Bioremediation of Complex Cyanide Contaminated Wastewater using *Pseudomonas Fluorescens* Pf-5

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Abstract

The biodegradation of some complex cyanide compounds, including sodium hexacyanoferrate (II) trihydrate ($Na_4Fe(CN)_6.3H_2O$), potassium hexacyanoferrate (II) decahydrate ($K_4Fe(CN)_6.10H_2O$), and potassium cyano-argentate ($KAg(CN)_2$) by free cells of Pseudomonas fluorescens Pf-5 (ATCC# BAA-477), was investigated in an aqueous suspension as a function of initial pH and glucose concentration. The minimum inhibition concentration (MIC) for each cyanide compound was determined, while bacterial growth, ammonia generation, and glucose utilization were monitored during the experimental tests. The results indicated that P. fluorescens Pf-5 is able to degrade both potassium and sodium ferrocyanides by using these cyanide compounds as sources of nitrogen and glucose as a source of carbon in M9 medium. However, potassium cyanoargenate ($KAg(CN)_2$) was observed to inhibit the growth of the same bacteria at all concentrations studied. The MIC values were determined to be 50 mM (21.1g/L) and 75 mM (36.3g/L) for $K_4Fe(CN)_6$ and $Na_4Fe(CN)_6$, respectively, while the maximum ammonia generated during cyanide biodegradation was observed at pH 7. As expected, bacteria growth increased with glucose concentrations.

1. Introduction

Cyanide compounds are used in different industries including gold and silver mining, steel and coal processing, synthetic fiber production, electroplating industries, etc. [1,2]. These industries discharge large quantities of wastewater containing cyanides. Most cyanide compounds are known to be highly toxic to human health and extremely harmful to the environment. In the presence of metal ions, such as nickel, silver, copper, zinc, and iron, cyanide forms complex compounds of varying toxicity and stability [2,3]. Industrial wastewater containing cyanide must be treated to reduce its concentration below 1 mg/L before discharging into the environment [2,4].

Cyanide contaminated wastewater are generally treated by physical and chemical methods such as natural degradation, alkaline chlorination process, hydrogen peroxide process, sulfur dioxide process, etc. [5,6]. However, these chemical-based processes are not always effective for total cyanide removal, and tend not to be cost effective when compared to the biological treatment methods for cyanide removal. Biological methods have now been proven to have the potential to effectively remove a wide range of cyanide compounds and produce high quality effluents [6-8]. In addition, biological methods show faster degradation kinetics compared to chemical and physical methods [9].

Biological treatment of cyanide has been shown to be a viable process for destroying cyanide in mining process waters due to its simplicity and ability to accommodate both large flows and wastewaters with elevated cyanide concentrations that are generated at commercial precious metals operations. The biological removal of cyanide has been shown to occur in two steps: the first step is the oxidative breakdown of

cyanide followed by a subsequent conversion into ammonia, carbonate, and sulfate [10]. In the second step, ammonia is converted to nitrite and nitrate through the conventional two step nitrification process [7,11].

The most commonly isolated microorganisms used for biodegradation of cyanide complex ions are *Pseudomonas* species. Metal cyanide complex ions can be used as a source of nitrogen for the growth of the microorganism [5], thus leading to the destruction of the CN^- ion. When bacteria use cyanide as nitrogen source, an external carbon source (usually glucose) may be added to the medium to allow appropriate bacterial cell growth. At high concentrations, cyanide compounds can generally interfere with bacterial growth rates and cause inhibition of bacterial growth. The maximum inhibition concentration (MIC) value is the concentration at which a chemical compound can completely inhibit bacterial growth. Other factors such as initial glucose concentration or initial pH of the medium can also influence bacterial growth or cyanide removal activities. Higher initial glucose concentrations will generally have a positive effect on bacterial growth up to a certain optimum level. Above this concentration, the amount of initial glucose will not have a significant effect on bacteria growth [5,12].

Although several studies have been conducted on the bioremediation of simple cyanide compounds, there is limited knowledge of the bioremediation of metal cyanide complex compounds, such as those studied here, by *Pseudomonas* species .Specifically no studies have been previously reported on the biodegradation of complex cyanide compounds by using *Pseudomonas fluorescens* Pf-5. Therefore, the main objective of this study was to ascertain whether or not *P. fluorescens* Pf-5 is capable of degrading metal-cyanide complex compounds in aqueous systems. The secondary objectives were to examine the growth kinetics of the bacteria in the presence of complex cyanide compounds studied, determine the MIC values for three cyanide compounds, and evaluate the effects of pH and initial glucose concentration on the degradation of the cyanide compounds. Since the cyanide compounds used were the only sources of nitrogen in our experiments, and previous studies have demonstrated ammonia formation in solution as the main destruction pathway during cyanide biodegradation, we employed the formation of ammonia as an indication of the occurrence of cyanide biodegradation by *P. fluorescens* Pf-5.

