Pharmacophore

ISSN-2229-5402

Journal home page: http://www.pharmacophorejournal.com

Pharmacophore
an International Journal
ISSN 2229-5402

EVALUATION OF BIOLOGICAL REMOVAL OF CYANIDE FROM WASTE OF GOLD REFUGE COMPLEX

Seyed Mansour Meybodi[1]*, Masood Mobini[2], Maryam Asghar Heydari[3]

1. *Assistant professor in Microbiology, Department of Biology, Islamic Azad University, Tonekabon, Iran.*
2. *Master of Young Researchers and Elites Club, University of Tonkabon, Iran.*
3. *Master of Young Researchers and Elites Club, University of Tonkabon, Iran.*

ARTICLE INFO

Received:
03th Jun 2017
Accepted:
29th Nov 2017
Available online:
14th Dec 2017

Keywords: *Cyanide, Biodegradation, Mute, Tailings Dam*

ABSTRACT

Background and Aim: Cyanide is a highly toxic compound used in many industries. There are several approaches to remove cyanide from the waste. Physical and chemical processes have been successfully used to purify the cyanide-forming elements. Today, cyanide biochemical analysis is considered as a cost-effective and environmentally compatible method compared to conventional chemical methods. The purpose of this study was to evaluate the cyanide removal from wastewater from Muteh golden complex using native bacteria.
Methods: After collecting soil samples, enrichment of cyanide degrading bacteria was carried out in a culture medium containing potassium cyanide. The ability of isolated bacteria was investigated using cyanide as the only source of carbon and nitrogen at different pH levels. The cyanide decomposition and production of ammonia and nitrate in the culture medium were measured. Finally, the genetic identification of the superior strain for bio-degradation was carried out.
Results: Seven strains were isolated from which 10 strains were able to withstand cyanide to a concentration of 1000 mg/L and use it as the sole source of carbon and nitrogen. The results showed that reduction of cyanide concentration was directly related to increasing the concentration of ammonia in growth medium and growth of isolates. After 72 hours, the cyanide content of the media in Mgt16 decreased from 1000 mg/l to 100 mg / l (90% removal efficiency). The highest efficiency was obtained at pH=10. The 16srDNA analysis indicated that the strain indicated the results of the strain.
Conclusion: The results of this study showed that bacteria isolated from Mutee wastewaters are an appropriate alternative for bioassay cyanide in alkaline conditions and can be used to decompose cyanide from industrial wastewater and contaminated sites.

Copyright © 2013 - All Rights Reserved - Pharmacophore

To Cite This Article: Seyed Mansour Meybodi, Masood Mobini, Maryam Asghar Heydari, (2017), "evaluation of biological removal of cyanide from waste of gold refuge complex", ***Pharmacophore, 8(6S),*** *e-1173322.*

Introduction

cyanidation method due to availability, low cost and high efficiency in the extraction of metals, is used in the extraction of precious metals such as gold, silver, copper, nickel, cobalt and molybdenum, lead and zinc ore (1). Followed by the use of cyanide compounds in the mining industry, a large amount of cyanide tailings entered to the dams. Concerns about environmental impacts and of cyanide poisoning have caused extensive studies to be performed on this matter and its removal methods (2). Physical adsorption and chemical oxidation are the most commonly used methods for the removal of cyanide. However, these methods are not most appropriate, because they have several disadvantages including cost, time-consuming, lack of efficiency on soil, replacement of cyanide with other toxic compounds and environmental contamination (3). Cyanide biodegradation, is an economical and cost effective technology to remove cyanide from waste water and mine tailing dam. Microorganisms are capable of oxidizing free cyanide to ammonia and carbon dioxide forms (4). Cyanide and its compounds are decomposed in the environment, by several types of bacteria, fungi, algae, plants and insects by different mechanisms (5). In Iran, little research has been done in this field. The aim of this study is to find native and efficient strains for biological

Corresponding Author: Seyed Mansour Meybodi, Master of Young Researchers and Elites Club, University of Tonkabon, Iran.
Email: Meybodi.SM.iau@gmail.com

oxidation of cyanide from gold tailings dam of mouteh gold complex, and optimize the use of these microorganisms in a laboratory system to achieve the technical knowledge to use on an industrial scale in the future.

