

# Cyanide Detoxification and Recovery of Gold from Gold Effluent

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## Abstract

Growing global concern on environmental health is forcing all the processing to adopt greener and cleaner manufacturing practices. Cyanide is one of the most potential toxic chemicals, which has a tremendous application in various fields and is also the main source of contamination of water basins with heavy metals. About 20% of cyanide is used in the gold mining industries. Mine wastes have heavy metals, acids which causes acute, chronic illness not only to human being but also to other organisms. The traditional treatment method followed for cyanide destruction was non profitable with more toxic by products such as liquid chlorine. Microbes have the ability to bind metals. Hence, to save the environment from pollution and find a method, which is rapid, efficient, inexpensive with low level of technology the biodegradation process of *Pseudomonas fluorescens* is to be carried out. The green algae *Chlorella vulgaris* bind noble metals like gold, silver, platinum regardless of the pH conditions with the use of microbes, not only the heavy metal is destructed but also recovery of noble metals can be achieved which is the main aim of our work. The strong bond link between metals and microorganisms helps in destruction and recovery of metals.

**Keywords:** Cyanide destruction, *Pseudomonas fluorescens*, gold recovery, *Chlorella vulgaris*.

## Introduction

Biodegradation is the earth's natural process of converting organic matter into environmentally safe by products such as water, carbon dioxide and ammonia. This process is accomplished through naturally occurring microorganism such as bacteria, algae, fungi, etc. These organic compounds such as heavy metals disturb the ecology. The toxicity of the effluent quality go up due to incomplete decomposition of the organic matter.

There are few microbes, which are able to work in highly toxic conditions, total dissolved solid levels (TDS), which are very high, and in some cases they are also able to withstand high temperature. A combination of sophisticated engineering and biotechnology can achieve preferential degradation of specific compounds especially cyanide by adding selected organisms, usually bacteria or

algae. Such strains improve the tolerance of toxic load.

Mining industry and in particular the gold mining industry, has been using cyanide as the chemical choice in the production processes for many decades. Cyanide complexes are more stable and effective, and do not require additional aggressive chemicals to effect gold recovery. Gold mining operations use very dilute solutions of sodium cyanide, typically in the range of 0.01% and 0.05% cyanide (100 to 500 parts per million). Gold recovery accounts for approximately 18% of total world cyanide production loads.

Mine wastes have been generated for several centuries and these constitute a potential source of contamination to the environment, as heavy metals and acid, which are released in larger amount. After gold is extracted via the hydrometallurgical processes, three principal types of cyanide compounds may be

present in wastewater or process solutions: free cyanide, weakly complexed cyanide and strongly complexed cyanide. Together, the three cyanide compounds constitute "total cyanide." The most toxic form of cyanide is HCN gas.

A great deal of public attention has recently been focused on the problem of heavy metal contamination of drinking water supplies; the ingestion of metals such as mercury, cyanide even in very small quantities, is known to cause acute and chronic illness in both humans and other animals. Cyanides being highly toxic, short term exposure can cause the following health effects: rapid breathing, tremors and other neurological effects; long term exposure can cause weight loss, thyroid effects and nerve damage. Cyanide tends to react readily with most other chemical elements, producing a wide variety of toxic, cyanide related compounds. Therefore, cyanide must be destroyed or removed from wastewater prior to discharge.

Managing risks associated with the use of cyanide involves sound engineering, careful monitoring and good management practices in order to prevent and mitigate potential releases of cyanide to the environment. The procedures required to accomplish this, such as carbon absorption, flocculation-sedimentation, and treatment with ion-exchange resins, may prove to be very costly. Thus, it would be desirable to develop a technique for removal of metals from water, which is rapid, efficient, and inexpensive, and at the same time requires a low level of technology.

Biological degradation of cyanide has often been offered as a potentially inexpensive, environmentally friendly alternative to conventional processes. Cyanide is highly

toxic for most living organisms because it forms very stable complexes with transition metals that are essential for protein function, i.e., iron in cytochrome oxidase. Consequently, organisms growing in the presence of cyanide must have a cyanide-insensitive metabolism, such as the alternative oxidase described for plants or the cytochrome bd (or cyanide-insensitive oxidase) in bacteria. The biological assimilation of cyanide needs, at minimum, the concurrence of three separate processes, i.e., a cyanide resistance mechanism, a system for metal acquisition, and a cyanide assimilation pathway. Although all of these factors in conjunction with one another have never been taken into account, a number of microorganisms are able to degrade cyanide and its metal complexes. From a chemical point of view, the biological treatment of industrial effluents contaminated with cyanide requires an alkaline pH in order to avoid the formation of the volatile HCN.