The bacterial concentration was determined at the beginning and after 24 hours of growth. A control experiment was set up by using M9 medium with ammonium chloride (5.0 g/L) as a nitrogen source rather than the cyanide compounds. The bacterial growth in the control broth was also assessed and

2. Materials and methods

2.1 Culture of *Pseudomonas fluorescens* Pf-5 strain

The bacteria *Pseudomonas fluorescens* Pf-5 (ATCC #BAA-477), purchased from ATCC in Manassas, VA, USA was used in this study. The bacteria cells were grown in a tryptic soy broth at a pH of 7.1, in 250 mL conical flask, and were incubated at 30°C in a shaker incubator at 230 rpm for 24 hours. The bacteria were then harvested by centrifugation at 4000×g for 5 min and washed twice in phosphate buffer solution. The harvested cells were then transferred (ration 1:10) into the medium containing the cyanide compounds of 6.25 mM.

2.2 Preparation of cyanide stock solutions

Three metal-cyanide complex compounds, potassium hexacyanoferrate (II) trihydrate ($K_4Fe(CN)_6.3H_2O$), sodium hexacyanoferrate (II) decahydrate ($Na_4Fe(CN)_6.10H_2O$), and potassium dicyanoargentate ($KAg(CN)_2$), purchased from SIGMA-ALDRICH (California, USA) were used in this study. Stock solutions of the cyanide compounds were prepared at a concentration of 0.5 M and pH of 10 to avoid the release of HCN gas [2]. A predetermined quantity of cyanide stock solution was added to 50 mL of the M9 medium to get the final desired cyanide concentration of the batch tests.

2.3 MIC tests

MIC tests were performed to identify the minimum concentration of cyanide compounds that will inhibit the growth of the bacteria. In these tests, cyanide compounds and glucose were added as the only nitrogen source, and a carbon source to the M9 medium, respectively. M9 medium with different concentrations of cyanide compounds ranging from 1.56 mM to 75 mM were introduced into culture tubes with a volume of 2mL for each sample. About 50µL of resting cells suspended in PBS solution (optical density (OD) ≈1) were added to each sample (4,13,14) of the 2 mL of M9 medium containing the cyanide compounds. The solution was then placed in the shaker incubator at 30°C and 230 rpm for 24 hours.

compared to the growth in M9 medium containing cyanide compounds.

2.4 Growth in M9 medium using cyanide as a sole source of carbon and nitrogen

To test the ability of the bacterial strain to use cyanide compounds as both carbon and nitrogen source, bacterial strains were added to the M9 medium which contains cyanide compounds. At the end of the 24 hours test, the bacterial growth was monitored by measuring the absorbance of the sample at 600 nm using the spectrophotometer.

2.5 Biodegradation test using cyanide as a nitrogen source and glucose as a carbon source

For the biodegradation studies, bacteria were grown in M9 medium supplemented with 6.25 mM of cyanide compounds and a predetermined quantity of glucose. The pH of the test solution was adjusted to the desired initial value by using sterile dilute and concentrated sulphuric acid or sodium hydroxide solutions, but was not controlled during fermentation. Sterilization of the medium was performed in an autoclave at 121°C for 15 min. About 5 mL of suspended resting cells at an optical density (OD) value of 0.5 was inoculated in 50 mL of the sterile medium, and grown at 30°C and 230 rpm for 72 hours. Some tests were conducted to assess the effect of the initial pH of the biodegradation medium on the bio-removal of cyanide. The biodegradation medium was prepared at constant cyanide and glucose concentrations of 6.25mM and 4 g/L, respectively. The initial pH values investigated were 5 [5], 7 [4,14] and 9 [12,15]. Resting cells were then added to the medium, and placed in the shaker incubator at 30°C and 230 rpm for 72 hours. For each initial pH, glucose concentration, and ammonia generation were measured during the test, and the results were then compared.

The effects of the initial glucose concentration of the biodegradation medium were also investigated. The medium was prepared with different glucose concentrations of 2, 4, 6, 8 g/L. The initial cyanide concentration of the medium was kept constant at 6.25 mM. The initial pH used here was the optimum pH value obtained from the study of pH effects outlined above. Resting cells were then added to the medium, which was placed in the shaker incubator at 30°C and 230 rpm for 72 hours. For each initial glucose concentration, bacterial growth, glucose concentration, and ammonia concentration in solution were determined during the test and the results were then compared.

2.6 Analytical methods

Bacterial growth was assessed by measuring the OD value of the medium at 600 nm wavelength using a spectrophotometer. This optical density technique has been widely employed in other studies to monitor bacteria growth and concentration trends as in this study [4,15,16]. A medium without inoculated bacteria was used as a blank solution. Glucose concentration determination was conducted by colorimetric technique using the "phenol-sulfuric acid assay" method [17]. Ammonia nitrogen was measured according to the Method 10023 (Salicylate method for Nitrogen, Ammonia Low Range (0.02 to 2.50 mg/L NH_3-N)) provided by HACH company (Loveland, CO, USA). pH of the medium was measured by using a pH meter. All the experimental data reported here were taken from an average of 3 measured values.

