

BIOLOGICAL REMEDIATION OF CYANIDE: A REVIEW

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ABSTRACT

Cyanide and its complexes are produced by industries all over the world as waste or effluents. Biodegradation is considered to be the cheapest and the most effective method to clean-up cyanide from the environment. Several studies on different types of microorganisms that can degrade cyanide in the environment have been carried out. Hydrolytic, oxidative, reductive and substitutive/transfer reactions are some of the common pathways used by microorganisms in cyanide degradation. Biodegradation of cyanide can occur aerobically or anaerobically depending on the environmental conditions. Immobilized enzymes or microorganisms prove to be very effective method of degradation. Microorganisms such as *Klebsiella oxytoca*, *Corynebacterium nitrophilous*, *Brevibacterium nitrophilous*, *Bacillus* spp., *Pseudomonas* spp. and *Rhodococcus* UKMP-5M have been reported to be very effective in biodegradation of cyanide.

Keywords: biodegradation, cyanide, environment, microorganisms

INTRODUCTION

Carbon and nitrogen elements form cyanide. They are ubiquitously found in the environment. Amygdalin, which is found in vegetables, fruits, seeds, cashew nuts, cherries, apricots and bean sprouts is a natural source of hydrogen cyanide (HCN) (Mark *et al.* 1999). Chemical treatment is the most widely used method in the degradation of industrial effluents containing cyanide. But, this treatment is very expensive and sometimes inefficient in the process and thus requires an alternative means of treatment (Patil & Paknikar 2000). Researches on microorganisms have been carried out to see if they can be an alternative means of cyanide degradation. Bioremoval has been reported to be less expensive than physical and chemical methods of cyanide degradation and faster than natural oxidation (Ozel *et al.* 2010; Dash *et al.* 2009). Destruction of cyanide in wastewaters and tailing solutions by capable microorganisms has been proved to be an alternative to the long-practiced chemical methods for the removal of cyanide. Biological methods of treating industrial effluents have been

reported to have high capital cost but low operating cost. Therefore, these methods are more profitable than the traditional process. Biological process that could satiate the need for extraction and environmental control is now practiced in some countries that understood the process (Dash *et al.* 2009).

Cyanide Degrading Microorganisms

Biological treatment process facilitates growth of microorganisms that are essential for treatment (Akcil 2003). It has been discovered that cyanide naturally occurs in the environment via the degradation of plant cyanogenic glycosides. A lot of microorganisms are able to detoxify simple forms of cyanide (Gadd 2001). *Pseudomonas fluorescens* NCIMB 11764 has been reported to be able to utilize potassium cyanide (KCN) in fed-batch culture (Kunz *et al.* 1998). *Pseudomonas fluorescens* has also been reported to degrade ferrocyanide (Arzu & Zumriye 2000). The growth of *Pseudomonas fluorescens* NCIMB 11764 on medium containing potassium cyanide (KCN) has also been reported (Kunz *et al.* 1998). Cyanide degradation by *Escherichia coli*, *Alcaligenes*,

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Acinetobacter and *Bacillus* species have also been reported in a number of studies.

It has been reported that strains of bacteria from genus *Klebsiella* are very effective in the bioremediation of cyanide and thiocyanate. *K. oxytoca* isolated from an industrial waste containing high level of cyanide proliferates well with the cyanide as the only nitrogen source (Kao *et al.* 2003). It has been reported that *K. oxytoca* is effective in biodegradation even at concentrations higher than 1 mM of cyanide (Chena *et al.* 1999). The microorganism biodegrades cyanide to products that are nontoxic using cyanide as the only nitrogen source aerobically or anaerobically (Kao *et al.* 2003; Stephen 2004). Seven bacterial strains isolated from gold mine environment in Korea have been found to be very effective in the biodegradation of thiocyanate with the initial concentration of 150 mg/L within sixteen days of incubation (Lee *et al.* 2003). Four strains from the genus *Bacillus*, one strain of *Corynebacterium nitrophilus* and two from *Brevibacterium* have been found to effectively degrade cyanide.