Gold is one of the rarest metals on earth, and its importance has been known since antiquity. Because of the increased demand for gold in industry and nanotechnology, exploration for new gold deposits in the natural environment has become very important. On the other hand, gold in waste solutions from several industrial processes, e.g., gold mining and gold electroplating effluents could be recovered and reused. Therefore, there is an essential need to develop alternative, cost effective and environmentally sound methods for recovering gold from waste solutions.

The chemical processes that exist are not economical for treating a large volume of water bodies of dilute metal concentration. In this endeavour, microbial biomass has emerged as an option for developing economic and eco friendly wastewater treatment processes. Non-living and dead microbial biomass may passively sequester metal(s) by the process of biosorption from dilute solutions.

The algae like *Anabaena* and *Chlorella* are used for copper, cadmium, lead and zinc extraction. A process, which could selectively recover the noble metals from old mining dumps, mineral leaching operations, and industrial processes using them would clearly have tremendous commercial potential. It has

now been unexpectedly discovered that certain microorganisms under controlled conditions of pH and salt concentration can be used to selectively bind gold, silver or platinum, while essentially preventing the binding or causing the release of a number of competing metals.

This biosorption technology has advantages of low operating cost, is effective in dilute solutions and generates minimum effluent. Here the dead microbial biomass functions as an ion exchanger by virtue of various reactive groups available on the cell surface such as carboxyl, amine, imidazole, phosphate, sulfhydryl, sulfate and hydroxyl. The process can be made economical by procuring natural bulk biomass or spent biomass from various fermentation industries.

This work employs the purification of cyanide-containing waste water using *Pseudomonas fluorescens* via bioaugmentation which can be applied in gold-mining industry as well as in non-ferrous metallurgy and then the noble metals such as gold, silver, platinum etc are also bioaccumulated or biosorped using *Chlorella vulgaris*. Both dead and living cells were used and provide a simple, inexpensive, low technology method of extracting valuable metals from even dilute aqueous solutions without the necessity of using costly ion-exchange resins.

This method also provides a simple procedure for eluting metals other than gold, silver, mercury and platinum from microbial cells to which they are bound. It has also been found that the metals like gold, silver and mercury can be selectively recovered from microbial cells to which they are bound, even if other

unwanted metals are also bound to the microbial cells, regardless of the pH at which binding occurs.

## Materials and methods

### 1. Cyanide Destruction Fermentation Broth:

300 ml of *Pseudomonas* culture of kings B medium was taken and to that 200 ml of autoclaved distilled water, 0.5 mg of cyanide and 1 g of glucose was added as carbon source (SAMPLE 1).

300 ml of *Pseudomonas* culture of kings B medium was taken and to that 200 ml of autoclaved distilled water, 0.5 mg of cyanide, 1 g of glucose was added as carbon source and 1 g of sodium chloride was added to enhance the growth. (SAMPLE 2)

300 ml of *Pseudomonas* culture of meat peptone broth-kings B medium was taken and to that 200 ml of autoclaved distilled water, 1 mg of cyanide, 1 g of glucose was added as carbon source and 1 g of sodium chloride was added to enhance the growth. (SAMPLE 3)

300 ml of *Pseudomonas* culture of meat peptone broth-kings B medium was taken and to that 200 ml of autoclaved distilled water, 1 mg of cyanide, 1 g of glucose was added as carbon source and 1 g of sodium chloride was added to enhance the growth. (SAMPLE 4)

For sample 1, and 2 the pH was adjusted to 7.2 and temperature was maintained as 30 degree Centigrade.

For sample 3, and 4 the pH was adjusted to 8.5 and temperature was maintained as 30 degree Centigrade.

