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Remediation of cyanide-contaminated environments through microbes and plants: a review of current knowledge and future perspectives

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ABSTRACT

Mining industry has been using cyanide for more than ten decades to recover precious metals such as gold and silver. The presence of cyanide in the environment has long been a matter of concern due to its high toxicity to human, animal, and aquatic life. The available treatment processes either physical or chemical are suffered with issues such as operating conditions, generation of secondary pollution, and lack of cost effectiveness. A number of micro-organisms are capable to consume cyanide as a source of carbon and nitrogen, and convert it into ammonia and carbonate. Some plants are also efficient in cyanide attenuation process. Bioremediation of cyanide might be an efficient, cost-effective, eco-friendly, and an attractive alternative to the conventional physical and chemical processes. This paper reviews the recent advances in remediation of cyanide contaminated tailings via micro-organisms and plants. Aspects such as speciation, toxicity, source, and degradation mechanisms of cyanide are discussed. Factors affecting functioning of micro-organisms and plants as bioremediation agents are also highlighted.

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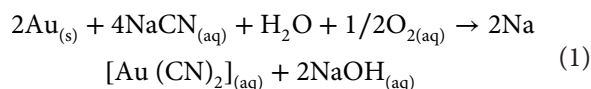
KEYWORDS

Mining; cyanide; leaching; soil/water contamination; bioremediation

1. Introduction

The term cyanides represent a group of compounds comprising hydrogen cyanide; simple inorganic salts including NaCN and KCN; complex metal cyanides, thiocyanates, and nitriles (cyanide bound with an organic radical). Hydrogen cyanide (HCN, gas) and sodium or potassium cyanide (NaCN or KCN, solid) are the major man-made cyanide forms. Cyanide ion can form complexes with most of the transition metals (Fe, Au, Cd, Co, Cu, and Ni); however, the complex with the iron and gold are the most stable (Table 1) (Meeussen, Keizer, Van Riemsdijk, & De Haan, 1992).

Cyanide has been using in mining industries for more than ten decades to recover gold (Ebel, Evangelou, & Schaeffer, 2007). Hundreds of tons of CN is consumed in gold mining annually (Hilson & Monhemius, 2006). The utilization of cyanide solution for gold extraction from fine-grounded ore can be presented as (Equation 1),



Cyanides are mainly contributed by anthropogenic activities but a range of organisms such as higher plants, fungi, and bacteria also produce cyanide (Baxter & Cummings, 2006a). About 2000 plant species are known to produce cyanide (mainly in the form of glucosides) for chemical defense (Larsen, Trapp, & Pirandello, 2004). Cyanide is also released in the environment due to coal combustion, refining of petroleum, iron and steel production, burning of plastics, incineration of household waste, combustion of fossil fuels, and cigarette smoking (Lottermoser, 2010). Significant amounts of cyanide may enter surface waters through municipal sewage treatment works and the dissolution of road salt. Cyanide is also synthesized and used for numerous industrial processes such as steel hardening and plastics production (Lottermoser, 2010). Biomass burning and agricultural practices are also reported as a major source of cyanide in the environment (Barber et al., 2003; Sharma & Mudhoo, 2011).

In soils, cyanide seldom remains biologically available because it is either complexed by trace metals, microbially metabolized, or lost through volatilization (Eisler, 1991; Gagnon, Zagury, & Deschênes, 2004; Nakles, Dzombak,

Table 1. Different cyanide complexes anions with stability constants.

| Anions of cyanide | IUPAC Name | Type of Complex | Stability Constant (log K at 25 °C) |
|--------------------------------------|--------------------------|-----------------|-------------------------------------|
| [Co(CN) ₆] ³⁻ | hexacyanocobaltate (III) | Strong | 64 |
| [Fe(CN) ₆] ³⁻ | hexacyanoferrate (III) | Strong | 43.6 |
| [Pd(CN) ₄] ²⁻ | tetracyanopalladate (II) | Strong | 42.4 |
| [Pt(CN) ₄] ²⁻ | tetracyanoplatinate (II) | Strong | 40 |
| [Hg(CN) ₄] ²⁻ | tetracyanomercurate (II) | Strong | 39 |
| [Au(CN) ₂] ²⁻ | dicyanoaurate (I) | Strong | 37 |
| [Fe(CN) ₆] ⁴⁻ | hexacyanoferrate (II) | Strong | 35.4 |
| [Ni(CN) ₄] ²⁻ | tetracyanonickelate (II) | Weak | 30.2 |
| [Cu(CN) ₄] ³⁻ | tetracyanocuprate (I) | Weak | 23.1 |
| [Cu(CN) ₃] ²⁻ | tricyanocuprate (I) | Weak | NA |
| [Ag(CN) ₂] ⁻ | dicyanoargentate (I) | Weak | 20.5 |
| [Zn(CN) ₄] ²⁻ | tetracyanozincate (II) | Weak | 19.6 |
| [Cd(CN) ₄] ²⁻ | tetracyanocadmium (II) | Weak | 17.9 |

NA: Not available.

Table 2. Sources of online information about different aspects of cyanide wastes handling and management.

| Organization/Source | Information available | Web address |
|--|---|---|
| International Cyanide Management Code for the Gold Mining Industry | Information on the use of cyanide in the gold mining industry | http://www.cyanidecode.org/ |
| InfoMine | Review on the use of cyanide in mining | http://technology.infomine.com/reviews/Cyanide/ |
| TechnoMine | Articles, news, reviews, and resources on mining technology and related topics | http://technology.infomine.com/reviews/Cyanide/ |
| MiningFacts.org | Present evidence-based mining facts and information in a way that permits balanced consideration of the impacts and opportunities that come from mining | http://www.miningfacts.org/environment/what-is-the-role-of-cyanide-in-mining/ |

Ghosh, Wong-Chong, & Theis, 2006; Oudjehani, Zagury, & Deschenes, 2002; Ozel et al., 2010). Cyanide salts in the soil are microbially degraded to nitrites or form complexes with trace metals under aerobic conditions (Kjeldsen, 1999). Iron cyanides are stable and loosely attached with the soil minerals and the possibility of leaching of iron cyanides into the surrounding surface and groundwater are high. Moreover, the soil has a tendency to reject anions (Mussato et al., 2007). Under anaerobic conditions, cyanides denitrify to gaseous nitrogen compounds. Cyanides being hazardous materials must be handled with extreme care. Different aspects of cyanide wastes handling and management are summarized in Table 2. Under alkaline conditions, cyanide may persist for at least a century in groundwater, mine tailings, and abandoned leach heaps (Eisler & Wiemeyer, 2004).

