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ANAEROBIC DISSOCIATION OF THIOCYANATE UNDER CONTROLLED PH AND TEMPERATURE

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ANAEROBIC DISSOCIATION OF THIOCYANATE UNDER CONTROLLED PH AND TEMPERATURE

by

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Abstract

Thiocyanate (SCN^-) is formed from both natural and industrial processes, including the use of cyanide to process gold and silver ores rich in sulfide minerals. In mining operations however, thiocyanate also plays a role as an alternative lixiviant to cyanide for leaching gold and silver out of their ores. Thiocyanate may be less toxic than cyanide but it is more stable and has proven to be more difficult to destroy in mining effluents. In addition, just like cyanide, an environmental contaminant of concern – nitrate – is the final product of the oxidation of thiocyanate.

This study investigated the chemical behavior of thiocyanate in the presence of oxidized nitrogen in an anoxic environment as well as under controlled pH and temperature conditions; with the possibility of generating nitrogen gas. If possible, this reaction will be similar to the anaerobic, microbe-mediated oxidation of ammonia (anammox) in the presence of nitrite or nitrate – a technique currently in use for wastewater treatment. The results obtained from this investigation can have possible application in the treatment of mining effluents; particularly of abandoned gold or silver mines (post-production) where SCN^- may have accumulated from the use of cyanide as a leaching agent.

Both low and high temperature experiments were conducted at different pH to examine the various mechanisms by which anaerobic thiocyanate oxidation (anSCNox) can occur. A 30 – 40% decrease in thiocyanate concentration was observed after thirty-one days in experiments performed at room temperature and ambient pH where thiocyanate was in association with nitrate or nitrite and gravel substrate obtained from a decommissioned mine tailings impoundment. It is believed that the microbes required to mediate anSCNox were present in the gravel substrate. Whether or not nitrogen gas was generated from this reaction is yet to be determined.

Hydrothermal experiments were conducted at pH between 2.0 –10.95 and temperatures between 60-300°C. The results from these experiments show that thiocyanate dissociation is both temperature and pH dependent. Thiocyanate is stable at temperatures of up to 150°C at ambient pH. However, thiocyanate will dissociate completely to form H_2S, NH_4^+ and CO_2 at pH 2.0 and temperature ≥ 150°C. The rates of thiocyanate dissociation were determined at different temperature and pH conditions and can be described by the following equation: $k = -0.3430 + 0.001525T - 0.04835pH$; where k and T represent reaction rate constant (per day) and temperature (Kelvin) respectively. The activation energies of thiocyanate dissociation at ambient pH (150 – 250°C) and pH 2.0 (25 – 200°C) were estimated to be 71.1kJ/mol and 32.7kJ/mol respectively.

Keywords: thiocyanate, cyanide, nitrogen, environmental contaminants, mine effluents, waste water treatment

Dedication

To those who dare to dream

To those who find strength in fear

Acknowledgements

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1. Introduction

1.1. Thesis Objective

The cyanidation process for the extraction of gold and silver from ore has been employed since the late nineteenth century. This extraction technique is still in use despite some environmental concerns because of its high extraction efficiency, relatively rapid extraction kinetics, wide availability of cyanide and the strength and solubility of the gold cyanide complex (Terry et al., 2001). However, mines that use cyanide as lixiviant generally have fairly high concentrations of a combination of toxic compounds which make their effluents very difficult to treat (Mudder and Botz, 2004). In oxidized environments, all byproducts of the breakdown of cyanide (including thiocyanate) are eventually converted to nitrate (NO_3^-), a stable molecule that has rigorous discharge criteria to protect human and aquatic health. Studies have shown that thiocyanate (SCN^-) forms at high concentrations if the ore being leached with cyanide is rich in pyrite or some other sulfide minerals (Gammons, 2010). In practice, SCN^- is sometimes used as an alternative lixiviant because gold dissolves in aqueous, acidified SCN^- solutions to form both the Au(I) and Au(III) complexes, depending on the electric potential of the solution (John and Iain, 1992). SCN^- may be less toxic than cyanide but it is more stable and therefore more difficult to destroy (Douglas et al., 2012).

This study investigates SCN^- behavior in the presence of nitrate or nitrite under anoxic conditions with changes in pH and temperature. Furthermore, other possible mechanisms by which SCN^- can be degraded anaerobically to produce a benign form of nitrogen (nitrogen gas) are explored. The results obtained from this study have possible applications in the treatment of mining effluents: particularly of abandoned gold or silver mines (post-production) where SCN^- might have accumulated from the use of cyanide as a leaching agent.

While a literature search revealed no previous research on this idea, an early hypothesis of the current study is that SCN^- might break down under anoxic conditions by a process analogous to the microbe-mediated anaerobic ammonia oxidation (anammox). Anammox is an alternative ammonia nitrogen removal process where ammonia is oxidized in the presence of nitrate and/or nitrite and the anammox bacteria in an anoxic environment to produce nitrogen gas (Bao-Ian et al., 2011; Tage et al., 2005). The reaction can be written as:

$$NO_2^- + NH_4^+ \rightarrow N_2(g) + 2H_2O \tag{1}$$

Several wastewater treatment systems use anammox solely or in combination with other treatment techniques (Op den Camp et al., 2006; Sri and Kurian, 2012). Studies have shown that the application of the anammox process results in significant energy reduction and decreased greenhouse gas emissions compared to traditional biological nitrogen removal processes (Ziye et al., 2013).

The analogous SCN^- reaction proposed in this thesis could be referred to as "anaerobic thiocyanate oxidation", or "anSCNox". The possible governing equations for the reaction between SCN^- and nitrate or nitrite are:

$$5SCN^- + 11NO_3^- + H^+ + 2H_2O \rightarrow 5SO_4^{2-} + 8N_2(g) + 5HCO_3^- \tag{2}$$

$$3SCN^- + 11NO_2^- + 5H^+ \rightarrow 3SO_4^{2-} + 7N_2(g) + 3HCO_3^- + H_2O \tag{3}$$

If a reaction scheme can be developed by which anSCNox proceeds at a rapid rate, then this would have tremendous benefits to several industries, including gold and silver mining, as it would convert two forms of dissolved nitrogen that are harmful to the environment (SCN^- and NO_3^- or SCN^- and NO_2^-) into nitrogen gas; a benign form of nitrogen.

1.2. Background

1.2.1. Chemical Properties

SCN^- is a free ion of thiocyanogen $(SCN)_2$; a pseudocyanogen. SCN^- is synthesized or manufactured as salt of ammonium, mercury, sodium, potassium and transition metals like copper. The SCN^- ion is a good ligand which readily forms several complexes that have similar stoichiometric behavior as halide complexes (Albert and Geoffrey, 1972). SCN^- is an ambidentate ion which coordinates either through the sulfur atom or the nitrogen atom, through monodentate or bidentate bonding. Molecular studies have shown that there are 10 potential bonding modes for SCN^- including bidentate complexes where it acts as a bridging ligand for two metal ions. It coordinates to metals of the first transition series through nitrogen and to metals of the second and third transition series through sulfur. SCN^- forms insoluble salts with silver, mercury (I) and lead (II) (John, 1990).

Thermodynamic calculations show that SCN^- is not a dominant aqueous species at equilibrium, but it can exist at low concentrations. Available free energy data for SCN^- (Wagman et al., 1982) were combined with data on aqueous species of S, C, and N (Drever, 1997) to construct an Eh-pH diagram that shows the equilibrium concentration of SCN^- in the presence of the more common forms of dissolved sulfur, carbon and nitrogen. As shown in Figure 1, the maximum stability of SCN^- is within the fields of bisulfide, ammonium, and bicarbonate ions. In this small Eh-pH region, the equilibrium concentration of SCN^- is approximately 10^{-6} molal and is independent of pH. A balanced reaction describing equilibrium between these 4 compounds can be written as:

$$SCN^- + 3H_2O \leftrightarrow HS^- + NH_4^+ + HCO_3^- \quad (4)$$

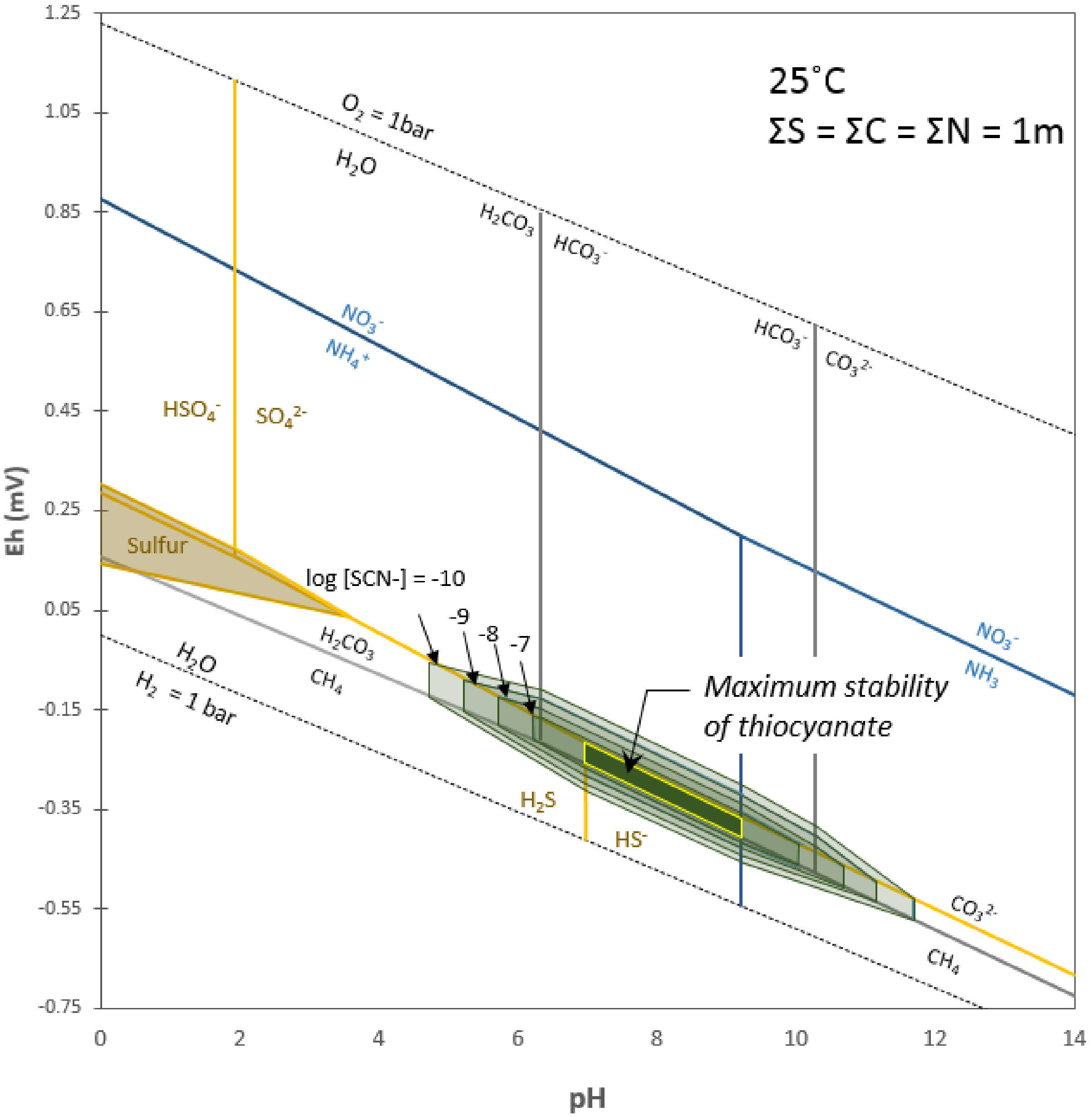

Figure 1: Stability field diagram showing SCN^- equilibrium concentration at 250ºC with total sulfur, carbon and nitrogen set at 1molal

1.2.2. Sources of Thiocyanate

Thiocyanate (SCN^-) is produced from both natural and industrial processes. The enzyme rhodanese (sulfotransferase), commonly identified with thiobacilli catalyzes the reaction between cyanide and thiosulfate to form SCN^- (Domenico et al., 2001). This reaction is considered relevant in some species of bacteria, algae, fungi, plants and animals that are physiologically suited for *in vivo* detoxification of cyanide. SCN^- can be produced naturally as a defense compound against microbial infection, during the decomposition of organic matter and in the metabolism of cysteine (Porter et al., 1996; Olea et al., 1988; Oh et. al, 1987). Industrial processes such as coke manufacturing for steel production and gold mining activities produce SCN^- in combination with other toxic compounds (Kononova et al., 2005). In mining gold, the cyanide could react with minerals rich in sulfide or partially oxidized sulfur intermediates to produce SCN^- (Gammons, 2010).

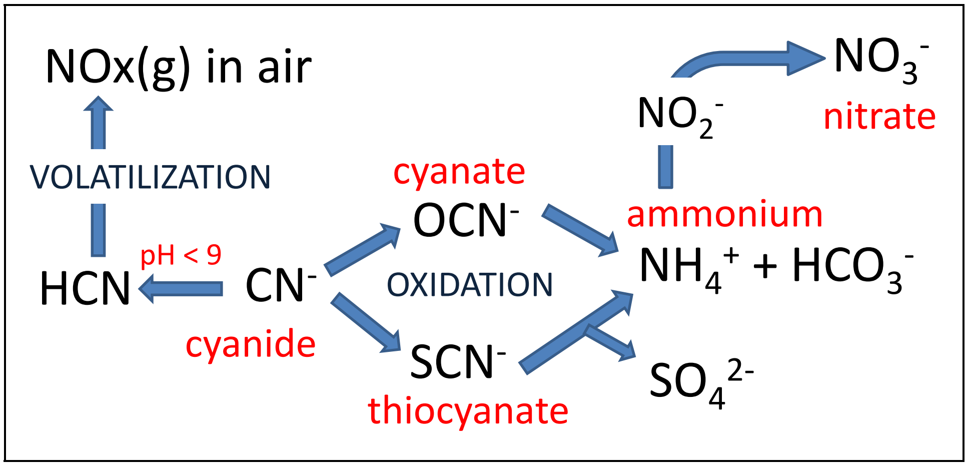

Figure 2: Simplified diagram showing the major transformations that occur during the breakdown of cyanide (from Gammons, 2010)

Environmental protection regulations require that all industrial effluents must be treated to reduce the free cyanide concentration to an acceptable limit before they are released to the environment. The various treatment processes employed have proved to be efficient; however a combination of other byproducts including thiocyanate, cyanate, ammonia, nitrate, sulfate and elevated metal concentrations can be formed as a result. The toxic effects of this mixture are not fully understood. Therefore, even when the free cyanide concentration is considerably reduced to meet discharge standards, the effluent may remain toxic to organisms and difficult to treat (Mudder and Botz, 2004).

1.2.3. Applications

Thiocyanates are used in different applications in the pharmaceutical, construction, metal and steel, textile and fiber, agriculture as well as photographic industries. Each thiocyanate has its own specific uses. For instance, ammonium thiocyanate is used for printing, dyeing and finishing operations, as acceleration in fixing baths in the photographic industry, and to stabilize various herbicides in the agriculture industry. Potassium thiocyanate is used as an analytical reagent and in the synthesis of antibiotics and other pharmaceutical products (Anonymous, 2016).

1.2.4. SCN^- Toxicity

Claude Bernard performed the first pharmacologic investigation of the thiocyanates in 1857 and since then there have been numerous instances of SCN^- toxicity. It was first discovered in 1903 that the drug reduces high blood pressure in humans. However, as a result of its unpredictable lethal effects, the drug fell out of use only to be reintroduced for the treatment of hypertension in 1925. The most common symptoms experienced by patients after exposure to the thiocyanates include vertigo, weakness of the arms and legs, toxic psychosis with disorientation, hallucination, mania, and sense of persecution (Akute et al., 1967; Curtis, 1939). In some cases, patients were relieved of the symptoms of the original ailment but became convulsive, comatose and eventually died. In other reports, even after the drug was discontinued, the patients still experienced most of the symptoms mentioned above over a period of time. Past literature reveals many fatalities from the administration of thiocyanates for medical purposes, poisoning and suicidal intent (Akute et al., 1967; Curtis, 1939; Grayson, 1957).

Sudden death may occur from SCN^- toxicity in animals but usually the effects develop gradually. Toxicity symptoms in animals may include vomiting, diarrhea, emaciation, general weakness, loss of equilibrium and buoyancy, tremors, convulsion, coma and death. From previous studies, the minimum lethal dose of sodium thiocyanate varies with different animals but it has been estimated to be about 500 mg per kg body weight (Curtis, 1939). Thomas and Karen (1989) observed the "sudden death syndrome" in SCN^- - exposed trout after different forms of strenuous exercises. That discovery led to the conclusion that SCN^- toxicity is influenced by the level of fish activity (Thomas and Karen, 1989). Further studies showed that SCN^- competes with other anions during bioaccumulation. At 100mg/L SCN^-, chloride ion at a concentration of 125mg/L reduced SCN^- accumulation by almost 95%. In one comparative study, toxicity effects of cyanide in rainbow trout manifested as mild goiter and bioaccumulation was in the form of SCN^-. No other symptoms of SCN^- toxicity were observed; most likely because the level of waterborne cyanide was insufficient to induce toxicity (Roman and Dixon, 1996).

1.2.5. Previous work on SCN^-

Most of the published studies for the breakdown of SCN^- were based on oxidative methods in which high concentrations of nitrate, nitrite and/or sulfate were formed as end products. The biodegradation of SCN^- in biological effluent treatment systems has been attributed largely to both autotrophic and heterotrophic bacterial species. One of the earliest discoveries described an autotrophic bacterium (*Thiobacillus thiocyanoxidans)* that utilized carbon dioxide as the sole source of carbon and inorganic sulfur-containing compounds, including SCN^-, as a source of sulfur (Happold et al., 1954). In another study, the metabolism of SCN^- by *Thiobacillus thiocyanoxidans* was difficult to quantify as this bacterium utilized SCN^- both as a source of energy and source of carbon and nitrogen. Therefore, it was difficult to distinguish between SCN^- utilization and carbon fixation. However, it was established that the end product of oxidation was sulfate (Jean, 1954).

Further microbial research revealed a novel chemolithotroph closest to *Paracoccus thiocyanatus* that oxidizes SCN^- as well as other reduced sulfur compounds like thiosulfate, tetrathionate, sulfide and elemental sulfur (Wriddhiman and Pradosh, 2007). Stafford and Calley (1969) discovered a gram negative heterotrophic pseudomonad which uses SCN^- as source of nitrogen as well as sulfur. Ammonium ion was an inhibitor of this reaction (Stafford and Calley, 1969). An indigenous gram negative heterotrophic bacteria belonging to the *Pseudomonas* genus was isolated from recycled water samples from a metallurgical plant wastewater. This species was found to degrade both cyanide and SCN^- while eliminating ammonia. Optimal conditions required for the survival of this bacterial community include temperature 34°C, pH 8.8 – 9.0, dissolved oxygen concentration 8 – 10 mg/L and organic matter concentration 5 g/L (Grigor'eva et al., 2008). Although SCN^- in high amounts is toxic to soil bacteria, it can be assimilated as a nitrogen source by an *Arthrobacter* isolated from soil. This bacterial species withstood SCN^- up to a concentration of 0.1M and SCN^- degradation continued even in the presence of nitrate and ammonia (Betts et al., 1979).

A new branch of chemolithoautotrophic sulfur-oxidizing bacteria capable of oxidizing SCN^- was discovered in some hyper-alkaline and saline lakes. These bacteria belong to the genus *Thioalkalivibrio* and were observed to thrive in sodium carbonate/bicarbonate buffered lakes. The high buffering capacity of sodium carbonate maintains the pH of the lakes around

9.5 – 10.5: a condition under which these bacteria function optimally. However, their growths are inhibited at pH < 7.5 and Na^+ concentration < 0.2M. The assimilation of SCN^- was mainly as a source of energy and nitrogen. Cyanate was noted as an intermediary product which slowly decomposed to ammonia (Dimitry et al., 2005; 2006; 2007).

It is important to emphasize that all of the mechanisms mentioned above rely on the oxidative degradation of SCN^-; the end products of which include sulfate and nitrate in relatively high concentrations.

