Cyanogenic Glycosides and the fate of cyanide in soil

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Abstract

Cyanogenic glycosides (CNgls), the precursor of cyanide (cyanogenesis) following their enzymatic breakdown. Although there are many natural sources of cyanide, including the plants, bacteria and fungi that synthesize and secrete it, the most significant sources of cyanide in the environment are industrial wastes. Soil as a weathered system does not contain cyanides nor does it generate cyanides, except indirectly in supporting the growth of microorganisms, plants and other intimate soil life and of course through anthropogenic activities. The loading rate in soil is the paramount factor determining toxicity to microorganisms or hazard for movement into groundwater and food chain. Cyanide played a primary role in the evolution of life on earth and remains an important form of nitrogen for microorganisms, fungi and plants. The co-evolution between plants, herbivores and pathogens may have afforded some insects and fungi the ability to overcome the defense system based on cyanogenic glycosides, either by their ability to transform the compounds into non-toxic constituents or by sequestration and further use in their own defense. Mobility of cyanide in soils is mostly influenced by volatilization and distribution. However, the rate of volatilization from soils is complex and depends on many factors. The author now reviews the above mentioned factors and with some emphasis on the biological elimination of cyanide.

Keywords: Biodegradation, biofilm, compartmentation, environment, microorganisms, rhizosphere, siderophores

Introduction

Cyanogenic glucosides (CNgls), the precursor of cyanide in many plants, arthropods and some bacteria are amino acid-derived β-glycosides of α-hydroxynitriles. They are widely distributed in more than 1000 species of food plants (notably cassava, peas, beans, and kernels of almonds) (Cade and Rubira, 1982 and Eiser, 1991). Generally, the level of cyanogenic glycosides produced is dependent upon the age and the variety of the plant, as well as environmental factors (Cooper-Driver and Swain, 1976, Woodhead and Bernays, 1977). More than 60 different CNgls are known to be present in more than 2,500 plant species including ferns, gymnosperms, and angiosperms (Bak et al., 2006, Moller and Seigler, 1998 and Poulton, 1990) and it is not uncommon to find cyanogenic and acyanogenic plants within the same species, where the function of cyanogenesis is revealed through their phenotypic characteristics (Francisco and Pinotti, 2000).

Cyanogenesis has been extensively studied in some bacteria. Amongst them are the fluorescent pseudomonads, especially Pseudomonas flourescens and Pseudomonas aeruginosa (Gallagher and Manoil, 2001). Cyanogenesis has also been reported in Chromobacterium violaceum and has often been reported to occur in the case of cyano-bacteria such as Anacystis nidulans, Nostoc muscorum and Plectonema boryanum (Vennesland et al, 1981 and Knowles and Buch, 1986). Some strains of Rhizobium leguminosarum have also been reported to produce cyanide as free-living bacteria (Antoun et al., 1998). Apart from producing various protein toxins, P. aeruginosa also produces small molecular toxins such as cyanide that facilitate the overall virulence of this opportunistic bacterium against multiple hosts (Lyczak et al, 2000, Terada et al., 1999, Britigan et al., 1999, Olivera et al., 1999 and Blumer and Haas, 2000). CNgls are also found in species within Diplopodia (millipedes), Chilopoda (centipedes) and particularly within Insecta (Davis and Nahrstedt, 1985). Siderophores and cyanide production ability in various pseudomonads are reportedly linked to antagonistic and disease suppressing activity against various plant pathogens (De Vleesschauwer et al., 2006). Accordingly, older arthropods and plant lineages contain aromatic cyanogenic glucosides while relatively more recent lineages like Lepidopteran species and angiosperms have acquired the capacity to contain aliphatic cyanogenic glucosides.