Cyanide is extremely toxic to aerobic lifeforms as it tightly binds to metals, inactivating metalloenzymes such as cytochrome c oxidase thereby inhibiting respiration (Luque-Almagro, Moreno-Vivian, & Roldan, 2016a). Human can come in contact with cyanide in their daily life through food, drink, smoking, medicines, etc. (Kuyucak & Akcil, 2013). In mammals, the lethal dose of cyanide is 0.5–3.5 mg kg⁻¹ body weight (Cipollone, Ascenzi, Frangipani, & Visca, 2006). The toxicity and associated hazards of cyanide for animals and plants have been discussed in details by Eisler and Wiemeyer (2004). Cyanide and its different forms in liquid, solid,

and gas phase (Figure 1), originated as a result of natural and anthropogenic activities are highly toxic, carcinogenic, and mutagenic in nature. Therefore, control and remediation of cyanide-contaminated water and tailings is necessary (Dzombak, Ghosh, & Wong-Chong, 2005; Moran, 1998; Patil & Paknikar, 2000; Zagury, Oudjehani, & Deschênes, 2004).

2. Remediation methodologies for cyanide-contaminated sites

Low concentrations (<0.2 mg/L) of weak acid dissociable cyanide (metal-bound cyanide dissociable in weak acids, WAD) are required to legally shut down the mining operations (Eisler & Wiemeyer, 2004). Natural destruction of cyanide by ore, soil, clay, and micro-organisms is a major pathway which revives a contaminated site (Gagnon et al., 2004; Oudjehani et al., 2002). Several bacteria and fungi utilize the toxicity of cyanide compounds to produce and secrete certain compounds to inhibit competitive organisms (Johnson, 2015). Cyanide treatment methodologies for liquid and solid media are physical, chemical, and biological in nature (Akci, 2003; Akci & Mudder, 2003). Cyanide treatment methods either physical or chemical in nature are suffered with certain inherent demerits pertaining to cost and eco-friendliness. Ion exchange and reverse osmosis are two major physical methods for cyanide treatment; using these methods, different species

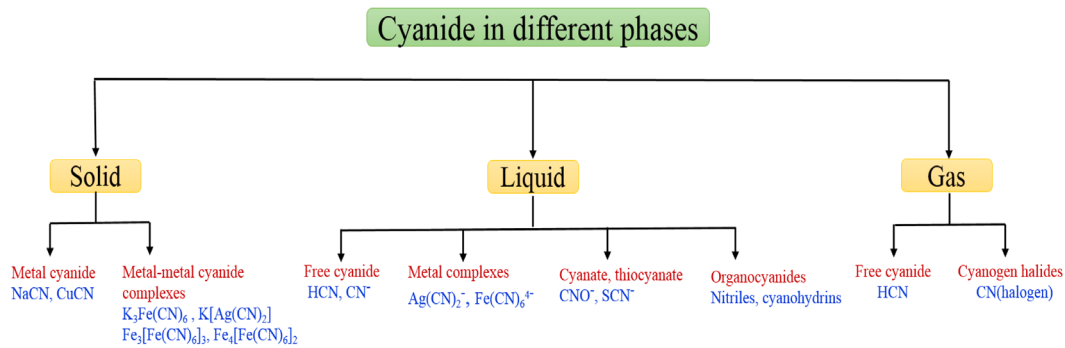


Figure 1. Cyanide speciation in solid, liquid, and gas phases.

such as cyanide, cyanates, thiocyanate, ammonia, and nitrate can be removed simultaneously (Botz, Mudder, & Akcil, 2005). The generation of byproduct (brine) is an associated concern with ion exchange and reverse osmosis processes. The disposal of byproducts is sometimes difficult and requires special handling and makes the process expensive. The chemical methods for cyanide treatment rely on the oxidation of cyanide to cyanate – a harmless form in itself. There are several methods for the treatment of CN⁻ rich effluents, such as alkaline chlorination, sulfoxide/air process and hydrogen peroxide process. The oxidants including chlorine, hypochlorite (sodium or calcium), ozone, hydrogen peroxide, Caro's acid or peroxymonosulfuric acid (H₂SO₅, sulfuric acid + hydrogen peroxide), sulfur dioxide + air (SO₂-INCO process), activated carbon are generally used for the oxidation of cyanide (Khodadad, Teimoury, Abdolahi, & Samiee, 2008). The reaction products include cyanate, carbon dioxide, bicarbonate, nitrogen, nitrate, ammonia, and sulfate. The hypochlorite ion (OCl⁻) oxidizes, for instance, cyanide to cyanate. A novel approach of five-compartment electrodialysis for the treatment of cyanide from gold mine effluent has recently been proposed (Zheng, Li, Wang, Gao, & Gao, 2015). The treatment of cyanide through adsorption has reported in a number of studies (Deveci, Yazıcı, Alp, & Uslu, 2006; Yazıcı, Deveci, & Alp, 2009). Photocatalytic degradation of cyanide in wastewater using a nano-thin film photocatalyst is also reported (Pala et al., 2015). Although the chemical methods are well established, they generally suffer from some serious shortcomings such as their inability to degrade stable CN⁻ metal complexes, requirement of expensive reagents and equipment (Patil & Paknikar, 1999), maintenance and royalty payments and generation of chlorinated compounds as byproducts (Akcil & Mudder, 2003; Khamar, Makhdoumi-Kakhki, & Mahmudy Gharaie, 2015; Kitis, Akcil, Karakaya, & Yigit, 2005). In such a scenario, the remediation of cyanide contaminated sites such as solid mine tailings, abandoned mining areas, and former gas manufacturing

plants (MGP) using biological agents (micro-organisms or plants) is a potential solution.

3. Bioremediation of cyanide contaminated tailings

Bioremediation might be attractive alternatives to conventional cleanup technologies not only due to relatively low capital costs but its inherent esthetic nature (Akcil, 2003; Akcil & Mudder, 2003). In the following sections, the potential of microbes and plants for cyanide degradation is reviewed.

3.1. Cyanide remediation through micro-organisms

Cyanide exposure is detrimental to human beings, but some (micro)organisms are tolerant to its toxic effects (Huertas et al., 2006, 2010; Khamar et al., 2015; McDonald & Vanlerberghe, 2005). These (micro)organisms convert cyanide to non-hazardous/less toxic materials. Several bacterial species, especially members of the genus *Pseudomonas* (Akcil & Mudder, 2003) and other taxa including *Bacillus*, *Alcaligenes*, *Acinetobacter*, and *Burkholderia* (Adjei & Ohta, 2000) have been reported for their potency to degrade cyanide (Table 3). The free cyanide in acidic medium, even at a slightly alkaline pH (pKa-9.2 at 25 °C) changes to toxic HCN, the bioremediation results should, therefore, be interpreted with caution, and the alkaline conditions are more desirable (Khamar et al., 2015). Microbial community can degrade cyanide. However, when produced HCN concentration reached to 0.3 mg HCN kg⁻¹ under anaerobic condition, the degradation efficiency decreased simultaneously (Eisler & Wiemeyer, 2004). These communities can acclimatize to cyanide followed by degradation of higher cyanide concentrations in wastes. Acclimatized micro-organisms in activated sewage sludge can often convert nitriles to ammonia at concentrations as high as 60.0 mg total CN kg⁻¹ (Eisler & Wiemeyer, 2004).