2. Methods

The experiments for this study were performed under anoxic conditions, as well as controlled pH and temperatures. Most of the experiments were performed under high temperatures in order to speed up the kinetics and to understand what types of SCN^- breakdown reactions are possible under these anoxic conditions. In the low temperature experiments, the presence of microbes that can degrade SCN^- under anoxic environments and at moderate to high pH was investigated using gravel substrate obtained from a decommissioned mine tailings impoundment where cyanide was used.

2.1. Experimental matrix

Appendix A and B summarize the experiments performed at various pH and temperatures.

2.2. Experimental methods

2.2.1. Hydrothermal experiments

The hydrothermal experiments for this study were conducted using sealed silica tubes in stainless steel autoclaves which were placed in a Fisher Isotemp high-precision oven. The silica tubes were made from 0.9mm O.D / 0.7mm I.D “quartz” tubing and were sealed using a hydrogen-oxygen flame. The autoclaves had an internal volume of 100mL which could hold up to 4 tubes at a time. Each quartz tube was filled with 2 to 4mL of experimental solution. After sealing, the tubes were roughly 50 to 70% solution and 30 to 50% vapor by volume. About 50mL of water was added to the autoclaves before sealing to equalize the pressure on either side of the silica glass wall. Thus, all of the experiments were conducted at pressures corresponding to the liquid/vapour pressure of water at temperatures up to 250°C.

Figure 3: Stainless steel autoclave

The autoclaves were placed horizontally inside a Fisher Isotemp high-precision oven with forced air convection, capable of maintaining temperatures at +/- 0.1°C at temperatures up to 300°C. At the end of the equilibration period, the autoclaves were removed from the oven using insulated gloves and immediately placed in cold water. After quench, the autoclaves were opened and the tubes extracted and examined for leakage. Some experiments at higher temperature had leak problems, possibly due to the generation of gas inside the tubes.

2.2.2. Creating the anoxic environment

Roughly 50 mL of the starting solution was transferred into a Büchner flask. With the cork of the flask in place, a vacuum was created within the flask using a vacuum pump. Initially, the solution bubbled as dissolved oxygen within the system was removed. The bubbling stopped when there was little or no dissolved oxygen left in the solution and within the flask. Approximately 3.0mL of the de-gassed solution was quickly transferred from the Büchner flask

into a silica tube. Thereafter, the tube was gently placed into a Dewar flask containing an instant freeze solution (acetone and dry ice) where the sample was frozen within a few seconds.
Once the sample was frozen, a vacuum was drawn within the silica tube using the vacuum pump. Afterwards, the silica tube was sealed over hydrogen-oxygen flame with the connecting vacuum tube locked in order to prevent air from entering the tube.

Creating a vacuum within the tubes was intended to remove the oxygen dissolved in the experimental solution as well as within the head space of the tube. However, even with this precaution in place, some oxygen probably remained in the tube after sealing. The possible oxidation of SCN^- in the starting solution and of H_2S generated during the experiment can explain the presence of sulfate, nitrate and nitrite in many of the run products. It is also important to mention that the degassing and freezing step in this oxygen removal method was not done for some of the early experiments.

Figure 4: Showing Dewar flask containing dry ice and acetone used for instant freezing

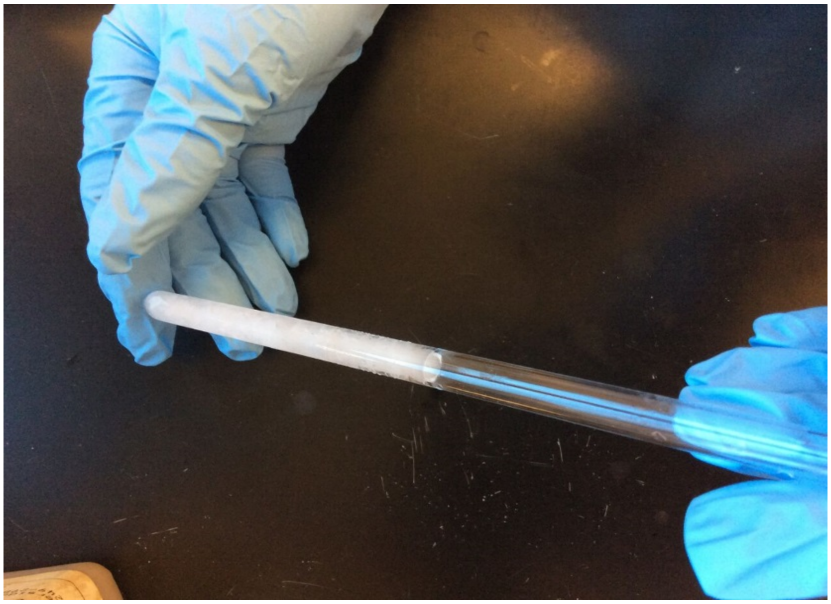

Figure 5: Showing frozen experimental solution before creating a vacuum and sealing

2.2.3. Experimental solutions set-up

Three different stock solutions were prepared for the experiments, as follows:

Solution 1: 100mg/L NaSCN as N

Solution 2: 100 mg/L NaSCN as N + 100mg/L $NaNO_3$ as N

Solution 3: 100mg/L NaSCN as N + 100mg/L $NaNO_2$ as N

2.2.3.1. Hydrothermal experiment 1: no pH adjustment at 150°C

Small amounts of solutions 1, 2 and 3 were transferred to three silica tubes without adjusting the pH of the solutions and then the tubes were sealed. For this experiment, 3 duplicate silica tubes were made so that each tube contained one of the 3 starting solutions. Each tube was placed in a separate autoclave and after the experiment, all 3 autoclaves were quenched on day 7. The hydrothermal experiment was set up at 150°C. The main objective was to see if SCN^- reacted with either NO_3^- (reaction 2) or NO_2^- (reaction 3) to form N_2 gas at 150°C.

2.2.3.2. Hydrothermal experiment 2: reduced pH and gold catalyst at 150°C

Two different tests were conducted in hydrothermal experiment 2. The first test was performed to see if SCN^- broke down at a faster rate at low pH in the presence of nitrate or nitrite at 150°C. In the second test, the conditions of Experiment 1 were repeated with the presence of a gold foil to establish whether or not SCN^- degradation without pH adjustment requires a catalyst to speed up the reaction.

For the first test, small volumes of solutions 1, 2, and 3 were transferred into three beakers where the pH of each solution was reduced to 2.0 by adding concentrated hydrochloric acid and a small amount of the acidified solution was added to three different silica tubes and sealed. In the second test, gold foils that weighed approximately 30mg were added to the tubes that contained solutions 2 and 3 and the tubes were sealed.

The 3 sealed tubes for the first test were placed in a separate autoclave from the tubes prepared for the second test. All 5 autoclaves were taken out of the oven on day 7.

The experiment was set up at 150°C as shown in Table I below.

Table I: Set up of hydrothermal experiment 2

Solution	Initial pH	Adjusted pH	Amount of Gold foil (mg)	Amount of solution (g)
1	5.60 – 7.00	2.00	-	2.7764
2		2.00	-	2.3045
3		2.00	-	2.2218
3		-	35.0	3.0790
2		-	25.0	2.6276
Solution 1 - 100mg/L NaSCN as N				
Solution 2 – 100mg/L NaSCN as N + 100mg/L $NaNO_3$ as N				
Solution 3 – 100mg/L NaSCN as N + 100mg?L $NaNO_2$ as N				

2.2.3.3. Hydrothermal experiment 3: increased pH at 150^{o}C

The objective of this experiment was to observe how the SCN$^-$ breakdown reaction vary at higher pH. Small volumes of solutions 1 -3 were transferred into beakers and the pH was adjusted as indicated below. For this experiment, 3 duplicate silica tubes were made and each tube contained one of the 3 starting solutions with adjusted pH. The tubes were placed in separate autoclaves so that 3 autoclaves were quenched at the end of day 7. The experiment was set up at 150^{o}C as shown in Table II below.

Table II: Set up of hydrothermal experiment 3

Solution	Adjusted pH	Amount of solution (g)
1	10.20	2.6512
2	10.07	2.4904
3	10.52	2.9398
Solution 1 - 100mg/L NaSCN as N		
Solution 2 -100 mg/L NaSCN as N + 100mg/L $NaNO_3$ as N		
Solution 3 - 100 mg/L NaSCN as N + 100mg/L $NaNO_2$ as N		

2.2.3.4. Hydrothermal experiment 4: no pH adjustment at 250^{o}C

Hydrothermal experiment 1 was repeated at 250^{o}C in order to establish temperature dependence of SCN$^-$ breakdown in the presence of nitrate or nitrite without adjusting the pH of solutions 1, 2 and 3.

No analysis was performed for hydrothermal experiment 4 because the silica tubes broke inside the pressure tubes while the experiment was on-going. This could have been as a result of possible accumulation of ammonia, carbon dioxide or hydrogen sulfide gas within the tubes at the relatively high concentrations of the starting solutions.

2.2.3.5. Hydrothermal experiment 5: no pH adjustment at 250°C

In hydrothermal experiment 5, the starting solutions used in hydrothermal experiment 4 were diluted 10 times as shown below. By diluting the starting solutions, it was reasoned that there would be less gas evolved and therefore less of a chance of the tubes breaking. For this experiment, three sets of 3 tubes each were prepared. Each set of three tubes that contained either solution 1, 2 or 3 (listed below) was placed in separate autoclaves; so that one autoclave was quenched at the end of day 1, 3 and 7. The experiment was set up at 250°C.

Solution 1: 10mg/L NaSCN as N

Solution 2: 10 mg/L NaSCN as N + 10mg/L $NaNO_3$ as N

Solution 3: 10mg/L NaSCN as N + 10mg/L $NaNO_2$ as N

2.2.3.6. Hydrothermal experiment 6: no pH adjustment at 200°C

Based on results obtained from hydrothermal experiment 5, it was apparent that the rate of SCN^- degradation was the same in the presence or absence of NO_3^- and NO_2^-. Consequently, hydrothermal experiment 6 was set up at 200°C using only solution 1 - 100mg/L NaSCN as N. Four silica tubes were made for this experiment. Each tube containing solution 1 was placed in a separate autoclave from the other tubes so that one autoclave was quenched at the end of day 1, 3, 7 and 14.

2.2.3.7. Hydrothermal experiment 7: no pH adjustment at 150°C

This is a repeat of experiment 1 using only solution 1 - 100mg/L NaSCN as N at 150°C wherein the analyses of the run products were performed more meticulously. Four silica tubes were made for this experiment. Each tube containing solution 1 was placed in a separate autoclave from the other tubes so that one autoclave was quenched at the end of day 1, 3, 7 and 14.

2.2.3.8. Hydrothermal experiment 8: pH reduction at 150°C

It was apparent from the analytical results obtained from hydrothermal experiment 2 (pH = 2.0) that the hydrothermal dissociation of SCN^- was pH dependent. Hydrothermal experiment 8 was conducted to further demonstrate the pH dependence of the reaction. This experiment was set up at 150°C using solution 1 - 100mg/L NaSCN as N at pH 2.0, 2.65, 3.56 and 4.01. Three tubes were prepared for each pH. Therefore, three sets of four tubes that contained solution 1 at the different pH stated above were placed in three separate autoclaves. One autoclave was quenched at the end of day 1, 3 and 7.

2.2.3.9. Hydrothermal experiment 9: pH reduction at 200°C

As a follow up to hydrothermal experiment 8, this experiment was set up at 200°C using solution 1 - 100mg/L NaSCN as N at pH 2.0, 2.65, 3.56 and 4.01 to show the temperature dependence of the SCN^- dissociation reaction under controlled pH. Three tubes were prepared for each pH. Therefore, three sets of four tubes containing solution 1 at the different pH stated above were placed in three separate autoclaves. One autoclave was quenched at the end of day 1, 3 and 7.

2.2.3.10. Hydrothermal experiment 10: pH reduction at 100°C

The results from hydrothermal experiment 9 revealed that the fastest rate of SCN^- dissociation occurred at pH 2.0. Therefore, hydrothermal experiment 10 was set up at 100°C using solution 1 - 100mg/L NaSCN as N at pH 2.0 only. Three silica tubes were prepared for this experiment and each tube contained solution 1 at pH 2. Each tube was placed in a separate autoclave so that 1 autoclave was quenched at the end of day 1, 3 and 7.

2.2.3.11. Hydrothermal experiment 11: pH reduction at 60°C

This experiment was set up at 60°C using solution 1 - 100mg/L NaSCN as N at pH 2.0. Three silica tubes were prepared for this experiment and each tube contained solution 1 at pH 2. Each tube was placed in separate autoclaves so that 1 autoclave was quenched at the end of day 1, 3 and 7.

2.2.3.12. Hydrothermal experiment 12 and 13: pH reduction at 300°C

Both experiments were set up at 300°C at different times using solution 1 - 10mg/L NaSCN as N at pH 2.0. However, even at the lower concentrations of the starting solutions, all of the tubes broke during the experiments.

2.2.4. Low temperature experiments

2.2.4.1. Low temperature experiment 1: pH reduction at room temperature

This experiment is an extension of the hydrothermal experiments but it was set up at approximately 25°C using solution 1 - 100mg/L NaSCN as N at pH 2.0. The degassed solution was loaded into sealed silica tubes, as in the high-T experiments, to replicate the procedures. Three silica tubes were prepared for this experiment and each tube contained solution 1 at pH 2. The solution in each tube was left to equilibrate at room temperature. One tube was opened at the end of day 1, 3 and 7.

2.2.4.2. Other low temperature experiments under anoxic conditions

As earlier discussed, previous studies reported the discovery of certain microorganisms that use SCN^- as source of energy. This served as the basis for the low temperature anaerobic experiments described below. Gravel substrate from a mine tailings impoundment presumed to harbor SCN^--degrading microorganisms was used to set up the following experiments.

2.2.4.2.1. Low temperature experiment 2: gravel substrate solution at ambient pH and temperature

Approximately 150g of the mine gravel were placed in three separate plastic bottles. Thereafter, the bottles were filled (no head space) with solutions 1, 2 and 3 at ambient pH (6.1 – 6.7) along with 1% methanol. The methanol was introduced as a source of organic carbon to be consumed by the microbes present so as to create an anoxic environment within the containers. The cap of each bottle was sealed tightly with parafilm to exclude air from the system. The experiment was set up at room temperature and was left to run for thirty-one days.

2.2.4.2.2. Low temperature experiment 3: gravel substrate solution at high pH and ambient temperature

This experiment was set up like low temperature experiment 2 above but the pH values of the starting solutions were adjusted to a range of 10.93 – 10.95. This experiment was set up at room temperature and was left to run for forty-nine days.

2.3. Analytical methods

The quantitative analyses of SCN^- - N, NH_3 - N and S^{2-} were performed using the HACH DR/2010 spectrophotometer. Ion chromatography was used for the analyses of SO_4^{2-}, NO_3^- - N and NO_2^- - N.

2.3.1. Thiocyanate analysis

There is no HACH method for the analysis of SCN^-. Consequently, the standard ASTM D4193-08 method was used. This method involves reaction between SCN^- and acidified ferric nitrate which forms a reddish-brown ferric thiocyanate complex with an absorption peak at 460nm. This procedure is simple, rapid and adheres to Beer’s law. However, possible interferences include ions such as CN^-, S^{2-}, SO_3^{2-}, Cr^{6+}, CrO_4^{2-}, Fe^{2+} and Cu^{2+}, reducing agents that reduce Fe(III) to Fe(II) and some colored organic compounds. Sulfide concentration above

50mg/L can interfere with the reaction between thiocyanate and ferric nitrate (ASTM Standard D4193). Sulfide interference was not significant in this study as the run products were sufficiently diluted before performing the SCN^- analyses. The concentrations of SCN^- - N (mg/L) were calculated from a standard calibration curve of absorbance at 460nm versus concentration. This method has an estimated detection limit of 0.05 mg/L SCN^- - N with a linear range of 0 - 1.5 mg/L SCN^- - N. All wastes generated from this procedure were reacted with hydrogen peroxide to convert SCN^- to CO_2, SO_4^{2-} and NO_3^- and then discarded.

2.3.2. Sulfide analysis

The USEPA-approved methylene blue method for wastewater analysis was used for sulfide analysis. In this method, hydrogen sulfide and acid-soluble metal sulfides react with N, N-dimethyl-p-phenylenediamine oxalate to form methylene blue. The intensity of the blue color is measured at 665nm and it is proportional to the sulfide concentration. Very high sulfide concentrations as well as reducing compounds like sulphite, thiosulfate and hydrosulfite could prevent the formation of the blue color (HACH Method 8131). The results from the sulfide analyses were recorded as S^{2-} in mg/L. This method has an estimated detection limit of 0.01 mg/L S^{2-} with a linear range of 0 - 0.60 mg/L S^{2-}.

2.3.3. Ammonium analysis

Ammonium was measured at 425nm using the Nessler method. In this method, any hardness in the sample is first neutralized by a mineral stabilizer. The Nessler Reagent reacts with ammonium ions to form a yellow-colored product; this step is aided by the prior addition of polyvinyl alcohol dispersing agent. The ammonium concentration is directly proportional to the intensity of the colored product. Possible interferences such as iron, sulfide, chlorine, glycine and some aromatic compounds could cause turbidity or some other off-color with the Nessler

Reagent (HACH; Method 8038). The results from the ammonium analyses were recorded as NH_3 - N in mg/L. This method has an estimated detection limit of 0.06 mg/L NH_3-N with a linear range of 0-2.50 mg/L NH_3 - N.

Nessler Reagent contains mercury; therefore the wastes from the analyses were stored in labeled containers as hazardous waste.

2.3.4. Ion chromatography

Samples of the experimental run products were analysed for other relevant anions like sulfate, nitrate and nitrite at the Montana Bureau of Mining and Geology (MBMG) analytical laboratory. The samples were preserved by refrigeration at 4°C and analysed within two weeks by ion chromatography (Metrohm 862 Compact IC plus; EPA method 300.0). For this technique, the limit of detection for sulfate is 0.5mg/L and 0.01mg/L for both nitrate and nitrite.

2.3.5. pH measurement

A WTW multi 340i pH electrode and a platinum tip redox microelectrode were used to measure the pH of the solutions in the experiments involving pH adjustment. The multi 340i pH electrode measured the pH of the solutions during the experiment set up while the platinum tip microelectrode measured the pH of the run products of hydrothermal experiments 8 and 9. Each electrode was calibrated with the appropriate buffer solution prior to use.

2.4. Constructing the SCN^- stability field diagram

The stability field diagram of SCN^- shown in Figure 1 was constructed “manually” with Microsoft Excel. Thermodynamic data for SCN^- and the relevant aqueous species of sulfur, carbon and nitrogen were obtained from Wagman et al. (1982) and Drever (1997).

2.5. Mathematically-derived rate constants

MATLAB was used to compute equations 6 and 7 presented in the discussion of results. These equations show the relationship between the reaction rate constant, pH and temperature of the different experiments. Equation (6) was derived from the experimentally-determined rate constants at pH 2.0 and 6.8, at temperatures between 25°C and 250°C. Equation (7) describes a similar relationship, however it also includes the rate constants of SCN^- hydrolysis at intermediate pH 2.65, 3.56 and 4.01.

3. Results

3.1. Hydrothermal experiment 1: no pH adjustment at 150°C

In this experiment, the pH of the three starting solutions was not adjusted and the hydrothermal experiment was set up at 150°C. The experiment was left to run for 7 days. The run products from this experiment were analysed for SCN^- and the results are shown below.

Table III: Run product analyses for hydrothermal experiment 1

Run product	Initial Composition	[SCN^- - N] in run product (mg/L)
1.1	Solution 1 - 100mg/L NaSCN - N	116
1.2	Solution 2 - 100mg/L NaSCN – N + 100mg/L $NaNO_3$ – N	104
1.3	Solution 3 - 100mg/L NaSCN – N + 100mg/L $NaNO_2$ – N	95

The results obtained from this experiment suggest that no reaction occurred within the tubes containing the starting solutions after 7 days. SCN^- did not break down at 150°C by itself or in the presence of nitrate or nitrite. Since all of the SCN^- remained intact after 7 days, no further analysis was performed to measure the amount of other possible products like ammonia and hydrogen sulfide that could form from the breakdown of SCN^-. However, this experiment was repeated more meticulously in hydrothermal experiment 7.

3.2. Hydrothermal experiment 2: reduced pH and gold catalyst at 150°C

Two different experiments were performed under hydrothermal experiment 2 at 150°C. In the first experiment, the pH dependence of the SCN^- dissociation reaction in the presence of nitrate or nitrite was observed. Here, the pH of the starting solutions 1, 2 and 3 were adjusted to 2.0 and no catalyst was used.

