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Isolation of Microorganisms from Gold Tailing Sample

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Gold tailings have high amount of toxic chemicals. Active cyanide is one of the most toxic chemicals found in the tailing samples. Cyanide is used as a laxiviant in Gold extraction, which helps in dissolution of Gold. Some amount of cyanide is left in the effluent after the extraction process. Inspite of being chemically treated before it is released into the environment, some active cyanide remain in the effluent, which are toxic to flora and fauna in that area. An attempt was made to isolate bacteria and fungi growing in these toxic Gold tailing samples. In the present study bacteria and fungi were isolated and identified and their efficiency to grow in toxic gold tailing samples are analyzed through their ability to degrade cyanide. The results can be exploited in the usefulness of these microorganisms in the biodegradation of cyanide.

Key words: Gold tailings, Bacteria, Fungi, Cyanide, Biodegradation, M9 minimal media.

Gold extraction involves use of cyanide which acts as a laxiviant that forms a complex and helps in the dissolution of Gold. It is the most efficient and economic method for gold extraction. There is no economical alternative available other then cyanide for dissolution of Gold. After extraction of Gold, the toxic cyanide is chemically treated by different processes to detoxify it[1]. Cyanide content has to be brought to environmentally safe levels before releasing into the environment. The chemical processes most commonly used by different mines do not completely detoxify the cyanide but instead add other toxic chemicals to the environment.

Cyanide is highly toxic to the living organisms acting as a respiratory poison. It binds to the enzyme Cytochrome C Oxidase and inactivates it, thus affecting the electron transport chain[2]. High amounts of cyanide can cause immediate death[3]. A lower dose of cyanide usually causes general weakness, giddiness, headache, vertigo, confusion and difficulty in breathing. Cyanide also affects the growth of microorganisms. Many of the microbial enzymes are sensitive to toxic cyanide. This reduces the growth of microorganisms in a medium containing cyanide. The microorganisms that are capable of withstanding cyanide are able to grow in the gold tailings.

Microorganisms either employ enzymes to degrade cyanide or they utilize alternative metabolic pathways which are insensitive to it. This helps the growth of microorganisms even in the presence of toxic cyanide. Isolation of such microorganisms from the Gold tailing samples can be commercially exploited for the biodegradation of cyanide. Biodegradation will not only be cost effective but also eco-friendly, which is the need of the hour.

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Microorganisms generally utilize one or more of the metabolic pathways to degrade cyanide[4].

MATERIALS AND METHODS

Gold Tailing Samples

Gold tailing samples were procured from Hutti Gold Mines Company Limited, Raichur, Karnataka, INDIA. Two types of gold tailing samples such as fresh gold tailings (that were recently processed) and old gold tailings (that were sorted in heaps at the mining site) were collected to be used in the present study. Gold tailing samples were analyzed for cyanide content and physical properties. Cyanide content was estimated by titrating against silver nitrate using Rhodanine indicator, p-dimethyl-amino-benzalrhodanine[5].

Isolation of Bacteria from Gold Tailing Samples

One gram of gold tailing sample was taken and mixed with 100ml distilled water. The soil was allowed to settle and then serially diluted. Pour plate and spread plate methods were used to isolate bacteria on nutrient agar. The serially diluted sample (0.1ml) was poured and spread on nutrient agar plates maintained at pH 7.2. The inoculated plates were incubated at temperature of 37^0 C for 24 hours. The bacterial cultures were characterized by Gram's staining.

Isolation of Fungi from Gold Tailing Samples

One gram of gold tailing sample of both fresh and old samples were taken and mixed with 100ml distilled water. The samples were then serially diluted, before inoculating in the potato dextrose agar. The pH of the media was adjusted to 6.0. The plates were then incubated at room temperature for 48-72 hours. Fungal cultures were identified by staining with cotton phenol blue.

Isolation of Bacteria and Fungi Growing in Presence of Cyanide

Bacterial and fungal cultures insensitive to cyanide were isolated from gold tailing samples, following dilution by both pour plate and spread plate method, with nutrient agar, potato dextrose agar and then M9 minimal media agar plates supplemented with increasing concentrations of sodium cyanide (5mM, 10mM, 15mM and 20mM). Sodium cyanide was added to the sterilized agar medium just before it solidified. Sodium cyanide was added as a sole source of Nitrogen to the M9 minimal media. Growth of bacterial colonies and fungi in cyanide containing media was taken as evidence of cyanide resistance, attributed either to their ability to biodegrade cyanide or presence of alternative metabolic pathways which are insensitive to cyanide.

RESULTS AND DISCUSSION

Cyanide content in the gold tailing was found to be 15mg in fresh sample and 10mg in old sample. Some of the properties of both the tailing samples are listed in table no.1 and table no.2.

A variety of microorganisms were isolated from gold tailings on nutrient agar medium and potato dextrose agar. Bacterial species were isolated on nutrient agar medium maintained at pH 7.2 and inoculated at 37^0C for 24hours. The isolated cultures were characterized by colony characters and Gram staining. Out of fourteen isolates of bacteria, six samples were Gram positive bacilli, four samples were Gram negative bacilli, two were Gram positive cocci and two were Gram negative cocci.