Materials and methods

Bacterial isolation and identification

Cyanide-degrading bacterium was isolated from "Mouteh Gold Mines Complex" and purified by repeatedly transferring the cells to buffer medium (BM). One liter of BM contained KH2PO4 2.7 g, K2HPO4 3.5 g, and 10 ml of trace salts solution (FeSO4•7H2O 300 mg, MgCl2•6H2O 180 mg, Co (NO3)2•6H2O 130 mg, CaCl2 40 mg, ZnSO4 40 mg, and MoO3 20 mg in 1 liter deionized water). Final pH was adjusted 7.2. The medium was autoclaved for 15 min at 15 PSI and 121°C before use. Inoculated medium was incubated for 7 days at 30°C on a rotary shaker (180 rpm). In order to screen cyanide-degrading bacterium and medium refresh, 10 ml of microorganisms were transferred into 500 ml Erlenmeyer flask containing 100 ml of buffer medium (BM). Final pH was adjusted 7.2. The medium was autoclaved for 15 min at 15 PSI and 121°C before use. Then Potassium cyanide (KCN) in different concentration will be added to BM for cyanide degrading experimental study and incubated at 30°C, 180 rpm. After 3 days, cyanide-degrading bacterial isolation was done by spreading plate technique on buffer medium containing potassium cyanide (BMK) agar (BMK and 18 g/L of Bacto agar) and incubated at 30°C for 3 days (6). After that the morphology and number of colonies were observed under a light microscope. Colony of bacterial morphology was analyzed using Gram stain (Bergey and John, 1994).

Analytical determinations

The isolated cyanide-degrading bacterium was inoculated in BM containing KCN at 25, 50, 150 to 1000 mg/L. The biodegradation of cyanide was set at 10:100 (inoculum's volume: BM's volume) in 500 ml Erlenmeyer flask and incubated at 30°C on a rotary shaker (180 rpm) for 3 days. After incubation times, bacterial growth, ammonia, nitrate and residual cyanide were analyzed according to standard method. The concentration of ammonia and nitrate was analyzed by titrimetric method (APHA, 1995). Residual cyanide was analyzed by picric acid method (Fisher et al., 1952). Bacterial growth was monitored by determining the absorbance at 600 nm (14).

Measurement of cyanide

Absorption of light dissolved at 520 nm with spectrophotometer was performed (Fisher et al., 1952). In order to measure the production of ammonia (640 nm) and nitrate (220 nm), the precursors were prepared from Wahab Company (VAHEB).

Cyanide removal efficiency

For calculation, the duplicate treatments were done for all. The removal efficiency (RE) of cyanide-degrading bacterium was calculated as shown in following formula (6).

$$\text{RE (\%)} = \frac{\text{Initial concentration(mg/L)} - \text{Residual concentration(mg/L)} \times 100}{\text{Initial concentration(mg/L)}}$$

An isolate which had the highest growth rate in the presence of cyanide as the only source of carbon and nitrogen, and exhibited the highest cyanide reduction efficiency, was genetically identified.

The 16S rDNA sequencing

Chromosomal DNA of the isolate was extracted manually by Marmur's procedure (17). The 16S rDNA genes were amplified using polymerase chain reaction (PCR) with the universal primers of 16S rRNA including For (5'-AGAGTTTGATCCTGGCTCAG-3') and 16S rRNA Rev (5'-ACGGCTACCTTGTTACGACTT-3') (Integrated DNA Technologies) and were sequenced at Xcelris Genomics. Related matching sequences were downloaded from the GenBank database (NCBI, USA) using BLAST search program (12). The sequences were aligned using multiple sequence alignment software, Clustal W version 2.0. A phylogenetic tree was drawn with MEGA align software version 5.1 (18) based on the partial 16S rDNA sequences of bacterial species similar to isolate bacteria name selected in the present study (11).

Results

Isolation and identification of cyanide-degrading bacteria

Cyanide-degrading bacterium was isolated from mouteh gold mines complex. The identification of bacterial strain was performed on the basis of its morphology by Bergey's manual (7). After that the DNA sequencing was performed. The result showed ……% of identity among bacterial isolates. This strain has never been reported as microorganisms capable for the removal of cyanide.

As of our observations, the highest tolerable concentration and growth rate, for bacteria isolated from 1 g/l concentration of potassium cyanide, the cyanide decomposition ability of 1 g/l potassium cyanide was used as the sole source of carbon and nitrogen in culture medium.

Cyanide-degrading bacterial growth

The growth of bacteria name was studied after the isolation. The growth of this bacterium is shown in Fig. 1. The highest growth rate was obtained on day 2 of the incubation time. OD of bacteria during this time were approximately 0.19.