Pseudomonas pseudoalcaligenes CECT5344 is an alkaliphilic strain of bacteria that is reported to be capable of biodegrading cyanide. The organism was isolated from sludge of Guadalquivir (Cordoba, Spain). It proliferates at an optimum pH of 9.5 and utilizes 2 mM of cyanide as the only hydrogen source (Luque-Almagro *et al.* 2005; Huertas *et al.* 2010). The organism can also utilize complex of cyano-metal, cyanate and residue from jewellery industry as the sole nitrogen source (Luque-Almagro *et al.* 2005). This strain has been described to possess a cyanide-insensitive respiration system, which includes cytochrome *bd*-type alternate oxidase (AOX) that replaces the cytochrome c oxidase (Quesada *et al.* 2007).

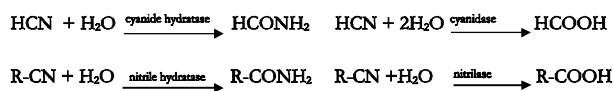
General Pathway Reaction for Biodegradation of Free Cyanide

There are four common pathways involved in the bioremoval of cyanide i.e. hydrolytic reaction, oxidative reaction, reductive reaction and substitution/transfer reaction. More than one pathway can be used for cyanide degradation by certain microorganisms (Raybuck 1992; Ezzi-Mufaddal & Lynch 2005). The pathway to be used depends on various factors such as oxygen availability, pH level of the environment, concentrations of the cyanide and cyanide

bioavailability and solubility in the soil water system (Aronstein *et al.* 1994).

Hydrolytic Reaction

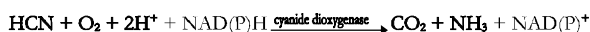
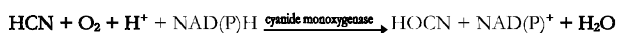
The following equations show the reactions that occur during hydrolytic pathway for cyanide degradation (David *et al.* 2006):



Hydrolytic reactions contain nitriles with R denotes either aliphatic or aromatic group. Hydrolytic reactions are catalyzed by cyanide hydratase, founding a formamide or cyanidase and yields formate and ammonia. Cyanide hydratase is principally a fungal enzyme and is extremely preserved between species (Barclay *et al.* 2002). Cyanide dihydratase (cyanidase) is produced chiefly by bacteria. Cyanide hydratase and cyanidase have, in recent times, been presented to have certain resemblances at both the amino acid and structural stages to nitrilase and nitrile hydratase enzymes (Reilly & Turner 2003). Enzymes that utilize nitrile have been established in a wide variety of fungal, plant and bacterial species. Nitrilases and nitrile hydratases modify both aliphatic and aromatic nitriles to the equivalent acid or amide, respectively, but indicate less substrate specificity than cyanide hydratase and cyanide dihydratase. For instance, conversion of *E. coli* with the cyanide hydratase gene from *Fusarium lateritium* permits the proliferation of nitriles as the only source of nitrogen. Site-directed mutagenesis of this gene stops the activity of both cyanide hydratase and nitrilase, signifying that cyanide hydratase possesses nitrilase activity, as well (Nolan *et al.* 2003). The variety of enzymes in this fantastic family and their diverse catalytic act and substrate specificities grant significant chance for biotechnological improvement, encompassing the bioremediation of industrial nitrile waste (Rezende *et al.* 2000; Dias *et al.* 2001).

Oxidative Reaction

In the oxidative reaction, the cyanate formed by the enzyme cyanide monooxygenase is changed to ammonia and carbon dioxide by the same pathway as cyanate and thiocyanate (David *et al.* 2006).

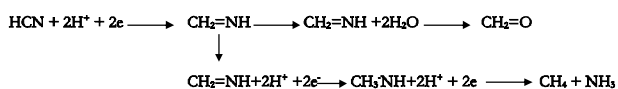


Cyanide is converted to cyanate by cyanide monooxygenase with cyanate standing as the catalyst for conversion of cyanate to ammonia and carbon dioxide dependent on bicarbonate. Cyanases have been identified in several bacteria, fungi, plants and animals (Guilloton *et al.* 2002). The assumed task of cyanase has, for a long time, been as a defense against poisoning by cyanate (Raybuck 1992). As cyanate is not a familiar metabolite, new essential roles in favor of cyanases in nitrogen and bicarbonate/carbon dioxide metabolism have been suggested. More proposed roles for plant cyanases comprise ammonia absorption as a result of cyanate bioremediation and a task in the concentration and deliverance of carbon dioxide for photosynthesis (Guilloton *et al.* 2002).

