**Bioremediation of soil and water polluted by Cyanide: a review**

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# Abstract

Cyanide is a chemical that is widely distributed in the environment, mainly as a result of anthropogenic activities. Only small quantities are naturally produced. Most industrial activities use this chemical compound for manufacturing a product as electroplating or for extracting gold. Exposure to cyanide results in negative health impacts to the wildlife and humans. In nature, cyanide occurs in several species, of which the free cyanide forms are the most toxic ones. Cyanide can be removed by chemical or biological processes. Biological treatment called “bioremediation”, which is cost-effective and eco-friendly, is the most applied process to remove cyanide from contaminated environments. This technology focused by the use of microorganisms to remove pollutants. Many microorganisms have been reported to transform the cyanide in another less toxic compound, or to consume cyanide for their growth. The reactions are influenced by environmental parameters such as pH and temperature and by the nutriment availability.

**Keywords:** Biotreatment, chemical compound, environment, microorganism.

# Introduction

Cyanide is a chemical product that is universally recognized as poison (Morocco 2005). It is highly toxic to living organisms (World Health Organization 2004). Many researches on the toxicity and the removal of cyanide have been published in the literature. Different technologies are available for cyanide removal. such as: alkaline chlorination or biological oxidation processes (Young and Jordan 1995; Patil and Paknikar 2000) and acidification and/or destruction by chemical oxidation (Young and Jordan 1995; Akcil 2003). Among these technologies, the most used one is chemical oxidation (Dubey and Holmes 1995,Young and Jordan 1995,Botz 2001,Parga *et al*. 2003, Roshan *et al*. 2009)

However, only few articles have reported the potential for bioremediation of cyanide. The present work reviews these articles by comparing the efficiency of microorganisms that have high potential for the bioremediation of cyanide-contaminated environments. It will first discuss on the sources, uses, and toxicity of cyanide, and then will address the bioremediation technologies completed with the bioremediation potential of investigated microorganisms.

# Cyanide

Cyanide is a group of compounds which contains a C≡N group: one atom of carbon linked with one atom of nitrogen by three molecular bounds. Cyanide compounds are usually categorized into 3 groups: the first group called free cyanide is related to the cyanide ion CN- (produced by the dissolution of sodium or potassium cyanide in water) and the hydrogen cyanide gas (HCN); the second group is related to weak and moderately strong complexes formed between cyanide ion and some metals such as Zn, Ni, Ag, Cd, Hg; the third group is related to strong complexes formed between cyanide ion and Fe ion (Botz *et al*. 2005, Nsimba 2009). Other forms of cyanide include cyanates and nitriles.

## Sources

The term cyanide refers to all of the cyanide compounds that can be determined as the cyanide ion (CN−) (Franson 1992, Donato *et al*. 2007). Cyanide is produced by both natural and anthropogenic processes.

### Natural processes

Cyanide is produced naturally in the environment by various bacteria, algae, fungi and numerous species of plants including beans, fruits, vegetables and roots. Today, cyanogenic compounds can be found in more than 3000 species of plants, animals, microbes and fungi (Ward and Lebeau 1962, Stevens and Strobel 1968). Many common plants contain the natural form of cyanide, cyanic glucoside (Aazam 2014). Several plants produce cyanides, however in most cases; cyanide is present in extremely small quantities.

Incomplete combustion during forest fires is believed to be a major environmental source of cyanide, and incomplete combustion of substances containing nylon produce cyanide through depolymerization (Li *et al*. 2000).