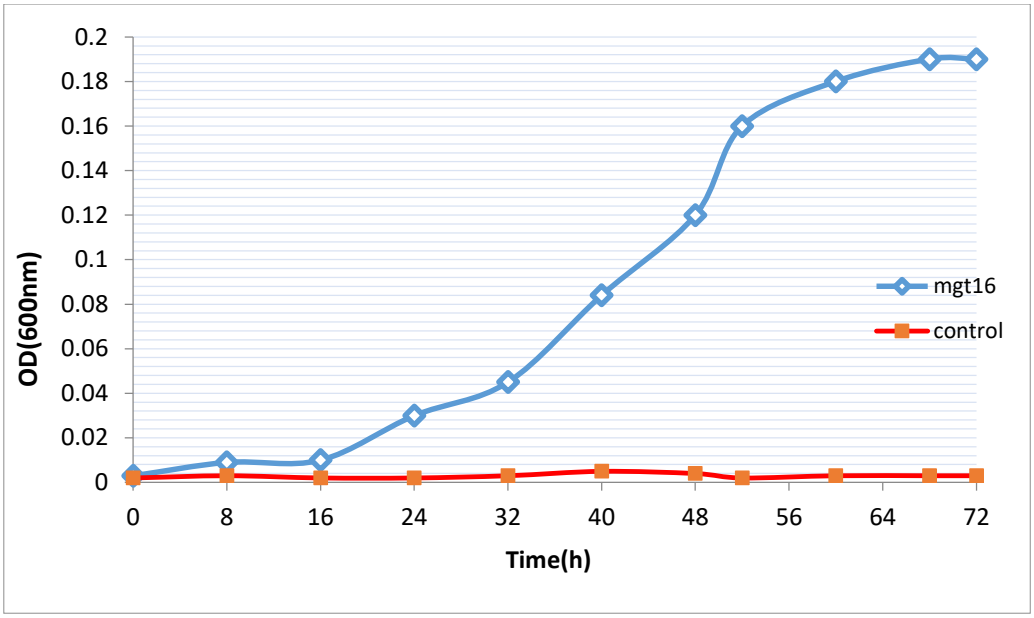

Fig 1. Growth curve of bacterial growth

Biodegradation of cyanide

The cyanide removal efficiency of bacteria name was observed equal to 1000 mg/L cyanide. The results showed that bacteria name obtained 90% removal efficiency with 100 mg/L residual cyanide within 3 days of study (fig 2).

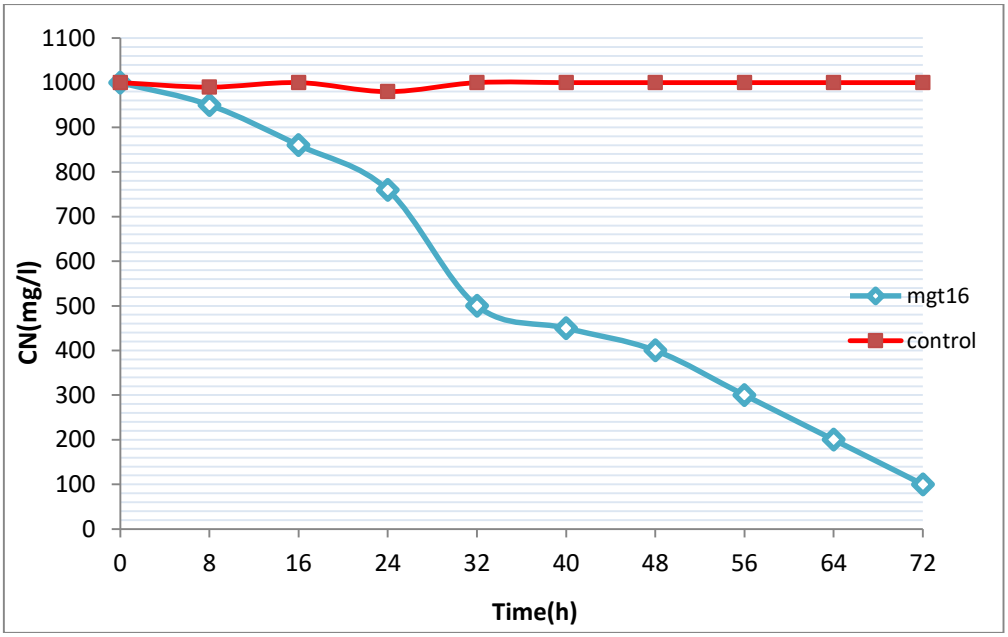

Fig 2. Curve of cyanide degradation

The ammonia concentration increased from 0.25 mg/l on 24h to 0.45mg/l on 60h and then unchanged to 72h. On the other site, the nitrate concentration was negligible changes but it increased from to 0.008 on day 1 to 0.22 mg/L on day 3.