Table 3. Different micro-organisms and their cyanide degradation efficiencies.

| Organisms | Sources of Cyanide | Concentration | Removal/Degradation efficiency (%) | Reference |
|---|--------------------------------------|-------------------------|------------------------------------|---|
| <i>Pseudomonas pseudoalcaligenes</i> | Gold mining wastewater | 39 mg/L | 60 | Tiong et al. (2015) |
| <i>Pseudomonas fluorescens</i> NCIMB 11764 (cell-free extracts) | Pure KCN | 2 mM in 0.25 mL | 90 | Kunz et al. (1994) |
| <i>Pseudomonas putida</i> | Sodium cyanide | 4 mM in 1.0 ml | NA | Babu, Vijaya, Ross, Wolfram, and Chapatwala (1996) |
| <i>Fusarium solani</i> and <i>Trichoderma polysporum</i> | Iron or Nickel cyanide | 0.5 mM in 50 mL | ~50 | Barclay et al. (1998) |
| <i>Fusarium oxysporum</i> , <i>Scytalidium thermophilum</i> , and <i>Penicillium miczynski</i> | | | | |
| <i>Pseudomonas pseudoalcaligenes</i> CECT 5344 | Lixivate from gold mining industry | 2 mM (20 mM = 520 mg/l) | ~30 | Igeño et al. (2007) |
| <i>Burkholderia cepacia</i> | KCN (Pure) | 10 mM | ~95 | Adjei and Ohta (2000) |
| <i>Fusarium solani</i> | KCN (Pure) | 2 mM | NA | Dumestre, Chone, Portal, Gerard, and Berthelin (1997) |
| <i>Cladosporium cladosporioides</i> | Copper cyanide, Nickel cyanide | 0.5 mM | 86 to 92 | Patil and Paknikar (1999) |
| Anaerobic sludge | KCN | 60 mg/L | ~50 | Gijzen, Bernal, and Ferrer (2000) |
| <i>Pseudomonas putida</i> strain 21, <i>Pseudomonas stutzeri</i> strain 18, <i>Pseudomonas sp.</i> strain 5 | KCN, NaSCN | 39 mg/L | 46 | Karavaiko et al. (2000) |
| Biofilm | Sodium cyanide | 20 mg/L | 96–100 | White et al. (2000) |
| <i>Trichoderma harzianum</i> | KCN | 0.125 M | NA | Ezzi and Lynch (2002) |
| <i>Trichoderma pseudokoningi</i> | | | | |
| <i>Pseudomonas</i> spp. | NaCN | 80 ppm | ~98 | Akcil et al. (2003) |
| <i>Klebsiella oxytoca</i> | Tetracyanonickelate (II) | 1 mM | ~70 | Kao et al. (2004) |
| <i>Trichoderma spp.</i> <i>Fusarium spp.</i> | CN ⁻ | 2000 ppm | ~95 | Ezzi and Lynch (2005) |
| <i>Pseudomonas pseudoalcaligenes</i> | [Cu(CN) ₄] ⁻² | 12.5 mM | NA | Luque-Almagro et al. (2005) |
| <i>Pseudomonas pseudoalcaligenes</i> | NaCN | 45 mg/L | ~99 | Huertas et al. (2010) |
| <i>Polyporus arcularius</i> | NA | 125 mg/L | 72.8 | Ozel et al. (2010) |
| <i>Pseudomonas stutzeri</i> | Soil cyanide | 0.218 mg/g | 66.9–72 | Nwokoro and Dibua (2014) |
| <i>Bacillus subtilis</i> | | | | |

Bioremediation of cyanide contaminated mining effluents is available in various reports (Kuyucak & Akcil, 2013; Luque-Almagro, Moreno-Vivián, & Roldán, 2016b). Most of the attempts for biotransformation of cyanides via microbes focused on fungi and bacteria and try to incorporate these natural attenuation processes into planned and more efficient treatment technologies. Yeasts' capability of degrading free and metalocyanides has been also reported in literature (Kwon, Woo, & Park, 2002). Coke-plant wastewater isolate *Cryptococcus humicola* can utilize tetracyanonickelate (II) ($K_2Ni(CN)_4$) as nitrogen source in presence of sufficient glucose with a degradation rate of 2.5 mM CN h⁻¹ for 60 h growth. Bacterial oxidation of free cyanide to CO₂ is the primary operator at shallow depths of disposal lagoons (~90% of total free cyanide degradation). Fungi and their strong oxidative enzymes are key players in degrading/decontaminating recalcitrant polymers and xenobiotic chemicals as well (Barclay, Hart, Knowles, Meeussen, & Tett, 1998; Baxter & Cummings, 2006a; Ebbs, 2004). Different biochemical reactions involved in the process of microbial-assisted

cyanide treatment are briefly summarized in the following discussion.

3.1.1. Biochemical reactions catalyzed by micro-organisms

The micro-organisms (bacteria and fungi) can break down cyanide in the environment through a number of chemical reaction namely hydrolytic, oxidative, reductive, and transformation reactions (Table 4).

3.1.1.1. Hydrolytic reactions. Cyanide reacts with H₂O in the presence of enzymes to produce formate or formamide and ammonia in hydrolytic reactions (Johnson, 2015). Enzymes involve in hydrolytic reactions are cyanide hydratase (CH), nitrile hydratase (NH), thiocyanate hydrolase (TCH, carbonyl pathways), nitrilase, and cyanidase. Cyanide hydratase, NH, and TCH have specific substrate and directly hydrolyze and cleave the carbon–nitrogen triple bond to form formamide. Nitrilase and cyanidase convert cyanide to ammonia and carboxylic acid, which are utilized in their

Table 4. Biochemical reactions catalyzed by different enzymes.