3. Results and discussion

3.1 Growth curve

The primary growth curve in tryptic soy broth showed three distinct phases during 72 hours: a lag phase which occurred within the first 4 hours, a logarithmic growth phase between 5 hours and 40 hours, and the stationary phase after 40 hours as shown in Figure 1. The near-classic growth curve observed is indicative of the bacterial viability and activity in the medium used, and demonstrated the adequacy of the use of the OD method for measuring bacteria growth and content in this study. The bacteria were harvested during the logarithmic phase (i.e. after 24 hours) and used in the cyanide biodegradation tests [18].

3.2 MIC tests

Figure 2 shows the plot for bacteria growth in M9 medium supplemented with potassium ferrocyanide ($K_4Fe(CN)_6$) at various concentrations, and with an initial OD value of 0.029 in the medium. The results showed increases in OD values of the media after 24

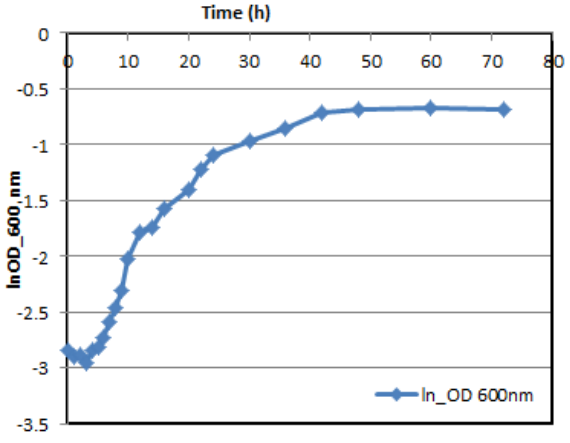


Figure 1. Growth of *P. fluorescens* Pf-5 in tryptic soy broth

hours for all cyanide concentrations except the one for 50 mM . The observed OD values were 0.075, 0.09 and 0.06 for $K_4Fe(CN)_6$ concentrations of 6.25 , 12.5, and 25 mM, respectively. These higher OD values for concentrations of up to 25 mM of $K_4Fe(CN)_6$ indicate that the bacteria is growing in the medium. For the sample containing 50mM of $K_4Fe(CN)_6$ a decrease in the observed OD value suggests an inhibiting effect of that cyanide concentration to the growth of the bacteria. Therefore, 50 mM (i.e. 21.1 g/L) is considered in this study as the MIC value for $K_4Fe(CN)_6$ in the growth of *P. fluorescens* Pf-5.

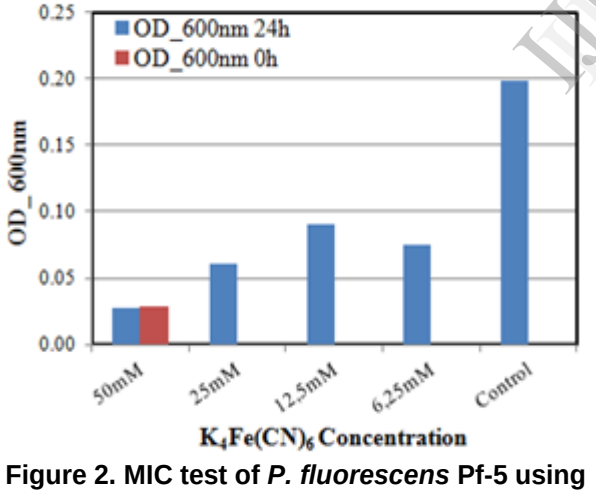


Figure 2. MIC test of *P. fluorescens* Pf-5 using $K_4Fe(CN)_6$ as a nitrogen source; red bar = initial bacteria content; blue bar = bacteria content after 24 hours

The growth of bacteria using M9 medium supplemented with sodium ferrocyanide ($Na_4Fe(CN)_6$) at different concentrations is shown in Figure 3. The initial OD value of the medium was 0.029. The observed OD values were 0.067, 0.09, 0.076 and 0.02 for concentrations of 12.5 mM, 25 mM, 50 mM and 75 mM of $Na_4Fe(CN)_6$, respectively after 24 hours. Since the OD at a $Na_4Fe(CN)_6$ concentration of 75 mM was 0.02, which was lower than the initial OD value of 0.029, we conclude that $Na_4Fe(CN)_6$ inhibited the bacterial growth at this higher concentrations. Therefore, the MIC value for $Na_4Fe(CN)_6$ was taken to be 75mM (i.e. 36.3 g/L) for the growth of *P. fluorescens* Pf-5.