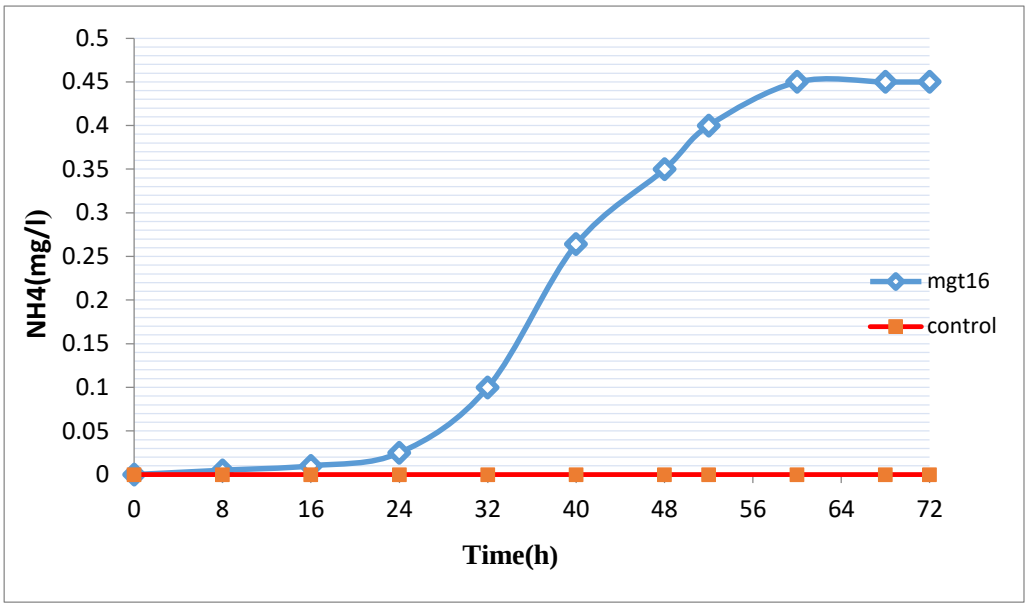

Fig 3. Curve of ammonia concentration changes

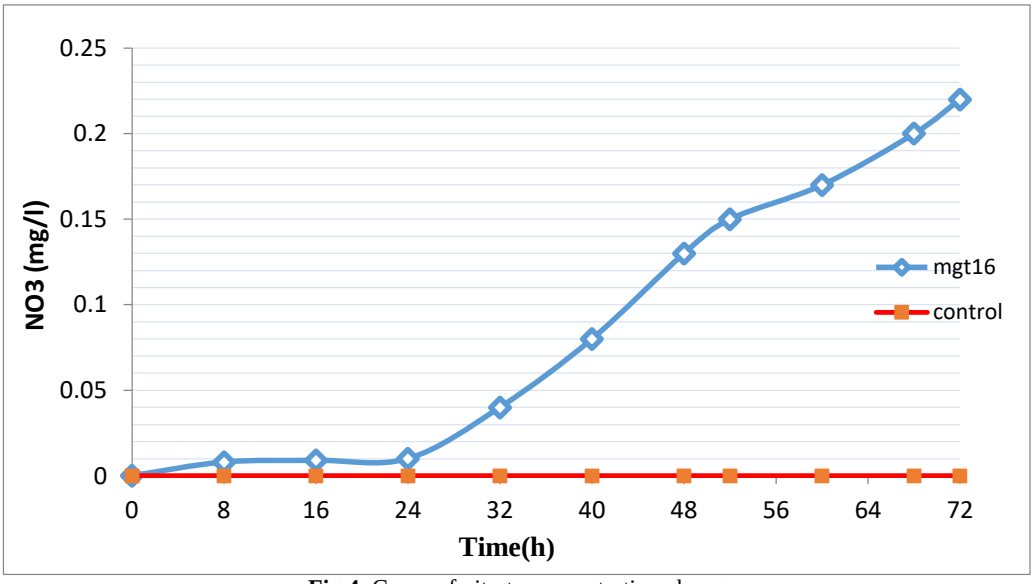

Fig 4. Curve of nitrate concentration changes

Effect of pH on the growth rate of bacteria

The results from the study of turbidity of samples containing bacteria are presented in Fig. 4-5. The results showed that the highest level of turbidity and the highest growth rate were observed at pH = 9. According to the results, pH = 5 showed the least opacity, indicating that the pH of the game is more effective than acidic and neutral pH in cyanide decomposition by bacteria isolated in this study. However, in the control sample, there was no significant change in the amount of opacity due to pH changes.