### Anthropogenic processes

Significant quantities of cyanide is a byproduct of various industrial processes, including coal coking, coal gasification and steel manufacturing as well as petroleum refining (Nsimba 2009). Cyanide also originates from metal finishing, ore extraction, and hydrometallurgical industries (Aazam 2014).The principal anthropogenic forms of cyanide are hydrogen cyanide (HCN), cyanogen sodium (NaCN) and cyanogen potassium (KCN). Anthropogenic inputs of cyanide into the environment are greater in quantity than natural inputs (Nsimba 2009).The process of degassing coal produces a raw gas containing hydrogen sulfide (H2S) and HCN. At US gas (work) sites it is typical to use 8–21 kg of gas purification material per 1000 m3 of gas produced (Theis *et al*. 1994, Kjeldsen 1999). The spent iron ore contains high quantities of sulfur (typically 40–50%) and substantial quantities of cyanide (typically 1- 2% by weight) (Young and Theis 1991, Theis *et al*. 1994, Kjeldsen 1999). During the electroplating process, the degreasing bath contains potassium or sodium cyanide and sodium hydroxide (Mohler 1969, Kjeldsen 1999). In gold mine extraction, tailing ponds containing gold mine wastes are sources of cyanide contamination (Alesii and Fuller 1976, Thompson and Gerteis 1990; Boucabeille *et al*. 1994, Kjeldsen 1999). Besides, in the artisanal small scale gold mining area, water and soil those have been analyzed were contaminated by the cyanide in all of sampling points that were heterogeneous distributed into a catchment area nerveless the few cyanidation ponds observed (Sawadogo 2015).

## Uses

Plants produce cyanide as a defense mechanism against herbivores (Jones 1998, Nsimba 2009, Randviir and Banks 2015). Cyanide is used by humans in many cases. Every year, in industry, massive quantities of cyanides are used in metal extraction, electroplating, pesticides, metal hardening, photography, printing, dyeing, and many other manufacturing processes. It is also used in the production of organic chemicals such as nitrile, nylon, and acrylic plastics (Aazam 2014).

The use of cyanide also facilitates the storage of salt. Potassium ferrocyanide (K4Fe(II)(CN)6) and sodium ferrocyanide (Na4Fe(II)(CN)6) in maximum concentrations of 200 mg kg−1 have been used as anti-clumping additives in road salt in order to facilitate handling and distribution (Ohno 1990, Kjeldsen 1999).

Cyanide is also used in the chemical extraction of gold from low-grade ores by the heap leach process (White and Markwiese 1994, Kjeldsen 1999). This is the predominant process in the gold extraction industry that has been applied commercially since 1887 (Adams and Lloyd 2008). Another use of cyanide is for war. Cyanide is a likely weapon for terrorists due to its notoriety, lethality, and availability. Battlefield use of cyanides was proposed by Napoleon III during the Franco-Prussian war, to improve the lethality of bayonets. The French introduced gaseous HCN to World War I in 1915, and used 4000 tons in battle (Morocco 2005). HCN gas was used in the gas chambers in the World War II holocaust, in prison for the execution of criminals with death sentences, and also as a chemical warfare agent (Nsimba 2009).

## Toxicity

In nature, various forms of cyanide are present depending on the environment. The most toxic form is free cyanide.

Humans and the environment are highly affected by cyanide. Cyanide is the most significant contaminant that affects wildlife mortality ( Henny *et al.* 1994, Donato *et al.* 2007). The most important exposure routes to humans are: ingestion and dermal contact, inhalation of volatilized cyanide, and groundwater exposure (Wiemeyer *et al.* 1985, Henny *et al.* 1994, Minerals Council of Australia 1996, Ryan and Shanks 1996, Kjeldsen 1999, Donato *et al.* 2007).

Other potential effects can occur on terrestrial species (plants and animals) and on surface water species (by recharge of cyanide containing groundwater to surface waters) (Henny *et al*. 1994, Kjeldsen 1999, Donato *et al.* 2007). Hydrogen cyanide and other cyano-compounds that liberate free cyanide ions are highly toxic to almost all forms of fauna (Souren 2000). The toxicity is related to the inverse of the bond strength of metal atoms and cyanide ligands (Klenk *et al*. 1996, Sadler 1990, Staunton and Jones 1989).