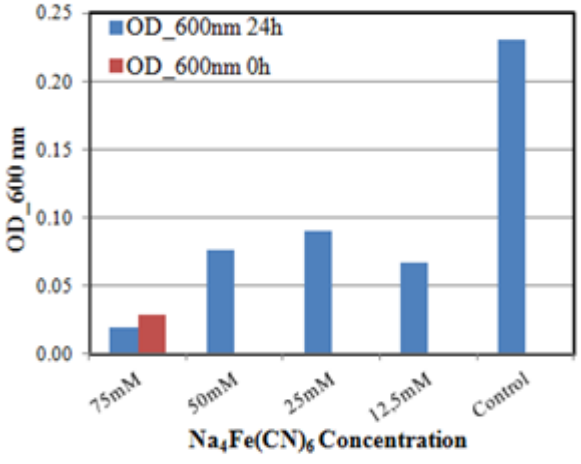


Figure 3. MIC test of *P. fluorescens* Pf-5 using $Na_4Fe(CN)_6$ as a nitrogen source; red bar = initial bacteria content; blue bar = bacteria content after 24 hours

Figure 4 shows that there was no growth of the bacteria for all concentrations of potassium cyanoargentate ($KAg(CN)_2$) studied. For each concentration of $KAg(CN)_2$ there was a decrease in the bacterial population after the 24 hours of the test compared to its initial bacterial population which suggests an inhibitory effect for $KAg(CN)_2$. This is probably due to the presence of silver in this compound. Silver is generally known as a bactericide and is used for disinfecting drinking water [19]. Therefore these results indicate that, bioremediation using *P. fluorescens* Pf-5 is not effective for the removal of the silver cyanide complexes.

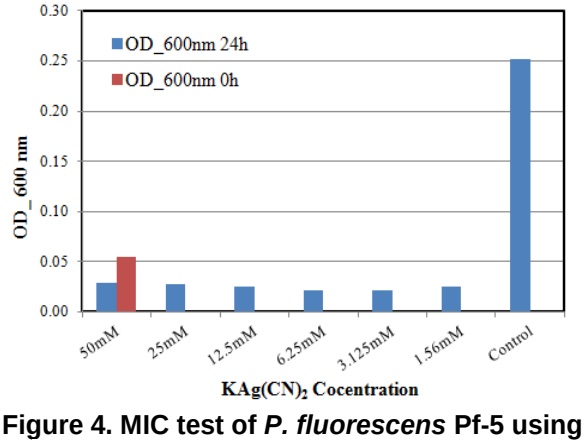


Figure 4. MIC test of *P. fluorescens* Pf-5 using $KAg(CN)_2$ as a nitrogen source; red bar =

initial bacteria content; blue bar = bacteria content after 24 hours

3.3 Biodegradation tests using cyanide as a sole source of carbon and nitrogen

The results of bacteria growth in the M9 medium supplemented with cyanide compounds as both carbon and nitrogen source are presented in Figure 5. After 24 hours, the OD values of the medium were 0.059, 0.062, and 0.038 for $K_4Fe(CN)_6$, $Na_4Fe(CN)_6$ and $KAg(CN)_2$, respectively which were equal or lower compared to the initial OD value of 0.061.Therefore, *P. fluorescence* Pf-5 was not able to grow in the M9 medium supplemented with $K_4Fe(CN)_6$, $Na_4Fe(CN)_6$, and $KAg(CN)_2$, because the bacteria was not able to use these cyanide compounds as both carbon and nitrogen sources. *P. fluorescens* P70 was also previously shown not to be able to use cyanide as a sole source of carbon and nitrogen for their growth [5]. The inhibitory or bactericidal effects of the silver cyanide compound is also clearly demonstrated in Figure 5 with a much lower bacteria concentration than the other two cyanide compounds after 24 hours. These results suggest that an external carbon source is needed to allow the growth of the *P. fluorescence* Pf-5.

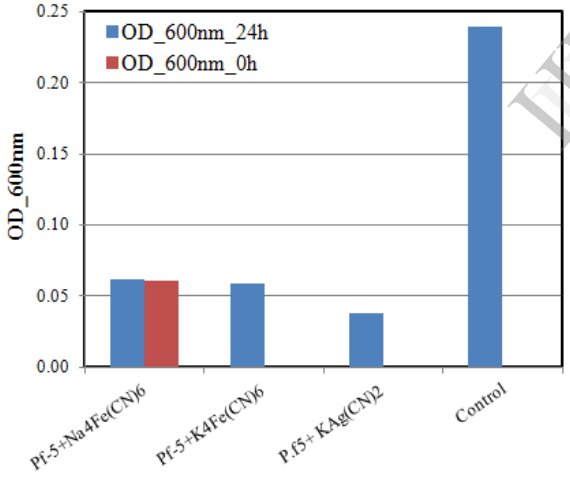


Figure 5. Growth of *P. fluorescens* Pf-5 using the different cyanide compounds as both carbon and nitrogen sources; red bar = initial bacteria content; blue bar = bacteria content after 24 hours

3.4 Biodegradation tests with external carbon source to observe the effect of initial pH

The plots of ammonia production as a function of initial pH are presented in Figures 6 and 7 for $K_4Fe(CN)_6$ and $Na_4Fe(CN)_6$, respectively. A concentration of 6.25mM for the cyanide compounds and glucose concentration of 4 g/L were used in these biodegradation tests. At pHs of 5 and 7 for the $K_4Fe(CN)_6$, ammonia presence in solution was first detected after 24 hours and 8 hours of fermentation, respectively . At a pH of 5, the ammonia concentration in solution increased slowly with time up to 0.44 mg/L at the end of the test. For the pH of 7, the ammonia concentration increased to 1.6 mg/L after 48 hours and after that, the concentrations remained stable. At a pH of 9, the ammonia was not detected during the entire experimental period except at the 24th hour when a transient ammonia concentration of 0.24 mg/L was observed. Since the only source of nitrogen in the broth was the cyanide compound, the formation of ammonia from cyanide nitrogen at pH of 5 and 7 clearly demonstrate that *P. fluorescens* Pf-5 is effectively degrading this complex metal-cyanide compound at these two pH values [4,10,11,20,21].