The second experiment involved the use of gold foil to investigate whether or not the SCN^- dissociation reaction without pH adjustment at 150°C requires a catalyst to speed up the reaction.

Both experiments were left to equilibrate for 7 days. The experimental set up and the results obtained from the analyses of the run products are shown below.

Table IV: Run product analyses for hydrothermal experiment 2

Run Product	Initial Composition	Adjusted pH	Gold foil	$[SCN^-$ - N] in run product (mg/L)	$[NH_4^+$ – N] in run product (mg/L)
2.1	Solution 2 - 100mg/L NaSCN – N + 100mg/L $NaNO_3$ – N	-	Yes	104	0
2.2	Solution 3 - 100mg/L NaSCN – N + 100mg/L $NaNO_2$ – N	-		99	0
2.3	Solution 1 - 100mg/L NaSCN - N	2.0	No	0	113
2.4	Solution 2 - 100mg/L NaSCN – N + 100mg/L $NaNO_3$ – N	2.0		0	138
2.5	Solution 3 - 100mg/L NaSCN – N + 100mg/L $NaNO_2$ – N	2.0		0	127

All of the SCN^- in the experiment set up at pH 2.0 (without catalyst) broke down at 150°C. This result was unexpected and considered important enough for follow-up work. At the end of this experiment, a fetid smell characteristic of hydrogen sulfide was perceived when the tubes containing solutions 1, 2 and 3 at pH 2.0 (without catalyst) were opened. The odor was stronger in the tube containing solution 1. Also, a pale yellow precipitate assumed to be elemental sulfur was formed in all three tubes.

The results obtained for the experiment set up with the gold foil (without pH adjustment) showed no effect of a catalyst on the dissociation of SCN^- at 150°C. The concentration of SCN^- in the run products with gold foil remained the same after 7 days. Since all of the SCN^- in the starting solution remained intact after 7 days, no further analysis was performed to measure the amount of other possible products like ammonia and hydrogen sulfide that could form from the breakdown of SCN^-.

3.3. Hydrothermal experiment 3: increased pH at 150°C

This experiment was performed in order to observe how SCN^- dissociation is affected by relatively higher pH. The pH of the starting solutions was increased from approximately 6.5 to a range of about 10.20 – 10.52. The experiment was left to run for 7 days at 150°C.

It was evident from the results obtained from the analyses of the run products that SCN^- did not break down significantly at high pH and 150°C as shown below. No further analysis was done to measure the amounts of other likely products since the concentration of SCN^- in all three starting solutions was almost the same after 7 days.

Table V: Run product analyses for hydrothermal experiment 3

Run Product	Initial Composition	Adjusted pH	$[SCN^-]$ in run product (mg/L)
1	Solution 1 - 100mg/L NaSCN – N	10.20	94
2	Solution 2 - 100mg/L NaSCN – N + 100mg/L $NaNO_3$ – N	10.07	93
3	Solution 3 - 100mg/L NaSCN – N + 100mg/L $NaNO_2$ – N	10.52	93

3.4. Hydrothermal experiment 4: no pH adjustment at 250ºC

In this experiment, the pH of the starting solutions were not adjusted just as in hydrothermal experiment 1 (at 150ºC) but this experiment was set up at 250ºC.

No results were obtained for hydrothermal experiment 4 because the silica tubes broke inside the pressure tubes while the experiment was on-going. This could have been as a result of possible accumulation of ammonia, nitrogen, carbon dioxide or hydrogen sulfide gas within the tubes. The concentrations of these gases might have been high enough to cause shattering of the silica tubes considering the high concentrations of the starting solutions.

3.5. Hydrothermal experiment 5: no pH adjustment at 250ºC

In this experiment, the three starting solutions were diluted by a factor of 10 in order to minimize gas accumulation in the tubes as was observed in hydrothermal experiment 4. The experiment was set up without pH adjustment of the starting solutions at 250ºC and allowed to run for 7 days. Three autoclaves, each containing an identical set of the three stock solutions in silica tubes, were placed in the oven at 250˚C. One autoclave was removed after 1 day, another after 3 days, and the third autoclave after 7 days.

After the 7-day experimental period, the last autoclave was quenched and the tubes containing the run products of the 3 starting solutions for the different quench-days were opened. The mass of the run product in each silica tube was measured before the run product was transferred to a plastic container where it was diluted as needed. Several analyses were performed to measure the concentrations of SCN^-, NO_3^-, NO_2^-, NH_4^+, S^{2-} and SO_4^{2-} in the run products. The analytical results obtained for each run product are presented below.

It was inferred from the results obtained that the SCN^- dissociation reaction is temperature dependent. In hydrothermal experiment 1, SCN^- did not break down at 150ºC without pH adjustment. However, SCN^- dissociation was evident for all three solutions set up at 250°C without pH adjustment. The presence of nitrate or nitrite did not influence the dissociation trend at 250°C (Table 3.4).

A large amount of NH_4^+ and S^{2-} and lesser amounts of NO_3^-, NO_2^- and SO_4^{2-} were detected upon analyses of the run products. The presence of NO_3^- and NO_2^- in the run products of Solution 1 - 10mg/L NaSCN as N as well as SO_4^{2-} in the run products of all 3 starting solutions could be as a result of residual oxygen in the tube after the experiment set up. In addition, some of the sulfate could have come from oxidation of H_2S after the tubes were opened and prior to IC analysis.

3.5.1. Hydrothermal experiment 5.1

Composition of starting solution: 10mg/L NaSCN as N

Table VI: Run product analyses for hydrothermal experiment 5.1

Day	[SCN^-] mol/L	[NO_3^-] mol/L	[NO_2^-] mol/L	[NH_4^+] mol/L	[S^{2-}] mol/L	[SO_4^{2-}] mol/L
0	7.14E-04	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00
1	5.78E-04	5.14E-05	0.00E+00	2.14E-04	3.28E-05	1.37E-04
3	3.65E-04	5.04E-05	1.09E-04	5.89E-04	2.20E-04	1.38E-04
7	3.05E-04	6.11E-05	0.00E+00	6.96E-04	3.35E-04	1.89E-04

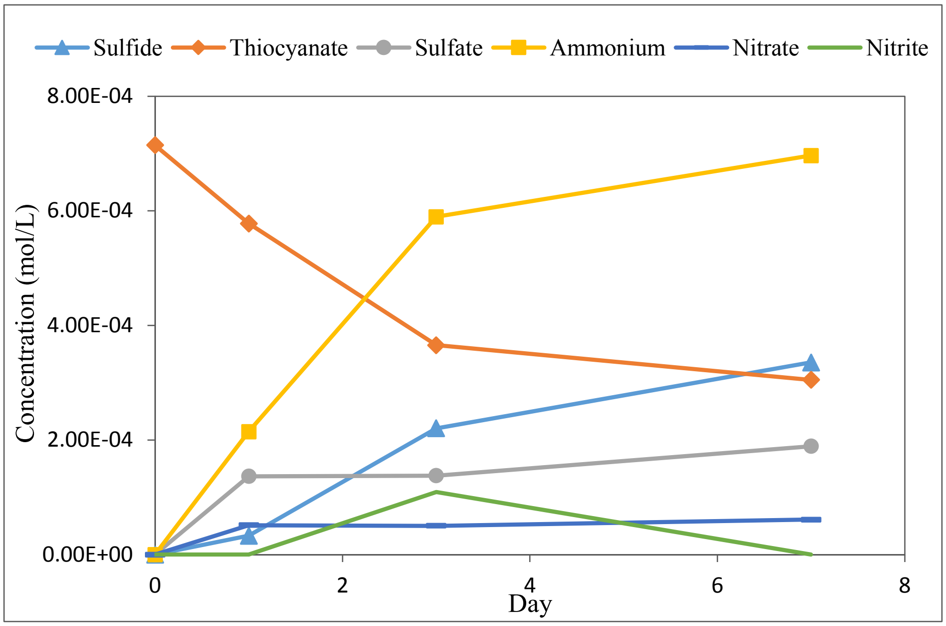

Figure 6: SCN$^-$ dissociation at 250°C and ambient pH (without nitrate or nitrite)

3.5.2. Hydrothermal experiment 5.2

Composition of starting solution: 10mg/L NaSCN as N + 10mg/L $NaNO_3$ as N

Table VII: Run product analyses for hydrothermal experiment 5.2

Day	[SCN^-] mol/L	[NO_3^-] mol/L	[NO_2^-] mol/L	[NH_4^+] mol/L	[S^{2-}] mol/L	[SO_4^{2-}] mol/L
0	7.14E-04	7.14E-04	0.00E+00	0.00E+00	0.00E+00	0.00E+00
1	4.94E-04	1.07E-04	0.00E+00	6.96E-04	2.34E-06	6.47E-04
3	3.43E-04	1.90E-04	1.26E-04	2.79E-03	2.34E-06	5.80E-04
7	3.20E-04	3.47E-04	3.19E-04	1.07E-03	0.00E+00	4.16E-04

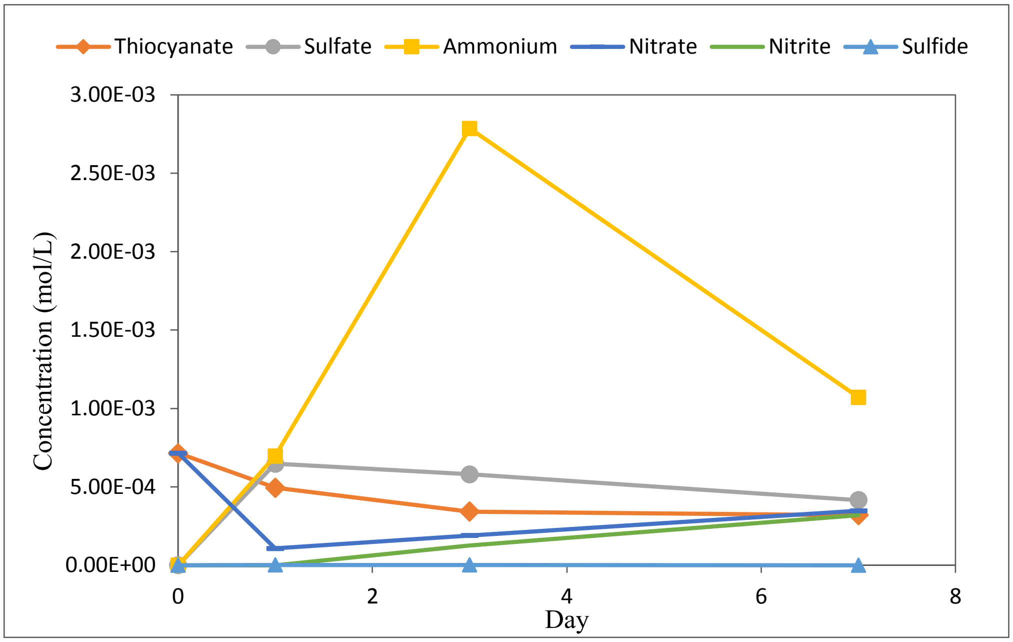

Figure 7: SCN^- dissociation in the presence of nitrate at 250ºC and ambient pH

3.5.3. Hydrothermal experiment 5.3

Composition of starting solution: 10mg/L NaSCN as N + 10mg/L $NaNO_2$ as N

Table VIII: Run product analyses for hydrothermal experiment 5.3

Day	$[SCN^-]$ mol/L	$[NO_3^-]$ mol/L	$[NO_2^-]$ mol/L	$[NH_4^+]$ mol/L	$[S^{2-}]$ mol/L	$[SO_4^{2-}]$ mol/L
0	7.14E-04	0.00E+00	7.14E-04	0.00E+00	0.00E+00	0.00E+00
1	7.60E-04	9.75E-05	0.00E+00	1.61E-04	2.34E-06	5.55E-04
3	4.79E-04	1.86E-04	5.57E-05	5.36E-04	1.64E-05	5.53E-04
7	4.11E-04	1.59E-04	1.33E-04	6.96E-04	1.29E-04	2.25E-04

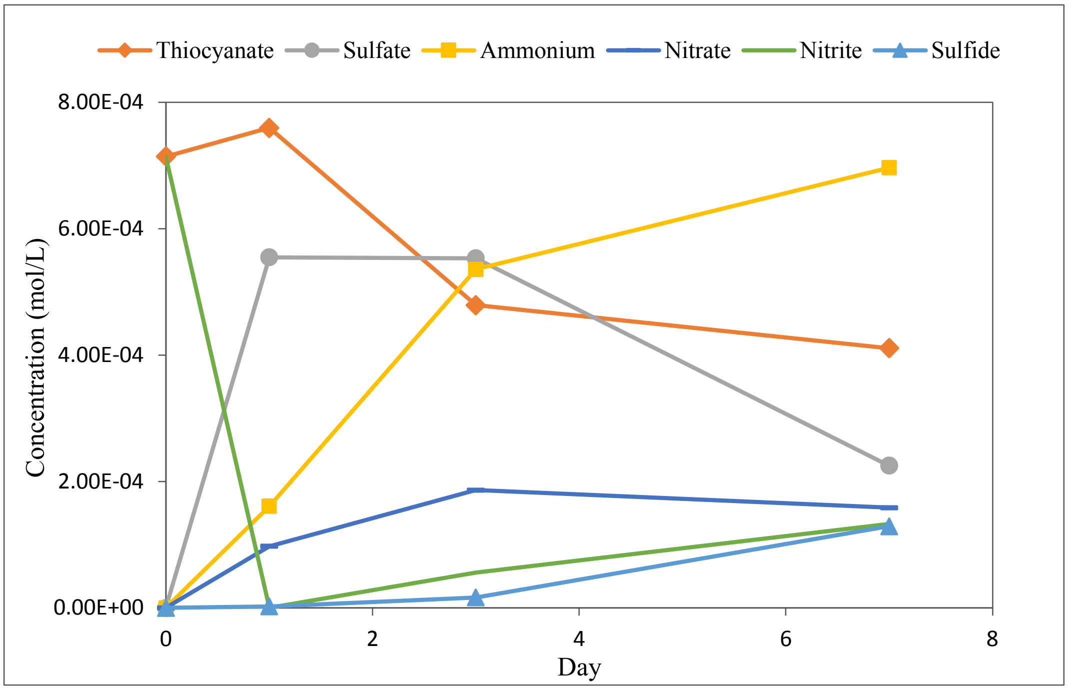

Figure 8: SCN$^-$ dissociation in the presence of nitrite at 250^{o}C and ambient pH

3.6. Hydrothermal experiment 6: no pH adjustment at 200^{o}C

Results obtained from hydrothermal experiment 5 suggest that the SCN$^-$ dissociation reaction follows the same trend in the presence or absence of nitrate or nitrite. Furthermore, the H_2S produced by SCN$^-$ breakdown reacted with NO_3^-/NO_2^- to form ammonia. Therefore, hydrothermal experiment 6 was set up using only Solution 1 - 100mg/L NaSCN as N at a lower temperature (200^{o}C) to further elucidate the temperature dependence of the reaction. No dilution or pH adjustment was made to the starting solution.

After the 7-day experimental period, the last autoclave was quenched and the tubes containing the run products of solution 1 – 100mg/L NaSCN for the different quench-days were opened. The mass of the run product in each silica tube was measured before the run product was transferred to a plastic container where it was diluted as needed. Several analyses were performed to measure the concentrations of SCN^-, NO_3^-, NO_2^-, NH_4^+, S^{2-} and SO_4^{2-} in the run products. The analytical results for each run product are presented below.

As expected, the results showed that the dissociation reaction of SCN^- is relatively slow at 200°C and without pH adjustment of the starting solution. These results can be compared with results obtained from hydrothermal experiment 5 (250°C, ambient pH) and hydrothermal experiment 7 (150°C, ambient pH). See Figure 24 in discussion of results.

The concentration of NH_4^+ in the various tubes increased with time and was inversely correlated with the decrease in SCN^- concentration. Small amounts of NO_3^-, NO_2^-, S^{2-} and SO_4^{2-} were also detected upon analyses of the run products. The presence of NO_3^-, NO_2^- and SO_4^2- in the run products of Solution 1 - 10mg/L NaSCN as N could be as a result of residual oxygen in the tube after the experiment set up.

Table IX: Run product analyses for hydrothermal experiment 6

Day	$[SCN^-]$ mol/L	$[NO_3^-]$ mol/L	$[NO_2^-]$ mol/L	$[NH_4^+]$ mol/L	$[S^{2-}]$ mol/L	$[SO_4^{2-}]$ mol/L
0	7.14E-03	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00
1	6.33E-03	3.34E-05	4.29E-06	4.71E-04	9.38E-06	4.01E-04
3	6.04E-03	5.36E-06	5.36E-06	9.11E-04	5.86E-05	4.11E-04
7	5.47E-03	3.86E-05	2.68E-05	1.98E-03	1.17E-04	3.98E-04
14	4.66E-03	5.63E-06	6.41E-05	3.15E-03	4.08E-04	5.38E-04

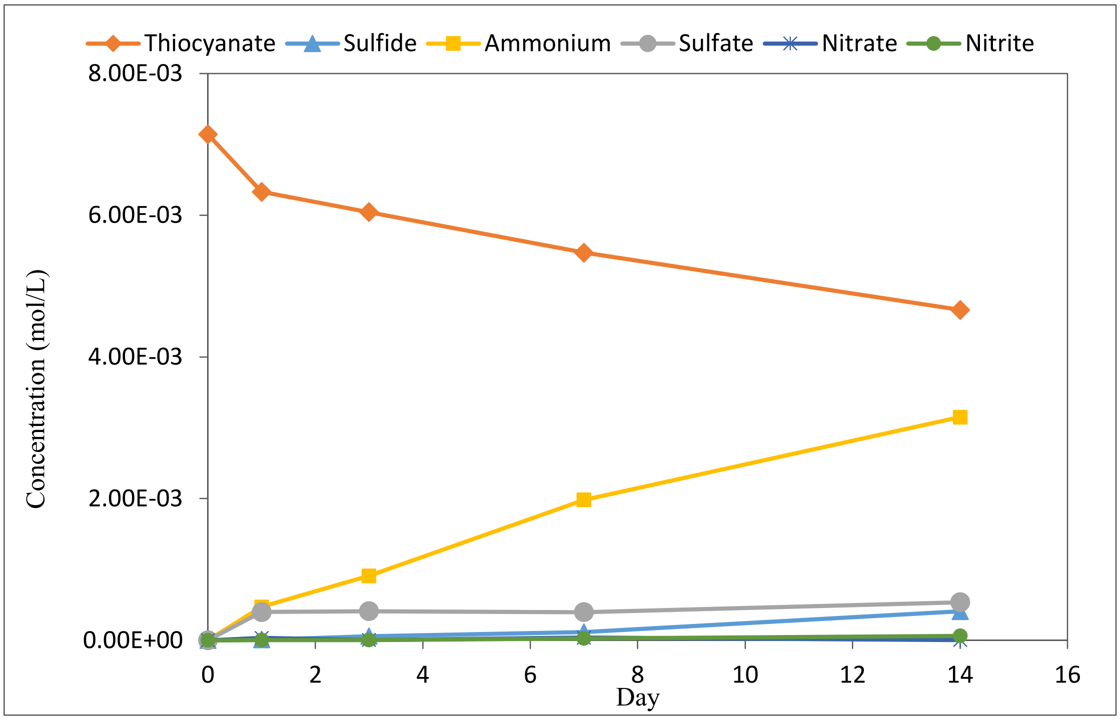

Figure 9: SCN$^-$ dissociation at 200ºC and ambient pH (without nitrate or nitrite)

3.7. Hydrothermal experiment 7: no pH adjustment at 150ºC

In order to obtain better analytical results, experiment 1 was repeated here but only Solution 1 - 100mg/L NaSCN as N was used as starting solution. The experiment was set up at 150ºC, at ambient pH and was left to run for 14 days.

At the end of the fourteenth day, the last autoclave was quenched and the tubes containing the run products of solution 1 – 100mg/L NaSCN for the different quench-days were opened. The mass of the run product in each silica tube was measured before the run product was transferred to a plastic container where it was diluted as needed. Several analyses were performed to measure the concentrations of SCN$^-$, NO_3^-, NO_2^-, NH_4^+, S^{2-} and SO_4^{2-} in the run products.