Many researchers have reported the lethal toxicity of several cyanide complexes to birds (Barcroft 1931, Davis 1981, Eisler 1991a,Eisler 1991b, Reece 1997). Lethal limits varied between species (Table 1 ) (Donato *et al.* 2007)

Table 1 : Effects of free cyanide on some birds and other animals (Christel and Eyer 1977, Bapat and Abhyankar 1984, Ballantyne 1987, Eisler 1991a, Eisler 1991b, Hagelstein 1997, Donato *et al.* 2007)

|  |  |  |
| --- | --- | --- |
| **Species** | **Dose** | **Comment** |
| Mallard Duck | 0.53 mg CN/Kg Bird Weight (BW)  1.43 mg CN/Kg BW | No deaths  Lethal Dose (LD) 50 (C.I at 95% 2.2 to 3.2) |
| Turkey Vulture | 36 mg NaCN/Kg BW | Average time of death was 19 min |
| Rock Dove | 1.6 mg CN/Kg BW | Minimum LD |
| Black Vulture | 2.54 mg CN/Kg BW  3.7 mg CN/Kg BW | Acute oral LD50  All dead within 16 min |
| Japanese Quail | 4.5 mg CN/Kg BW | Acute oral LD50 for adult females |
| American Kestrel | 2.12 mg CN/Kg BW | Acute oral LD50 |
| Domestic Chicken | 11.1 mg CN/Kg BW | Acute oral LD50 |
| European starling | 9.0 mg CN/Kg BW | Acute oral LD50 |
| Cattle | 200 mg HCN/kg BW | Lethal |
| Dog | 24 mg NaCN/Kg BW | Lethal single dose |
| Mouse | 8.5 mg CN/Kg BW | LD 50 lethal single dose |
| Rat | 5.1-5.7 mg NaCN/Kg BW | LD 50 lethal single dose |

**Humans**

Human can be exposed to cyanides by breathing air and drinking water, touching soil or water containing cyanide, or eating foods that contain cyanide (ATSDR 2006), with potentially lethal results (table 2).

Table 2 : Lethal dose of cyanide for human depending on exposure way

|  |  |  |
| --- | --- | --- |
| **Exposure way** | **Lethal dose** | **Sources** |
| Inhalation | 200 – 314 mg HCN/m3 | (Chaumont and Weil 1960 , Yacoub *et al.* 1974) |
| Ingestion | 0.56 – 1.52 mg CN-/kg | (Gettler and Baine 1938 , United State Environmental Protection Agency 1987) |
| Dermal contact | 100 mg CN-/kg | (Rieders 1971) |

## Physical and chemical treatments of cyanide

Cyanide could be removed by physical, chemical or biological treatments. Natural cyanide attenuation is also possible.

The physical and chemical treatments of cyanide operate on the principle of converting cyanide into a less toxic compound through an oxidation reaction. Several destruction processes are well proven to produce treated solutions or slurries with low levels of cyanide as well as many metals: alkaline chlorination process (Dubey and Holmes 1995,Young and Jordan 1995, Botz 2001b, Parga *et al*. 2003, Dash *et al.* 2009), sulfur dioxide and air process, copper-catalyzed hydrogen peroxide process, Caro‘s acid process, the iron-cyanide precipitation, activated carbon polishing, ion exchange, reverse osmosis, ozonation, etc. (Ackil 2003).

Most of these methods are expensive and have several disadvantages (Wild *et al*. 1994). For example, alkaline chlorination process is not effective in the case of cyanide species complexed with metals such as nickel, silver, etc. due to slow reaction rates (Patil and Paknikar 2000). The process also produces sludge, which requires specific license for disposal. Another disadvantage is that it is relatively expensive due to the quantity of chlorine required. Further, the addition of excess chlorine increases the total solids content of water, making it undesirable for recycling and reuses purposes and leaves a residue with a high chlorine content which is toxic to aquatic life (Kao *et al.* 2003, Kao *et al.* 2006). In addition, various chlorinated organics may be produced if the wastewater contains organic substances (Dash *et al.* 2009).