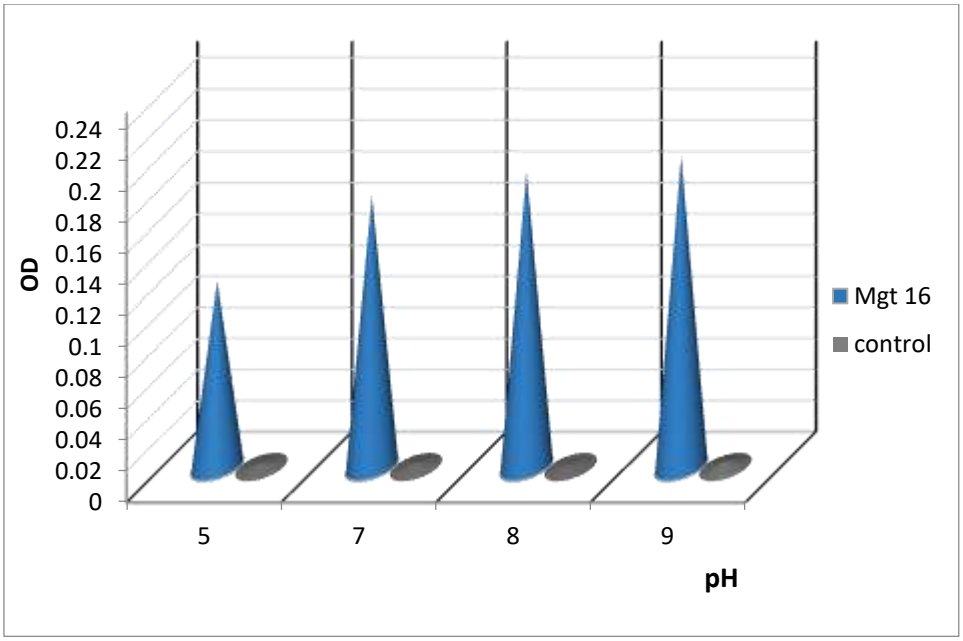

Figure 5. Results of the cyanide decomposition bacterial growth rate at different pH values after 72 hours

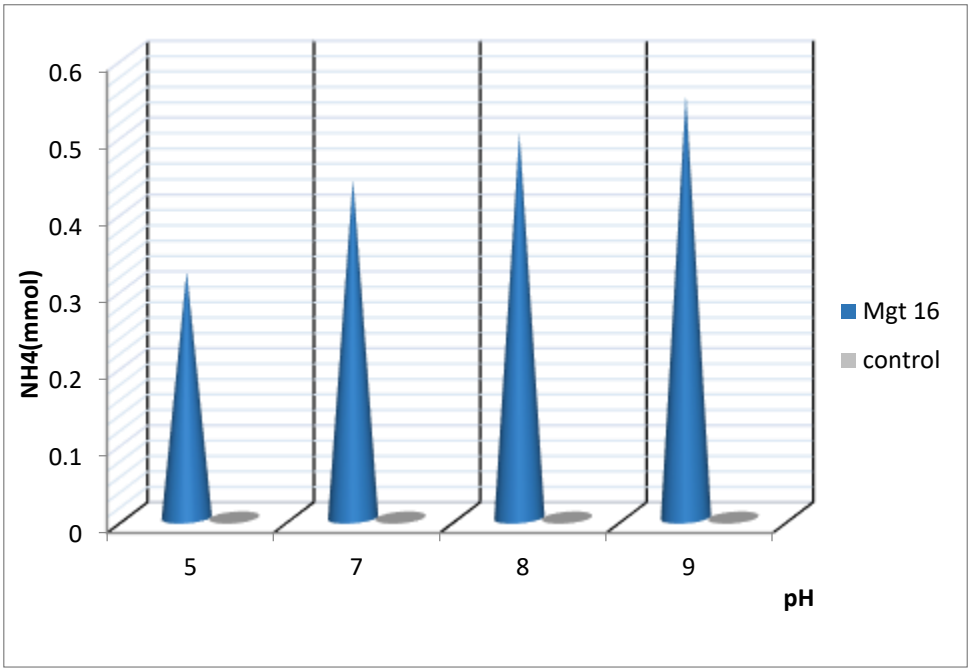

Figure 6. The results of a comparative study of ammonia production by cyanide degrading bacteria at different pH values after 72 hours

The highest ammonia production was observed at pH 9, 8 and 7, respectively. In the control sample, ammonia production was not observed and pH changes did not significantly differ from the amount of ammonia produced in the control sample.

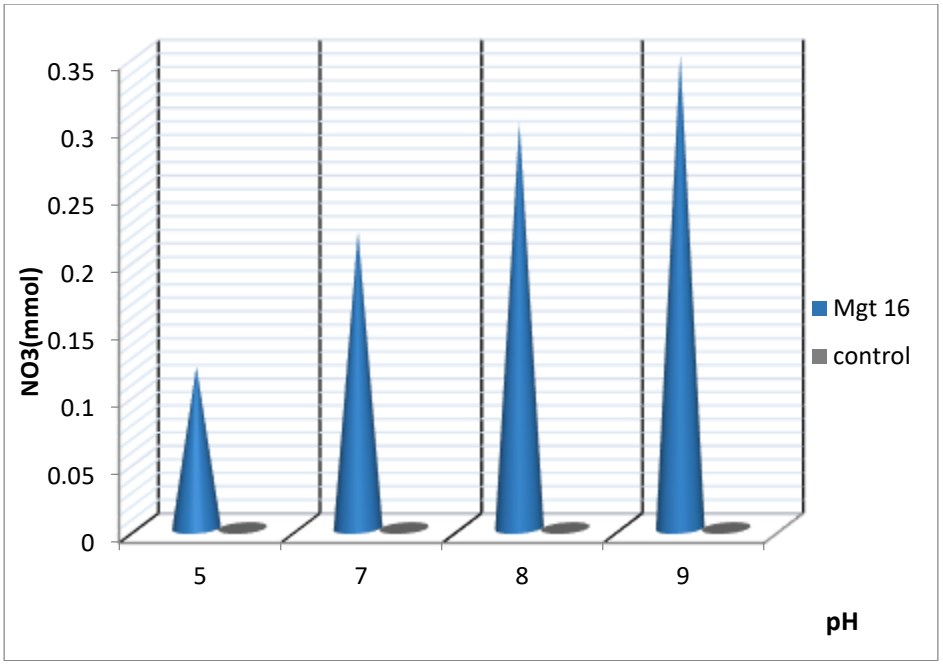

Figure 7. Results of the study of the primary pH of the culture medium in the amount of nitrate produced by the cyanide degrading bacteria after 72 hours

The pH = 9 was the best level calculated for nitrate production.