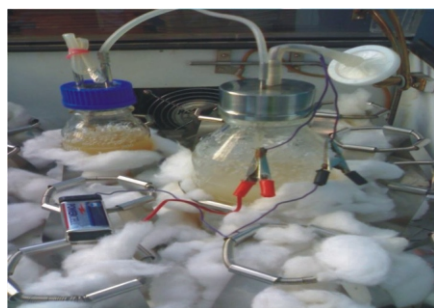
SAMPLES	I	II	III	IV
Medium	Kings B	Kings B	Meat peptone kings B	Meat peptone kings B
Cyanide	0.5	0.5	1.0	1.0
Glucose	1.0	1.0	1.0	1.0
NaCl	-	1.0	1.0	1.0
pH	7.2	7.2	8.5	8.5
Temperature	30	30	30	30
Days	3	3	5	5
Time interval	1 min/ 1 hour	45sec/ 1 hour	1 min/ 1 hour	45sec/ 1 hour
Volts	9	9	9	9

- ⇒ Centrifuged at 10,000 rpm for 10 minutes and supernatant was collected
- ⇒ Absorbance was measured at 580 nm

It was agitated in shaker and aeration was passed through membrane filter continuously during daytime for three days (10 hours per day) for sample 1 & 2 and five days for sample 3 & 4 and 9 volts current was passed for 1 minute at 1-hour interval.

It was then centrifuged at 5,000 rpm for 10 minutes and then supernatant was collected.

Then absorbance at 580 nm was measured by various biochemical tests.



Experimental Set-up For Cyanide Destruction

## 2. Methods for Analysis of Cyanide Concentration:

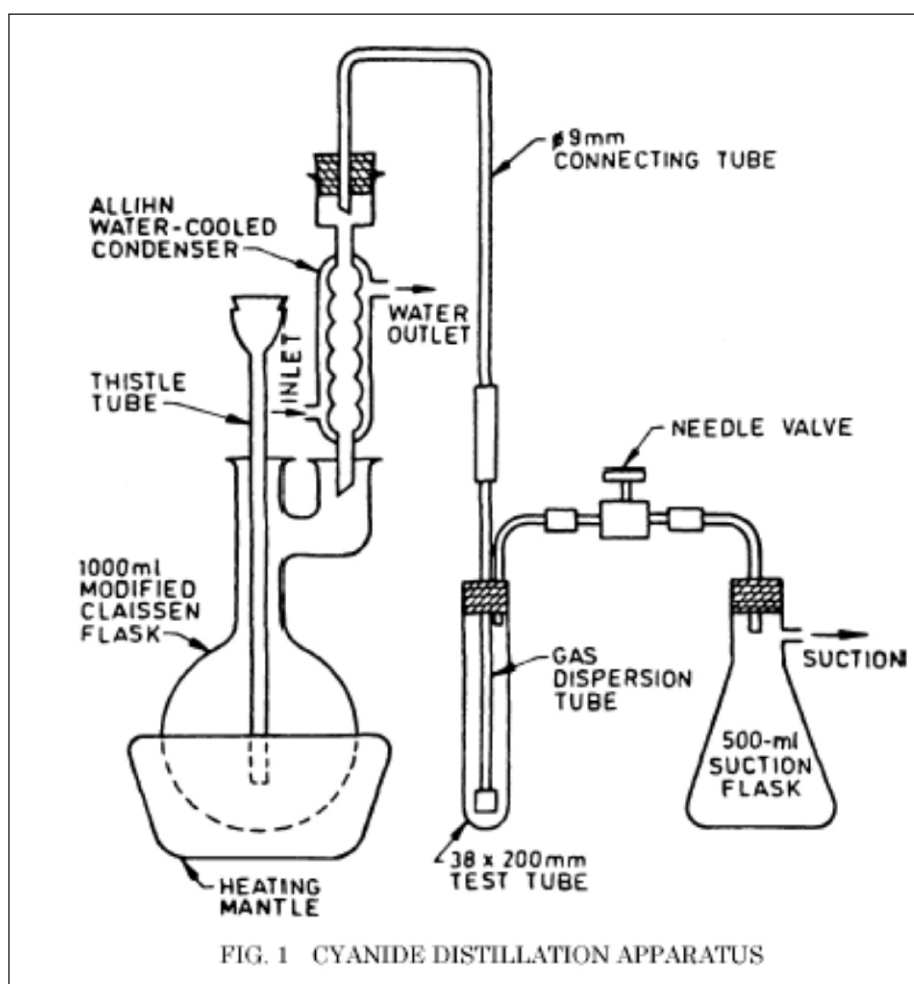
### 2.1 Cyanide Distillation

1. 100ml sample, containing not more than 2mg CN/L was added to the boiling flask.
2. Since, a higher concentration of cyanide is anticipated, SPOT TEST was done to approximate the level of cyanide at least amenable to chlorination and the level was diluted.
3. 4ml of Sodium Hydroxide solution was added to the gas scrubber, and diluted with distilled water, to get an adequate liquid depth of about 45ml solution.
4. Suction was adjusted such that 1 air bubble/second enters boiling flask
5. This air rate will carry HCN gas from flask to absorber and it prevents backflow.
6. 0.4g sulfamic acid was added through the air inlet tube and washed with distilled water.