| Reaction type | Chemical reaction | Enzyme | Micro-organism |
|---------------------------------------|---|---|---|
| Hydrolytic reactions | $\text{HCN} + \text{H}_2\text{O} \rightarrow \text{HCONH}_2$ | Cyanide hydratase | Pathogenic fungi |
| | $\text{R-CN} + \text{H}_2\text{O} \rightarrow \text{R-CONH}_2$ | Nitrile hydratase | <i>Pseudomonas</i> , <i>Corynebacterium</i> , <i>Brevibacterium</i> |
| | $\text{HCN} + 2\text{H}_2\text{O} \rightarrow \text{HCOOH}$ | Cyanidase | <i>Alcaligenes xylosoxidans</i> |
| | $\text{R-CN} + 2\text{H}_2\text{O} \rightarrow \text{R-COOH}$ | Nitrilase | <i>Klebsiella ozaenae</i> , <i>Arthrobacter</i> sp., <i>Pseudomonas aeruginosa</i> , <i>Norcadia</i> sp. |
| Oxidative reactions | $\text{HCN} + \text{O}_2 + \text{H}^+ + \text{NAD(P)H} \rightarrow \text{HOCN} + \text{NAD(P)}^+ + \text{H}_2\text{O}$ | Cyanide monooxygenase | <i>Pseudomonas</i> sp. |
| | $\text{HCN} + \text{O}_2 + 2\text{H}^+ + \text{NAD(P)H} \rightarrow \text{CO}_2 + \text{NH}_3 + \text{NAD(P)}^+$ | Cyanide dioxygenase | <i>Pseudomonas fluorescens</i> , <i>Bacillus cereus</i> , <i>Bacillus pumillus</i> |
| | $\text{HCN} + \text{O}_2 + \text{H}^+ + \text{NADPH} \rightarrow \text{CO}_2 + \text{NH}_3 + \text{NADP}^+$ | Cyanase | <i>Escherichia coli</i> , <i>Rhodococcus Rhodochrous</i> |
| Reductive reactions | $\text{HCN} + 2\text{H}^+ + 2\text{e}^- \rightarrow \text{CH}_2=\text{NH} + \text{H}_2\text{O}$ $\rightarrow \text{CH}_3=\text{O}$ | | |
| Substitution/Transformation reactions | $\text{CH}_2=\text{NH} + 2\text{H}^+ + 2\text{e}^- \rightarrow \text{CH}_3-\text{NH} + 2\text{H}^+ + 2\text{e}^- \rightarrow \text{CH}_4 + \text{NH}_3$ | | |
| | Cysteine + $\text{CN}^- \rightarrow \beta$ -cyanoalanine + H_2S | Cyanoalanine synthase | <i>Bacillus megaterium</i> |
| | $\text{OAS} + \text{CN}^- \rightarrow \beta$ -cyanoalanine + CH_3COO^- | Thiosulfate:cyanide sulfurtransferase <i>Rhodanese</i> | <i>Thiobacillus denitrificans</i> , <i>Bacillus subtilis</i> , <i>Bacillus stearothermophilus</i> |
| Thiocyanate biodegradation | $\text{CN}^- + \text{S}_2\text{O}_3^{2-} \rightarrow \text{SCN}^- + \text{SO}_3^{2-}$ | | |
| | $\text{SCN}^- + 2\text{H}_2\text{O} \rightarrow \text{COS} + \text{NH}_3 + \text{OH}^-$ | Carbonyl pathway (thiocyanate hydrolase) | |
| | $\text{SCN}^- + 3\text{H}_2\text{O} + 2\text{O}_2 \rightarrow \text{CNO}^- + \text{HS}^- \rightarrow \text{HS}^- + 2\text{O}_2 \rightarrow \text{SO}_4^{2-} + \text{H}^+$ $\text{CNO}^- + 3\text{H}^+ + \text{HCO}_3^- \rightarrow \text{NH}_4^+ + 2\text{CO}_2$ | Cyanate pathway (cyanase) | |

metabolic activities (Ebbs, 2004; Gupta, Balomajumder, & Agarwal, 2010).

3.1.1.2. Oxidative reactions. In the presence of NADPH (nicotinamide adenine dinucleotide phosphate) cyanide reacts with dioxygen (O_2) to produce ammonia (NH_3) and carbon dioxide (CO_2) in oxidative reactions (Ebbs, 2004). Oxidative pathways involve three different enzymes namely cyanide monooxygenase (CMO), cyanase, and cyanide dioxygenase. Cyanide dioxygenase converts cyanide to cyanate, with cyanase then catalyzing the bicarbonate-dependent conversion of cyanate to ammonia and carbon dioxide. Cyanase is present in various bacteria, fungi, plants, and animals and its main role is protection against cyanate poisoning (Guilloton, Espie, & Anderson, 2002; Raybuck, 1992). The cyanate formed by cyanide monooxygenase is converted to NH_4 and CO_2 by the same pathway as the cyanate from thiocyanate. Micro-organisms require an extra carbon source with cyanide for their initial growth to exhibit oxidative reaction.

3.1.1.3. Reductive reactions. Nitrogenase enzyme induces the reductive reactions as a result of electrons transfer. Anaerobic conditions are generally considered as suitable for the reductive degradation of cyanide. The enzyme nitrogenase utilizes HCN and produces methane and ammonia as end products. Bacterial strains from the genus *Klebsiella* have been shown to degrade both

cyanide and thiocyanate. Strains of *Klebsiella oxytoca* isolated from cyanide-enriched industrial wastewaters grew on cyanide as the sole nitrogen source, and produced methane in cell culture by showing increased activity of nitrogenase, suggesting a reductive pathway for cyanide biodegradation (Kao, Liu, Lou, Lin, & Chen, 2003).

3.1.1.4. Substitution/transformation reactions. The substitution/transfer reaction catalyzed by cyanoalanine synthase use O-acetylserine (OAS) as a substrate. These reactions prevent the cells from cyanide toxicity through cyanide assimilation and provide extra nitrogen source for the growth of micro-organism. The synthesis of amino acid, β -cyanoalanine, and γ -cyano- α -aminobutyric acid takes place using amino acid residues as precursor that reacts with cyanide compounds (Ebbs, 2004).

3.1.2. Factors influencing the cyanide degradation efficiency of microbes

The success of biodegradation depends upon the presence of microbes with the physiological and metabolic capabilities to degrade the pollutants in the contaminated environment. Thus, the first step in the biological treatment process is the selection of microbial species which can tolerate and degrade cyanide in alkaline medium (Igeño et al., 2007). A bacterial strain, *Pseudomonas pseudoalcaligenes* uses cyanide as the sole nitrogen source under alkaline conditions was isolated (Luque-Almagro et al., 2005). The bacterium was able to grow

in alkaline media, up to an initial pH of 11.5, and tolerated free cyanide in concentrations of up to 30 mM, which makes it a good candidate for the biological treatment of cyanide contaminated residues. Cyanide can be utilized as carbon and nitrogen source for the growth of several micro-organisms under specific conditions. Nonetheless, a number of factors including concentration of cyanides, availability of nutrients, availability of oxygen, and presence of additional pollutants significantly influence their performance toward cyanide degradation, which are briefly discussed in this section.