The cyanide content was measured after 72 hours of inoculation of the bacteria in a BM medium containing 1000 mg / l of cyanide. The highest amount of cyanide removal was observed in alkaline pH, so that at pH 9 = the cyanide content decreased to 50 mg.

Discussion

Today, cyanide biochemical analysis is considered as a cost-effective and environmentally compatible method compared to conventional chemical methods. In the present study, a cyanide decomposing bacterium was isolated from the Moute gold mine effluent and was identified as ..... before which was in genus ..... studied in microbiology research. However, the destruction of cyanide by this bacterium has not been reported so far. In this research, in order to identify efficient microorganisms in cyanide removal, cyanide resistant bacteria were isolated from the waste dump of the Muteh-Isfahan golden complex (Golpayegan city) and the strain ..... with the highest resistance (tolerance mg / L000 potassium cyanide) was isolated. Suza et al. (2004) reported a minimum inhibitory concentration of NaSCN cyanide composition growth with Pseudomonas aeruginosa at 45 mM. In a study by Kharm et al. (2013), 313 isolates of cyanide decomposing bacteria were isolated from the tailings dam of Zarmahr golden complex (Torbat Heydarieh city), of which 9 strains were able to withstand cyanide up to 600 ppm. (8) in a study to isolate and purify the cyanide degrading microorganisms and to determine their removal rate isolated a gram negative strain M3 from Takab gold mining waste samples which displayed 93.5% tolerance at 500 ppm cyanide in culture media. In another study by (10), a strain isolated from the Takab mining waste land could withstand more than seven mM of potassium cyanide in a culture medium. The results of this study showed that the strain had the ability to use cyanide as the only source of carbon and nitrogen in addition to its ability to withstand cyanide up to a concentration of 1000 mg / liter and could well grow in an environment free of carbon and nitrogen sources and containing cyanide.

(13) and his colleagues used Pseudomonas fluorescens for decomposition of cyanide, and their results showed that the bacterium used cyanide ions as the only source of nitrogen. In another study in 1999, Adjie and O'Hatha studied cyanide decomposition by Burkhulderia Sepasia in alkaline conditions. The results of this study indicated that the bacterium was able to use cyanide as the only source of nitrogen, but needed the addition of glucose as a carbon source. Also, bacteria such as Pseudomonas fluorescence and Pseudomonas pseudoalkaligenes (Luke et al., 2005) identified the ability to use cyanide compounds as the only source of nitrogen. In 2005, an isolate called Pseudomonas pseudoalkaligenes was isolated from Spain and its colleagues from the wastewater of the Spanish jewelry industry. The bacterium was able to use cyanide as the only source of nitrogen in alkaline conditions, but it also needed acetate as an additional carbon source. Also, the isolated bacteria in (15) study were able to degrade 0.1mM cyanide by adding additional carbon and nitrogen sources to the growth medium. In a study by Luke and colleagues in 2011, the Pseudomonas alkaliphylic bacterium CECT5344 was able to decompose cyanide and use it as the sole source of nitrogen. Firozyar et al. Identified isolates of Acinetobacter and Bourkholderia Sepasia that demonstrated the ability to use various cyanide compounds (cyanide-metal and nitrile compounds) as the only source of carbon and nitrogen

Lack of carbon source growth environment is an inhibitor of biodegradation of cyanide in contaminated soils. Therefore, strains isolated in this study with the ability to decompose cyanide without adding carbon and nitrogen source would be suitable for use in bio-degradation processes on a semi-industrial and industrial scale.

In addition to using more economical resources, in large-scale biodegradation processes, the speed of the analytical response along with high efficiency is of great importance. Therefore, in previous studies, the study of bioavailability of cyanide in a unit time has been considered.