The analytical results obtained for this experiment suggested that the SCN^- dissociation reaction is very slow at 150ºC and ambient pH when compared to the reaction rates at 250ºC and 200ºC (hydrothermal experiments 5 and 6 respectively). See figure 24 in discussion of results.

Small amounts of NO_3^-, NO_2^-, NH_4^+ and SO_4^{2-} were detected upon analyses of the run products. The presence of NO_3^-, NO_2^- and SO_4^{2-} in the run products of Solution 1 - 10mg/L NaSCN as N could be as a result of residual oxygen in the tube after the experiment set up

Table X: Run product analyses for hydrothermal experiment 7

Day	$[SCN^-]$ mol/L	$[NO_3^-]$ mol/L	$[NO_2^-]$ mol/L	$[NH_4^+]$ mol/L	$[S^{2-}]$ mol/L	$[SO_4^{2-}]$ mol/L
0	7.14E-03	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00
1	6.84E-03	3.96E-05	4.39E-05	0.00E+00	0.00E+00	1.59E-04
3	6.82E-03	-	5.36E-06	0.00E+00	0.00E+00	1.69E-04
7	6.78E-03	3.86E-05	5.36E-06	1.61E-04	0.00E+00	2.02E-04
14	6.76E-03	-	5.36E-06	1.07E-04	0.00E+00	2.75E-04

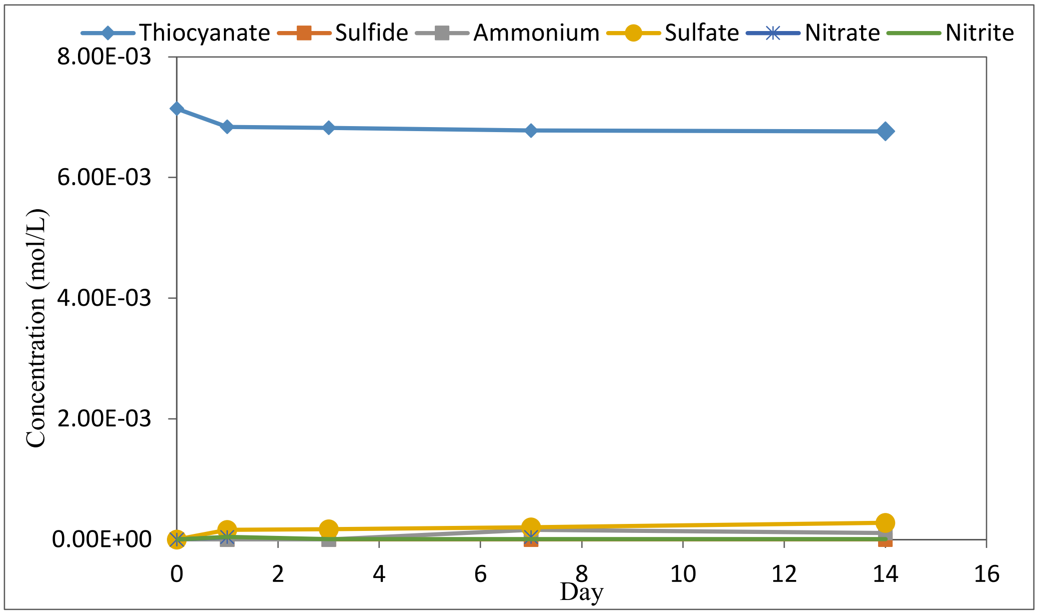

Figure 10: SCN^- dissociation at 150ºC and ambient pH (without nitrate or nitrite)

3.8. Hydrothermal experiment 8: pH reduction at 150°C

Referring to the results obtained from hydrothermal experiment 2 (pH 2.0), it was inferred that the SCN^- dissociation reaction is pH dependent. As a follow up to hydrothermal experiment 2, hydrothermal experiment 8 was set up at 150°C using solution 1 - 100mg/L NaSCN as N adjusted to 4 different pH: 2.0, 2.65, 3.56 and 4.01 for 7 days.

At the end of the seventh day, the last autoclave was quenched and the tubes containing the run products of solution 1 – 100mg/L NaSCN for the various quench-days and pH were opened. The pH and mass of the run product in each silica tube were measured before the run product was transferred to a plastic container where it was diluted as needed. Several analyses were performed to measure the concentrations of SCN^-, NH_4^+, S^{2-} and SO_4^{2-} in the run products. The analytical results for each run product are presented in Tables XI – XIV below.

Just as in hydrothermal experiment 2 (pH = 2 at 150°C), a fetid smell characteristic of hydrogen sulfide was perceived when the tubes for hydrothermal experiments 8.1 (pH 2.0) and 8.2 (pH 2.65) were opened. The pungent smell was strongest in the day 7 tubes for both experiments. A pale yellow precipitate assumed to be elemental sulfur was also observed in the pH 2 tubes as well as the day 3 and 7 tubes for pH 2.65. Even though the pH and measurements were performed quickly, some hydrogen sulfide was probably lost to the environment when the tubes containing hydrogen sulfide were opened.

The pH dependence of SCN^- dissociation is evident in the results obtained from hydrothermal experiment 8. Thiocyanate was completely broken down at pH 2.0 and 150°C. Complete dissociation of SCN^- was not observed at the other pH and the rate of the reaction decreased with increasing pH. See figure 25 in discussion of results.

The concentration of NH_4^+ in the various tubes increased with time and was inversely correlated with the decrease in SCN^- concentration. Small amount of SO_4^{2-} was detected upon analyses of the run products. The presence of SO_4^{2-} in the run products of Solution 1 - 10mg/L NaSCN as N could be as a result of oxidation by the residual oxygen in the tube after the experiment set up.

3.8.1. Hydrothermal experiment 8.1

Composition of starting solution: Solution 1 - 100mg/L NaSCN as N at pH 2.0

Table XI: Run product analyses for hydrothermal experiment 8.1

Day	$[SCN^-]$ mol/L	$[NH_4^+]$ mol/L	$[S^{2-}]$ mol/L	$[SO_4^{2-}]$ mol/L
0	7.14E-03	0.00E+00	0.00E+00	0.00E+00
1	5.36E-04	6.60E-03	1.08E-03	7.98E-04
3	5.08E-04	6.88E-03	1.23E-03	1.25E-03
7	5.15E-04	6.59E-03	1.18E-03	3.20E-03

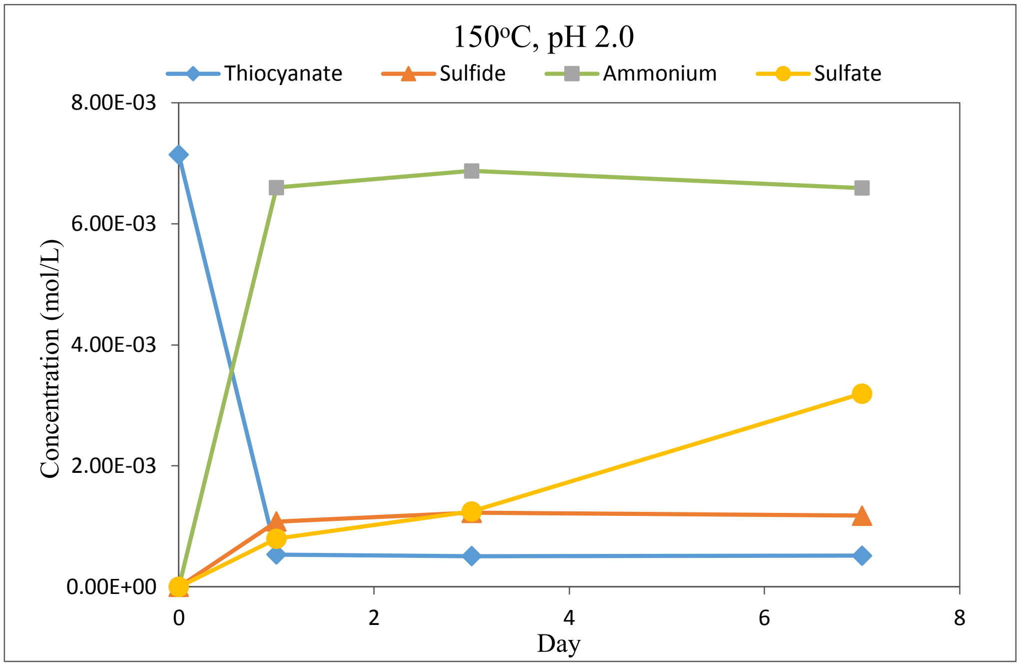

Figure 11: SCN^- dissociation at 150ºC and pH 2.0

3.8.2. Hydrothermal experiment 8.2

Composition of starting solution: Solution 1 - 100mg/L NaSCN as N at pH 2.65

Table XII: Run product analyses for hydrothermal experiment 8.2

Day	[SCN^-] mol/L	[NH_4^+] mol/L	[S^{2-}] mol/L	[SO_4^{2-}] mol/L
0	7.14E-03	0.00E+00	0.00E+00	0.00E+00
1	5.73E-03	1.98E-03	3.94E-06	1.14E-03
3	5.31E-03	1.61E-03	1.52E-04	1.80E-03
7	5.42E-03	1.70E-03	2.15E-04	3.49E-03

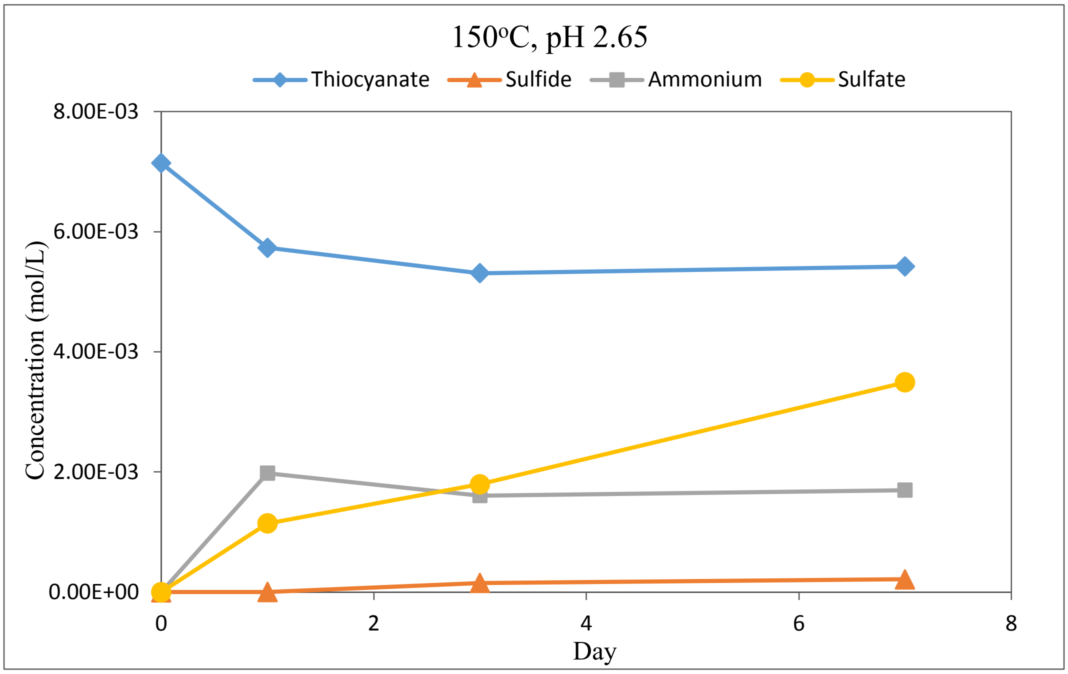

Figure 12: SCN^- dissociation at 150°C and pH 2.65

3.8.3. Hydrothermal experiment 8.3

Composition of starting solution: Solution 1 - 100mg/L NaSCN as N at pH 3.56

Table XIII: Run product analyses for hydrothermal experiment 8.3

Day	[SCN^-] mol/L	[NH_4^+] mol/L	[S^{2-}] mol/L	[SO_4^{2-}] mol/L
0	7.14E-03	0.00E+00	0.00E+00	0.00E+00
1	6.79E-03	1.79E-04	7.81E-06	5.18E-04
3	6.79E-03	2.68E-04	3.52E-05	1.01E-03
7	6.75E-03	9.04E-05	1.19E-05	1.22E-03

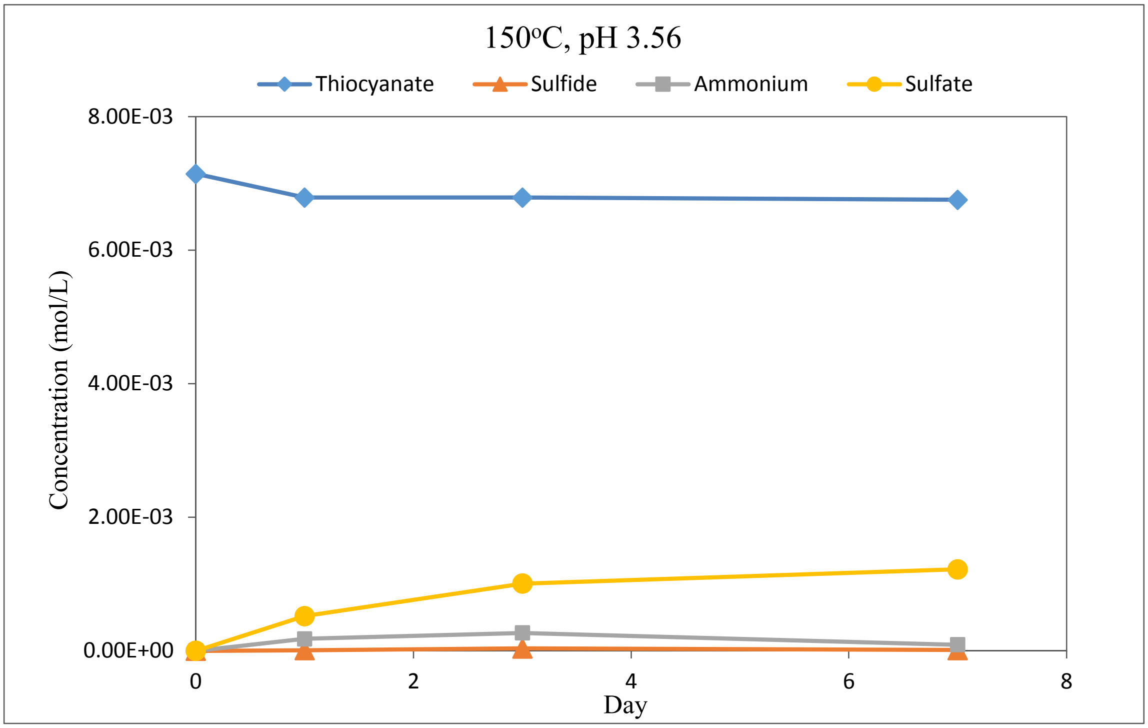

Figure 13: SCN^- dissociation at 150ºC and pH 3.56

3.8.4. Hydrothermal experiment 8.4

Composition of starting solution: Solution 1 - 100mg/L NaSCN as N at pH 4.01

Table XIV: Run product analyses for hydrothermal experiment 8.4

Day	[SCN^-] mol/L	[NH_4^+] mol/L	[S^{2-}] mol/L	[SO_4^{2-}] mol/L
0	7.14E-03	0.00E+00	0.00E+00	0.00E+00
1	7.15E-03	8.93E-05	0.00E+00	4.11E-04
3	6.48E-03	8.93E-05	0.00E+00	8.15E-04
7	6.58E-03	0.00E+00	7.81E-06	7.29E-04

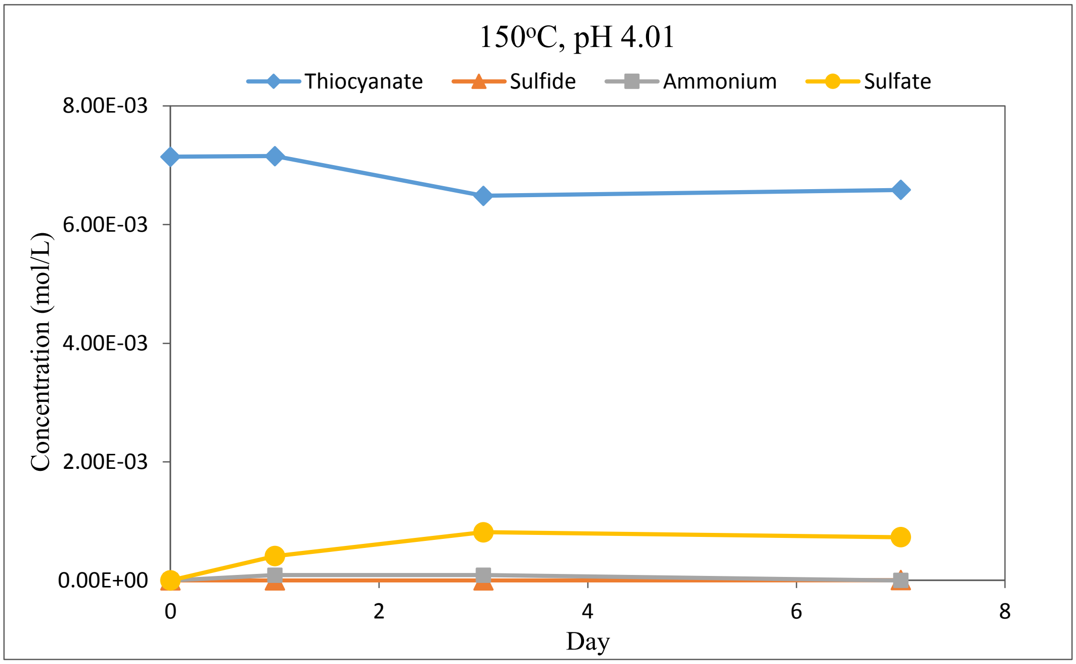

Figure 14: SCN^- dissociation at 150ºC and pH 4.01

3.9. Hydrothermal experiment 9: pH reduction at 200°C

Hydrothermal experiment 9 was performed to investigate how temperature affects the SCN^- dissociation reaction when the pH of the starting solution is reduced. This experiment was set up at 200°C (compare with hydrothermal experiments 2 and 8 at various pH at 150°C) using solution 1 - 100mg/L NaSCN as N at pH 2.0, 2.65, 3.56 and 4.01 to show the temperature dependence of the reaction at different pH.

At the end of the seventh day, the last autoclave was quenched and the tubes containing the run products of solution 1 – 100mg/L NaSCN for the different quench-days and pH were opened. The pH and mass of the run product in each silica tube were measured before the run product was transferred to a plastic container where it was diluted as needed. Several analyses were performed to measure the concentrations of SCN^-, NH_4^+, S^{2-} and SO_4^{2-} in the run products. The analytical results for each run product are presented in Tables XV - XVIII below.

Just as in hydrothermal experiments 2 and 8 (various pH at 150°C), a fetid smell characteristic of hydrogen sulfide was perceived when the tubes for hydrothermal experiments 9.1 (pH = 2.0) and 9.2 (pH = 2.65) were opened. The pungent smell was strongest in the day 7 tubes for both experiments. A pale yellow precipitate assumed to be elemental sulfur was also observed in the pH 2 tubes and for the day 3 and 7 tubes for pH 2.65. Even though the pH measurements were performed quickly, some hydrogen sulfide was lost to the environment when the tubes containing hydrogen sulfide were opened.

The temperature and pH dependence of the anerobic dissociation reaction of SCN^- was confirmed with the results obtained from this experiment. As with hydrothermal experiments 2 and 8, SCN^- was completely broken down at pH 2.0 at 200°C. The rates of the reaction at pH 2.65, 3.56 and 4.01 increased at 200°C when compared with the rates at 150°C. It was also clear that the reaction was slower with increasing pH. See figure 26 in discussion of results.

The analytical results for each run product are presented below. No results were obtained for day 3 at pH 2.0 and 3.65 because the silica tubes broke inside the pressure while the experiment was on-going. This could have been as a result of the accumulation of carbon dioxide, ammonia or hydrogen sulphide gas. The concentration of NH_4^+ in the various tubes increased with time and was inversely correlated with the decrease in SCN^- concentration. Small amounts of SO_4^{2-} was detected upon analyses of the run products. The presence of SO_4^{2-} in the run products of Solution 1 - 10mg/L NaSCN as N could be as a result of oxidation by the residual oxygen in the tube after the experiment set up.