## Natural cyanide attenuation

It is well reported that cyanide solutions placed in ponds or tailings impoundments undergo natural attenuation reactions, which result in the decrease of the cyanide concentration. These attenuation reactions are dominated by natural volatilization of hydrogen cyanide, but other reactions such as biological degradation, oxidation, hydrolysis, photolysis and precipitation also occur (Botz *et al.* 2005). At several sites, ponds or tailings impoundments are intentionally designed to maximize the rate of cyanide attenuation. Advantages of natural attenuation include lower capital and operating costs when compared to chemical-oxidation processes. (Ackil 2003)

# Bioremediation of cyanide

Cyanide is a chemical compound that microorganism or plants can transform to another compound less toxic. Usually, microorganisms or plants are used for remediating environments polluted by cyanide. Bioremediation refers to the use of microorganism (Elkins 2013) and phytoremediation refers to the use of plants. Biological methods are preferred for cyanide removal because of their low operation cost, their ability to remove a wide range of cyanide compounds, and their ability to produce high quality effluents (Botz *et al.* 2005).

## Biodegradation mechanism

There are many groups of microorganism discovered which can transform simple or complex cyanide compounds, including bacteria such as *Klebsiella oxytoca* (Chen *et al*. 2008), *Pseudomonas fluorescens* P70 (Dursun *et al*. 1999),fungus such as *Fusarium solani* (Barclay *et al*. 1998), *Fusarium oxysporum (Akinpelu et al.* 2015) and algae such as *Scenedesmus obliquus* (Gurbuz *et al.* 2009)

Cyanide is used as a nutrient by the bacteria for their growth, acting as nitrogen source. Some bacteria are able to use cyanide compounds as both a carbon and nitrogen source. Therefore, supply of external carbon source is no longer needed for these bacteria. Other bacteria need glucose as carbon source for survival in presence of cyanide (Dursun *et al.* 1999, Bouari 2012).

The biodegradation occurred into two steps:

The first step is the oxidative breakdown of cyanides, and subsequent sorption and precipitation of free metals into the biofilm.. Cyanide and thiocyanate are then converted to ammonia, carbonate and sulfate (Ackil 2003)

In the second step, ammonia is converted to nitrate through the conventional two step nitrification process shown below:

The ease of degradation of metal cyanides depends on their chemical stability: free cyanide is the most readily degradable, followed by metal cyanide complexes of Zn, Ni, and Cu; iron cyanide the least degradable (Mudder *et al.* 1998).

## Bioremediation capacity

Most of the reported studies on bioremediation have focused on: correlation of the growth kinetics of the bacteria and the rate of cyanide removed, evaluation of the environmental parameters on the degradation of different cyanide compounds or determination of the minimum inhibitory concentration (MIC) cyanide compounds for the microorganism. Table 3 and 4 below show a comparison of studies evaluating cyanide biological transformation, respectively in water and in soil

In tables 3 and 4, the same bacteria were used for biological treatment of cyanide but the potential effectiveness varies depending on the composition of the medium, type and initial concentration of cyanide and organic matter, pH, and temperature.

In the water, the optimal condition is formed by a pH ranging from 5.2 to 10.5 for bacteria, from 6 to 8.5for fungus and pH 12 for plant. Temperature is usually held between 25 – 50°C, with the majority around 30°C for bacteria, 43°C for fungus and 40°C for plants. Cyanide hydratase is often used by microorganism as enzyme for degrading cyanide. The potential cyanide degrading bacteria is formed by *Pseudomonas Fluorescens* NCIMB 11764, its performance is bewteen 105 – 706 µmol min-1 for degrading free cyanide with or without enzyme as catalyzer (Kunz *et al.* 1992; Kunz *et al.* 1998). The mixed bacteria composed by *Klebsiella pneumoniae* and *Ralstonia sp.* have also a high potential with a velocity 1042 µg L-1 min-1 for degrading thiocyanate (Chaudhari and Kodam 2010). For Fungus, *Gloeocercospora sorghi* is the most effective. Its maximal velocity is 4.4 mmol min-1 mg-1(Jandhyala 2002) (Basile 2008).