In 1983, by Harris et al., 79% of cyanide in the growth medium was removed in acidity 5 and within 48 hours by bacteria. In 2003, Cao et al. investigated the cyanide degradation with Klebsiella oxytoca. The results showed that this isolate split 0.9 mM cyanide over 80 hours. In a similar study in 2010, Azal et al. (2010) examined the ability of cyanide decomposition by different isolates of the fungi from the basidiomy group and identified five different cyanide degrading isolates. These isolates had the ability to decompose 60 to 80% of cyanide in medium for 60 hours. Also, in this study, Fusarium solani was able to decompose 50 mg /l of cyanide in 96 hours in alkaline conditions. The strain isolated in the Quito (2013) study was able to reduce cyanide from 500 mg /L to 155 mg / L. In another study by (16) using Bacillus spp., Bacillus ligniferamus isolated from the electron plating solution over a period of 8 days reduced 65.5% and 44.3% of the whole cyanide. In 2013,(15). examined cyanide biodegradation by Rhodococcus bacteria, which resulted in cyanide degradation by this bacterium in 40 hours. The results of the study by (10) showed that Acinetobacter pitiy and Bacillus saphensis could cyanide in the growth medium in alkaline conditions after 40 hours. The highest decomposition occurred at 12 h before the bacterial maximum growth. In another study by Mohseni and Firoozyar, isolate MF1 with 99% homology with Sarajevo Nematodephyla was able to completely disassemble 132 mg / L of cyanide in a culture medium (2 mM cyanide) after 40 hours.

The results of this study indicate that Mgt 16 strains, in addition to good ability for complete cyanide breakdown, decomposed at approximately 48-48 hours, had the highest decomposition rate during the first 24 hours. After 72 hours, the amount of cyanide in Mgt16 from 1000 mg / l to 100 mg / l (90% removal efficiency) increased to 95% in alkaline pH. The results show that the strain is effective.

Metal-cyanide compounds based on their dissolution in different acidity can be divided into two weakly acid-soluble (CN_WAD) and strong acid (CN_SAD) soluble groups. The cyanide composition with metals such as iron and cobalt is in the CN_SAD category. These compounds are very stable and release their cyanide group in zero acidity. Hence, depending on the cyanide composition and the type of bacteria, pH can be effective on the cyanide breakdown efficiency.

In Maniam et al. (2013), it was found that optimal growth and biochemical decomposition of cyanide by the UKMP-5M Rodococcus at 30 ° C and pH = 6.3 were carried out in a culture medium with glucose as a carbon source. Also, the results of (11) showed that the maximum cyanide decomposition rate was determined by the bacterial superficial bacteria at pH = 6 and temperatures of 35 ° C.

However, according to studies, cyanide ion is converted to neutral hydrogen and to hydrogen cyanide and evaporates. Hence, microbial strains with the ability to use cyanide in alkaline conditions are good alternatives for the elimination of these compounds.

In 1997, Devasteri and his colleagues isolated the IHN 8026 fungus from the cyanide effluent, which showed a good potential for biochemical decomposition under alkaline conditions (pH = 7.2). The isolated bacteria in the study, Luke et al. (2005), were able to grow in an alkaline medium with an initial pH of 11.5. In the study, (6) reported an increase in the pH of cyanide removal efficiency. In Horace et al. (2010), it was shown that the removal of cyanide by Pseudomonas alkylated bacteria is related to pH, so that at pH = 10, the cyanide elimination efficiency increased. In the study of Khmer et al. (2013), optimal pH for the removal of cyanide by isolated bacteria was determined pH = 10.5-10. Furthermore, the results of the study by (9) indicated that Serratia nematodephyla isolated from the Takab gold mine waste deposit soil at pH = 9 were completely capable of removing cyanide from the medium. In a study by Wu et al in 2014, it was reported that the highest percentage of cyanide removal for Bacillus sp. CN-22 occurred at pH = 3/10 = pH.

According to previous studies, alkaline pH greater than 9 will have the risk of producing hydrogen cyanide, a combination of alkaline pH and very low soluble oxygen (less than 10%) can reduce the production of hydrogen cyanide, however These conditions will reduce the growth of bacteria.

In this study, pH 5, 7, 8, and 9 were used to investigate the effect of pH on the removal efficiency of cyanide from the culture medium by microorganisms isolated from the mud wastewater of the Muteh golden complex. According to the results, the highest amount of cyanide removal was observed in alkaline pH, so that at pH= 9, the cyanide content decreased to less than 100 mg / L. The amount of cyanide in the culture medium containing Mgt 16 to 50 mg / L decreased. In other words, alkaline pHs were more effective in eliminating cyanide relative to acidic and neutral pH. However, in the control sample, there was no significant change in the amount of cyanide.

Generally, cyanide is converted into less toxic products, such as ammonia, after being degraded by microorganisms. In the next step, ammonia can be converted to a final product such as nitrate, or other products such as methane, carbon dioxide, or nitrite. The results of the study by Cao et al. (2003) showed that biological processes of cell growth, biochemical decomposition of cyanide, and ammonia production by the Klebsiella oxytoca bacteria occur simultaneously. In the study of (6), Agrobacterium tuberculin SUTS 1 was reported to reduce cyanide to ammonia and nitrate. It was also observed that with increasing ammonia, with increasing cyanide removal efficiency, the concentration of ammonia also increased. However,

nitrate concentration could only be calculated when ammonia concentration was reduced or not. Similar results were reported in Firozyar et al. (2012) that reduction of cyanide concentration was directly related to the increase of ammonia concentration in the culture medium as well as the growth of the isolates.