Another oxidative pathway makes use of cyanide dioxygenase to produce ammonia and carbon dioxide directly (Stephen 2004). Moreover, in *E. coli* strain BCN6 and *P. fluorescens* NCIMB 11764, the production of cyanohydrin complexes is reportedly essential for oxygenase-mediated cyanide biodegradation (Kunz *et al.* 1998; Figueira *et al.* 1996).

Reductive Reaction

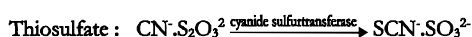
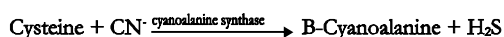
The reductive pathway results from the act of nitrogenase enzyme and the products ensuing from transfer of pair of electrons (Stephen 2004; David *et al.* 2006).



Studies have been carried out in two bioreactors in cassava wastewaters and synthetic wastewater. This indicates a clear reductive process on biodegradation (Paixao *et al.* 2000; Annachhatre & Amornkaew 2000). The studies indicate that the anaerobic bioreactors prop up the proliferation of methanogenes which can result in the production of biogas. The increase in the cyanide concentration inhibits methanogenesis from anaerobic biogranules. This effect could be a result of high cyanide concentration in the feed stock (Annachhatre & Amornkaew 2000).

Substitution/ Transfer Reaction

This is referred to as assimilatory pathway. Several genera of bacteria are said to assimilate cyanide. This reaction is more extensively researched in *Chromobacterium violaceum*. Other bacteria that utilize this reaction include *Escherichia coli*, *Bacillus megaterium*, *Citrobacter freundii* and *Enterobacter aerogenes* (David *et al.* 2006). The substitution/transfer reaction uses cyanoalanine synthase enzyme as the catalyst using O-acetylserine (OAS) as substrate.

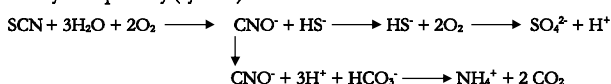


The cyanate produced by cyanide monooxygenase is changed to NH_4^+ and CO_2 by very similar pathway as the cyanate from thiocyanate (Stephen 2004).

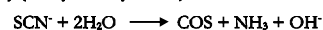
Thiocyanate Biodegradation

Reactions of cyanide with pyretic materials in effluents lead to the production of thiocyanate. The enzyme that is responsible for the production of thiocyanate *in vivo* is sulfurtransferase via the action of thiosulfate-cyanide. Its biodegradation can be achieved by at least two pathways namely, cyanate and carbonyl pathways (David *et al.* 2006; Kwon *et al.* 2002; Plessis *et al.* 2001; Sorokin *et al.* 2001; Yamasaki *et al.* 2002).

i. Cyanate pathway (cyanase):



ii. Carbonyl pathway (thiocyanate hydrolase):

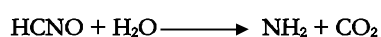


Three species of thiocyanate-degrading bacteria obtained from very high alkaline soda lagoon soils and sediments produce high amounts of cyanate when grown at pH 10 with thiocyanate as the only source of nitrogen. The activity of cyanase that converts cyanate to carbon dioxide and ammonia is also high (Sorokin *et al.* 2001). The fungus *Acromonium strictum* produces sulphate and ammonia from thiocyanate devoid of the production of cyanate (Kwon *et al.* 2002). In the

carbonyl pathway, the thiocyanate is transformed to ammonia and carbonyl sulphide. The confirmation for this pathway is as a result of the identification of thiocyanate hydrolase that is responsible for the transformation in the chemolithotroph *Thiobacillus thioparus* and the genes encoding this enzyme have been acknowledged in other thiocyanate-degrading bacterial cultures (Yamasaki *et al.* 2002).