7. 10ml of H<sub>2</sub>SO<sub>4</sub> was added through the air inlet tube and rinsed with distilled water.
8. 4ml of MgCl<sub>2</sub> reagent was also added and rinsed with distilled water.
9. It was heated with rapid boiling adequate reflux is indicated by a reflux rate of 40 to 50 drops/min.
10. Heating was stopped and cooled for 15 minutes. The connecting tube

between the condenser and gas scrubber solution was rinsed with distilled water and made up to 50ml with distilled water in a volumetric flask.

11. The cyanide concentration in the scrubbing solution is estimated using any one of the methods stated above.



### 2.2 Spot Test

The pH of the solution was tested and if it is greater than 10, neutralise a 20 to 25 ml portion.

About 250 mg sodium carbonates and was added and dissolved.

1 drop of phenolphthalein indicator was added.

HCl was added drop wise with constant swirling until the solution becomes colourless.

3 drops sample and 3 drops distilled water was added in separate cavities of the spot plate.

To each cavity add 1 drop chloramines-T solution was added and mixed with a clear stirring rod.

1 drop pyridine-barbituric acid solution was added to each cavity and mixed again.

1 min later, the sample spot will turn to either red or yellow.

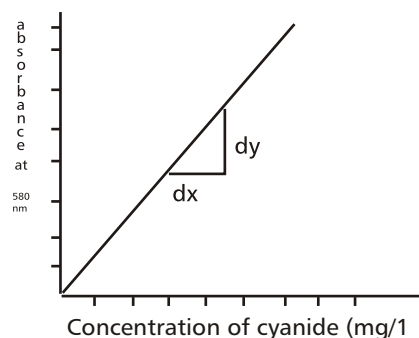
Red colour-50 microgram or more  
Yellow colour-less than 50 microgram

### 2.3 Colorimetric Method

CN in the alkali distillate from preliminary treatment is converted to CNCL by reaction with chloramine T at pH<8 without hydrolyzing to CNO. After the reaction is complete, CNCL forms a red-blue dye on addition of a pyridine-barbituric acid reagent. If the dye is kept in an aqueous solution, the absorbance is read at 580 nm.

## Procedure

### 1. Preparation of Calibration Curve



Concentration of cyanide (mg/l)	1	2	3	4	5
Absorbance at 580 nm	27	56	84	110	135

Blank of NaOH dilution solution was prepared. From the standard CN solution a series of standards containing different CN concentration in 20 M ml using the NaOH dilution solution was prepared. Plotted the absorbance of standard against concentration. From the calibration curve the slope obtained was 27.2

## 2. Colour development

Blank: NaOH dilution + all reagents  
10 ml of **sample** was taken

↓

5 ml of **phosphate buffer** was added to the sample

↓

2 ml of **chloramine-T solution** was also added and mixed

↓

5 ml of **pyridine-barbituric acid** solution was added

↓

Then solution was made to 50 ml using **NaOH solution**.

↓

**Absorbance** was measured at **580 nm** after 8 min but within 15 min from the time of addition of the pyridine-barbituric acid reagent.

## 3. Bioaccumulation of the gold from effluent samples

### 3.1 Inoculation And Maintenance Of Algal Culture

A loop full of chlorella vulgaris algae was inoculated in the BG 11 medium and it was maintained at 37°C room temperature with partial sunlight for 12 hours. It was maintained for 3-4 weeks to attain the growth phase.

### 3.2. Sample 1

#### Algal preparation

1g of lyophilised cells of Chlorella vulgaris was weighed and was diluted in 0.01 M (100ml) of Con. HCl.

Then pH of the solution was adjusted to 3 with Con. Nitric acid and allowed to stand for 1 hour.

It was then centrifuged at 10,000 rpm for 10 min.

The pellet was discarded and the supernatant was collected and measured.

### Gold Precipitation work

10ml of supernatant was taken. To that 5ml of gold chloride solution was added in the ratio 2:1.

The mixture was stirred well for 10 minutes and allowed to stand for 1 hour.

It was then centrifuged at 5000 rpm for 10 min.