3.9.1. Hydrothermal experiment 9.1

Composition of starting solution: Solution 1 - 100mg/L NaSCN as N at pH 2.0

Table XV: Run product analyses for hydrothermal experiment 9.1

Day	[SCN^-] mol/L	[NH_4^+] mol/L	[S^{2-}] mol/L	[SO_4^{2-}] mol/L
0	7.14E-03	0.00E+00	0.00E+00	0.00E+00
1	4.96E-04	9.29E-03	1.32E-03	3.10E-03
7	4.96E-04	6.96E-03	1.73E-03	3.23E-03

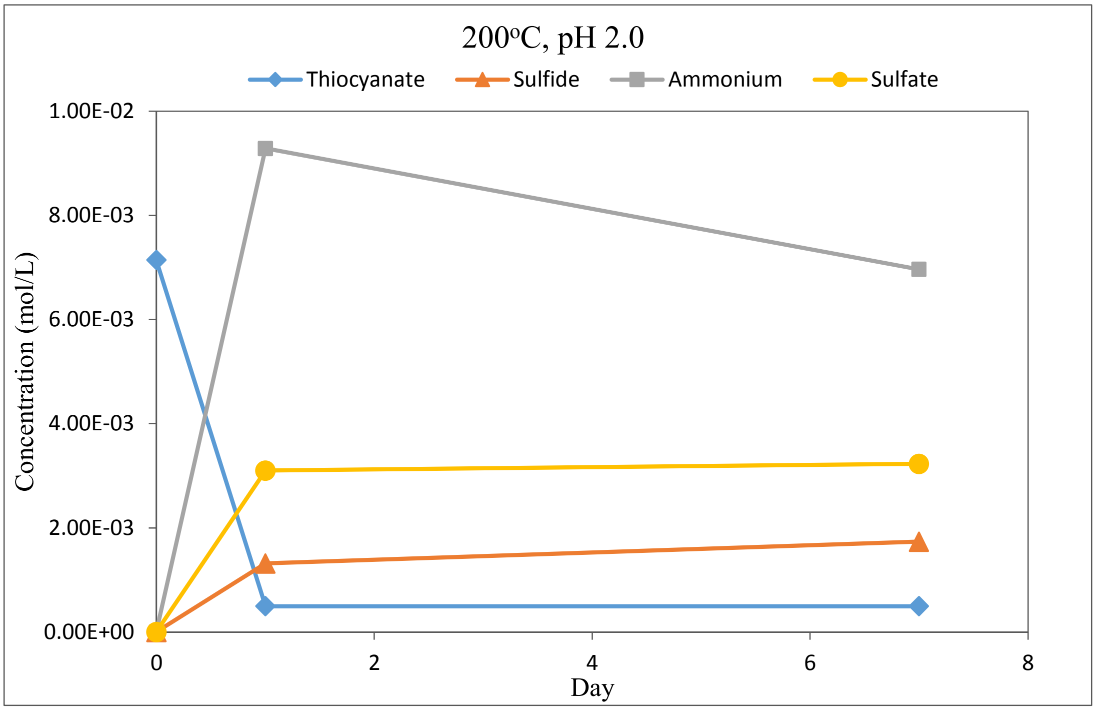

Figure 15: SCN$^-$ dissociation at 200ºC and pH 2.0

3.9.2. Hydrothermal experiment 9.2

Composition of starting solution: Solution 1 - 100mg/L NaSCN as N at pH 2.65

Table XVI: Run product analyses for hydrothermal experiment 9.2

Day	[SCN$^-$] mol/L	[NH_4^+] mol/L	[S^{2-}] mol/L	[SO_4^{2-}] mol/L
0	7.14E-03	0.00E+00	0.00E+00	0.00E+00
1	4.40E-03	3.48E-03	6.56E-04	1.58E-03
3	4.07E-03	4.20E-03	1.08E-03	6.56E-04
7	2.53E-03	6.52E-03	1.92E-03	1.33E-03

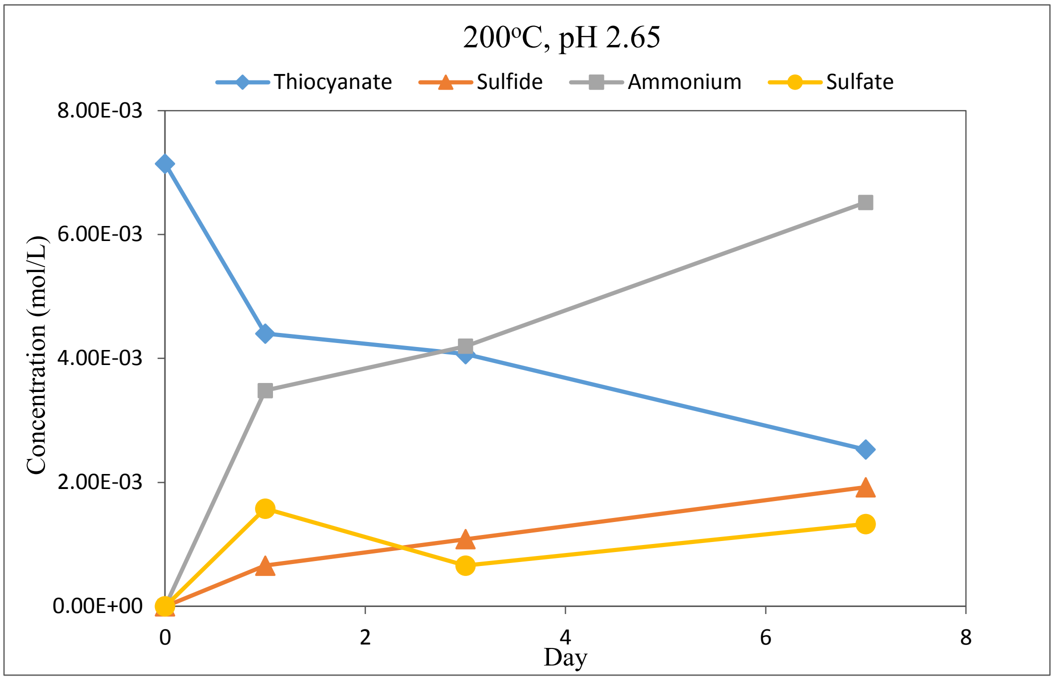

Figure 16: SCN$^-$ dissociation at 200°C and pH 2.65

3.9.3. Hydrothermal experiment 9.3

Composition of starting solution: Solution 1 - 100mg/L NaSCN as N at pH 3.5

Table XVII: Run product analyses for hydrothermal experiment 9.3

Day	[SCN$^-$] mol/L	[NH$_4^+$] mol/L	[S^{2-}] mol/L	[SO$_4^{2-}$] mol/L
0	7.14E-03	0.00E+00	0.00E+00	0.00E+00
1	6.27E-03	1.17E-03	1.54E-04	7.25E-04
7	4.20E-03	3.90E-03	1.04E-03	1.19E-03

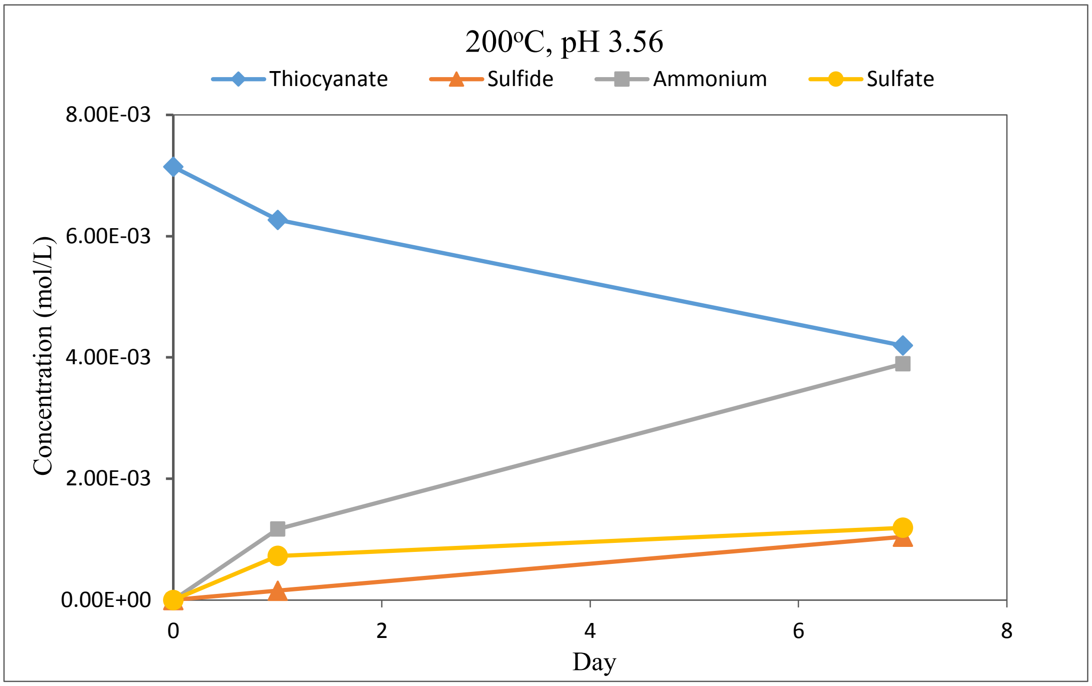

Figure 17: SCN⁻ dissociation at 200ºC and pH 3.56

3.9.4. Hydrothermal experiment 9.4

Composition of starting solution: Solution 1 - 100mg/L NaSCN as N at pH 4.01

Table XVIII: Run product analyses for hydrothermal experiment 9.4

Day	[SCN$^-$] mol/L	[NH$_4^+$] mol/L	[S^{2-}] mol/L	[SO$_4^{2-}$] mol/L
0	7.14E-03	0.00E+00	0.00E+00	0.00E+00
1	6.77E-03	7.14E-04	8.20E-05	4.71E-04
3	6.15E-03	1.07E-03	1.80E-04	5.86E-04
7	5.47E-03	2.05E-03	4.88E-04	5.16E-04

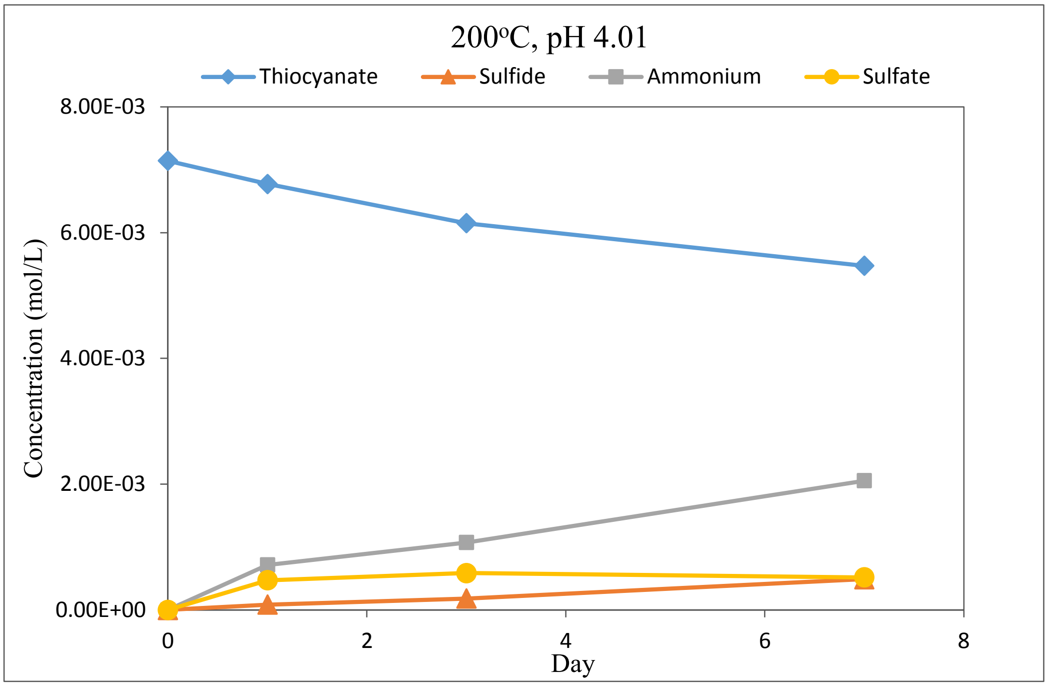

Figure 18: SCN$^-$ dissociation at 200ºC and pH 4.01

3.10. Hydrothermal experiment 10: pH reduction at 100ºC

From hydrothermal experiments 8 and 9, it was evident that the SCN$^-$ dissociation reaction was fastest at pH 2.0. Therefore, subsequent hydrothermal experiments were conducted using Solution 1 - 100mg/L NaSCN as N at pH 2.0 only.

Hydrothermal experiment 10 was set up at 100ºC and was left to run for 7 days. At the end of the seventh day, the last autoclave was quenched and the tubes containing the run products of solution 1 – 100mg/L NaSCN for the different quench-days were opened. The mass of the run product in each silica tube was measured before the run product was transferred to a plastic container where it was diluted as needed. Several analyses were performed to measure the concentrations of SCN$^-$, NH_4^+ and S^{2-} in the run products. The concentrations of SO_4^{2-}, NO_3^- and

NO_2^- in the run products were not measured for this experiment. The analytical results for each run product are presented in Table XIX below.

The rate of SCN^- dissociation at pH 2.0 and 100ºC was slower when compared to the rates observed in hydrothermal experiments 2 (150ºC), 8 (150ºC) and 9 (200ºC). At pH 2.0 and 100ºC, SCN^- did not break down completely within 7 days.

Table XIX: Run product analyses for hydrothermal experiment 10

Day	$[SCN^-]$ mol/L	$[NH_4^+]$ mol/L	$[S^{2-}]$ mol/L
0	7.14E-03	0.00E+00	0.00E+00
1	3.77E-03	0.00E+00	1.03E-05
3	2.53E-03	7.86E-05	0.00E+00
7	1.84E-03	7.86E-05	0.00E+00

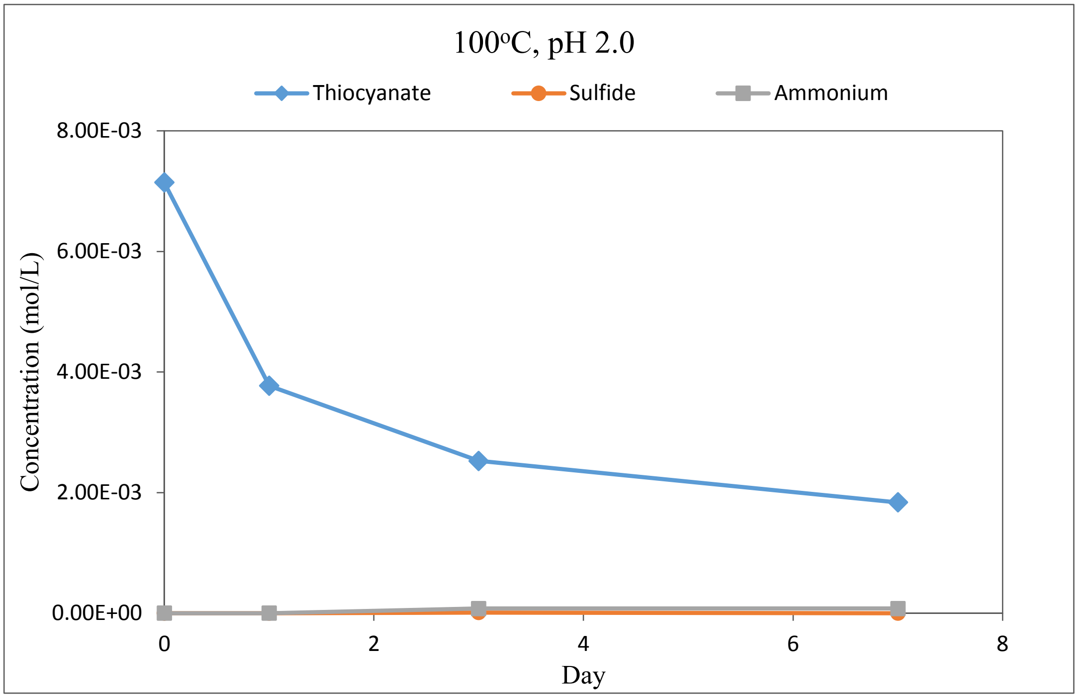

Figure 19: SCN^- dissociation at 100°C and pH 2.0

3.11. Hydrothermal experiment 11: pH reduction at 60°C

This experiment was conducted as a follow up to hydrothermal experiment 10 (pH 2.0, 100°C). The setup of this experiment required Solution 1 - 100mg/L NaSCN as N at pH 2.0 and 60°C.

At the end of the seventh day, the last autoclave was quenched and the tubes containing the run products of solution 1 – 100mg/L NaSCN for the different quench-days were opened. The mass of the run product in each silica tube was measured before the run product was transferred to a plastic container where it was diluted as needed. Several analyses were performed to measure the concentrations of SCN^-, NH_4^+ and S^{2-} in the run products. The concentrations of SO_4^{2-}, NO_3^- and NO_2^- in the run products were not measured for this experiment. The analytical results for each run product are presented in Table XX below.

As expected, the rate of SCN^- dissociation was slower at 60°C when compared to the rates observed in experiments 2 (150°C), 8 (150°C), 9 (200°C) and 10 (100°C). At pH 2.0 and 60°C, SCN^- did not break down completely within 7 days.

Table XX: Run product analyses for hydrothermal experiment 11

Day	$[SCN^-]$ mol/L	$[NH_4^+]$ mol/L	$[S^{2-}]$ mol/L
0	7.14E-03	0.00E+00	0.00E+00
1	6.66E-03	0.00E+00	1.25E-05
3	6.33E-03	7.32E-05	3.20E-06
7	5.94E-03	0.00E+00	3.13E-06

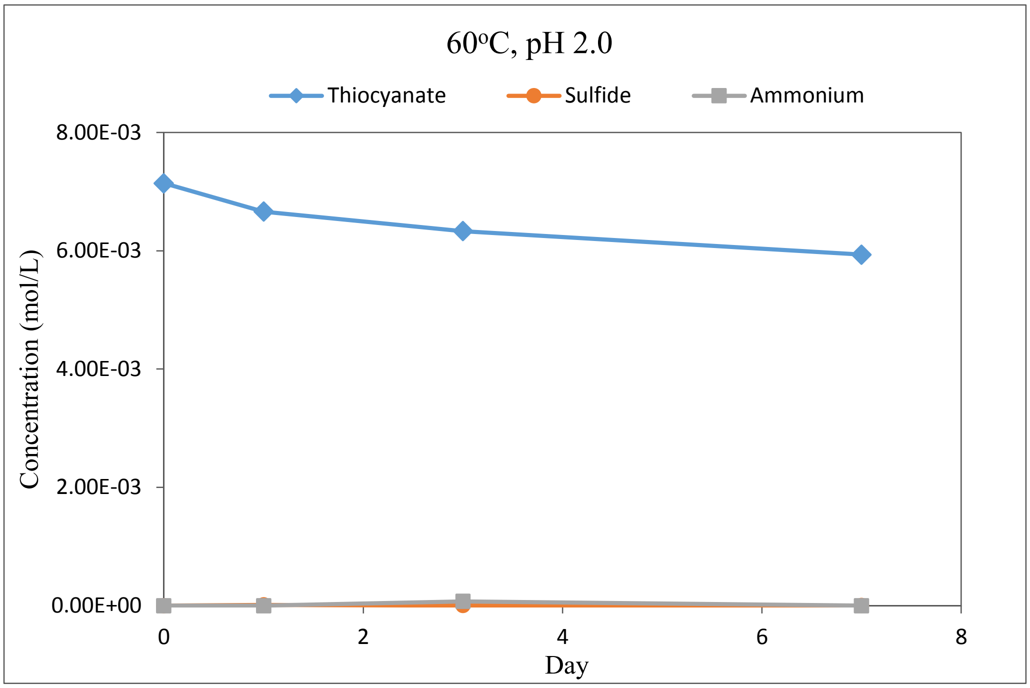

Figure 20: SCN^- dissociation at 60°C and pH 2.0

3.12. Hydrothermal experiment 12 and 13: pH reduction at 300°C

Both hydrothermal experiments 12 and 13 were set up at 300°C at different times using Solution 1 - 10mg/L NaSCN as N at pH 2.0.