Aerobic Biodegradation of Cyanide

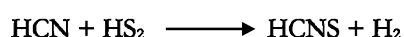
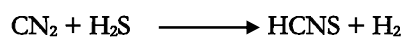
The process of biodegradation that requires the use of oxygen is termed as aerobic system of biodegradation. Under aerobic conditions, cyanide is broken down by cyanide-oxidizing bacteria into harmless compound. The process degrades hydrogen cyanide and produces hydrogen cyanate, which then undergoes hydrolysis to form ammonia and carbon dioxide as shown in the equation below:



Reports have indicated that aerobic process of biodegradation is many times faster and better than anaerobic degradation (Stephen 2004). Algae such as *Arthrospira maxima*, *Scenedesmus obliquus*, and *Chlorella* spp. have also been reported in the detoxification of cyanide (Dwivedi *et al.* 2011; Gurbuz *et al.* 2009).

Anaerobic Biodegradation of Cyanide

Anaerobic system of biodegradation is the process that occurs in the absence of oxygen (Dwivedi *et al.* 2011). Fedorak and Hurudey in 1989 was the first to report biotreatment of cyanide anaerobically using semi continuous batch cultures and Hydraulic Retention Time (HRT) of 25 days for one liquid volume replacement (Fallon *et al.* 1991). Anaerobic biodegradation can only occur in the presence of HS_2 or H_2S and is restricted to reduce portion of the heap environment. The sulphur species that is present depends on the pH. At $\text{pH} \geq 7$, HS_2 is the species that is dominant and at $\text{pH} \leq 7$ although H_2S is the more dominant species of sulphur in other pHs (Dwivedi *et al.* 2011).



The HCNS will undergo hydrolyses to produce NH_3 , H_2S and CO_2 .

Anaerobic cyanide biodegradation has been proven to be a concomitant method of biogas generation that is of economic benefit (Stephen 2004). Under anaerobic condition, biodegradation results in the formation of nitrogen as the end product. Furthermore, anaerobic treatment is not fast and it is more vulnerable to toxic upsets ensuing from exposure to other elements present in the solution undergoing the treatment. In addition, cyanide toxicity threshold for anaerobic bacteria is only 2 mg/L, while aerobic bacteria have 200 mg/L threshold. Therefore, anaerobic bioremoval is considered to be less effective method of bioremoval mechanism (Stephen 2004).

Effect of Immobilization on Cyanide Biodegradation

Immobilization has been reported to be a very effective and efficient tool of biodegradation. This tool offers many economic and technical advantages over the free cell type as it offers the possibility of maintaining the cells in a stable and viable condition with high specific surface area for microbial proliferation (Dursun & Aksu 2002). Immobilized cells are less vulnerable to compounds that are toxic and exhibit high tolerance towards distress in the reaction environment (Chen *et al.* 2008). Moreover, immobilized cells are more advantageous when compared with the use of free cells because of the ability to support higher concentration of cell density and eliminate the difficult, time-consuming and expensive process of cell recovery and recycling (Zhou *et al.* 2007). Bioremediation by *Candida guilliermondii* CCT 7207 was very effective on immobilized cells (Dias *et al.* 2001). This is a remark that reiterates previous researches indicating that immobilized microorganisms or enzymes make available an active stage for bioremediation (Stephen 2004). Effective biodegradation of cyanide compounds by *P. fluorescens* immobilized on zeolite has been reported (Suh *et al.* 1994). M. Graca Campos in 2005 reported effective, feasible and efficient detoxification of cyanide by immobilized *Fusarium oxysporum* CCMI 876 and *Methylobacterium* sp. RXM CCMI 908 using a packed bed reactor for integrated biodegradation

of cyanide (Campos *et al.* 2006). Maegala *et al.* (2012) reported effective biodegradation of cyanide by immobilized cells of *Rhodococcus* UKMP.

Oxygenation in Immobilized Cell Culture

Oxygenation is a major challenge in immobilization. Researchers have reported critical challenges in supplying sufficient and adequate concentration of oxygen in order to keep high amount of viable and productive cells throughout a culture period (Meuwly *et al.* 2005). In packed bed reactors it was observed that if there is low supply of oxygen, there will be decrease in viability of culture, metabolic activity and productivity until optimal supply of oxygen is achieved. This is because oxygen has a very poor solubility in cell culture medium (Fassnacht & Portner 1999).