Cyanide metabolism in microorganisms have been investigated and described. Cyanide toxicity to a wide spectrum of organisms is as a consequence of its ability to form complex with metals (Fe²⁺, Mn²⁺ and Cu²⁺) that are functional groups of many enzymes, inhibiting processes like the reduction of oxygen in the cytochrome respiratory chain, electron transport in the photosynthesis and the activity of enzymes like catalase, oxidase (Cheeke, 1995, McMahon et al., 1995). An organism can only metabolize cyanide only when it possesses a biodegradable pathway to
convert cyanide into an assimilative product (NH$_4^+$), cyanide resistance mechanism and a system for taking up Fe$^{3+}$ from the medium (siderophores). Although some organisms synthesize cyanide, a greater number are capable of cyanide biodegradation. The existence of pathways in these organisms allowed for the development of biotechnologies to degrade cyanide compounds in industrial waste streams (Ebb, 2004). These degradation pathways are sensitive to the form and concentration of the cyanide compound, the physiochemical conditions of the media, and the presence of interfering and inhibitory compounds. The lethal single dose of cyanide for vertebrates has been reported to be between the ranges of 35-150 µmol/kg, though much higher amounts of HCN can be tolerated if consumed over a long period (Zagrobelny et al., 2008).

Although cyanide is ubiquitous in the environment, the highest environmental levels are found in the vicinity of combustion sources (automotive exhaust, fires, cigarette smoke and solid waste incineration); in waste waters from chemical industries; in landfills and associated ground water; and in areas of road salt applications and runoff (Fiskel et al., 1981, ATSDR 1997). Cyanide can be present in environmental matrices and waste streams as simple cyanides (e.g. HCN, CN-, NaCN), metal cyanide complexes, cyanates and nitriles (Ebb, 2004). Soils, the worlds underfoot, are the most abundant natural system with which beings make contact not only directly but indirectly. The soil has proven to be an acceptable waste receptacle and will always play an important part in waste disposal despite trends toward recycling of waste constituents. It is arguably the oldest and most effective chromatographic column in the history of the world. Soil is the unconsolidated outer cover of the earth and represents the weathered product of environmental factors at any specific location. They differ in characteristics just as do plants and animals and also differ greatly from one place to another, yet they perform the same unique activities of biodegrading, precipitating, attenuating, sorbing/desorbing, structuring and integrating every chemical behaviour known to mankind (Fuller, 1984).

Soil-agricultural wastes interactions are a complex set of relationships that are dependent on the soil environment, microbial populations and the chemical and physical properties of the soil and wastes materials (Ubalua, 2007). Many toxic waste waters entering the environment as a result of anthropogenic activities are potentially biodegradable to less toxic compounds (Ezeronye and Ubalua, 2005). Cyanide is highly toxic for most living organisms because it forms very stable complexes with transition metals that are essential for protein function, i.e., iron in cytochrome oxidase (Luque-Almagro et al., 2005). Consequently, organisms growing in the presence of cyanide must have a cyanide-insensitive metabolism, such as the alternative oxidase described for plants (Berthold et al., 2000) or the cytochrome bd (or cyanide insensitive oxidase) in bacteria (Jüinemann, 1997, and Richardson, 2000). The exploitation of cyanides by a variety of taxa, as a mechanism to avoid production or to inhibit competitors has led to the evolution in many organisms of enzymes that catalyse degradation of a range of cyanide compounds (Cummings and Baxter, 2006). The presence of cyanide in the environment causes an additional problem, the formation of extremely stable metal-cyanide complex that make essential metals unavailable to the organisms. Therefore, bacterial proliferation in the presence of cyanide requires specific metal uptake systems. The strategy for iron uptake consists of the production of organic compounds, generically called siderophores which strongly bind iron and are subsequently transported and assimilated (Andrews et al., 2003 and Faraldo-Gomez and Sansom, 2003).

