibration graph.

As the metal concentration of the bioleaching solutions was known, the theoretical amount of base required to raise the solution pH to 12, and to account for metal-hydroxide precipitation, was determined by multiplying the molar concentration of each metal that formed an insoluble hydroxide complex by its stoichiometric equivalent of hydroxide e.g. the moles of OH removed by Fe(II) precipitation are equivalent to double the Fe(II) concentration.

The concentrations of OH removed from solution are shown in the Table 9. Al exhibits amphoteric behaviour and will therefore dissolve in acidic and basic solutions but not in neutral solutions (House, 2008). Therefore, $Al(OH)_3$ will precipitate as the pH is increased toward 7 but will dissolve again once a basic solution is produced. NaOH, as a group 1 hydroxide, is also soluble and will therefore also not remove OH from solution.

Table 9: Metal hydroxide species and moles of hydroxide absorbed.

Metal	**Conc. of metal (M)**	**Hydroxide species**	**Conc. of hydroxide absorbed during precipitation (M)**	**Solubility at pH 12**
Al	0.185	$Al(OH)_3$	0.00	Soluble
Fe(II)	0.179	$Fe(OH)_2$	0.358	Insoluble
Fe(III)	0.179	$Fe(OH)_3$	0.537	Insoluble
Mg	0.617	$Mg(OH)_2$	1.23	Insoluble
Mn	0.091	$Mn(OH)_2$	0.182	Insoluble
Na	0.022	NaOH	0.00	Soluble
Total OH removed with Fe(II)			**1.8**	
Total OH removed with Fe(III)			**2.0**	

The theoretical concentration of base addition required to account for the metal content was therefore approximately 2 M, which is equivalent to 60 mL of 1 M base addition to a 30 mL assay. This amount of base addition was approximately 1.5 to 2 times the experimentally observed suitable addition of 30 to 40 mL needed to obtain sharp instrument response. It was possible that the quickly poured addition of 30-40 mL of base to the bioleaching assays was sufficient enough to liberate ammonia from the assay and into the electrode's internal solution and to produce a reading before the added hydroxide was precipitated from the assay solution as metal-hydroxides complexes. This was in line with the observation that the assay solutions became turbid due to the OH precipitation slightly after the instrument began to respond.

The recovery factors of 0.92 and 0.96 for ammonium in the two additional bioleaching solutions provided by CSM were satisfactory and showed that the instrument was accurate to approximately between 4 and 8% of a known value. However, due to the approximations made with regard to the estimation of the calibration curve, more assessments would need to be performed before a reliable characterisation of the instrument's accuracy could be stated, such as repeat determinations using a certified reference material.

In context the method is appropriate. As samples can easily be drawn from CSM's bioleaching columns, assays can be performed at their laboratory. Once the electrode apparatus is set up and calibration solutions are prepared, a calibration series can be analysed in an hour and individual potential difference measurements can then be made in approximately 10 minutes using the method outlined in in this paper.

Electrode Precision and Scaling of Instrument Error

The precision of the new electrode provided by CSM was determined to be ± 1.1 mV. This was calculated by doubling the average of the standard deviations of the potential difference measurements in the Fe(III) bioleaching solution.

The scaling of error when using an instrument with a logarithmic response must be considered. For example, if we consider an electrode sensitivity of -60 mV/decade, with the observed precision of ± 1.1 mV, the y error bars would remain at ± 1.1 mV but the x error bars would increase logarithmically with the ammonium concentration. The impact of this is most plainly seen when logarithmic error bars are plotted on a linear scale, as shown below. For the measurements of 10 and 100 mg/L-NH_4^+, the error bars are barely visible, however, for 1000 mg/L-NH_4^+, they are considerable and easily seen on a linear scale.