In the soil, the optimal condition is formed by a pH around 7 for bacteria and pH 4 for fungus. The cyanide degrading bacteria have a temperature between 30-37°C. But, for fungus, it stays around 30°C. For degrading cyanide, bacteria and fungus use various enzymes as: thiocyanate hydrolase, cyanidase and hydratase amidase. Microorganisms have a faculty to degrade strong acid dissociable cyanide in the soil than in the water. The potential cyanide degrading bacteria is formed by *Pseudomonas putida.* (Bipinraj *et al.* 2003). The fungus, *Fusarium oxysporum N-10* is the most effective with a velocity 0.02 mM Day-1 and 1 mM Day-1 respectively in the mixed (Barclay *et al.* 1998)and single culture(Yanase *et al.* 2000)*.*

Glucose was often used as organic matter in water and soil and the final product of biodegradation is formed by ammonia or ammonium. While earlier studies only focused on the microorganism application, after the discovery of co-culture bacteria or fungus, many current studies are focused on the addition of agricultural wastes or wastes in the microorganism mixed culture.

Research about phytoremediation is not investigating deeply.

Table 3 : Comparison of potential cyanide bioremediation in water

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Microorganism | Enzyme | Origin | Compound to be removed | Optimum condition | Degradation efficiency | Final Product |
| Bacteria | | | | | | |
| *Thiobacillus intermedius*  (Singleton and Smith 1988) | Rhodanese | Salt swamp  Salt water | CN- / 50mM | Salt swamp : pH 8.1  Salt water : T 25°C | 0.021µmol/min (without enzyme)  0.042 µmol /min (with enzyme)  0,015 µmol/min | SCN-  Sulfite ) |
| *a. Klebsiella sp.*  *b. Klebsiella pneumoniae*  *c. Pseudomonas putida*  (Silva-avalos *et al.* 1990) | Nhase | Creek water | Tetracyanonickelate (II){K2[Ni(  CN)4] (TCN)  KCN | 0.25 – 16 mM TCN  0.25 mM KCN / T 41°C |  | Ni(CN)2 |
| *Pseudomonas Fluorescens* NCIMB 11764(Kunz *et al.* 1992) |  |  | KCN/50mM | 20 - 50 mM  pH 7 / T 31°C | 85%/6h (aerobic condition)  89%/12h (anaerobic condition) | Formamide(HCNOH2)  or formate (HCOO-) |
| *Pseudomonas Fluorescens* NCIMB 11764(Kunz *et al.* 1998) | Cyanide oxygenase Keto-acid 23mM |  | HCN | pH 7  T 30°C | 760 µmol /min ml (after 72 hours) (without acid ) | NH3 |
| *Pseudomonas ﬂuorescens* NCIMB 11764 (Fernandez *et al.* 2004) | cyanide oxygenase |  | KCN | 10 – 50 µmol  T 30°C | 90 – 100% | HCOO- |
| a. *Neurospora crassa,*  *b.Gibberella zeae,*  *c.Aspergillus nidulans,*  (Basile 2008) | Cyanide hydratase | Waste water | KCN 20mM  Metal-cyanide complexes | pH: 5,2 – 9 (a) / 6 – 8,5 (b)  7,5 (c) / 6 – 7 (a), (b), (c)  T 27- 43°C | < 80% (a), (b), (c) (after 48 hours) |  |
| *Thiobacillus thioparus* THI115  (Yamasaki *et al.* 2002) | thiocyanate hydrolase | Lake water | SCN- | T 30°C | 93% (in 38 h) | Carbonyl sulﬁde(COS) |
| *Bacille sp*.(*Bacille safensis, Bacille lichenformis, et Bacille tequilensis*  (Mekuto *et al.* 2013) |  |  | Cyanide compounds | T 37°C | 65.5% (200 mg CN-/L)  44.3% (400 mg CN-/L) |  |
| *Micromonospora braunna*  (Shete and Kapdnis 2012) | Cyanide hydratase | Garden soil | KCN (N source) : 10-1000ppm  Dextrose (C source) | T 30°C (aerobic condition) | 98.79 % (pour 100ppm in 18hours) | HCOOH  NH3 |
| *Bacillus safensis + Bacillus lichenformis + Bacillus tequilensis*  (Mekuto *et al.* 2013) |  | Wastewater | KCN: 200 and 400 mg CN-/L | T 37°C | 65.5% (over 8 days) for 200 mg CN-/L  44.3% (over 8 days) for 400 mg CN-/L |  |