The biological assimilation of cyanide needs, at minimum, the concurrence of three separate processes, i.e., a cyanide resistance mechanism, a system for metal acquisition and a cyanide assimilation pathway. Although all of these factors in conjunction with one another have never been taken into account, a number of microorganisms that are able to degrade cyanide and its metal complexes have been described to date (Barclay et al., 1998, Dubey and Holmes, 1995, Goncalves et al., 1998, Harris and Knowles, 1983 and Raybuck, 1992). Dumestre et al., (1997) reported that some phytopathogenic fungi, like Piasarium solani are able to degrade cyanide, but that bacterial biodegradation shows considerable advantages since bacteria are more easily manipulated both at biochemical and generic levels. Harris and Knowles, (1983) also reported that Pseudomonas fluorescens NCIMB11764 is capable of growth on cyanide (CN-/HCN) as the sole nitrogen source. Industrially generated cyanide waste water contains free cyanide, in addition to cyano-metal complexes, making it even more poisonous (Huertas et al., 2006). In spite of cyanide toxicity, there are organisms able to survive in its presence and some of them are able to use it as a nitrogen source (Dubey and Holmes, 1995). At present physicochemical treatments are available for these residues, but they are expensive and also present some collateral effects, thus since cyanide is a natural biodegradable compound, it is therefore technically suggestive that biological treatments may be a better alternative for its elimination.

**Evolution of cyanogenic glycosides**

A major goal of modern evolutionary biology is to understand the molecular underpinnings of adaptation. Cyanide played a primary role in the evolution of life on earth and remains an important form of nitrogen for microorganisms, fungi and plants (Oro and Lazcano-Araujo, 1981). Many pathogens have non-pathogenic, plant-associated relatives that share many of the same attributes. Pathogenic and non-pathogenic microorganisms live on plant surfaces and inside plant tissues, and these common habitats provide frequent opportunities for recombination, and horizontal gene transfer, facilitating the evolution and acquisition of common plant colonization mechanism (Beattie and Lindow, 1995, 1999, Bjorklof et al., 2000, Lindow and Brandl, 2003). Studies on the intricacies on plant-Pseudomonas interactions offers the possibility of understanding not only how plants distinguish between closely related bacteria with different pathogenic potential, but also of understanding the factors that affect the evolution of pathogenic and beneficial relationships between animals, plants and bacteria (Preston et al., 1998). Individual Pseudomonas strains may have biocontrol activity, plant growth-promoting activity, the ability to induce systemic plant defense responses or the ability to act as pathogens. It has been hypothesized that plants,
herbivores and pathogens may have co-evolved in a constant chemical warfare for about 430 million years, thus plants do not rely on a single defense mechanism, but rather express multiple defenses comprising the constitutive and induced synthesis of many chemical compounds as well as the production of structural traits (Romeo 1998, Paul et al., 2000, Walling 2000 and Becerra et al., 2001). Presumably, such a combination of different traits may have lead to the evolution of multiple defense syndromes, since the association with specific ecological interactions results in co-variation of defensive traits (Kursar and Colley, 2003 and Agrawal and Fishbein, 2006). The co-evolution between plants, herbivores and pathogens may have afforded some insects and fungi the ability to overcome the defense system based on CNGls, either by their ability to transform the compounds into non-toxic constituents or by sequestration and further use in their own defense (Zagrobelny et al., 2008).

Evidence of CNGls have been documented in more than 2,650 higher plant species distributed among 130 families and further use in their own defense (Zagrobelny et al., 2008). Bak and his colleagues, (2006) proposed that the widespread occurrence of cyanogenic glycosides in nature implies that they are ancient biomolecules in terrestrial plants and that the specific presence of aromatic cyanogenic glycosides in plants do not rely on a single defense mechanism, but rather express multiple defenses comprising the constitutive and induced synthesis of many chemical compounds as well as the production of structural traits (Romeo 1998, Paul et al., 2000, Walling 2000 and Becerra et al., 2001). Presumably, such a combination of different traits may have lead to the evolution of multiple defense syndromes, since the association with specific ecological interactions results in co-variation of defensive traits (Kursar and Colley, 2003 and Agrawal and Fishbein, 2006). The co-evolution between plants, herbivores and pathogens may have afforded some insects and fungi the ability to overcome the defense system based on CNGls, either by their ability to transform the compounds into non-toxic constituents or by sequestration and further use in their own defense (Zagrobelny et al., 2008).