Factors Affecting Cyanide Biodegradation in Environment

The presence of microorganisms that have the physical and metabolic abilities to degrade the contaminants in the polluted environment ensures the success of biodegradation. Cyanide compounds are found broadly in natural surroundings and the metabolic degradation of these compounds by microorganisms is thus possible. However, the following factors affect the process (Kao *et al.* 2006; Baxter & Cummings 2006; Dash *et al.* 2009):

- Cyanide concentration in the environment can have significant effect in the treatment. For instance, high concentration of acetonitrile has been proved toxic to *Klebsiella oxytoca* by causing damage to nitrile hydratase, which is the nitrile-degrading enzyme and by preventing bioremoval of the compound by the microorganism.
- Biodegradation of cyanide compounds can be affected by the availability of nutrients. Carbon has been recognized as the restrictive factor in the biodegradation of cyanide compounds, which may make the biodegradation of industrially polluted soils not feasible.
- Aeration is very important in the biodegradation of cyanide as oxygen is required during the degradation pathways.
- Cyanide toxicity can be paramount to anaerobic bacteria principally methanogens.

Other contaminants present at the polluted areas may also affect bioremoval.

- Existence of high concentration of other pollutants can have negative effect on degradation of cyanide by swaying the native population and possibly hindering the proliferation of specific organism.

Biodegradation of Free/ complex Cyanide and Thiocyanate

In the biotreatment of cyanide, bacteria have the capacity to change free and metal cyanides to bicarbonate and ammonia, whereas the free metals are adsorbed within the biofilm or unconfined as precipitates in solution. Alkali metal cyanides such as potassium cyanide (KCN) and sodium cyanide (NaCN₂) are easily being degraded by different forms of bacteria across the globe. In 1969 in USA, free cells of *Bacillus megaterium* was reported to degrade potassium cyanide (Castric & Strobel 1969). In the same year in Canada, free cells *Bacillus pumilus* was reported to degrade 2.5 mg/L of potassium cyanide at the pH of 8.5–9.0 and temperature of 40 °C (Skowronski & Strobel 1969). In 1972, free cells of *Stemphylium loti*, which is a pathogenic fungus of the cyanogenic plant 'Bird's Foot-Trefoil' (*Lotus corniculatus* L.) (Fry & Mills 1972) was reported to degrade 0.97 M of potassium cyanide at pH of 6.5–7.5 and temperature of 25 °C with the removal efficiency of 77 nM (Fry & Mills 1972). Several reports have been reviewed across the world up to the year 2012. In Malaysia *Rhodococcus* UKMP-5M in both free and immobilized forms was reported to degrade potassium cyanide at 30 °C temperature condition with the removal efficiency of 64 and 96%, respectively (Maegala *et al.* 2011; Maegala *et al.* 2012). This removal capacity is not only limited to bacteria but other fungal organisms such as *Fusarium solani*, which in 1997 its free cells were reported to degrade potassium cyanide concentration of 0.5–0.8 mM at the pH of 9.2–10.7 and temperature condition of 30 °C in France (Dumestre *et al.* 1997). In 1975, free cells of *B. stearrowthermophilus* NCA 1503 was reported to degrade NaCN₂ and NaHSO₃ with initial concentration of 5 mM and 50 mM at a pH of 7.8 and temperature of 27±2 °C with the removal efficiency of 5–8 g/L/hour (Atkinson 1975). The simplicity within which the complexes of metal

cyanide are being degraded commonly follows the sort of general stability, with free cyanides being the readily degradable while cyanide of iron being the least degradable. Zinc (Zn), nickel (Ni) and copper (Cu) are moderate in terms of degradability of their metal cyanides (Young & Jordan 1995; Botz 2001). A study conducted in Korea in 1994 reveals that *P. fluorescens* immobilized on zeolite was able to degrade Tetra-cyano Nickelate II at 30 °C (Suh *et al.* 1994). Studies in Turkey in 1999 showed that immobilized *P. fluorescens* was able to degrade ferrous (II) cyanide complex (Dursun *et al.* 1999) while free cells of *P. fluorescens* was able to degrade 100 mg/L ferrous (II) cyanide complex at pH 5 and temperature of 25 °C at degradation rate of 30 mg/g/hour (Dursun *et al.* 1999). Free cells of *Citrobacter* spp. and *Pseudomonas* spp. were reported in India to degrade 52 mg/L metal cyanide complexes such as copper cyanide and zinc cyanide with the removal efficiency of > 99.9% (Patil & Panikar 2000). In the United Kingdom in 2005, free cells of *Trichoderma* sp. and *Fusarium* sp. were reported to degrade 2,000 mg/L metallo cyanide at pH 6.5 and temperature of 25 °C, with the removal efficiency of 2,000 ppm (Ezzi-Mufaddal & Lynch 2002). Degradation of complex cyanide is not limited to only bacteria because in the United Kingdom in 1998, mix cultures of *Fusarium solani*, *T. polysporum*,