| *Bacillus safensis + Bacillus lichenformis + Bacillus tequilensis + Agrowaste (Ananas comosus extract: 1% v/v, Beta vulgaris extract:1% v/v, Ipomea batatas extract: 1% v/v, spent brewer’s yeast: 1% v/v, and whey: 0.5% w/v)*  (Mekuto *et al.* 2013) |  | Wastewater | KCN: 200 and 400 mg CN-/L | T 37°C pH = 9.5 | 89.5% (over 8 days) for 200 mg CN-/L  59.75% (over 8 days) for 400 mg CN-/L |  |
| *Burkholderia cepacia C-3*(Adjei and Ohta 2000) |  |  | Free cyanide 260 mg/L  Fructose | T 30°C pH =10 | 80% |  |
| *Pseudomonas sp.*(Kao *et al.* 2003) |  |  | Free cyanide 100 mg/L  Lactate, Sucrose | T 28-30°C pH 9-9.2 | 60% |  |
| *Pseudomonas sp.*(Akcil *et al*. 2003) |  |  | Free cyanide 400 mg/L  Whey | T 30°C pH 9-9.2 | 89% |  |
| *Klebsiella oxytoca*(Kao *et al.* 2003) |  |  | Free cyanide 21 mg/L  Glucose | T 30°C pH 7 | 99.9% |  |
| *Trametes versicolor* |  |  | Free cyanide 400 mg/L  Citrate | T 30°C pH 10.5 | 30%(Cabu *et al.* 2006)  100% (after 42 hours)(Akinpelu *et al.* 2015) | Ammonium Nitrogen (NH4+-N) |
| *Klebsiella oxytoca*(Chen *et al.* 2008) | Nitrogenase |  | Free cyanide 157 mg/L  Glucose | T 30°C pH 7 | 26% |  |
| *Pseudomonas*  *pseudoalcaligenes CECT5344*(Huertas *et al.* 2010) |  |  | Free cyanide 40 mg/L  Acetate | T 30°C pH 9.5-10 | 99.9% |  |
| *Klebsiella pneumoniae + Ralstonia sp.*  (Chaudhari and Kodam 2010) | Thiocyanate hydrolase | Wastewater (Effluent industrial sites) | KSCN (Thiocyanate) | T 37ºC pH 6.0 | 500 - 2500 mg /L/day | H2S |
| *Bacillus sp. CN-22*  (Wu *et al.* 2014) | Cyanide dihydratase | Wastewater (Electroplating effluent) | HCN 700 mg/L | T 31 °C pH, 10.3 | 200 - 6.62 mg/L/72h | HCOOH  NH3 |
| *Bacteria + cassava peels*  (Siller and Winter 1998) |  | Wastewater | KCN | T 25-37°C  pH 6-7.5 | 400 mg CN-/L/day | HCOO- (formate)  NH3 |
| *Enterobacter sakazakii* (a)  *Azotabacter sp* (b)  *Rhizobium sp* (c)  (Ninan *et al.* 2013) |  | Wastewater (effluent de Sago) | KCN | MIC 5000ppm (a)  MIC 50 ppm (b), (c) | 99% (after 96hours) |  |
| *Pseudomonas fluorescens+ Chlorella vulgaris.*  (Kiruthika 2008) |  |  | Cyanide 0.5mg + glucose 1g (a)  Cyanide 0.5mg + glucose 1g + NaCl 1g (b)  Cyanide 1mg + glucose 1g + NaCl 1g (c)  Cyanide 1mg + glucose 1g + NaCl 1g (d) | T 30°C pH 7.2 (a), (b)  T 30°C pH 8.5 (c), (d) | 60% (a)  58% (b)  54% (c)  51% (d) |  |
| Fungus | | | | | | |
| *Fusarium solani*  (Barclay *et al.* 1998) | Cyanide hydratase |  | KCN 80mM |  | Km: 4.7mM  Vmax : 1.7 microM min-1 mg-1, |  |
| *Gloeocercospora sorghi*  (Jandhyala 2002) | Cyanide hydratase |  | KCN 30mM |  | Km: 90mM  Vmax: 4.4 mmol min-1 mg-1 |  |
| *Gloeocercospora sorghi*  (Basile 2008) | Cyanide hydratase | Wastewater | KCN 20mM  Metal-cyanide complexes | pH: 6 – 8,5 / T: 27- 43°C | < 80% (after 48 hours) |  |
| *Aspergillus awamori*  (Santos *et al.* 2013) | Nitrilase | Wastewater | KCN 0-475 ppm | Citrus peel , T : 45 -50 ˚C and pH: 4.0 to 5.5 |  |  |
| *Fusarium oxysporum + Beta vulgaris(Agrowaste)*  (Akinpelu *et al.* 2015) |  | Gold mining wastewater | Metal cyanide +  KCN 500 mg CN-/L |  | 83 – 263 mg F-CN/L | 120- 210 mg NH4  +-N/L |
| Plants | | | | | | |
| *Citrus sinensis*  ( Santos *et al.* 2013) |  | Citrus sinensis solide waste | Free cyanide (F-CN) 100 mg /L + 0.1% (w/v) of unhydrolysed *Citrus sinensis* (a)  Free cyanide (F-CN) 100 mg F-CN/L + 0.1% (w/v) of unhydrolysed *Citrus sinensis* (b)  F-CN + heavy metals 10 mg/L (c) | T 50°C pH 12 (a) et (b)  T 40°C pH12 (c) | 17.82% (a)  62.48%, (b)  26.35% (c) |  |