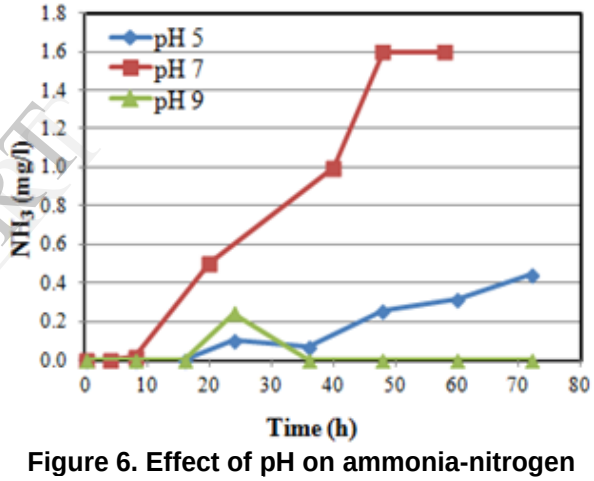


Figure 6. Effect of pH on ammonia-nitrogen generation using $K_4Fe(CN)_6$ as a nitrogen source

For the $Na_4Fe(CN)_6$ biodegradation tests shown in Figure 7, the trends for the ammonia production were similar to the $K_4Fe(CN)_6$ results, except for the actual concentrations. At a pH of 5, the ammonia was first detected after 16 hours of experimentation with 0.11 mg/L. The ammonia concentration increased with time up to 1.08 mg/L at the 48th hour, but remained constant after. At a pH of 7, the ammonia concentration was 0.2 mg/L at the 12th hour and increased to 1.3 mg/L after 20 hours. Then the concentration was relatively stable during the rest of

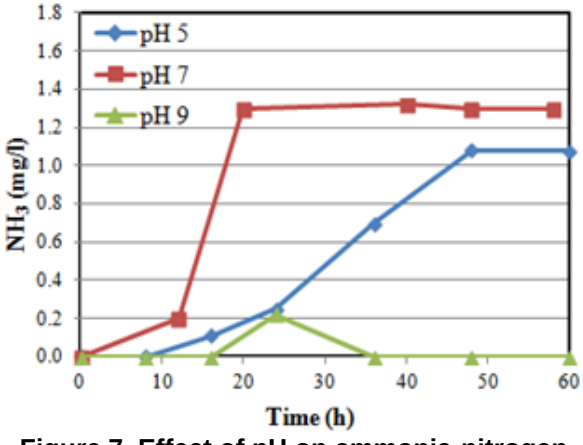
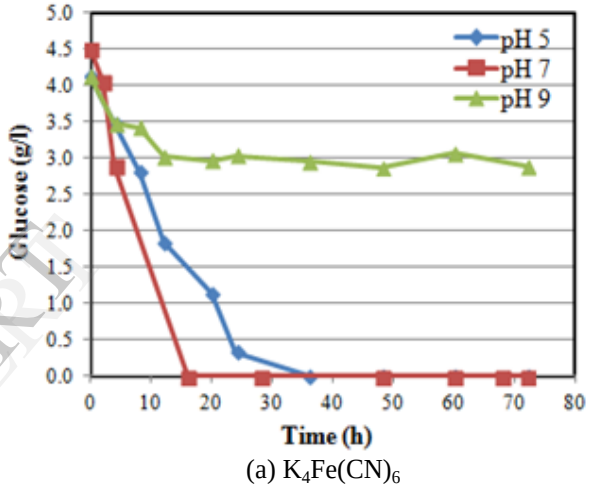


Figure 7. Effect of pH on ammonia-nitrogen generation using $Na_4Fe(CN)_6$ as a nitrogen source

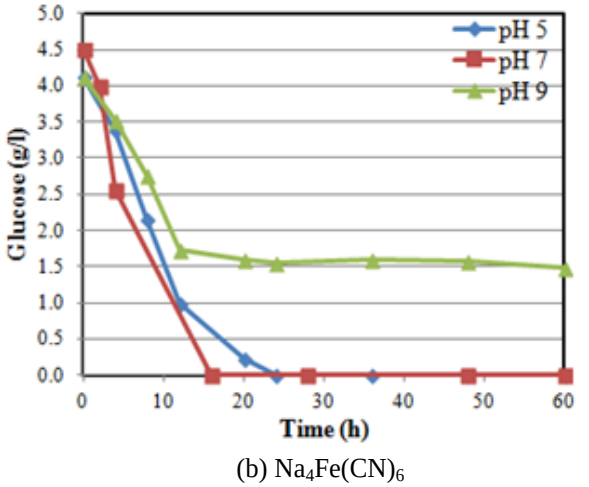
the test. At pH 9, the pattern or trend observed for $Na_4Fe(CN)_6$ is similar to the results observed for $K_4Fe(CN)_6$. Several other researchers have also reported the production of ammonia during the degradation of cyanide compounds in aqueous media [4,20-22]. The production of ammonia is also indicative of a reductive hydrogenation pathway for the CN^- biodegradation by the microbes.