The results of this study showed that, with time, ammonia production increased compared to control. In the sample, no measurable amount of ammonia was obtained. The results also showed a decrease in the amount of ammonia. In addition, an increase in the amount of ammonia was observed with an increase in the pH to alkalinity, indicating a greater cyanide breakdown. The highest ammonia production was observed at pH 9, 8 and 7, respectively. In the analysis of nitrate content during bio-degradation, the increase in nitrate content was observed from 0 to 0.25 mg / liter. The results demonstrated that the highest amount of nitrate was observed during 48-72 hours after inoculation of bacteria with cyanide culture medium, indicating a large conversion of ammonia to nitrate.

In conclusion, the results of this study suppose that the Mgt 16 bacteria isolated from the Mutee wastewaters are suitable alternatives for the biochemical analysis of cyanide in alkaline conditions and can be used to decompose cyanide from industrial wastewater and contaminated sites.

Refrences

1. Akcil, A., & Mudder, T. (2003). Microbial destruction of cyanide wastes in gold mining: process review. Biotechnology Letters, 25(6), 445-450

2. Dash, R. R., Gaur, A., & Balomajumder, C. (2009). Cyanide in industrial wastewaters and its removal: A review on biotreatment. Journal of Hazardous Materials, 163(1), 1-11.

3. Naveen, D., Majumder C.B, Mondal P and Dwivedi Shubha. (2011). Biological Treatment of Cyanide Containing Wastewater. Vol. 1(7), 15-21, Oct.

4. Pickett, T.M., Van Komen, J., Adams, D.J. (2009). Biological Cyanide Degradation. Applied and Environmental Microbiology. 50(2): 450-456.

5. Gurbuz, F., Ciftci, H., Akcil, A. (2009). Biodegradation of cyanide containing effluents by Scenedesmus obliquus. HubJournal of Hazardous Materials. 162(1) : 74–79.

6. Potivichayanon, S., Kitleartpornpairoat, R. (2010). Biodegradation of Cyanide by a Novel Cyanide degrading Bacterium. World Academy of Science, Engineering and Technology. 42: 1362-1365.

7. Holt, J. G., Krieg, N. R., Sneath, P. H., Staley, J. T., & Williams, S. T. (1994). Bergey's Manual of determinate bacteriology.

8. Moradkhani, M, Yaghmaei S, Ghobadi, Z. (2013). Isolation and purification of cyanide degrading microorganisms and assessment of the level of their elimination. Third International Conference on Management and Environmental Planning

9. Firooyiar S, Mohseni M, Nazari L. (2013). Biological elimination of cyanide by strains isolated from contaminated soils of Iran. The 8th Biotechnology Conference of the Islamic Republic of Iran and the 14th National Biological immunity Conference. University of Tehran

10. Firoozyar S, Mohseni, M, Nazari L. (2013). Isolation and molecular analysis of cyanide degrading bacteria from soil. Master's thesis at Mazandaran University

11. Kumar, V., Kumar, V., & Bhalla, T. C. (2015). Statistical enhancement of cyanide degradation using microbial consortium. Journal of Microbial & Biochemical Technology, 2015.

12. Altschul, S. F., Gish, W., Miller, W., Myers, E. W., & Lipman, D. J. (1990). Basic local alignment search tool. Journal of molecular biology, 215(3), 403-410.

13. Harris, R. E., & Knowles, C. J. (1983). The conversion of cyanide to ammonia by extracts of a strain of Pseudomonas fluorescens that utilizes cyanide as a source of nitrogen for growth. FEMS microbiology letters, 20(3), 337-341.

14.Luque-Almagro, V. M., Huertas, M. J., Martínez-Luque, M., Moreno-Vivián, C., Roldán, M. D., García-Gil, L. J., Blasco, R. (2005). Bacterial degradation of cyanide and its metal complexes under alkaline conditions. Applied and environmental microbiology, 71(2), 940-947.

15.Maniyam, M. N., Sjahrir, F., Ibrahim, A. L., & Cass, A. E. (2013). Biodegradation of cyanide by Rhodococcus UKMP-5M. Biologia, 68(2), 177-185.

16. Mekuto, L., Ntwampe, S. K. O., & Jackson, V. A. (2013). Biodegradation of free cyanide using bacterial species isolated from cyanide waste water.

17. Sambrook, J., Russell, D. W., & Sambrook, J. (2006). The condensed protocols from Molecular cloning: a laboratory manual. NY: Cold Spring Harbor Laboratory Press v.

18.Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M., & Kumar, S. (2011). MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods.Molecular biology and evolution, 28(10), 2731-2739.