Table 4 : Comparison of reports on cyanide biological transformation in soil

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Microorganism/plants | Enzyme | Source | Compounds to be removed | Optimum condition | Degradation efficiency | Final Product |
| Bacteria | | | | | | |
| a*.Pseudomonas putida*  *b.Pseudomonas picketti*  *c.Klebsiella pneumonia*  (Silva-avalos *et al.* 1990) |  | Sewage sludge (a)  Soil (b), (c) | TCN  KCN | 0.25 – 16 mM TCN  0.25 mM KCN  T 41°C (with use of benzyl-amine for (a) ad |  | Ni(CN)2 |
| *Thiobacillus thioparus* THI115  (Yamasaki *et al.* 2002) | thiocyanate hydrolase | Soil | SCN- | T 30°C | 93% (in 38 h) | COS |
| *Pseudomonas putida*  (Bipinraj *et al.* 2003) |  | Wet soil | SCN- 2mM, KCN 0.2 mM, cyanocuprate (TCC) 0.5 mM, tetracyanonickelate (TCN) 0.5 mM  Glucose 2mM or  Ferrous sulphide 1% or  Thiosulphate 1% | Alkali condition (4% Nacl)  pH 7,5 ,T 30°C | With glucose :  99% TCC (in 109 cells/ml, 4 h)  92% TCN (in 109 cells/ml, 4 h)  95% KCN (in 109 cells/ml, 6h)  96% SCN-  (in 109 cells/ml, 6h)  With ferrous sulphide :  81% KCN (in 6h)  91% TC (in 9h)  With thiosulfate :  40% TC (in 72 h) |  |
| *Alcaligenes xylosoxidans subsp*  (Ingvorsen *et al.* 1991) | cyanidase | soil | HCN | T 37°C | 1% (in 55h) | HCOO-  NH3 |
| Fungus |  |  |  |  |  |  |
| *Fusarium oxysporum N-10*  (Yanase *et al.* 2000) | Hydratase  Amidase | Soil | Tetracyanonickelate II (TCN) 0.5mM and 20mM | T 30°C | 20-30% (1 week) (for 0.5mM TCN)  30% (6 days) (for 20 mM TCN) | HCOOH, HCOO-, NH3 |
| *Fusarium solani + Trichoderma polysporum*  (Barclay *et al.* 1998) |  | Gasworks site soil | Tetracyanonickelate K2Ni(CN)4 0.25mM (a)  Hexacyanoferrate K4Fe(CN)6 0.5mM (b) and K3Fe(CN)6 0.5mM (c) | pH 4 | 95% (b), (c): after 28 days  90% (a): after 28 days |  |
| *Fusarium oxysporum + Scytalidium thermophilum + Penicillium miczynski*  (Barclay *et al.* 1998) |  | Gasworks site soil | Hexacyanoferrate K4Fe(CN)6 0.5mM | pH 4 | 32% after 28 days |  |