For the two ferro-cyanide compounds studied, the maximum ammonia generated in solution (1.6 mg/L for $K_4Fe(CN)_6$, and 1.32mg/L for $Na_4Fe(CN)_6$) occurred at pH 7, thus suggesting an optimum bacterial activity for cyanide conversion at this pH. However, Dursun et al. [5] have reported a maximum ferrocyanide degradation rate at pH 5 when using a different strain of bacteria, *P. fluorescens* P70. They also observed that the bacterial activity was significantly reduced at pH 7 and was almost inhibited at pH 9. The *P. fluorescens* Pf-5 used in this study was very active at pH 7, but showed reduced activity at pH 5. A near total inhibition of the activity was observed at pH 9. Therefore, pH 7 was considered as the optimum value for the biodegradation of both cyanide compounds. Chen et al. [13] and Chen et al. [14] also reported maximum cyanide removal at pH 7 while using *Klebsiella oxytoca*. However, Huertas et al. [2] found effective cyanide removal at pH 9.5 using *P. pseudoalcaligenes* strain CECT5344.

Figures 8a and b present the results of glucose utilization at the three pH values studied with the two ferrocyanide compounds at initial cyanide concentrations of 6.25mM. For the $K_4Fe(CN)_6$, the glucose utilization was completed at both pH values of 5 and 7 in 36 hours and 16 hours, respectively (Figure 8a). However, at pH 9, the glucose utilization rate was much lower as shown in Figures 8a and b. The glucose concentration at this pH was reduced from an initial value of 4.12 to 3.03 g/L in 12 hours, and then, it was relatively constant until the end of the test. The observed trends for glucose utilization during the biodegradation of $Na_4Fe(CN)_6$ (Figure 8b) were similar to the trends for the $K_4Fe(CN)_6$ of Figure 8a except the actual concentrations. These glucose utilization results support the enhanced production of ammonia at pH values of 5 and 7 discussed earlier, and also in terms of the higher viability of the microbes at these pH values. In line with the ammonia generation results at pH 7, glucose utilization rate was observed to be slightly faster at pH 7 than at pH 5. At pH 9, the glucose utilization was significantly reduced which explains the non-generation of ammonia during the test. Some amounts of glucose were used, thus suggesting that the bacterial activity was not completely inhibited at pH 9.



(a) $K_4Fe(CN)_6$

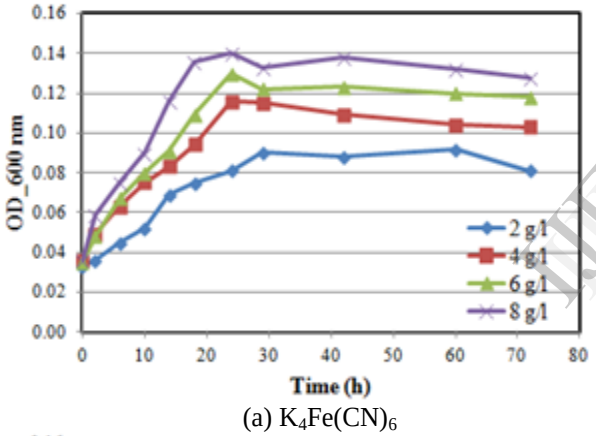


(b) $Na_4Fe(CN)_6$

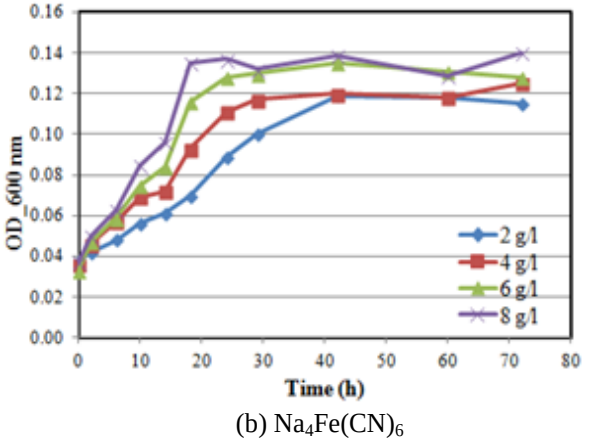
Figure 8. Effect of pH on glucose utilization with; (a) $K_4Fe(CN)_6$ and (b) $Na_4Fe(CN)_6$ as a nitrogen source