Even with the 10 times dilute solutions, the silica tubes containing the starting solutions broke within the pressure tubes. This could be due to pressure build up due to the accumulation of gases like carbon dioxide, hydrogen sulfide and ammonia within the tubes as the reaction progressed. Consequently, no analysis was performed for these experiments.

3.13. Low temperature experiment 1: pH reduction at ambient temperature

This experiment is an extension of the hydrothermal experiments discussed above. However, it was set up at room temperature. The composition of the starting solution for this experiment was Solution 1 - 100mg/L NaSCN as N at pH 2.0.

At the end of day 1, 3 and 7, the mass of the run product in each silica tube was measured before the run product was transferred to a plastic container where it was diluted as needed. Laboratory analyses were performed to measure the concentrations of SCN^- and S^{2-} in the run products.

The analytical results obtained from this experiment suggest that the rate of SCN^- dissociation is extremely slow at pH 2.0 and room temperature. See figure 27 in discussion of results. The analytical results for each run product are shown below. Although the rate of decrease in concentration of SCN^- could not be measured within errors, the presence of up to 0.02 mM H_2S suggests that a small amount (roughly 0.3%) of the initial SCN^- may have dissociated after 7 days.

Table XXI: Run products for low temperature experiment 1

Day	[SCN^-] mol/L	[S^{2-}] mol/L
0	7.14E-03	0.00E+00
1	7.18E-03	0.00E+00
3	7.18E-03	3.44E-06
7	7.07E-03	2.06E-05

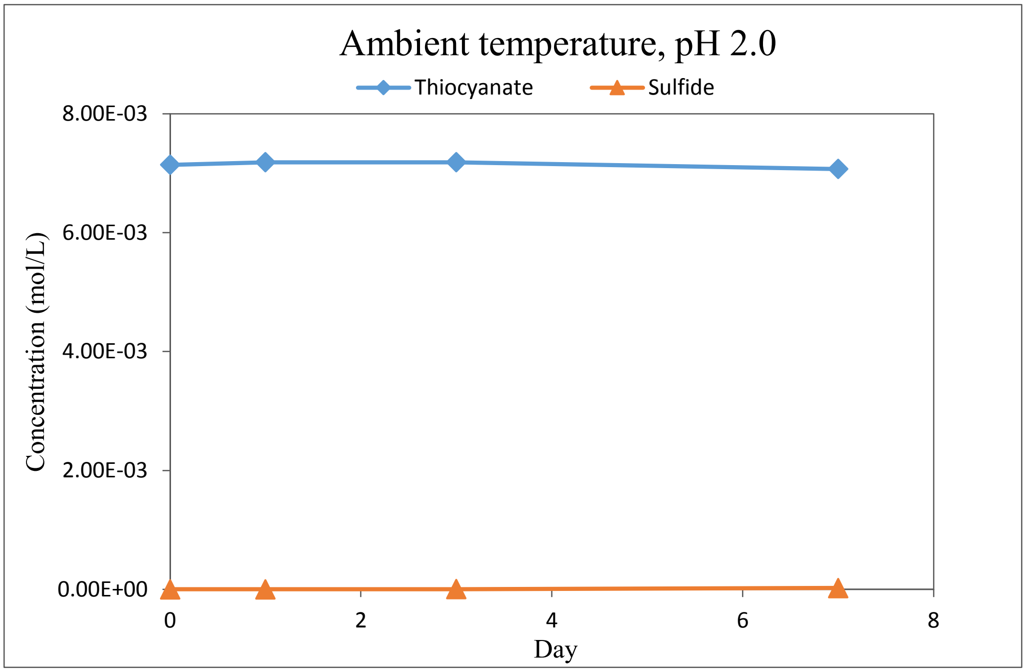

Figure 21: SCN^- dissociation at ambient temperature and pH 2.0

3.14. Low temperature experiment 2: gravel substrate solution at ambient pH and temperature

This experiment involving gravel substrate collected from a mine tailings impoundment was performed in order to investigate whether or not the microorganisms that use SCN^- as energy source are present in the gravel substrate and whether they can be activated under anoxic environments, ambient pH and temperature.

The experimental setup involved Solutions 1, 2 and 3 at ambient pH (6.1 – 6.7) with 1% methanol. The methanol was introduced as a source of organic carbon to be consumed by microbes present within the system so as to create the anoxic environment. The solution was shaken gently once every 2-3 days and left to equilibrate in the fume hood at room temperature. The experiment ran for thirty-one days.

The results obtained from this experiment suggest that the microbes presumed to use SCN^- as energy source were present in the gravel substrates obtained from the mine. Under the conditions employed for this study, a 10 - 43% decrease in the concentration of SCN^- in association with the gravel substrates was observed after thirty-one days. The most reduction in SCN^- concentration was observed in the bottle containing nitrite. A noticeable bulge of the plastic bottles containing the three solutions indicates that a gas or gas mixture evolved during the experiment, possibly CO_2, CH_4, and/or N_2. No attempt was made to analyse the composition of this gas. The analytical results obtained for this experiment are as follows:

3.14.1. Low temperature experiment 2.1

Composition of starting solution: Solution 1 - 100mg/L NaSCN as N with 1% methanol at ambient pH

Table XXII: Run product analyses for low temperature experiment 2.1

Day	[SCN^-] mol/L	NH_4^+] mol/L	[S^{2-}] mol/L
0	7.14E-03	0.00E+00	0.00E+00
31	6.42E-03	3.14E-04	3.13E-06

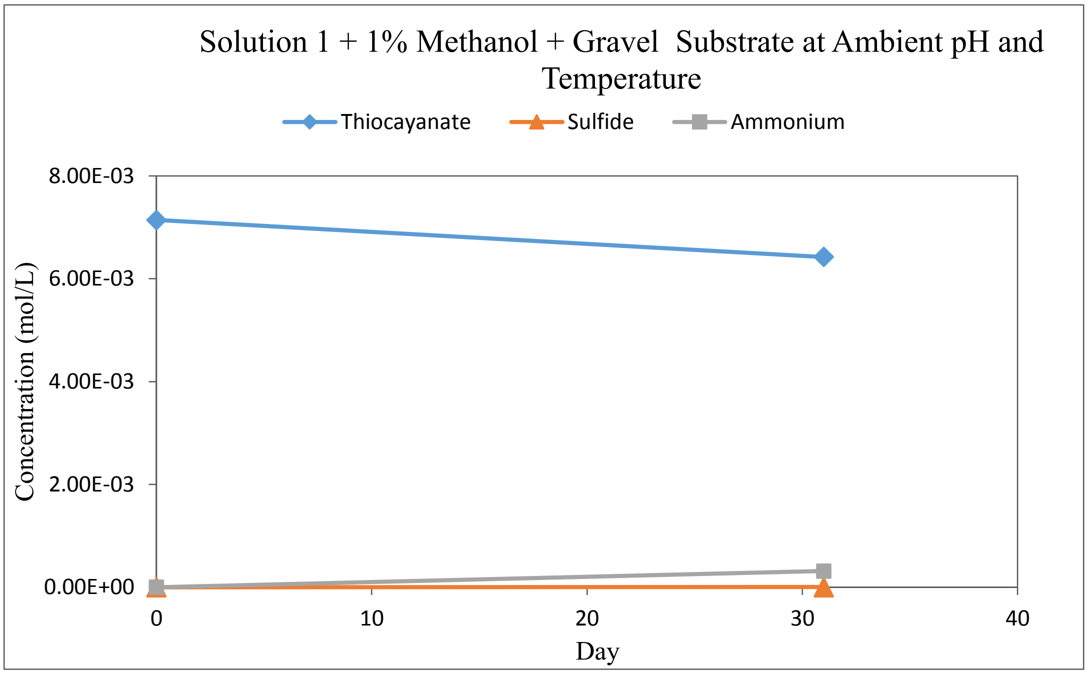

Figure 22: Dissociation of SCN$^-$ (Solution 1) in association with gravel substrate at ambient pH and temperature after thirty-one days

3.14.2. Low temperature experiment 2.2

Composition of starting solution: Solution 2 - 100mg/L NaSCN + 100mg/L $NaNO_3$ as N with 1% methanol at ambient pH

Table XXIII: Run product analyses for low temperature experiment 2.2

Day	[SCN$^-$] mol/L	NH_4^+] mol/L	[S^{2-}] mol/L
0	7.14E-03	0.00E+00	0.00E+00
31	4.62E-03	1.51E-03	1.88E-06

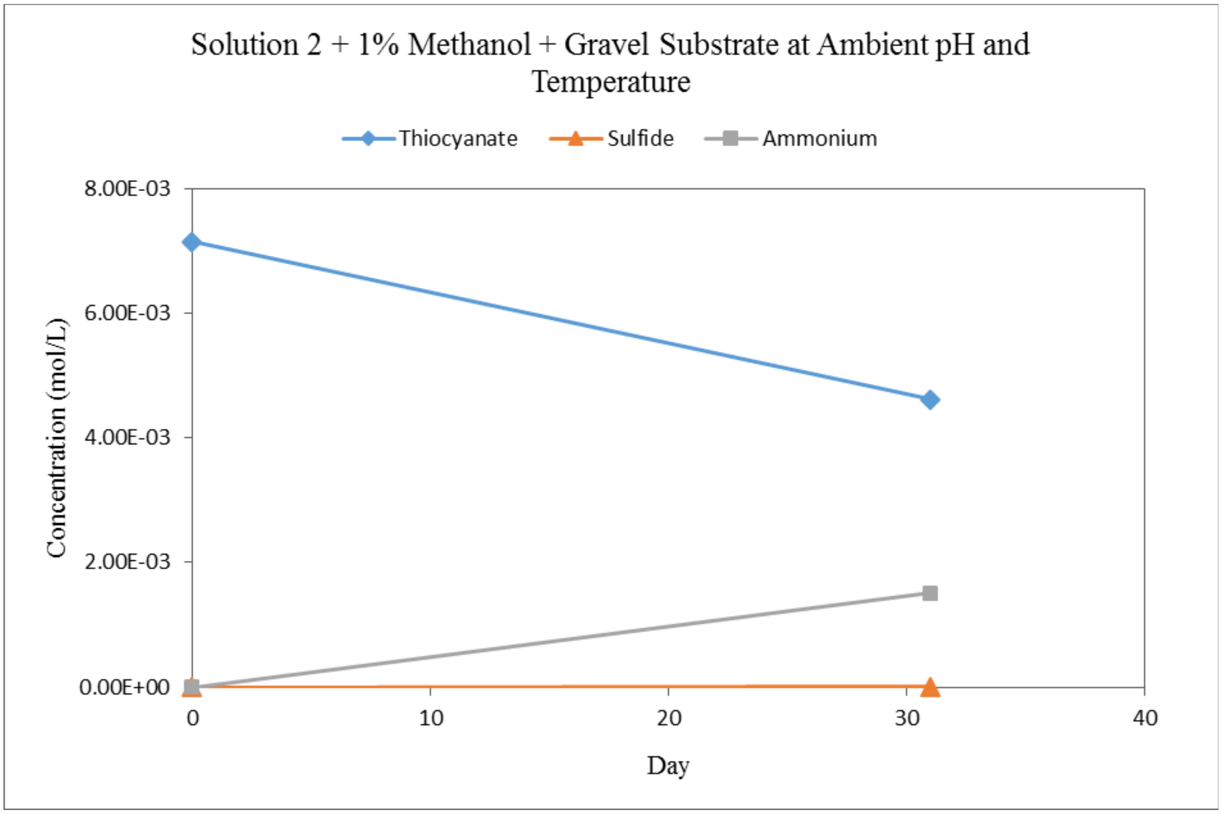

Figure 23: Dissociation of SCN^- (Solution 2) in association with gravel substrate at ambient pH and temperature after thirty-one days

3.14.3. Low temperature experiment 2.3

Composition of starting solution: Solution 3 - 100mg/L NaSCN + 100mg/L $NaNO_2$ as N at ambient pH and 1% methanol.

Table XXIV: Run product analyses for low temperature experiment 2.3

Day	$[SCN^-]$ mol/L	$NH_4^+]$ mol/L	$[S^{2-}]$ mol/L
0	7.14E-03	0.00E+00	0.00E+00
31	4.08E-03	9.14E-04	9.38E-07

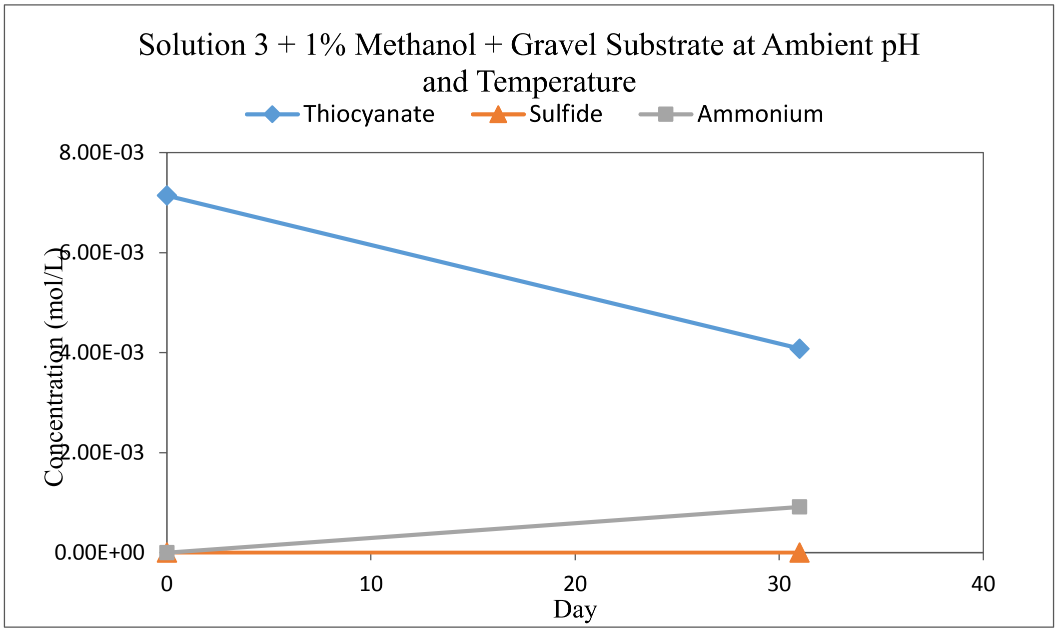

Figure 24: Dissociation of SCN$^-$ (Solution 3) in association with gravel substrate at ambient pH and temperature after thirty-one days

3.15. Low temperature experiment 3: gravel substrate solution at high pH and ambient temperature

This experiment involving gravel substrates collected from a mine tailings impoundment was performed to investigate whether or not the microorganisms that use SCN$^-$ as energy source are present in the gravel substrate and if they can be activated under anoxic environments, at relatively high pH and ambient temperature.

The experimental set up involved Solutions 1, 2 and 3 at a pH range of 10.93 – 10.95 with 1% methanol. The methanol was introduced as a source of organic carbon to be consumed by microbes present within the system so as to create the anoxic environment. The solution was shaken gently once every 2-3 days and left to equilibrate in the fume hood at room temperature. The experiment ran for forty-nine days.

The results obtained from this experiment suggest that the microbes presumed to use SCN^- as energy source were present in the gravel substrates obtained from the mine. Under the conditions employed for this study, a 5-10% decrease in the concentration of SCN^- in association with the gravel substrates was observed after forty-nine days. The most reduction in SCN^- concentration was observed in the bottle containing nitrate. A noticeable bulge of the plastic bottles containing the three solutions indicates that a gas evolved, as was the case for the similar experiment at neutral pH. However, in this case, it is clear that the gas could not have been CO_2, since it would have dissolved into the solutions at pH 10.9. Instead, the gas is speculated to have been methane and/or N_2. The analytical results obtained for this experiment are as follows:

3.15.1. Low temperature experiment 3.1

Composition of starting solution: Solution 1 - 100mg/L NaSCN as N with 1% methanol at pH 10.93

Table XXV: Run product analyses for low temperature experiment 3.1

Day	$[SCN^-]$ mol/L	$NH_4^+]$ mol/L	$[S^{2-}]$ mol/L
0	7.14E-03	0.00E+00	0.00E+00
31	6.50E-03	1.79E-04	4.06E-06

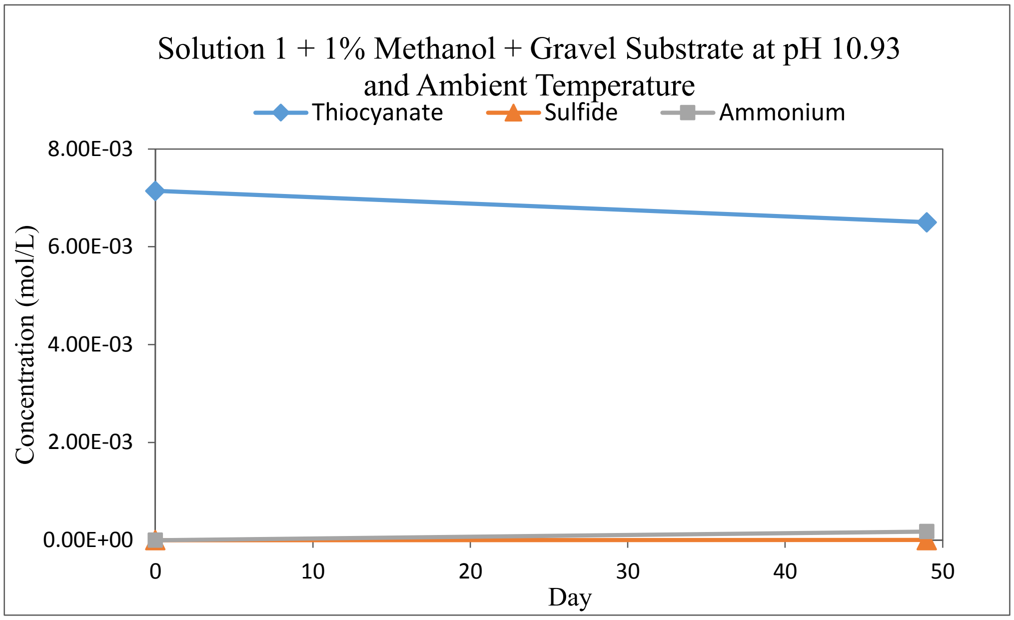

Figure 25: Dissociation of SCN^- (Solution 1) in association with gravel substrate at pH 10.93 and ambient temperature after forty-nine days

3.15.2. Low temperature experiment 3.2

Composition of starting solution: Solution 2 - 100mg/L NaSCN + 100mg/L $NaNO_3$ as N with 1% methanol at pH 10.95

Table XXVI: Run product analyses for low temperature experiment 3.2

Day	[SCN^-] mol/L	NH_4^+] mol/L	[S^{2-}] mol/L
0	7.14E-03	0.00E+00	0.00E+00
31	6.18E-03	2.50E-04	2.19E-06

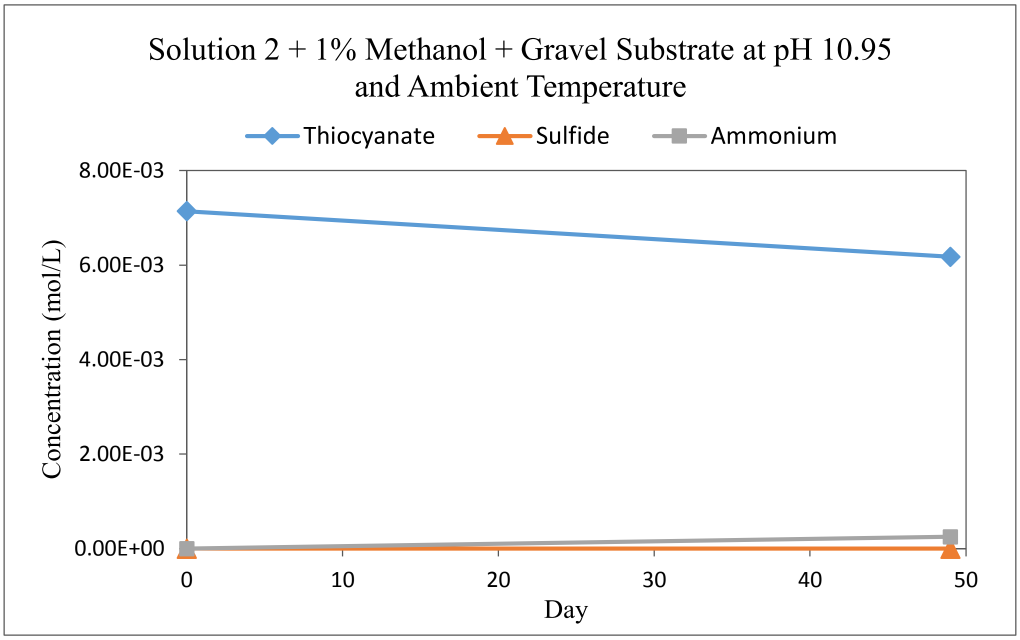

Figure 26: Dissociation of SCN$^-$ (Solution 2) in association with gravel substrate at pH 10.95 and ambient temperature after forty-nine days