3.1.2.1. Concentration of cyanides. The concentrations of cyanides in contaminated soil have a significant impact on passive biodegradation (Alfani, Cantarella, Spera, & Viparelli, 2001; Kao, Chen, Liu, Chou, & Chen, 2006). The higher concentrations of acetonitrile are reported to be toxic to *Klebsiella oxytoca*, by causing damage to the nitrile degrading enzyme nitrile hydratase, and inhibiting the biodegradation of the compound by the organism (Kao et al., 2006). Out of the five strains, which were able to use cyanide as the sole nitrogen source under alkaline conditions, only one strain showed higher efficiency of cyanide degradation at concentrations of up to 500 mg L⁻¹ (Mirizadeh, Yaghmaei, & Nejad, 2014). The bacterium was able to tolerate free cyanide, which makes it a potential candidate for the treatment of cyanide. In an another study, acrylonitrile at high concentrations proved toxic to *Brevibacterium imperialis* CBS 489–74, by causing irreversible damage to the nitrile degrading enzyme hydratase and inhibited the biodegradation of the compound (Alfani et al., 2001).

3.1.2.2. Availability of nutrients. The availability of nutrients affects the biodegradation of cyanide compounds. Nutrient as the source of carbon and nitrogen for the enhancement of microbial growth induces the microbial uptake and degradation of cyanide compounds. Carbon has been identified as a limiting factor in the microbial degradation of metal-cyanides, which may prevent the bioremediation of industrially contaminated soils (Dash, Balomajumder, & Kumar, 2009a; Kao et al., 2006). The functional group on the natural organic material (humus) such as aldehyde and ketones (carbonyl groups) react with cyanide at natural condition (40 °C and neutral pH) to form carboxylic salt (a stable cyanide species) in the presence of metal ion; which make it out of reach by microbial strain (Barany, 2004). The soil isolate of *Burkholderia cepacia* strain prefers KCN for their growth as nitrogen source instead of low-molecular weight proteins (Adjei & Ohta, 2000). The authors noticed an increase (3–3.5-fold and 2-fold) in cyanide degradation in presence of nitrogen (inorganic/organic). The presence of fructose (0.25%) significantly affected cyanide degradation by

Burkholderia cepacia (1.85 mg CN/h). *Pseudomonas* species have specific fascination toward thiocyanate and cyanide compounds as their sole carbon-nitrogen requirements due to the presence of enzyme Cyanidase, which mediates the production of formate and ammonia from cyanide compounds. The effectiveness of cyanide degradation was induced to 100 mg L⁻¹ in presence of lactose (White, Jones, Huang, & Gauthier, 1988). Potassium lactate and sucrose as sole carbon source for *Pseudomonas* community can induce the uptake and utilization of cyanide and thiocyanate as nitrogen source (Karavaiko, Kondrat'eva, Savari, Grigor'eva, & Avakyan, 2000). An increase in the cyanide removal rate (0.1–0.5 mg L⁻¹ h⁻¹) is also reported in the presence of glucose (~155 mg L⁻¹) as a nutrient source in cyanide-contaminated wastewater (White, Pilon, & Woolard, 2000). Similar to other carbon sources, glucose has a promising effect on the degradation of metal cyanide complex by facultative anaerobe *Klebsiella oxytoca* (Kao et al., 2004). Though metal cyanide bonds are strong enough to be biodegraded, presence of nickel cyanide promotes the secretion of cyanide oxygenase in *Pseudomonas fluorescens* to endorse the utilization of nickel cyanide as nitrogen source. *Fusarium solani* (a fungal species) can consume up to 90% of hexacyanoferrate (0.5 mM) in 34 days as sole nitrogen source when glucose is supplied (20 mM) (Barclay et al., 1998). The strains of *Trichoderma* and *Fusarium* can utilize cyanide for their growth within 32 days with glucose, which is comparable with the growth and cyanide degradation by these strains in the absence of glucose within 90 days of incubation (Ezzi & Lynch, 2005).

3.1.2.3. Availability of oxygen. Oxygen is consumed in most of the cyanide degrading pathways, and hence the availability of oxygen is a significant factor in the microbial mineralization of cyanide (Baxter & Cummings, 2006a). On the other hand, cyanide can be toxic to anaerobic bacteria, particularly methanogens. The metal cyanide degradation efficiency of *Klebsiella oxytoca* is reported to be lower in the presence of less than 100% of dissolved oxygen (Kao et al., 2004). Cell-free extracts of *Pseudomonas fluorescens* mediated cyanide degradation by the enzymatic action of cyanide oxygenase is oxygen depended. The supply of oxygen and NADH induced the formation of CO₂ from KCN (Kunz, Wang, & Chen, 1994). The effect of oxygen in cyanide degradation by the strains of *Pseudomonas pseudoalcaligenes* in alkaline pH is also reported (Huertas et al., 2010). The aerobic degradation of cyanide is faster than the anaerobic one for most of the aerobic bacteria.

3.1.2.4. Presence of additional pollutants. The presence of additional inorganic and organic pollutants

at contaminated sites may influence the cyanide bioremediation process in a negative or positive way. Sometimes, xenobiotics act as a source of nutrients for bacteria involved in biodegradation (Ubalua, 2010). *Klebsiella oxytoca*, isolated from cyanide-containing wastewater, was able to utilize nitriles as a sole source of nitrogen. The presence of additional pollutants in high amount can impact on cyanide degradation by influencing the indigenous microbial population and the growth of particular organisms (Baxter & Cummings, 2006a; Kao et al., 2006).

3.1.2.5. Temperature & pH. Cyanide degrading enzymes are generally produced by mesophilic micro-organisms. The biodegradation rate of cyanide is temperature dependent as these micro-organisms are generally isolated from soil and work efficiently with temperature typically ranging between 20 and 40 °C (Akcil, Karahan, Ciftci, & Sagdic, 2003; Baxter & Cummings, 2006a; Kao et al., 2003; Ubalua, 2010). The effect of pH (a master variable) specifically on the bioremediation of cyanide-contaminated soil is significant. The optimum pH for the growth of bacteria (6–8), fungus (4–5), and cyanide degrading enzymes (6–9) is typical during cyanide degradation. Thiocyanate needs pH 10 for biodegradation (Akcil & Mudder, 2003). Therefore, extreme pH may have a significant negative effect on the biodegradation process. However, *Fusarium solani* and mixed cultures of fungi, including *F. solani*, *Fusarium oxysporum*, *Trichoderma polysporum*, *Scytalidium thermophilum*, and *Penicillium miczynski* are capable of degrading iron cyanides at pH 4 (Barclay et al., 1998). Iron cyanides are largely stable in acidic soils and have little environmental mobility. However, at alkaline pH, the solubility of precipitated iron cyanide complexes such as Prussian blue, greatly increased and allows cyanide to become mobile. It is therefore possible for iron-cyanide complexes to dissociate into free cyanide under these conditions and contaminate groundwater. Prussian blue can persist for decades in alkaline soil, this is thought to be because the dissolution rate is determined by the buffering capacity of the soil solution (Baxter & Cummings, 2006a). In order to avoid volatile HCN formation, alkaline pH is preferential ($pK_a = 9.2$). Thus, from a chemical point of view, the biological treatment of industrial effluents contaminated with cyanide requires an alkaline pH (Luque-Almagro et al., 2005).