3.5 Biodegradation tests with external carbon source to observe the effect of initial glucose concentrations

The results of the effects of initial glucose concentration on bacteria growth, glucose utilization, and ammonia-nitrogen production are presented in Figures 9, 10 and 11, respectively. The biodegradation medium was prepared at a cyanide concentration of 6.25 mM and pH of 7. The bacteria growth pattern is similar for both the $K_4Fe(CN)_6$ (Figure 9a) and $Na_4Fe(CN)_6$ compounds (Figure 9b) at all glucose concentrations except the actual concentrations. At 2g/L of initial glucose concentration, the OD value increased from 0.033 to 0.09 in 29 hours for the $K_4Fe(CN)_6$ while the OD value increased from 0.038 to 0.119 in 42 hours for the $Na_4Fe(CN)_6$, and then remained relatively stable until the end of the test. At 4 g/L, 6 g/L and 8 g/L of initial glucose concentrations, the OD values increased up to the 24th hour, and remained constant after that for the both $K_4Fe(CN)_6$ and $Na_4Fe(CN)_6$. The OD values generally increased



(a) $K_4Fe(CN)_6$



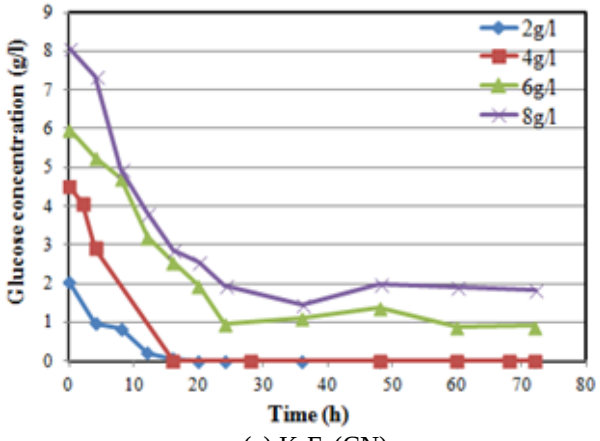
(b) $Na_4Fe(CN)_6$

Figure 9. Effect of initial glucose concentration on the growth of bacteria at pH of 7.0 and cyanide concentration of 6.25 mM with; (a) $K_4Fe(CN)_6$ and (b) $Na_4Fe(CN)_6$ as a nitrogen source.

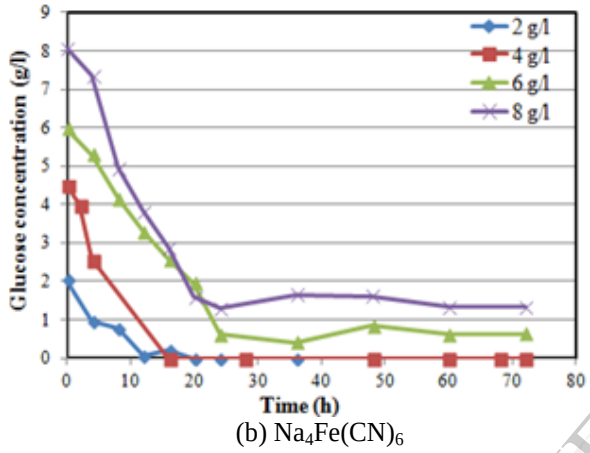
when the initial glucose concentration was increased. The maximum observed OD value, which indicates the maximum bacteria concentration, was about 0.14 after 24 hours of fermentation at 8g/L of initial glucose concentration for the two cyanide compounds used. These results clearly indicate increasing bacteria growth rate with increasing glucose concentration in the log growth phase.

Initial glucose concentration had a significant effect on glucose utilization by *P. fluorescens* Pf-5 using both $K_4Fe(CN)_6$ (Figure 10a) and $Na_4Fe(CN)_6$ (Figure 10b). The glucose utilization pattern was similar for the both $K_4Fe(CN)_6$ and $Na_4Fe(CN)_6$ in the sense that a complete glucose utilization was observed in 16 hours at 2g/L and 4g/L of initial glucose concentrations. At 6g/L and 8 g/L of initial glucose concentrations, a rapid glucose concentration drop was observed for the first 24 hours, after which the concentration remained relatively constant for the rest of the experimental period. However, the concentration at which glucose utilization levels off is higher as the initial glucose concentration increases. For 6 g/L and 8 g/L of initial glucose concentrations, the glucose concentrations rapidly decreased to 0.94 g/L and 2 g/L, respectively for $K_4Fe(CN)_6$ (Figure 10a) while the glucose concentrations reduced to 0.6 g/L and 1.28 g/L, respectively for $Na_4Fe(CN)_6$ (Figure 10b). For both ferrocyanides, glucose utilization pattern explains the bacteria growth in the medium as shown in Figures 9a and b. The bacteria growth stopped with the complete utilization of glucose at 2 g/L and 4 g/L of initial glucose concentrations. For 6 g/L and 8 g/L of initial glucose concentration, the bacteria did not utilize the glucose completely and stopped growing when glucose utilization was stopped.

The results of the effects of initial glucose concentration on ammonia generation is presented in Figures 11a and b. The figure clearly indicate that for both cyanide compounds studied, ammonia production begins after about 12 to 24 hours, which corresponds with the period at which glucose utilization becomes insignificant. The production of ammonia is clearly indicative of the beginning of the biodegradation of the cyanide compounds. Ammonia formation was observed to increase rapidly in both Figures 11a and b after 16 to 24 hours, rising in solution to a maximum concentration after 48 to 60 hours, and remained constant untill the end of the experiment except for the $Na_4Fe(CN)_6$ at a 4 g/L of initial glucose concentration.