3.15.3. Low temperature experiment 3.3

Composition of starting solution: Solution 3 - 100mg/L NaSCN + 100mg/L $NaNO_2$ as N with 1% methanol at pH 10.94

Table XXVII: Run product analyses for low temperature experiment 3.3

Day	[SCN$^-$] mol/L	NH_4^+] mol/L	[S^{2-}] mol/L
0	7.14E-03	0.00E+00	0.00E+00
31	6.62E-03	2.48E-04	2.45E-06

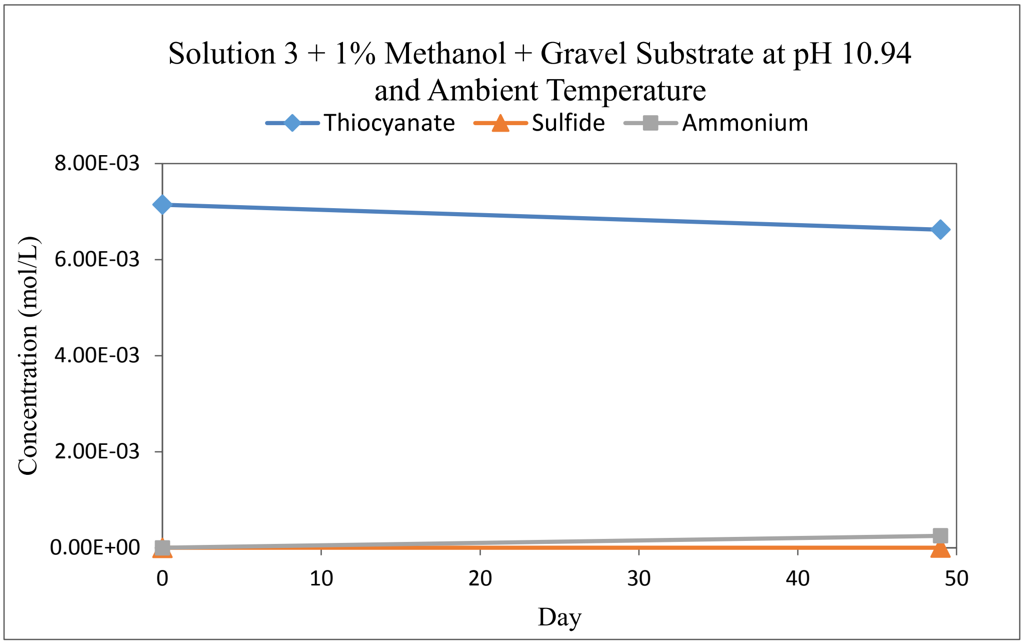

Figure 27: Dissociation of SCN^- (Solution 3) in association with gravel substrate at pH 10.94 and ambient temperature after forty-nine days

4. Discussion

Under abiotic and anoxic conditions, SCN^- does not break down at a time-scale of weeks at temperatures below 150˚C. Between 150 and 250˚C, SCN^- was observed to break down at a rate that increased with increase in temperature. However, at any given temperature, the rate of SCN^- degradation was similar in the presence or absence of nitrate and nitrite (Figure 28). Therefore, the initial hypothesis of this study, i.e., that SCN^- would react with oxidized nitrogen species to form nitrogen gas, does not appear to be valid, even at high temperature. Instead, SCN^- was observed to decompose to its constituent S, C, and N species, via the following reaction:

$$SCN^- + 2H_2O + 2H^+ \rightarrow H_2S + NH_4^+ + CO_2 \qquad (5)$$

The above reaction is essentially a hydrolysis reaction. It is similar to reaction (4), presented in the introduction to this thesis, except that it is written in terms of H_2S and dissolved CO_2. A preliminary literature search for “thiocyanate hydrolysis” found no previous studies that made reference to reaction (5). Therefore, determining the temperature and pH dependence of the rate of this reaction became a priority in the current study. A later, more exhaustive literature search uncovered a paper by Crowell and Hankins (1968), who measured the rate of thiocyanic acid hydrolysis at 25˚C and 90˚C at extremely low pH values (< 0).

The following discussion will first focus on the results of the present study, and will then make a comparison to the findings of Crowell and Hankins (1968). The discussion will end with some ideas regarding the possibility of bacterially-catalyzed reaction of SCN^- with nitrate/nitrite, based on the results of the low temperature experiments of this study.

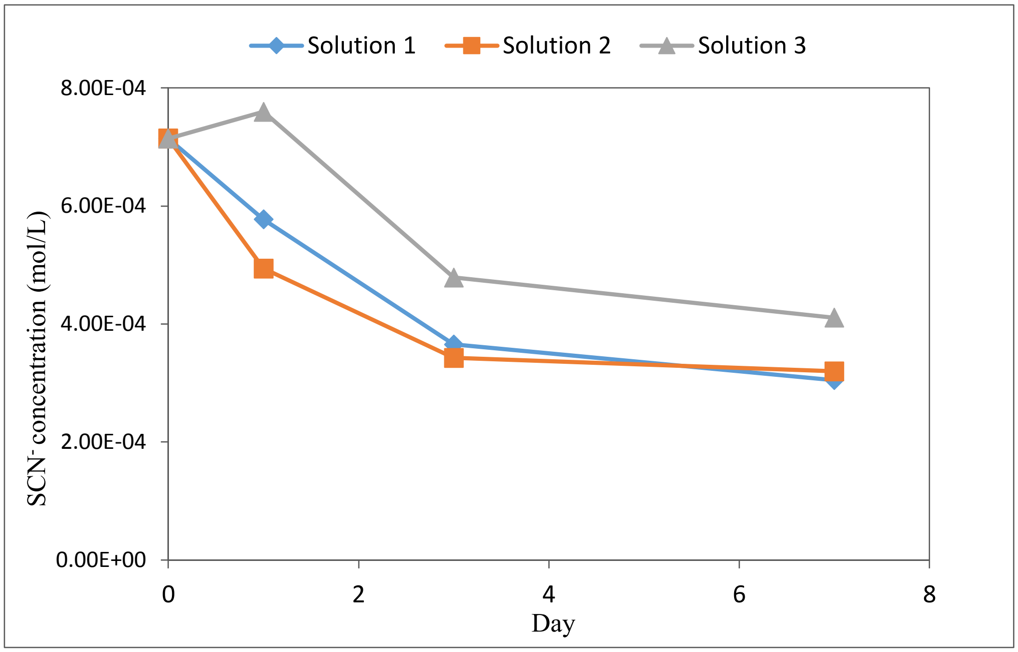

Figure 28: SCN$^-$ dissociation trend in the presence of nitrate/nitrite at 250^{o}C and ambient pH

4.1. Temperature and pH dependence of the rate of thiocyanate hydrolysis

The complete hydrolysis of SCN$^-$ in hydrothermal experiment 2 (at 150˚C, pH 2) provided the first indication that the anaerobic dissociation of SCN$^-$ is pH-dependent. Results from hydrothermal experiments 5 – 7 also indicate that the dissociation reaction is temperature dependent (Figure 29). With these findings, subsequent experiments were focused on demonstrating the temperature and pH dependence of SCN$^-$ dissociation under anoxic conditions.

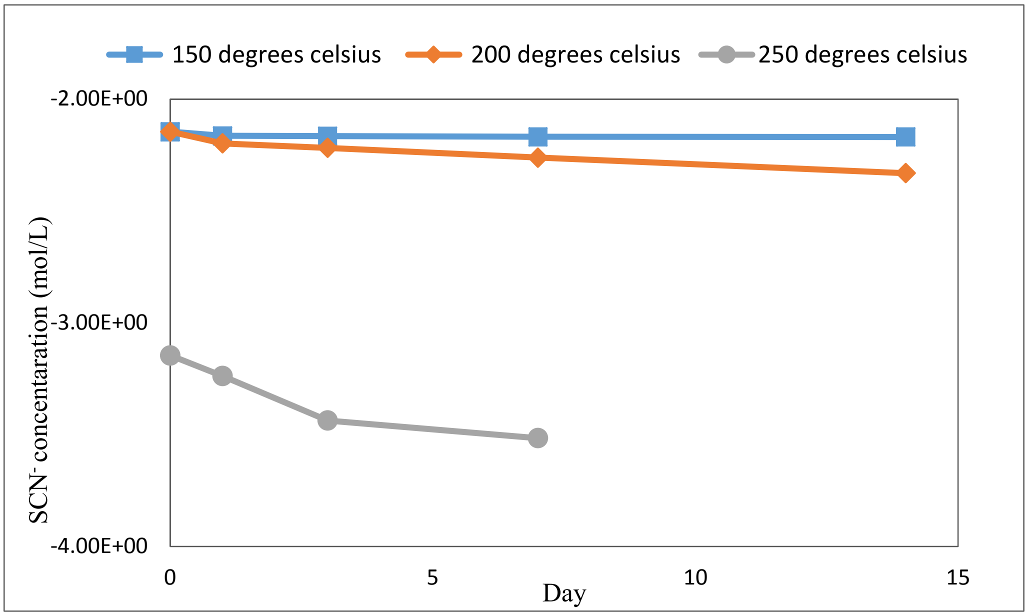

Figure 29: SCN$^-$ dissociation trend at various temperatures and ambient pH

It was evident from hydrothermal experiments 8 (at 150ºC) and 9 (at 200ºC) that the SCN$^-$ dissociation reaction is pH-dependent as the rate of the reaction decreased significantly as pH increased (Figures 30 - 32). Thiocyanate at pH 2 was completely broken down by the end of day 1. The final products were sulfide, ammonia and most likely CO_2 for all pH levels; the concentrations of which increased with decreasing pH. The small amounts of sulfur, sulfate, nitrate and nitrite detected were formed from oxidation reactions by the residual oxygen in the tubes. The results obtained from hydrothermal experiments 8 and 9 clearly followed reaction 5 above; especially at pH 2.

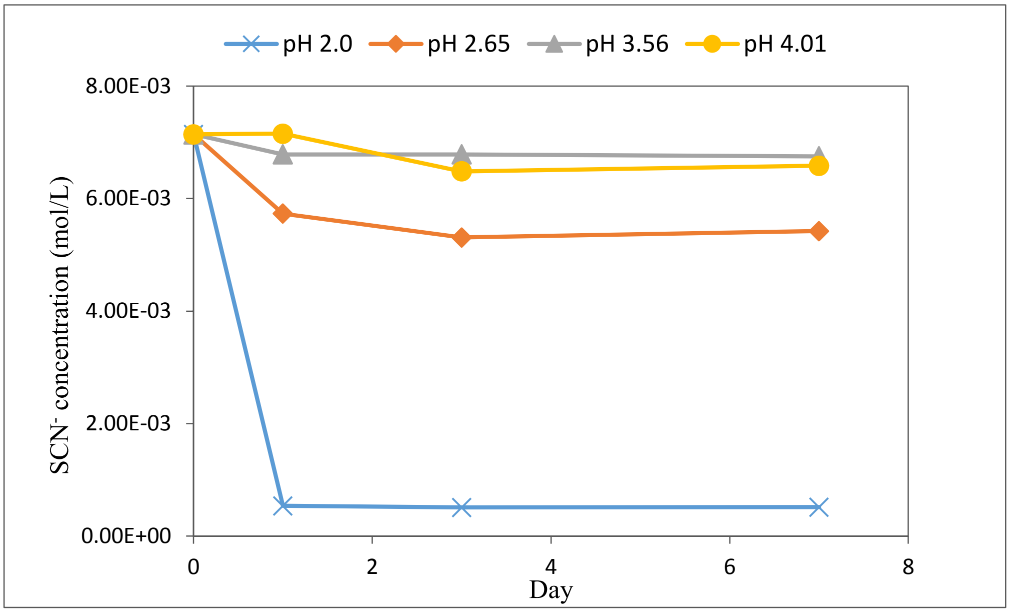

Figure 30: SCN$^-$ dissociation trend at 150ºC and various pH

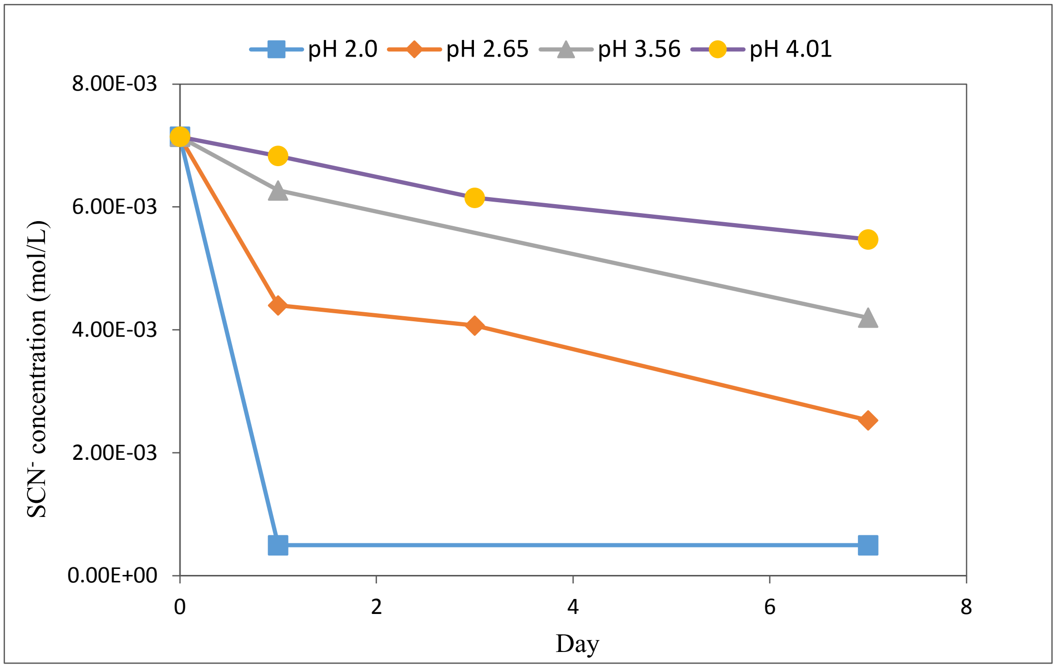

Figure 31: SCN$^-$ dissociation trend at 200ºC and various pH

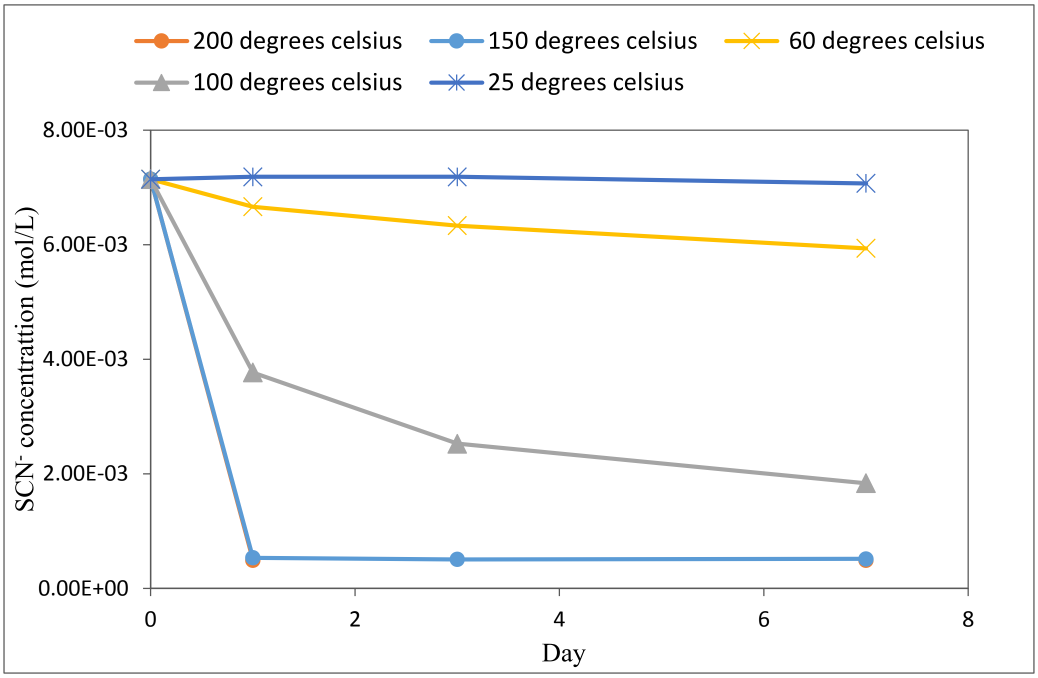

Figure 32: SCN$^-$ dissociation trend at pH 2 and various temperatures

For each abiotic experiment conducted for this study, the rate of the breakdown of SCN$^-$ was first order with respect to SCN$^-$ The rate constant (k) of the reaction was determined from an exponential trendline ($[SCN^-]_f = [SCN^-]_o \cdot e^{-kt}$) on the plot of SCN$^-$ concentration versus time as shown in Figure 33. Thiocyanate hydrolysis occurred so fast at pH 2 and temperatures above 150°C that the determination of the rate constants for the reaction under these conditions may not be as accurate as those obtained for the reaction at pH > 2 and temperature below 150°C.

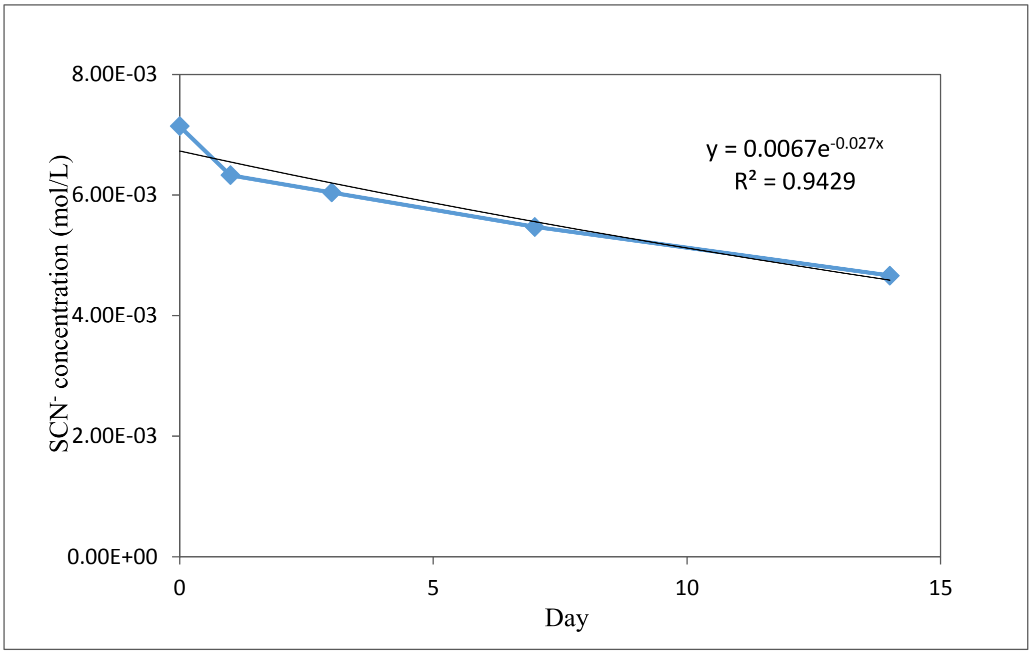

Figure 33: Determination of rate constant for hydrothermal experiment 6 set up at 200°C and ambient pH ($k = 0.027d^{-1}$)

Matlab was used to compute equations 6 and 7. These two best-fit equations describe the relationship between the rate of SCN^- break down, pH and temperature. Equation (6) was derived from the rate constants of SCN^- hydrolysis at pH 2 and 6.8; at temperatures between 25°C – 250°C. Equation (7) describes a similar relationship, however it also includes the rate of the SCN^- hydrolysis at intermediate pH 2.65, 3.56 and 4.01.

$$k\,(d^{-1}) = -0.3430 + 0.001525T(K) - 0.04835pH \tag{6}$$

$$k\,(d^{-1}) = -0.214 + 0.000982T(K) - 0.0348pH \tag{7}$$

Both Tables 4.1 and 4.2 show experimentally-determined and mathematically-derived rate constants at various temperatures and pH for equations 6 and 7 respectively.