## Bioremediation technologies

More choice of bioremediation technologies are existed for removing cyanide. It could be conducted in-situ or ex-situ or by using bioreactors (Sharma 2012). Each method has its specificity and most of them are cost-effective as shown as in Table 5, which summarize the advantages of the different technologies, and their conditions of application.

Table 5 : Methods applied in bioremediation (Vidali 2001, Shukla *et al.* 2010, Sharma 2012)

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Technique | Examples | Advantages | Conditions of application | References |
| In situ | Biosparging  Bioventing  Bioaugmentation | Most efficient Non Invasive  Relative passive  Naturally attenuated process, treat soil and water | Biodegradation abilities of indigenous microorganisms  Presence of metals and inorganic compounds  Environmental parameters Biodegradability of pollutants  Chemical solubility  Geological factors Distribution of pollutants | (Bouwer and Zehnder 1993, Colberg and Young 1995, Niu *et al*. 2009) |
| Ex-Situ | Land farming (Solid-phase treatment system)  Composting (Anaerobic, converts solid organic wastes into humus-like material)  Biopiles | Cost efficient ,Simple, Inexpensive ,self-heating  Low cost Rapid reaction rate, Inexpensive, self-heating  Can be done on site | Surface application, aerobic process, application of organic materials to natural soils followed by irrigation and tilling  To make plants healthier good alternative to land filling or incinerating practical and convenient.  Surface application, agricultural to municipal waste | (Antizar-Ladislao *et al.* 2007, Antizar-Ladislao *et al.* 2008) |
| Bioreactor | Slurry reactors  Aqueous reactors | Rapid degradation kinetic Optimized  environmental  parameters  Enhances mass transfer Effective use of inoculants and surfactant | Bioaugmentat Toxicity of  amendments  Toxic concentrations of contaminants | (Behkish *et al.* 2007) |

# Conclusions

This review summarizes the bioremediation technologies applied for cyanide decontamination. Potentiality of bioremediation technologies depends on the existence of cyanide degrading bacteria population; the availability of cyanide as contaminant and the environment factors. Bioremediation is a natural process; it takes a little time, as an acceptable waste treatment process for contaminating material such as soil. Bioremediation also requires a very less effort and can often be carried out on site. It is a cost effective process than the other conventional methods that are used for cleanup of hazardous waste and it does not use any dangerous chemicals. Bioremediation technologies could be applied in large scale and in different contaminated unit by cyanide as liquid, solid and gas industrial wastes. Nevertheless, the choice of single or mixed microorganism is very important for applying bioremediation.

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