The pellet was discarded and the supernatant was collected and stored. The pH was adjusted to 3 and the colour change of bright yellow was noted.

The supernatant was analysed for free gold ions.

### 3.3 Sample 2

#### Algal Preparation

1.1g of Lyophilised cells of Chlorella vulgaris was taken and washed 3 times in NaCl.

2. It was then diluted with 0.01M of Con. HCl.

3. The pH of the solution was measured.

#### Gold Precipitation Work

0.100 g of gold metal was weighed

The gold solution was prepared with 100ml concentration (100ml = 100µg Au)

Then the gold solution was mixed with algal culture at 10mg/ml for 15 minutes

It was then centrifuged at 5000 rpm for 10 minutes

The supernatant was transferred to a fresh portion of algae

It was shaken well for 15 minutes

It was then repeated twice with fresh algae at 10mg/ml

The pH of the supernatant was adjusted to 3 with concentrated nitric acid. The colour change was noted.

A slight precipitate was formed

The solution was transferred to fresh beaker with lid

It was kept in a boiling water bath at 75°C for 4 hours

The gold particles settle down at the bottom of the beaker.

It was then filtered with a help of Whitman filter paper<sup>42</sup>.

The filter paper was dried in the incubator at 75°C for 20 min.

The dried gold particles were weighed.

### 3.4 Sample 3

#### Algal precipitation

1.250 ml of algal culture was taken with BG 11 medium

#### Gold Precipitation work

30 g of gold industry soil was weighed and added to the algal culture it was kept in a shaker for 24 hours at 30 °C

Then it was centrifuged at 5000 rpm for 10 minutes

The supernatant was collected and the pellet was discarded

It was again centrifuged at 5000 rpm for 10 minutes

The supernatant was collected and the pellet was discarded

The pH of the supernatant was adjusted to 3 with concentration nitric acid

The colour change was noted

A large excess clear solution of ferrous sulphate was added

It was then transferred to beaker with lid

#### Tabulation:

Samples	Absorbance at 578 nm	Initial concentration of cyanide (mg)	Final concentration of cyanide (mg)
I	0.111	0.050	0.0302
II	0.106	0.050	0.0288
III	0.184	0.100	0.0544
IV	0.186	0.100	0.0506

The gold particles settle down at the bottom of the beaker.

It was then filtered with a help of whattman filter paper<sup>42</sup>.

The filter paper was dried in the incubator at 75 °C for 20 min.

The dried gold particles was weighed

## 4. Quantitative Analysis of free Gold Ions

Transfer the supernatant to a fresh beaker with a lid.

The solution was allowed to boil for 4 hrs in water bath at 100°C

The gold particles settle down at the bottom of the beaker.

It was then filtered with a help of whattman filter paper<sup>42</sup>.

The filter paper was dried in the incubator at 75°C for 20 min.

The dried gold particles were weighed.

## Results and Discussion

The cyanide was destructed by bio degradation using the microorganism *Pseudomonas fluorescens*. The 4 different samples were allowed for destruction with different pH and with and without the addition of glucose and NaCl

From the Spot test, a spot of yellow colour was observed this confirmed the presence of less than 50 mg of cyanide in the solution free cyanide (CN) combines with chlorine and forms cyanogen chloride (CNCl). This combination helps to find the amount of cyanide present in the effluent sample.

The first sample was destructed with cyanide amount to 0.032 mg without the addition of glucose as nutrient with more aeration and pH 7 and with the passage of current of about 9 volt for 1 minute for every 1 hour.

The second sample was destructed with cyanide amount to 0.0288 mg/l with the addition of glucose as nutrient and Nail with more aeration and pH 7 and with the passage of current of about 9 volt for 1 minute for every 1 hour.

The third sample was destructed with cyanide amount to 0.05448 mg/l with the addition of glucose as nutrient and NaCl with more aeration and pH 8 and with the passage of current of about 9 volt for 1 minute for every 1 hour.

But in the fourth sample the pH was maintained in 8.5 with the carbon source of glucose and NaCl with more oxygen content, nutrient sources and high alkaline condition, the degradation was faster and the concentration was found to be 0.0506 mg/l.