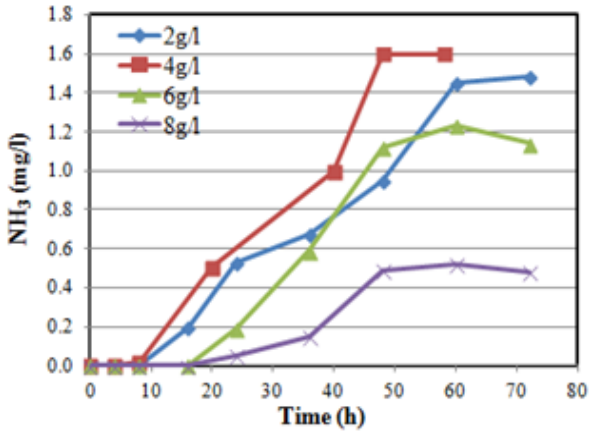
(a) $K_4Fe(CN)_6$



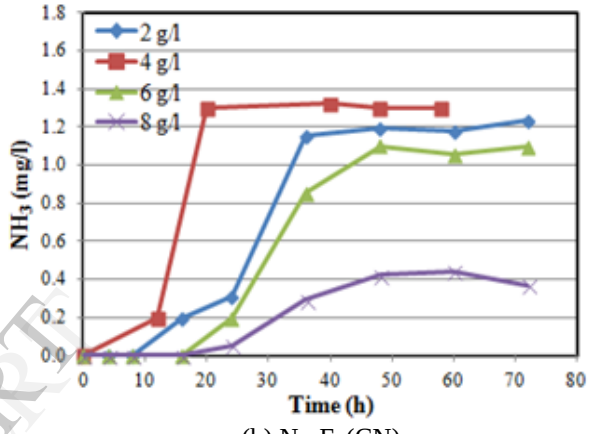
(b) $Na_4Fe(CN)_6$

Figure 10. Effect of initial glucose concentration on the glucose utilization at pH of 7.0 and cyanide concentration of 6.25 mM with; (a) $K_4Fe(CN)_6$ and (b) $Na_4Fe(CN)_6$ as a nitrogen sources.

For both ferrocyanide compounds, the maximum ammonia concentration was detected at 4 g/L of initial glucose concentration (i.e. 1.6 mg/L for $K_4Fe(CN)_6$ and 1.3 mg/L for $Na_4Fe(CN)_6$). The ammonia concentrations decreased with the increase of initial glucose concentrations except for 4 g/L, and it might be due to the increase of bacteria population in the medium as shown in Figures 9a and b. The bacteria might have sufficient cyanide as a nitrogen source up to 4 g/L of initial glucose concentration resulting in an increase of the ammonia generation. However, above 4 g/L of initial glucose concentration, as the bacteria population increased in the medium, they utilized the ammonia generated by the biodegradation of cyanide as a supplemental nitrogen source. The results of this study also indicated that *P. fluorescens* Pf-5 might degrade $K_4Fe(CN)_6$ better than that of $Na_4Fe(CN)_6$ due to the higher generation of ammonia for $K_4Fe(CN)_6$.



(a) $K_4Fe(CN)_6$



(b) $Na_4Fe(CN)_6$

Figure 11. Effect of initial glucose concentration on ammonia-nitrogen generation at pH of 7.0 and cyanide concentration of 6.25 mM with; (a) $K_4Fe(CN)_6$ and (b) $Na_4Fe(CN)_6$ as a nitrogen source.

4. Conclusions

In conclusion, the results of this study has demonstrated that P. fluorescens Pf-5 was not able to use the complex $K_4Fe(CN)_6$ and $Na_4Fe(CN)_6$ as both carbon and nitrogen sources in M9 medium when the initial complex cyanide concentrations are less than their MIC values. However, P. fluorescens Pf-5 can degrade the compounds as a nitrogen source in M9 medium when glucose was added as a supplemental carbon source. $KAg(CN)_2$ inhibited the growth of the bacteria at all concentrations studied, and this is attributed to the presence of silver, which is a bactericide, in the compound. The MIC values were 50 mM (21.1 g/L) and 75 mM (36.3 g/L) for $K_4Fe(CN)_6$ and $Na_4Fe(CN)_6$, respectively . In the biodegradation of $K_4Fe(CN)_6$ and $Na_4Fe(CN)_6$, an initial pH of 7.0 was considered as an optimum value because the maximum ammonia production was observed during *P. fluorescens* Pf-5 degradation of the

cyanide compounds studied. The growth rate of bacteria was higher when initial glucose concentration was increased. However, the ammonia concentration decreased with the increase of initial glucose concentration which might be due to the effect of higher bacteria population present in the medium at higher initial glucose concentration. The results of this study also indicated that $K_4Fe(CN)_6$ might be degraded better compared to $Na_4Fe(CN)_6$ when P. fluorescens Pf-5 is employed. The findings of this study provide additional valuable information for the application of bioremediation for cyanide contaminated wastewater treatment.

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