In nutshell, the application of micro-organisms to remove the cyanide from solid and liquid industrial waste has several benefits such as low operation cost, ability to remove a wide range of cyanide compounds, ability to produce high-quality effluent, and eco-friendly process

(Dash, Gaur, & Balomajumder, 2009b; Dash et al., 2009a; Gurbuz, Ciftci, Akcil, & Karahan, 2004).

3.2. Cyanide remediation through plants

Species of fungi, micro/macro algae, and mostly vascular plants are well known for their remediation mechanisms. Several remediation methods are employed by plants to remediate pollutants naturally. Bioremediation through plant has been classified as mainly, Rhizoremediation and Phytoremediation. Onsite plantation could be effective alternative for chemical-based treatment methods for the exploration of plant-based bioremediation. The constructed wetland is a typical example of plant-based pollutant stabilization, degradation, accumulation, and sometimes volatilization (Pilon-Smits, 2005).

Bioremediation of cyanide includes the removal of cyanide by terrestrial and aquatic plants (Table 5). Root exudates produced by cyanogenic plants may contain compounds that stimulate or regulate the activity of cyanide-degrading micro-organisms. This method, being cost effective, is attractive and useful in dealing with this detrimental chemical (Ashraf, Qztürk, & Ahmad, 2010). Plant having high biomass production, wide distribution, and tolerant to cyanide and metal cyanide in the effluents from the mines could possibly be useful agents for the cyanide removal. A variety of plants including natural, transgenic, and/ or associated with rhizosphere micro-organisms can be effective in cleaning up pollutants (Hong, Banks, & Schwab, 2008). Many studies (Table 6) have shown the effectiveness of plants for cyanide removal from aqueous and solid phases (Ebel et al., 2007).

Cyanide contamination of rivers located nearby large-scale industrial mines was studied and possible solution of cleaning up using green technology of phytoremediation was proposed earlier (Hidayati, Juhaeti, & Syarif, 2009). Researchers grew some plant species at the contaminated sites and observed a high tolerance and effectiveness in accumulating cyanide or mercury in their roots and portions above the ground. Indian mustard (*Brassica juncea*) and muskgrass (*Chara canescens* a macroalga,) have also shown their potency in selenocyanate remediation in upland and wetland situations. Removal efficiency of 9 and 30% were accounted for muskgrass and Indian mustard, respectively, for cyanide compounds through roots and shoots; although all the accumulated Selenocyanate has been degraded into organic Se form (de Souza, Pickering, Walla, & Terry, 2002). Remediation of cyanide by grasses (*Sorghum bicolor*) could be another possible way of phytotreatment of gold mining effluents. In the year 2003, Trapp et al. observed that grasses could degrade cyanide and cyanide complexes effectively up to the concentration

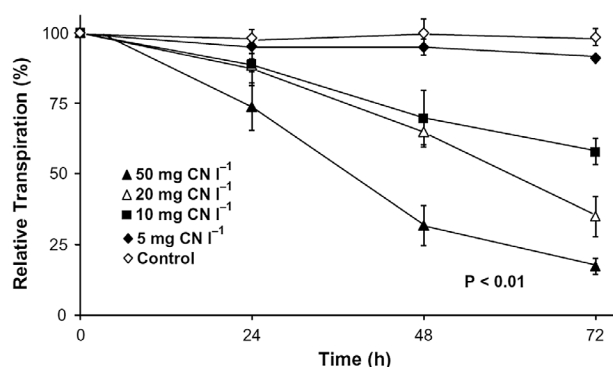


Figure 2. Relative transpiration of *E. crassipes* growing in hydroponic solution supplied with cyanide at different concentrations.

Notes: Transpiration is expressed relative to the initial transpiration of each plant (=100%) without cyanide treatment. Error bars denote standard deviation ($n = 5$). P -values are derived from T -tests. Results were compared with the relative transpiration at the end of the experiment (72 h) (Ebel et al., 2007).

of 125 mg L⁻¹ of irrigation water through roots and leaves (Trapp, Larsen, Pirandello, & Danquah-Boakye, 2003).

Woody plants have the capacity to degrade cyanide efficiently. Basket hybrids (*Salix viminalis* × *schwerinii*) have showed the removal capacity of 1100 kg of free cyanide/hectare in 200 days of growth period. Cyanide was converted to asparagine by β-cyanoalanine synthase and β-cyanoalanine hydrolase into these willows (Larsen et al., 2004). Woody plants are also effective for the removal of cyanide from aqueous phase. Larsen et al. (2004) reported *in vivo* capacity of woody plants (willow, poplar, elder, rose, birch) taking detached leaves and roots to remove cyanide. The highest removal capacity was obtained for basket willow hybrids (*Salix viminalis* × *schwerinii*) when the tests were performed with KCN solutions of varying concentrations (Larsen et al., 2004). Birch (*Betula pendula*) was the only plant capable to survive on gas works waste with a pH of 3.3 and 3219 mg total CN kg⁻¹.

The water hyacinth *Eichhornia crassipes* can remove cyanide in the effluents from the mines (Ebel et al., 2007). The phytotoxicity for *Eichhornia crassipes* due to NaCN was quantified as between 5 and 50 mg CN L⁻¹. Only a slight reduction in transpiration but no morphological changes were observed at 5 mg CN L⁻¹ (Figure 2). Cyanide at 5.8 and 10 mg L⁻¹ was completely eliminated after 23–32 h. Metabolism of K¹⁴CN in batch systems with leaf and root cuttings indicated that leaf cuttings removed about 40% of the radioactivity from solution after 28 h and 10% was converted to ¹⁴CO₂; root cuttings converted 25% into ¹⁴CO₂ after 48 h but only absorbed 12% in their tissues.

Eichhornia Crassipes – an obnoxious and invasive weed is omnipresent in wetlands of India. *E. crassipes* is reported as useful in treating cyanide containing water (Malik,

2007). The cyanide degrading microbial activity in the soil of the cyanogenic plants exposed to highly contaminated soil was significantly higher than in uncontaminated soil. Similar responses were identified in switchgrass pots. Although this study was conducted with an artificially contaminated soil, the results suggest that cyanide removal by plants and rhizosphere micro-organism is a potentially viable approach for soil contaminated by iron cyanide complexes (Hong et al., 2008).