Out of fourteen bacterial isolates only two samples grew on M9 minimal agar medium supplemented with increasing concentrations of cyanide. Cyanide alone as the sole source of carbon and nitrogen in M9 minimal media was not sufficient to support the growth of these bacteria.

Table 1. Analysis of Gold Tailing (Fresh Sample)

S. No.	Parameters	Units (mg/ml)
1.	Color	Grey
2.	Odor	Odorless
3.	pH	7.5
4.	Cyanide Content	15
5.	Particle size	75µm

Table 2. Analysis of Gold Tailing (Old Sample)

S. No.	Parameters	Units (mg/ml)
1.	Color	Brown
2.	Odor	Odorless
3.	pH	7.0
4.	Cyanide Content	10
5.	Particle size	75µm

Table 3. Characteristics of Bacterial Isolates from Gold Tailing Samples

Colony No.	Shape & Margin	Elevation & Texture	Color	Opacity	Gram nature	Growth in presence of Cyanide
1	Circular & irregular	Flat & rough	White	Opaque	Gram positive bacilli	Negative
2	Circular & regular	Lobed & smooth	White	Opaque	Gram positive bacilli	Negative
3	Circular, irregular & dented	Flat & smooth	Cream	Opaque	Gram positive bacilli	Negative
4	Circular & regular	Flat & rough	White	Opaque	Gram positive bacilli	Negative
5	Circular & regular	Lobed & smooth	Cream	Opaque	Gram positive strepto bacilli	Negative
6	Circular & irregular	Flat & rough	Cream	Opaque	Gram positive bacilli	Negative
7	Circular & irregular	Lobed & smooth	White	Opaque	Gram negative bacilli	Positive*
8	Circular & regular	Flat & smooth	Orange	Opaque	Gram negative bacilli	Negative
9	Circular & regular	Flat & smooth	Cream	Opaque	Gram negative bacilli	Positive*
10	Circular & regular	Lobed & smooth	Cream	Opaque	Gram negative bacilli	Negative
11	Circular & irregular	Flat & Lobed	Cream	Opaque	Gram positive cocci	Negative
12	Circular & regular	Flat & Lobed	Cream	Opaque	Gram positive cocci	Negative
13	Circular & irregular	Lobed & smooth	Yellow	Opaque	Gram negative cocci	Negative
14	Circular & regular	Lobed & smooth	Cream	Opaque	Gram negative cocci	Negative

*Bacterial culture grew in presence of cyanide

Table 3. Fungal genera isolated from gold tailing samples

Sample No	Type of Fungi
1	*Aspergillus***
2	*Fusarium*
3	*Penicillium*
4	*Cladosporium***

**Fungal genera grew luxuriantly in presence of cyanide

Hence 50mM sodium acetate was added to the culture medium as a source of carbon. Cyanide is probably used as a source of nitrogen by microorganisms. The pH of the media was maintained at 9, to avoid loss of cyanide due to natural volatilization. Gram staining revealed that both the cultures were Gram negative bacilli. Out of the two bacterial samples, one of the species showed efficient degradation of cyanide in batch culture in M9 minimal broth containing cyanide in

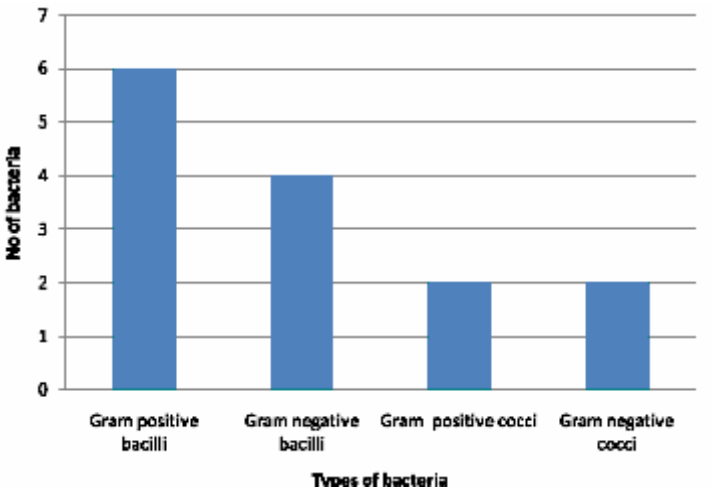

Fig. 1. Types of bacteria isolated from gold tailing sample

alkaline pH. Bacteria are mainly known to utilize hydrolytic and oxidative pathways to degrade cyanide and utilize it as nitrogen source[6, 7].

The fungal species which grew on potato dextrose agar medium at pH 6 and inoculated at room temperature for 48 to 72 hours included *Penecillium*, *Aspergillus*, *Fusarium* and *Cladosporium*. Out of the four fungi, only *Aspergillus* and *Cladosporium* were able to grow in the M9 minimal media in presence of sodium cyanide at alkaline pH of 9. Alkaline pH was maintained to minimize loss of cyanide due to natural volatilization. Fungal cultures grew in M9 minimal media supplemented with 50mM sodium acetate as source of Carbon. The above two fungal cultures were able to withstand cyanide toxicity. *Cladosporium* is reported to bind to metal cyanides[8]; hence it might be useful in biosorption, for metal cyanide recovery. Fungi mostly utilize hydrolytic pathway to degrade cyanide[9, 10].

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