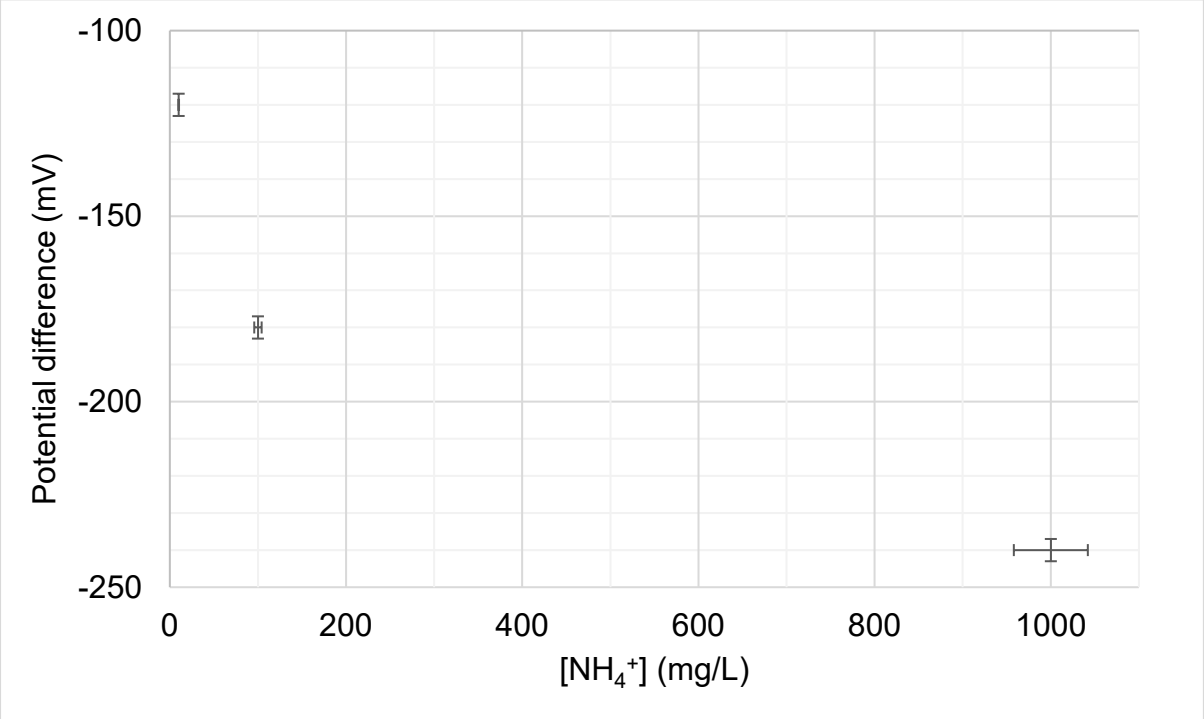

Figure 12: Logarithmic scaling of electrode error shown on a linear scale.

At 10 mg/L with an instrument response of -120 ± 1.1 mV, the determined ammonium concentration is 10 ± 0.42 mg/L, a spread of 0.84 mg/L. However, at a

higher concentration of 100 mg/L, the instrument response is 180 ± 1.1 mV and the determined ammonium concentration is 100 ± 4.2 mg/L, a spread of 8.4 mg/L. At 1000 mg/L the total spread increases to 84 mg/L. Therefore, it is shown that the precision of the instrument logarithmically decreases with an increase in ammonium concentration. No trend in precision level was observed with increasing ammonium concentration in the Fe(III) bioleaching solution assays.

The precision can also be stated in terms of a percentage of the potential difference measurement, in this case the precision was determined to be ± 4.2 %, which is in line with literature values of ±4 % (Horvai and Pungor, 1983). This was advised to CSM who agreed that it would be an acceptable level or precision for their experiments, which typically do not require determination of >50 mg/L-NH_4^+ and would therefore mean their lowest precision value, at 50 mg/L, would be ± 2.1 mg/L-NH_4^+.