The root exudates produced by cyanogenic plants contain compounds that stimulate the activity of cyanide-degrading micro-organisms. Therefore, the soil containing cyanogenic plants (*Sorghum bicolor* and *Linum usitatissimum*) can degrade cyanide effectively than soil with noncyanogenic plant (*Panicum virgatum*). Higher cyanide degrading microbial activity in the cyanogenic plants' rhizosphere soil was compared with the activity in switchgrass rhizosphere and the unvegetated soil which showed difference in degradation efficiency between different species of switchgrass (Hong et al., 2008). Cyanide occurs naturally in plant cells as a byproduct in the last step of ethylene synthesis and is rapidly detoxified by reacting with cysteine to form asparagine. The enzyme β-cyanoalanine synthase (CAS) catalyzes the conversion of cyanide and cysteine to β-cyanoalanine and sulfide (Figure 3). CAS is widely distributed in higher plants and plays an important role in cyanide metabolism. Free cyanide in micro-organisms is oxidatively detoxified. Cyanide monooxygenase converts free cyanide to cyanate, which is further mineralized by cyanase to form carbon dioxide and ammonia. Cyanide dioxygenase directly mineralizes cyanide (Akciil & Mudder, 2003). Various plants have been shown to tolerate and eliminate cyanides from nutrient solutions (Ebel et al., 2007; Larsen et al., 2004; Trapp et al., 2003; Yu, Trapp, Zhou, & Hu, 2005; Yu, Trapp, Zhou, Wang, & Zhou, 2004).

Free water surface wetlands have been used for the reduction of complex and free cyanide, and associated pollutants in water from a groundwater spring (Gessner, Kadlec, & Reaves, 2005). Shallow basins, initially planted with cattail (*Typha latifolia*) and bulrush (*Schoenoplectus tabernaemontani*), subsequently converted to coontail (*Ceratophyllum demersum*) and pondweed (*Potamogeton spp.*), respectively. Both total and free cyanide were effectively reduced during 7 d detention, by 56 and 88%, respectively.

3.2.1. Factors influencing the cyanide degradation efficiency of plants

A number of factors might affect efficiency of phytoremediation process undertaken for cyanide removal and the selection of plant species is one of them (Tables 5 and 6).

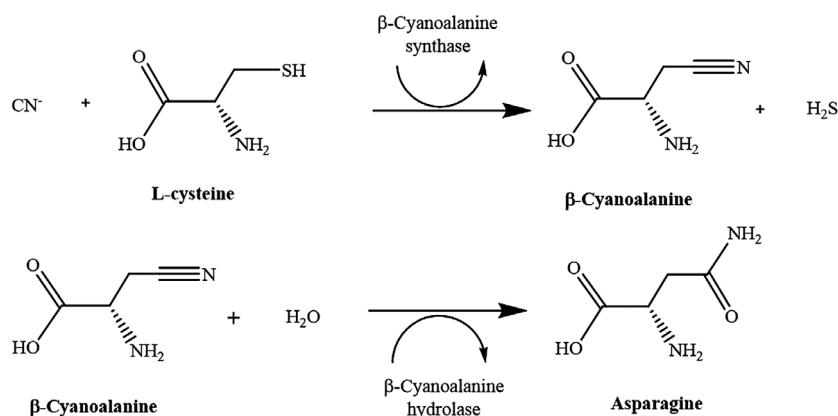


Figure 3. Microbial degradation pathway of cyanide through plant enzymes (Larsen et al., 2004).

Table 5. Examples of plants applied in cyanide phytoremediation function.

| Plant name | Common name | Phytoremediation function |
|------------------------------|-------------|--|
| <i>Papaver dubium</i> | Poppy | Cyanide tolerance |
| <i>Phaseolus vulgaris</i> | Bean | Cyanide phytotoxicity, dichlorobenzoate rhizodegradation |
| <i>Plantago major</i> | Plantain | Cyanide tolerance |
| <i>Rumex obtusifolius</i> | Dock | Cyanide |
| <i>Salix alba</i> | Willow | Cyanide metabolism |
| <i>Scrophularia nodosa</i> | Figwort | Cyanide tolerance |
| <i>Sonchus arvensis</i> | Thistle | Cyanide tolerance |
| <i>Taraxacum offi cinale</i> | Dandelion | Cyanide tolerance |

Source: Prasad (2011).

Table 6. Cyanide removal capacity of different plants.

| Species | Plant family | CN removal capacity ($\text{mg CN kg}^{-1} \text{ h}^{-1}$) |
|---|---------------------|---|
| <i>Sambucus chinensis</i> Lindl. | Caprifoliaceae | 8.77 |
| <i>Torilis japonica</i> (Houtt.) DC | Umbelliferae | 7.52 |
| <i>Prunus persica</i> Sleb. | Rosaceae | 2.83 |
| <i>Prunus pseudocerasus</i> Lindl. | Rosaceae | 6.28 |
| <i>Salix babylonica</i> L. | Salicaceae | 6.08 |
| <i>Glycine max</i> (L.) Merr. | Leguminosae | 3.45 |
| <i>Chimonanthus praecox</i> (L.) Link | Calycanthaceae | 5.97 |
| <i>Liquidambar formosana</i> Hance | Hamamelidaceae | 5.22 |
| <i>Metasequoia glyptostroboides</i> Hu & Cheng | Taxodiaceae | 5.32 |
| <i>Capsicum frutescens</i> L.cv. 'Hexiniujiao'jiao' | Solanaceae | 4.93 |
| <i>Euonymus alatus</i> (Thunb.) Sieb. | Celastraceae | 4.27 |
| <i>Zea mays</i> L. | Poaceae | 4.75 |
| <i>Cudrania tricuspidata</i> (Carr.) Bur. | Moraceae | 4.33 |
| <i>Sorghum vulgare</i> Pers. | Poaceae | 3.98 |
| <i>Calendula officinalis</i> L. | Compositae | 4.02 |
| <i>Nymphaea teragona</i> | Nymphaeaceae | 3.6 |
| <i>Salix matsudana alba</i> | Salicaceae | 3.15 |
| <i>Alternanthera philoeroides</i> Griseb | Amaranthaceae | 3.97 |
| <i>Populus deltoides</i> Marsh. | Salicaceae | 3.43 |
| <i>Iris tectorum</i> Maxim | Iridaceae | 3.05 |
| <i>Prunus persica</i> (L.) Batsch | Rosaceae | 2.83 |
| <i>Buxus sinica</i> (Rehd. & Wils.) M. Cheng | Buxaceae | 2.72 |
| <i>Trachycarpus fortunei</i> (Hook.f.) H. Wendl | Areacaceae | 1.63 |
| <i>Viburnum odoratissimum</i> Ker-Gawl | Caprifoliaceae | 2.28 |
| <i>Gossypium hirsutum</i> L. | Malvaceae | 1.52 |
| <i>Pterocarya stenoptera</i> C.DC. | Juglandaceae | 1.75 |
| <i>Podocarpus macrophyllus</i> (Thunb.) D.Don | Podocarpaceae | 0.6 |
| <i>Credrus deodara</i> (Roxb.) Loud | Pinact of plantseae | 0.23 |

Courtesy: Yu et al. (2004).