F. oxysporum, *Scytalidium thermophilum* and *Penicillium miczynski* were reported to degrade $K_2Ni(CN)_4$ and $K_4Fe(CN)_6$ at a concentration of 0.75 mM and 0.25–1 mM concentrations and pH 4.5–7.0 and temperature conditions of 25 °C with the removal efficiency of 50–56%, respectively (Barclay *et al.* 1998). In 2001 in South Africa, some free cells of microorganisms were reported to degrade thiocyanate at the concentration of 500 mg/L with the removal efficiency of 0.5 mg/L/h (Sorokin *et al.* 2001). In the same year in Russia, free cells of Alkaliphilic bacteria were reported to degrade 40 mM initial concentration of thiocyanate at a pH 10 and temperature of 28 °C with removal efficiency 4 mM (Plessis *et al.* 2001). In 2002 in Korea, *Acremonium strictum* was reported to degrade 7.4 g/L concentration of thiocyanate at a pH 6 with 100% removal efficiency in 85 hours (Kwon *et al.* 2002). In 2002 in Tokyo, Japan, free cells of *Thiobacillus thioparus* THI115 was reported to degrade thiocyanate with initial concentration of 0.1 g/L at a temperature of 30 °C (Yamasaki *et al.* 2002). There is no doubt from this review that *Pseudomonas* sp. has an upper hand in the degradation of both free and complex cyanides in quite a number of countries across the globe. Table 1 summarizes the degradation potentials of some microorganisms on free and complex cyanides and thiocyanate.

Table 1 Some microorganisms and their biodegradation potential of free/complex cyanides and thiocyanate

No	Name of microorganism	Cyanide compound	Concentration	Free/immobilized cells	pH	Temperature	Removal efficiency	Country	Reference
1	<i>Bacillus megaterium</i>	Potassium cyanide	1 mM	Free cells	-	35 °C	-	USA	(Castric & Strobel 1969)
2	<i>Bacillus pumilus</i>	Potassium cyanide	2.5 M	Free cells	8.5 – 9.0	40 °C	-	Canada	Skowronski & Strobel 1969)
3	<i>Stemphylium loti</i>	Potassium cyanide	0.97 M	Free cells	6.5 – 7.5	25 °C	77 nM	USA	(Fry & Mills 1972)
4	<i>Bacillus stearothermophilus</i> NCA 1503	NaCN ₂ and NaHSO ₃	5 mM, 50 mM	Free cells	7.8	27±2 °C	5–8 g/L/hour	-	(Atkinson 1975)
5	Granular Cyanidase	Sodium cyanide	300 ppm	Enzymes	7.4	26 °C	0.1–0.2 ppm CN ⁻	Switzerland	(Basheer <i>et al.</i> 1992)
6	<i>Pseudomonas putida</i>	Sodium cyanide	100–400 mg/L	Immobilized	6.7	25 °C	-	USA	(Babu <i>et al.</i> 1992)
7	<i>Pseudomonas fluorescens</i>	Tetra-cyano nickelate II	26 mg/L	Immobilized (Zeolite)	-	30 °C	100%	Korea	(Suh <i>et al.</i> 1994)
8	<i>Pseudomonas acidovorans</i>	Potassium cyanide	31 mg/L (12, 26, 53 and 75 mg/L)	Free cells	7.1, 7.9, 9.1	30 °C	-	India	(Shivaraman & Parthad 1985)
9	<i>Escherichia coli</i> BCN6	Potassium cyanide	50, 100 and 200 mg/L	Free cells	9.2	30 °C	-	Brazil	(Figueira <i>et al.</i> 1996)
10	<i>Fusarium solani</i>	Potassium cyanide	0.5 – 0.8 mM	Free cells	9.2–10.7	30 °C	-	France	(Dumestre <i>et al.</i> 1997)
11	Mix culture of <i>F. solani</i> , <i>T. polysporum</i> , <i>F. oxysporum</i> , <i>Scytalidium thermophilum</i> and <i>Penicillium miczynski</i>	(K ₂ Ni(CN) ₄ K ₄ Fe(CN) ₆)	K ₄ Fe(CN) ₆ - 0.25–1.0 mM, K ₂ Ni(CN) ₄ - 0.75 mM	Free cells	4.5–7.0	25 °C	50–56%	UK	(Barclay <i>et al.</i> 1998)