Conclusions

Ammonium determination in bioleaching solutions using an ammonia gas selective electrode yielded promising results, with recovery factors of up to 0.96 in provided bioleaching solutions using Camborne School of Mines' newly purchased electrode. A limit of quantification of 0.8 mg/L-NH_3 was determined and the instrument response was linear over five orders of magnitude of concentration. However, it was recommended to dilute samples with ammonium concentrations >50 mg/L due to logarithmic scaling of error. Therefore, the working range of the electrode advised was between 0.8 and 50 mg/L-NH_4^+. The electrode provided precision of ±4.2 %, which was in line with literature data.

Electrode condition was found to be important and a modern electrode capable of producing literature sensitivity of between -58 and -62 mV/dec was required to achieve usable results. The concentration and species of metals in a bioleaching assay heavily impacted the amount of base addition required to liberate gaseous ammonia from the assay but did not impact the selectivity of the electrode for ammonium in the form of ammonia gas.

In summary, the main objective of this research project of producing a standard operating procedure for ammonium determination in bioleaching samples was met. This allowed for Camborne School of Mines to further their research in relation to Project NEMO. Ammonium determination in bioleaching solutions by method of ammonia gas selective electrode provided a quick, cost-efficient analysis method with a low initial financial outlay of around £600.

Future work

Further laboratory works which would have been conducted if more research time were available included to investigate the impact of assay solution temperature on electrode performance, as temperature is closely linked to both electrode sensitivity and to ammonia's solubility. More analyses using real bioleaching solutions would have also been undertaken to further refine the method and better understand electrode performance characteristics. Repeat analyses of a certified reference material would have allowed for a more accurate recovery factor with a defined

uncertainty to have been stated and this would have led to a better understanding with regard to the accuracy of the electrode.

Another area of interest would have been to investigate the accuracy of the electrode when using an estimated calibration curve based solely on assay temperature and addition of a large excess of base to produce instrument response. This approach could potentially eliminate the need for production of a calibration graph and preparation of a blank solution of assay matrix, as all ammonium in the solution would be liberated. It could potentially be possible that the electrode would then only require preliminary assessment to verify that it provided a within-specification level of sensitivity before ammonium measurements could be performed.

Investigation and comparison of methods other than potentiometric determination of ammonium, namely Kjeldahl digestion, would also have been of interest for further work. Kjeldahl digestion could potentially offer superior ammonium determination in bioleaching solutions that are extremely saturated with metal, where it may be the case that electrode performance is impacted due to extreme turbidity affecting the availability of ammonia to the electrode's membrane. Further works would need to be completed to determine whether this investigation would be appropriate, as the turbidity associated with the metal concentrations assessed in this research did not impact the electrode's performance as literature sensitivity was observed.

If a higher research budget were available, it would have allowed for more technological approaches toward ammonium determination to be investigated. For example, use of an automated Kjeldahl instrument could potentially provide a quick, 'one button press' method for the distillation and determination of ammonium in a typical bioleaching solution.

A less expensive addition would be the purchase of a second ammonia gas selective electrode to operate in tandem with the first. This could potentially allow for quicker determination times, as the second electrode could provide a live measurement of a blank and act as a reference solution. This could potentially allow for seamless movement between ammonium assays with different matrices without the need for the zeroing associated with using a single electrode for multiple measurements.

Acknowledgements

Throughout the process of writing this paper, I have received much support from both academic and non-academic sources. I would first like to thank my research supervisor, Dr Simon Ussher, for his guidance and timely feedback, which was much appreciated. I would next like to thank the laboratory staff at the University of Plymouth who always offered me assistance and practical advice. I would also like to thank Dr David Dew and Dr Carmen Falagan-Rodriguez of Camborne School of Mines for lending me their expertise, especially in relation to their vast knowledge of not just bioleaching but of the mining industry in general, which proved invaluable when completing the literature review of this paper.

I would finally like to thank my parents for providing me with the home support network, which allowed for me to return to education several years after leaving college. I would not be submitting this paper without their support.

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