3.2.1.1. Nature of plant. The selection of an appropriate plant species with the desired properties is necessary for the desired remediation results. Screening under uniform conditions is a supportive strategy to

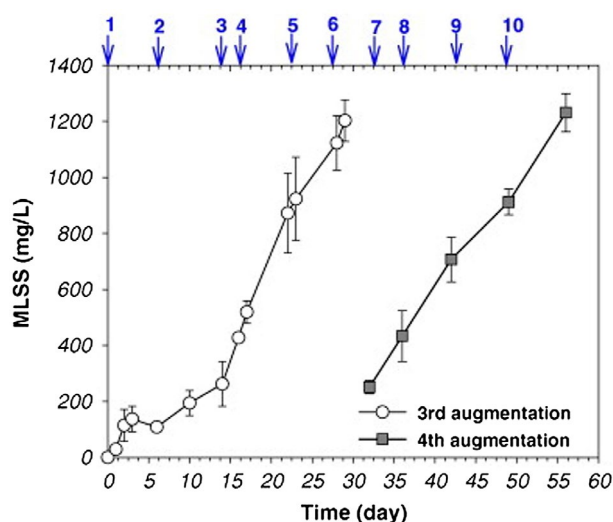


Figure 4. Increase of cyanide-degrading micro-organisms' concentration with periodic supply of cyanide compound (blue arrows) (Park et al., 2008).

compare cyanide uptake characteristics of different species. Agronomic practices may also be employed to maximize cyanide removal. Plant species may be selected for suitable root depth and morphology (Prasad, 2011). Plant roots can be guided to grow into the polluted zone via deep planting in a casing, forcing the roots to grow downward into the polluted soil and to tap into cyanide polluted water rather than rain(surface)water (Pilon-Smits, 2005).

3.2.1.2. Chelation in roots. Chelation in roots can affect phytoremediation efficiency as it may facilitate root sequestration, translocation, and/or tolerance. Root sequestration may be desirable for phytostabilization (less exposure to wildlife), whereas export to xylem is desirable for phytoextraction. Bioavailability of cyanide can be enhanced using chelators that are released by plants and bacteria. Chelators such as siderophores, organic acids, and phenolics can release various metal cations from soil particles. This usually increases availability of the toxic substances for plant uptake (Taiz & Zeiger, 2002), although in some cases, it can also prevent uptake.

Cyanide bioavailability could be affected by various plant and/or microbial activities. Some bacteria are known to release biosurfactants (e.g. rhamnolipids) that make hydrophobic compounds more water soluble. Plants' exudates or lysates may also contain lipophilic compounds that can increase solubility or promote biosurfactant-producing microbial populations in cyanide polluted water. Furthermore, plant and enzymes derived from microbes can affect the solubility and thus bioavailability of cyanide via modification of side groups. Endophytic bacteria also detoxify the contaminants and make those available

for plant uptake and degradation. Detoxifying activity of *Pseudomonas* species toward cyanide compounds promotes plant uptake through root from soil or water.

3.2.1.3. pH and Cation Exchange Capacity (CEC). The pH of the soil is a controlling factor of adsorption-desorption and precipitation-solubilization reactions. In addition to this, CEC of soil also depends on soil pH (Canter, Fairchild, & Knox, 1986). Ejecting H^+ ions via ATPase action, plants make metal cations more bioavailable in soil (Taiz & Zeiger, 2002). This leads to the detoxification of cyanide-metal complexes in soil.

3.2.1.4. Bio-augmentation. If the microbial consortia responsible for the remediation process are known, it may be possible to increase the abundance of these species by the choice of vegetation. An alternative approach is to grow these microbial isolates in large amounts and add them to the soil, a process called bioaugmentation (Pilon-Smits, 2005). Bioaugmentation is the addition of biocatalysts (generally bacteria, but it also could involve the addition of fungi, genes, or enzymes) to degrade target pollutants, either *in situ* or *ex situ* (Stroo, Leeson, & Ward, 2012). The application of nonnative microbes is considered as ineffective as the new microbes might be outcompeted by the specialized microbial populations. The specialized-micro-organisms include indigenous or allochthonous wild types or genetically modified organisms.

The inoculation of isolated *Rhodococcus spp.* into cyanide-contaminated coke works soil influenced the dominant presence of cyanide-degrading microbes with active degradation; though, removal rate of $K_2Ni(CN)_4$ was not significant (Baxter & Cummings, 2006b). The cyanide removal from the cokes wastewater treatment facility in a steel company of Korea has been found to be enhanced with the simultaneous increase in the cyanide-degrading micro-organisms (1203 mg/L) in 29 d (Figure 4) when these organisms are cultured through periodic increase in cyanide concentration as nitrogen source in long time augmentation process (Park, Lee, Kim, & Park, 2008). Hence, bioaugmentation can play an effective role for the enhancement of cyanide-degrading capability in microbes or microbial consortium.

In a nutshell, rhizosphere processes favoring bioremediation can be optimized by the choice of plant species (plants with large and dense root systems for phytostimulation, or aquatic plants for metal precipitation). Secondly, if a certain exudate compound is identified to enhance phytoremediation (e.g. a chelator or a secondary metabolite that stimulates microbial degradation), plants can be selected or genetically engineered to produce large amounts of this compound. In one such study,

overexpression of citrate synthase in plants conferred enhanced aluminum tolerance, probably via enhanced citrate release into the rhizosphere, which prevented aluminum uptake due to complexation (Pilon-Smits, 2005).

4. Conclusions and recommendations

Cyanides are ubiquitously present in nature, and microbial metabolism of these compounds by various micro-organisms and plant species is possible. Specific microbes and plant species and/or the associated rhizosphere can successfully remove strong acid dissociable (SAD) including iron cyanide complex and Prussian blue from contaminated soil. The soil containing certain cyanogenic plants (*Sorghum bicolor* and *Linum usitatissimum*) can effectively remove cyanide.

Bioremediation is cost effective (low reagent and capital costs) than chemical and physical methods, and faster than natural oxidation process. Reagents to be added to maintain a viable culture are simply the nutrients. Capital costs are low since the bacteria are mobile, and chemical handling systems are minimum. Bacteria can degrade cyanide effectively within the heap (heap as a reactor), hence the total volume of solution that must be added to the heap to meet the leachate standard can be smaller than for conventional chemical treatments. The feasibility for concomitant biogas generation due to biodegradation of cyanide under anaerobic conditions is an economic benefit of bioremediation.

Relatively high cost of testing and developing a process tailored for each application are the major challenges to be addressed to make bioremediation more attractive. Further studies must consider the factors such as interactions of cyanides with the environment, improvement in bio-augmentation strategies, degradation enzymes, biochemical, and genetic aspects for the better application in field-scale bioremediation. The search for effective candidates for bioremediation of contaminated tailings or other wastes, micro-organisms need to be selected not only on their ability to degrade cyanide compounds, but also to tolerate the additional stresses and compete effectively with indigenous microbial populations within the environment in which they will be operating. Some other bio-sorbent such as biochar can be explored for the remediation of cyanide contaminated soil and effluents.

Disclosure statement

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