As expected, the rate constants were comparatively higher for SCN^- dissociation at low pH and high temperatures. The rate constants calculated from the best-fit equations are somewhat close to what was determined experimentally. However, a closer look at Table 4.2 shows that at 200°C and 150°C, the fitted log k was significantly higher than experimental log k for pH 2.65, 3.56 and 4.01 reactions. This difference can be attributed to pH drifting effects. An increase in the pH of the solution during the course of the experiment is reflected in the relatively lower experimental log k. Due to the experimental setup, the pH of the solution could not be kept constant as the experiment progressed. It was observed upon measurement that the pH of the run product was higher than the pH of the starting soluton. Only a few of such measurements were done for this study. Therefore, the starting pH was used to derive equations 6 and 7 instead of the final pH of the solution and this resulted in an elevated log k; especially with equation 7.

Table XXVIII: Experimentally-determined and mathematically-fitted rate constants for SCN^- hydrolysis at pH 2.0 *and* 6.8

pH	Temperature (°C)	Temperature (K)	Experimentally determined rate constant (k) d^{-1}	log k	Fitted log k (Equation 6)
6.8	250	523.15	0.119	-0.92	-0.90
6.8	200	473.15	0.027	-1.57	-1.30
2.0	200	473.15	0.248	-0.61	-0.55
2.0	150	423.15	0.254	-0.60	-0.69
2.0	100	373.15	0.171	-0.77	-0.89
2.0	60	333.15	0.024	-1.62	-1.17
2.0	25	298.15	0.002	-2.70	-1.82

Table XXIX: Experimentally-determined and mathematically-fitted rate constants for SCN^- hydrolysis at pH between 2.0 and 6.8

pH	Temperature (°C)	Temperature (K)	Experimentally determined rate constant (k) d^{-1}	log k	Fitted log k (Equation 7)
6.80	250	523.15	0.119	-0.92	-1.20
6.80	200	473.15	0.027	-1.57	-1.85
2.00	200	473.15	0.248	-0.61	-0.74
2.65	200	473.15	0.129	-0.89	-0.80
3.56	200	473.15	0.073	-1.14	-0.90
4.01	200	473.15	0.037	-1.43	-0.95
2.00	150	423.15	0.254	-0.60	-0.88
2.65	150	423.15	0.030	-1.52	-0.96
3.56	150	423.15	0.006	-2.22	-1.11
4.01	150	423.15	0.013	-1.89	-1.21
2.00	100	373.15	0.171	-0.77	-1.08
2.00	60	333.15	0.024	-1.62	-1.36
2.00	25	298.15	0.002	-2.70	-2.04

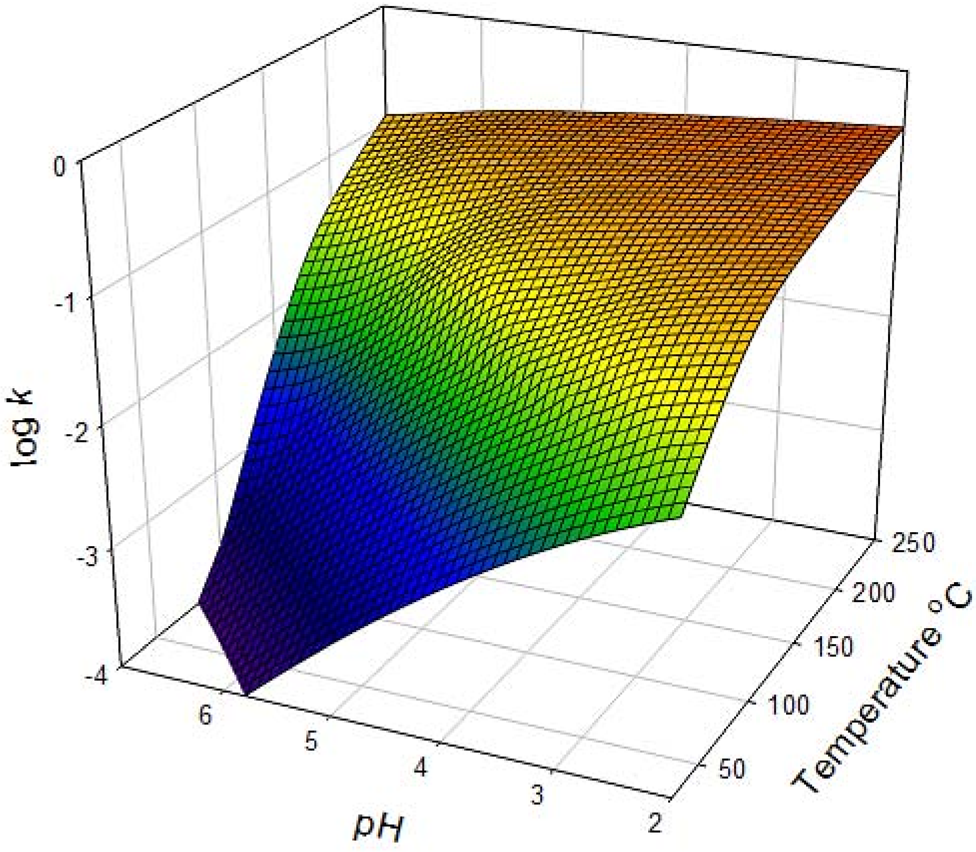

Figure 34: 3-D plot of log k for SCN$^-$ breakdown as a function of pH and temperature. Sigmaplot (v.11)

The activation energies (Ea) for experiments performed at ambient pH (71.1 kJ/mol) and pH 2 (54.9 kJ/mol) were calculated from the slope of the plot of log k versus 1/T (K^{-1}) for these experiments as shown in Figures 35 and 36.

$$\text{Slope} = -\frac{\text{Ea}}{2.303\text{R}}; \quad \text{R} = 8.314\text{E-03kJmol}^{-1}\text{K}^{-1} \qquad (8)$$

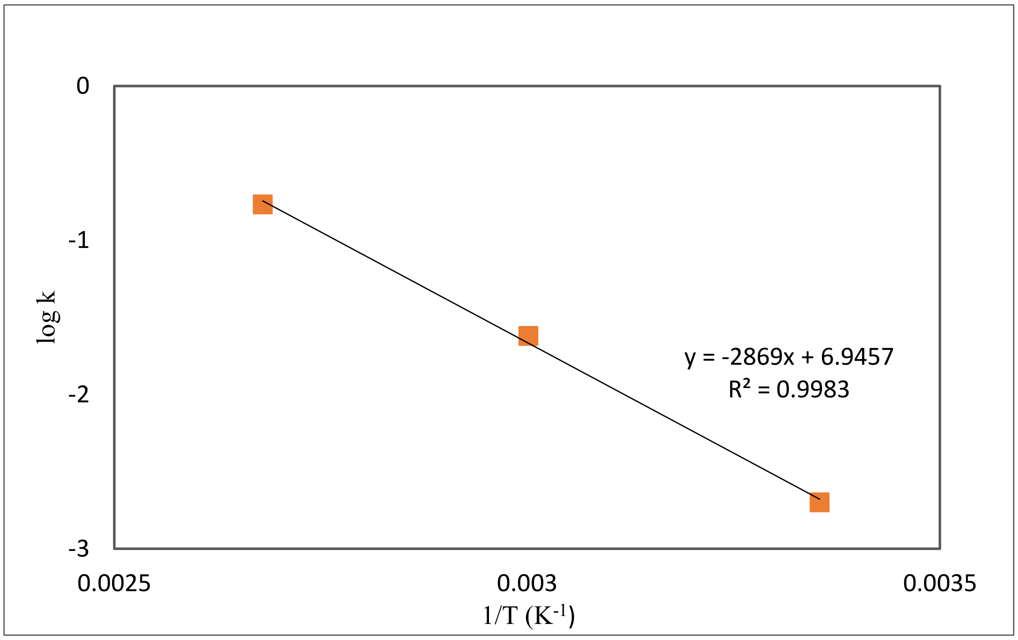

Figure 35: Plot of log k versus 1/T to determine the activation energy of SCN^- dissociation at pH 2 using data obtained from experiments performed at 25ºC, 60ºC and 100ºC

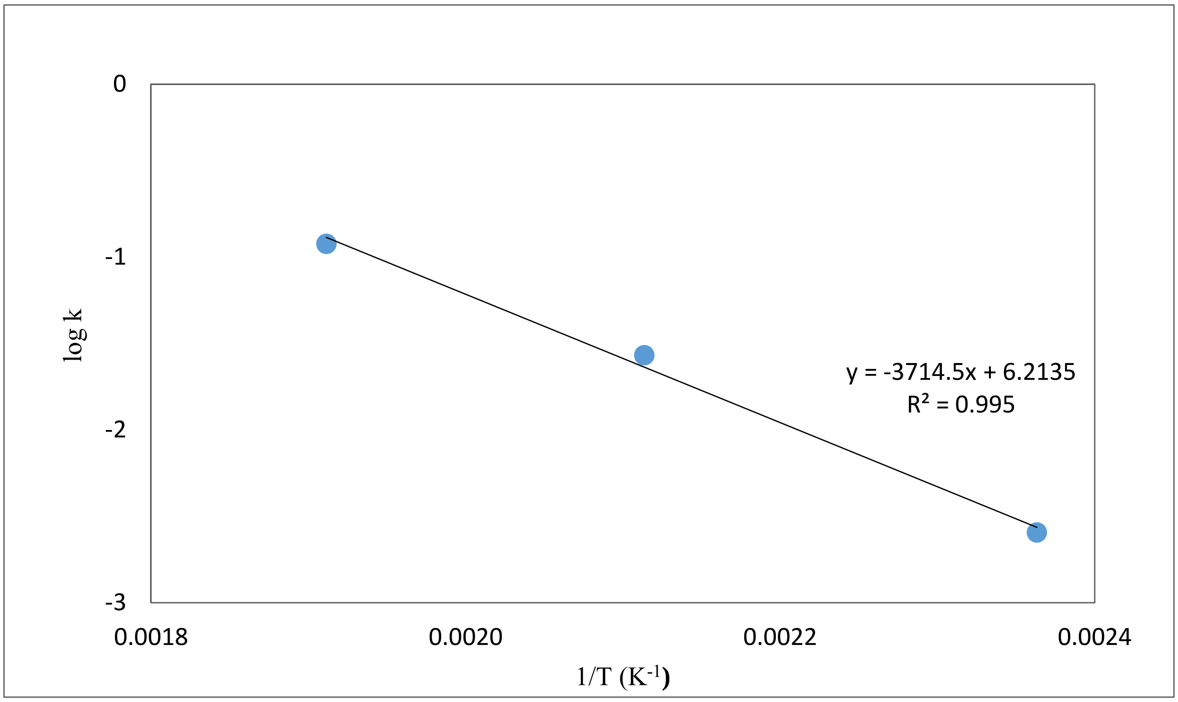

Figure 36: Plot of log k versus 1/T to determine the activation energy of SCN^- dissociation at ambient pH using data obtained from experiments performed at 150ºC, 200ºC and 250ºC

4.2. Comparison to Crowell Hankins (1968)

Crowell and Hankins (1968) conducted a kinetics study to observe the dependence of the rate of thiocyanic acid (HNCS) hydrolysis on acidity function (Daniel, 2010) at temperatures between 13 – 90°C. The pH of the starting solution (potassium thiocyanate in 0.005 – 11.0 M hydrochloric acid, phosphoric acid and sulfuric acids) for this study was below zero. The pK_A of thiocyanic acid was estimated from this study to be between -2.3 to -2.0 at 25°C depending on the ionic strength of the solution as well as the strength of the acid used. In order to determine the rate constants of thiocyanic acid hydrolysis at different temperatures and pH, the volume of carbon dioxide evolved was measured over mercury in a gas buret surrounded by 25°C water jacket and the amount of ammonia produced by the reaction was measured by titration with an acid. This approach is different from how the rate constants where determined in the present study which involved measuring the decrease in SCN^- concentration over time.

It is important to compare some of the results obtained by Crowell and Hankins (1968) to the results of the present study even though HNCS was used in the referenced study instead of SCN^- and the methods of rate determination were different. In the referenced investigation, when the acidity function of the starting solution was -0.52 and -0.99, the rate constants of HNCS hydrolysis at 25°C were determined to be approximately 0.0042 d^{-1} and 0.017 d^{-1} respectively. The rate constant for the hydrolysis of SCN^- determined from the present study at pH 2 and 25°C is 0.002 d^{-1}. An activation energy of 75kJ/mol was obtained for temperatures between 13 – 40°C for thiocyanic acid hydrolysis at acidity function close to -3.86. The activation energy for HSCN hydrolysis obtained by Crowell and Hankins (75 kJ/mol) is similar in magnitude to the activation energy obtained in the present study at ambient pH (71 kJ/mol), but higher than the value of 32.7 kJ/mol obtained in this study at pH 2 and temperatures between 25 – 200°C. The latter Ea estimate has a high uncertainty due to the fact that the rate of SCN- breakdown was so fast at pH

2 and T ≥ 150°C. Overall, the similarity in Ea estimates in this study and in the work of Crowell and Hankins suggests that the mechanism of hydrolysis is similar for HSCN at pH < 0 compared to SCN^- at pH ≥ 2. From Crowell and Hankins' study, the hydrolysis of HSCN proceeds according to a reaction similar to what was observed in the present study (Compare equation 7 and equation 9). The combined results from both studies can provide an insightful approach to accurately understand the complex behavior of thiocyanate; especially in low pH environments.

$$\mathrm{HNCS} + \mathrm{H^+} + 2\mathrm{H_2O} \leftrightarrow \mathrm{H_2S} + \mathrm{NH_4^+} + \mathrm{CO_2} \tag{9}$$

4.3. Supporting evidence for microbially catalysed anSCNox reaction

Similar to the anammox reaction, the anaerobic oxidation of SCN^- (anSCNox) to yield nitrogen gas (reactions 2, 3 of the Introduction) may have occurred at ambient temperature and pH during this study. In the presence of unsterilized gravel from the substrate of a decommissioned tailings impoundment that used cyanide, approximately 35% and 42% of SCN^- was lost after 31 days when the SCN^- was in solution with nitrate and nitrite respectively (see section 3.14). Only about 10% of SCN^- was lost after 31 days in the absence of nitrate and nitrite. It is believed that any microbes capable of breaking down SCN^- in association with nitrate/nitrite under ambient temperature and pH would have been present in the mine-gravel substrates and that the presence of nitrate and nitrite sped up the reaction. Small amounts of NH_4^+ were detected but H_2S was below detection, suggesting that SCN^- did not break down via simple hydrolysis. Furthermore, a bulge in the plastic bottles after a few days suggests that a gas evolved in the sealed containers. It is possible that this gas was a mixture of CO_2 and N_2, both being products that are predicted to accumulate as the anSCNox reaction proceeds (see reactions 2, 3). These discoveries are exciting and promising but were made late in the study.

Unfortunately, the results for these experiments are inconclusive as the final solutions were not analysed for anions, i.e., sulfate, nitrate, or nitrite.

When the low temperature gravel experiments were repeated at pH 10.93-10.95, only a 10 to 15% decrease in SCN^- concentration was observed in the presence of nitrate or nitrite. This suggests a possible pH dependence to the microbially catalysed decomposition of SCN^-.

Due to lack of time, no attempt was made in this thesis to repeat the gravel experiments at low pH. This could be an area to explore in the future.

5. Conclusions and Recommendations

The following are some of the important findings of this study:

- Contrary to the early hypothesis, SCN^- does not react with nitrate or nitrite to yield nitrogen gas under abiotic, anoxic conditions even at high temperatures. If this reaction is possible, it must be microbially catalyzed like the anammox reaction. At temperatures below 150°C and ambient/high pH, SCN^- does not break down to form nitrogen gas in the presence or absence of nitrate or nitrite. However, SCN^- will dissociate rapidly to hydrogen sulfide, ammonia and carbon dioxide at temperatures above 150°C and low pH; at pH 2 all of the intitial SCN^- had broken down at 150°C.
- Under anoxic conditions and ambient or high pH, SCN^- can withstand temperatures of up to 150°C. This finding can be useful in mining operations where SCN^- is used as lixiviant for extraction of gold or silver from their ores. Without breaking down SCN^-, the high temperature can serve to increase the rate at which the SCN^- - gold complexes form.
- The results obtained from the later part of this study show the possibility of biotic, anaerobic degradation of SCN^- at ambient temperature and pH to form nitrogen gas by a reaction that may be more complex than hydrolysis. This aspect of the study was not fully explored and could serve as the basis of future work.

The following are some recommendations for future work:

- Include identification and characterization of the microbes on the gravel substrate thought to be responsible for anSCNox in the scope of future work.
- Certain microbes may not have a high tolerance for 1% methanol added to the gravel substrate solutions of the low temperature experiments. Therefore, subsequent

experiments that explore the microbially-catalyzed anSCNox reaction should use ≤ 0.1% methanol or an alternative “food” such as sugar or methane.

- The gravel substrate may have the right bacteria to mediate anSCNox, however it may also be relevant to use the ground water obtained from the tailings impoundment for low-temperature experiments.
- It is equally important to know which nitrogen and sulfur species are formed at ambient temperature and pH and at what concentrations. Therefore, the concentrations of sulfate, nitrate and nitrite (if possible, nitrogen gas) formed during the course of future experiments set up at low temperatures should be measured accurately.
- The pH drifting effect observed in the hydrothermal experiments could be eliminated or minimized with an automated pH system that can serve to maintain the pH of the solution as the experiment progresses. However, this would require an entirely different hydrothermal experiment setup from what was used in this study.

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Appendix A: Hydrothermal Experiment Matrix

Experiment	Initial composition	Initial pH	Temperature (°C)	Duration (days)
1	Solution 1, 2 and 3	6.50 – 7.00	150	7
2	Solution 1, 2 and 3	6.50 – 7.00	150	7
3	Solution 1, 2 and 3	10.20, 10.07, 10.52	150	7
4	Solution 1, 2 and 3	6.50 – 7.00	250	7
5	Solution 1, 2 and 3*	6.50 – 7.00	250	7
6	Solution 1	6.50 – 7.00	200	14
7	Solution 1	6.50 – 7.00	150	14
8	Solution 1	2.0, 2.65, 3.56, 4.01	150	7
9	Solution 1	2.0, 2.65, 3.56, 4.01	200	7
10	Solution 1	2.00	100	7
11	Solution 1	2.00	60	7
12 & 13	Solution 1*	2.00	300	1

Solution 1 - 100mg/L NaSCN as N

Solution 2 -100 mg/L NaSCN as N + 100mg/L $NaNO_3$ as N

Solution 3 - 100 mg/L NaSCN as N + 100mg/L $NaNO_2$ as N

*10 times dilution of solutions 1, 2 and 3

Appendix B: Low Temperature Experiment Matrix

Experiment	Initial composition	Initial pH	Temperature (ºC)	Duration (days)
1	Solution 1(100mg/L NaSCN as N)	2.0	room temperature	7
2	Solution 1 + 1% Methanol + gravel substrate obtained from mine tailings Solution 2 + 1% Methanol + gravel substrate obtained from mine tailings Solution 3 + 1% Methanol + gravel substrate obtained from mine tailings	6.1 – 6.7		31
3	Solution 1 + 1% Methanol + gravel substrate obtained from mine tailings Solution 2 + 1% Methanol + gravel substrate obtained from mine tailings Solution 3 + 1% Methanol + gravel substrate obtained from mine tailings	10.93 - 10.95		49
Solution 1 - 100mg/L NaSCN as N				
Solution 2 -100 mg/L NaSCN as N + 100mg/L $NaNO_3$ as N				
Solution 3 - 100 mg/L NaSCN as N + 100mg/L $NaNO_2$ as N				

SIGNATURE PAGE

This is to certify that the thesis prepared by **Alero Jennifer Gure** entitled "**Anaerobic Dissociation of Thiocyanate under Controlled pH and Temperature**" has been examined and approved for acceptance by the Department of Chemistry and Geochemistry, Montana Tech of The University of Montana, on this 15th day of April, 2016.

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