Table 1 Continued

No	Name of microorganism	Cyanide compound	Concentration	Free/immobilized cells	pH	Temperature	Removal efficiency	Country	Reference
12	Bacteria Mixed Cultures	Cyanide	20 mg/L	Free cells	7	22 °C	<0.5 mg/L	USA	(White & Schnabel 1998)
13	<i>Pseudomonas putida</i>	Phenol and cyanide	5 mg/dm ³	Immobilized		27 °C	20.30%	Poland	(Kowalski <i>et al.</i> 1998)
14	<i>Pseudomonas putida</i>	Sodium cyanide, Thiocyanate and Cyanates	4 mM	Non Immobilized & Immobilized	7.5	25 °C	79.75 & 98%	USA	(Chapatwala <i>et al.</i> 1998)
15	<i>Pseudomonas fluorescens</i>	Ferrous II cyanide	100 mg/L	Immobilized	4–7	25–35 °C	78.9	Turkey	(Dursun <i>et al.</i> 1999)
16	<i>Asotobacter vinelandii</i> TISTR 1094	Cyanide	86 mg/L	Free cells	7.4	30 °C	90%	Thailand	(Kaewkannetra <i>et al.</i> 2009)
17	<i>Pseudomonas fluorescens</i>	Ferrous II cyanide complex	100 mg/L	Free cells	5	25 °C	30.7 mg/g/hour	Turkey	(Dursun <i>et al.</i> 1999)
18	Anaerobic Waste Water Treatment	Cyanide	125 mg/L	Free cells	7	33 °C	91–93%	Colombia	(Huub <i>et al.</i> 1999)
19	Bacterial Consortium Viz: <i>Citrobacter</i> sp. MCM B-181, <i>Pseudomonas</i> sp. MCM B-182, <i>Pseudomonas</i> sp. MCM B-183 and <i>Pseudomonas</i> sp. MCM B-184	Potassium cyanide	52 mg/L	Free cells	7.5	35 °C	99.90%	India	(Patil & Panikar 2000)
20	<i>Citrobacter</i> spp. and <i>Pseudomonas</i> spp.	Copper and Zinc cyanide	52 mg/L	Free cells	7.5	35 °C	>99.9%	India	(Patil & Panikar 2000)
21	Fermentative bacilli and methanogenic bacteria.	Cyanide	7,500, 9,000, 11,000, 14,000 mg/L	Free cells	-	Room temperature	96–98%	Brazil	(Paixao <i>et al.</i> 2000)