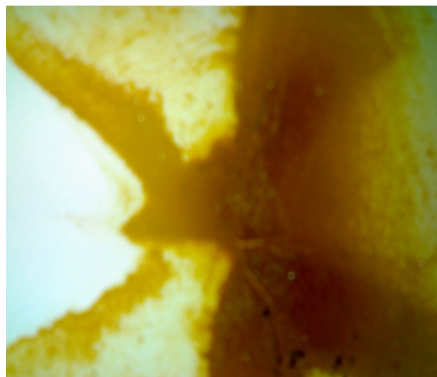
Therefore, this micro-organism offers new perspectives in the bioremediation of industrial effluents contaminated with cyanide as its enzymes have the ability to degrade cyanide and metal cyanide complexes (also stable iron cyanide complexes) into less toxic compound like ammonia, formic acid and formamide. Cyanide degradation by *Pseudomonas fluorescens* seems to be an assimilative process since cyanide removal coincided with the exponential growth phase as well as with the maximal rate of oxygen consumption. These results clearly show that cyanide can be mostly degraded by this microorganism and suggests that the strain may also use cyano-metal complexes, according to the high quantity of heavy metal-cyanide complexes present in the residue from the jewellery industry, in addition to free cyanide.

In addition, the tolerance of the bacterium to heavy metals is also a clear improvement over other microorganism that can degrade cyanide under alkaline conditions but are very sensitive to the presence of heavy metals. This capability, in combination with the ability to use cyanide in the presence of heavy metals and the unusual resistance to cyanide, make this microorganism a very good candidate for the biotreatment of cyanurated residues.

Gold Precipitation:		
Samples	Initial concentration of gold (mg)	Final concentration of gold (mg)
I	100	80
II	100	70
III	Unknown	88



Gold Precipitation In Standard Solution



Gold Precipitation In Effluent

From the calorimetric test, the conformation for the presence of gold was carried out by dissolving the gold in an HCl solution, and adding a limited

amount of tin (stannous chloride) to the solution. Finally, the gold precipitated as colloid of purple gold particles.

By reducing the pH of the solution containing the cells to about 2 or less the binding of gold occurs more. Thus it was found that the adsorption occurs through interaction of the metal ions with functional groups that are found in the cell wall biopolymers of either living or dead organisms.

### Large Scale Testing: Pilot Scale Up

#### Large Scale Proposal

- All the sewages are collected in a collection tank below the ground level.
- Screen grids of various sizes are fixed in different locations in order to segregate the particles entering into the treatment systems and would avoid damages to the mechanical equipment
- The wastewater from the collection well was pumped by means of a sludge pump to the aeration tank
- Submersible diffused aeration systems are used to provide the required dissolved oxygen levels.
- The heating circuit of 0.5 V/cm is passed for 1 min every once in a hour.
- The microorganisms are transferred to the aeration tank.
- The aerobic bacteria utilize the oxygen to break down the cyanide into simpler compounds with a lesser retention time.
- The microorganisms along with semi and fully degraded matter become the biomass at that instant and are the powerhouse of wastewater.
- Retention time is provided in the primary aeration tank for the

microbial culture to degrade the substances.

- The end product of this process is water, natural gases and small amount of biomass.
- The overflow from the aeration tank goes to the settling tank, where large amount of silt is being settled.
- The supernatant from the settling tank goes to the secondary aeration tank where enhanced degradation is achieved.
- The supernatant is allowed to pass to the next tank, which contains the live *Chlorella vulgaris* impregnated with the glass wool culture.
- The pH of the tank was adjusted to 3.
- The glass wool impregnated with the green algae should be replaced once in a month to recover the noble metals.

### Conclusion

This combination of sophisticated engineering and biotechnological effluent treatment methods ensures a hazard free sludge-less and an eco friendly solution for gold mining and milling effluent with less sludge, sludge handling cost will be lowered. This engineering of microorganism has proved to be highly cost effective and successful and these microorganisms' works synergistically to lower the toxicity level in the system and also to recover the precious metals from effluent and to create a healthier and better environment. The use of microorganisms for the purification of gold wastewater allows developing ecologically pure processes to clean cyanide-containing wastewater. This then provides an extremely versatile method for selective precious metal recovery. The successful removal of cyanide in the biological column provides a concept for the biological detoxification of cyanide. The

results indicated that a biological treatment could attain cyanide removal rates superior to other treatment. The recovery of noble metals is a further, considerable benefit of a biological approach. This capability, in combination with the ability to use cyanide in the presence of heavy metals and the unusual resistance to cyanide, make these microorganisms a very good candidate for the bio treatment of cyanurated residues. Thus this is the most promising proposal for treatment of gold effluents at the current scenario.

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