Table 1 Continued

No	Name of microorganism	Cyanide compound	Concentration	Free/immobilized cells	pH	Temperature	Removal efficiency	Country	Reference
22	Biogranules	Cyanide	100 mg/L	-	-	30 °C	35–40 mg/L/day	Thailand	(Annachhatre & Amornkaew 2000)
23	Proteobacteria, <i>Thiobacillimicrobium</i> sp. and <i>Thiodalkalibrio</i> sp.	Thiocyanate	200–500 mg/L	Free cells	10	28 °C	16 mg/g/hour	South Africa	(Sorokin <i>et al.</i> 2001)
24	<i>Ralstonia eutropha</i> , <i>Bacera thiooxidans</i> and <i>Sphingomonas paucimobilis</i>	Thiocyanate	40 mM	Free cells	7.2	28 °C	4 mM	Russia	(Plessis <i>et al.</i> 2001)
25	<i>Acremonium strictum</i>	Thiocyanate	7.4 g/L	Free cells	6	25 °C	7.4 g/L	Korea	(Kwon <i>et al.</i> 2002)
26	<i>Thiobacillus thioparus</i> TH115	Potassium Thiocyanate	0.1 g/L	Free cells	7	30 °C	-	Tokyo	(Yamasaki <i>et al.</i> 2002)
27	<i>Pseudomonas</i> spp CM5, CMN2	Cyanides (WAD)	100–400 mg/L	Free cells	9.2–11.4	30 °C	-	Turkey	(Akçil <i>et al.</i> 2003)
28	<i>Trichoderma</i> sp. and <i>Fusarium</i> sp.	Cyanide	2000 mg/L	Free cells	6.5	25 °C	2000 ppm/CN	UK	(Ezzi-Mufaddal & Lynch 2004)
29	<i>Fusarium oxysporum</i>	Cyanide and Formamide	1–7 mM	Immobilized	8	25–30 °C	96%	Portugal	(Campos <i>et al.</i> 2006)
30	<i>Klebsiella oxytoca</i>	Potassium cyanide	5 mM	Immobilized Free cells	7	30 °C	0.224–0.192 nm/hour	-	(Kao <i>et al.</i> 2003)
31	<i>Senedesmus obliquus</i>	WAD	77.9 mg/L	Free cells	10.3	> 20 °C	92.30%	Turkey	(Fatma <i>et al.</i> 2009)
32	<i>Pseudomonas pseudoalcaligenes</i> CECT5344	Potassium cyanide	2 mM	Free cells	9.5	30 °C	2.31 mg/CN/ L/ O.D/ hour	Spain	(Luque-Almagro <i>et al.</i> 2005)
33	<i>Agrobacterium tumefaciens</i> SUTS 1	Potassium cyanide	25, 50 and 150 mg/L	Free cells	7.2	30 °C	87.5%, 97.9%	Thailand	(Potivichayanon & Kitleartpornpairat 2010)

Table 1 Continued

No	Name of microorganism	Cyanide compound	Concentration	Free/immobilized cells	pH	Temperature	Removal efficiency	Country	Reference
34	<i>Rhodococcus</i> UKMP-5M	Potassium cyanide	3–15 mM	Free cells	7	30 °C	47.78 – 64.0%	Malaysia	(Maegala <i>et al.</i> 2011)
35	<i>Rhodococcus</i> UKMP-5M	Potassium cyanide		Immobilized	Unadjusted	30 °C	64 & 96%	Malaysia	(Maegala <i>et al.</i> 2012)
36	<i>Serratia marcescens</i>	Potassium cyanide	25 mg/L	Free cells	7	30 °C	97%	Malaysia	(Karamba <i>et al.</i> 2015)

CONCLUSIONS

Certain economic and physical factors chiefly limit the use of biotechnologies in the degradation of cyanide. Biodegradation is potentially the cheapest means to get rid of cyanide but factors such as pH, temperature and nutrients concentration of the cyanide affect the process. Bioremoval can be achieved under aerobic and anaerobic conditions. Free and immobilized cells have proven to be very effective and efficient methods of biodegradation. The microbes potentially have certain enzymes that can change cyanide into naturally occurring compounds. Four types of pathways are used by the microorganisms to biodegrade and one or combination of two pathways can be employed in the process by microorganisms (Raybuck 1992; Ezzzi-Mufaddal & Lynch 2002). *Pseudomonas* spp. shows great capability for cyanide removal. In 2011 and 2012, *Rhodococcus* UKMP-5M obtained from Culture Collection Unit, Institute of Bio-IT Selangor was reported to be used to degrade potassium cyanide in free and immobilized forms and it has been proven to be efficient in the degradation (Maegala *et al.* 2011; Maegala *et al.* 2012). Table 1 indicates that from 1969 to date, researches have been carried out on the bioremoval of cyanide. However, despite all the investigations that have proved microorganisms can degrade cyanide in the laboratory; it has not been accomplished in a large scale (Fatma *et al.* 2009).

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