Cyanogenic glycosides are a group of nitrile-containing plant secondary compounds that yields cyanide (cyano-genesis) following their enzymatic break down. The functions of cyanogenic glycosides remain to be fully determined in many plants; although in some plants they have been implied as herbivore deterrents and as transportable forms of reduced nitrogen (Belloti and Arias, 1993, Selmar, 1993, and McMahon et al., 1995). Recent experiments have further accentuated the possibility that cyanogenic glycosides and cyanolipids might serve as nitrogen storage compounds (Selmar et al., 1990). In *Hevea brasiliensis* seeds, the endosperm represents almost 85% of the seed dry matter and contains more than 90% of the cyanogenic glycoside, linamarin. During germination and plantlet development, the cyanogenic potential of the entire seedling declines by 85% as cyanogenic compounds are metabolized to non-cyanogenic substances and negligible amounts of gaseous HCN are liberated during this process. Since highest levels of the cyanide detoxifying enzyme β-cyanoalanine synthase occur in young seedling tissues, Selmar et al., (1988), proposed that linamarin is transported from the endosperm via the apoplast to the young, growing tissues for further catabolism. The lability of this glycoside to apoplastic and intracellular linamarase dictates the need for a protected transport form of resistant to linamarase action. A suitable candidate may be the disaccharide linustatin, derived from linamarin by glucosylation. Moving safely via the apoplast and vascular system to target tissues, linustatin would be degraded there by disaccharidase to HCN. Detoxification of HCN to asparagines by β-cyanoalanine synthase would allow this nitrogen to reenter general metabolic pools. Much evidence supports this attractive hypothesis: (a) that linustatin is not hydrolyzed by linamarase; (b) linustatin levels in *Hevea* seeds increase upon storage; (c) at that developmental stage when the linamarin content is decreasing, linustatin occurs in endosperm exudates, and increasing levels of β-cyanoalanine synthase and a linustatin-splitting disaccharidase are found in seedling tissues; and (d) linustatin is present in leaf nectary and phloem exudates. Whether linamarin metabolism and utilization occur in other cyanogenic species via this so-called ‘linustatin pathway’ is still under investigation (Selmar et al., 1998). Concurrently, cyanogenic glycosides are synthesized but reach levels equal to only one-fourth of the original cyanolipid content. This large decrease in cyanogenic potential points to major utilization of cyanolipids for synthesis of non-cyanogenic compounds.
Arguably, the most agronomically important of all the cyanogenic crops, may be the tropical root crop cassava (Manihot esculenta, Crantz). All cassava tissues, with the exception of the seeds, contain the cyanogenic glycosides linamarin (>90% total cyanogens) and lotaustralin (<10% total cyanogens). The leaves have the highest cyanogenic glycoside levels (5.0 g linamarin/kg fresh weight), whereas the roots have approximately 20-fold lower linamarin levels. In addition to tissue-specific differences, there are cultivar-dependent differences in root cyanogens levels. Total root linamarin levels range between 100 and 500 mg linamarin/kg fresh weight for low and high cyanogenic cultivars, respectively (Okafor, 2005). A common feature of cyanophoric plants is that cyanogenic glycoside hydrolysis occurs at a significant rate only after their tissues have been disrupted by herbivores, fungal attack, or mechanical means. Although other explanations are possible, it is generally assumed that the glycosides and their catalytic enzymes are separated in the intact plant by compartmentation at either tissue or subcellular levels (Poulton, 1988). These possibilities have been extensively tested in the leaves of 6-d-old light-grown sorghum seedlings (Kojima et al., 1979). The authors demonstrated that the substrate and its catalytic enzymes were localized within different tissues. The cyanogenic glycoside dhurin was sequestered in the vacuoles of epidermal cells, whereas the β-glycosidase and hydroxynitrile lyase were present almost entirely in the underlying mesophyll cells. These two enzymes were located in the chloroplasts and cytosol, respectively and it therefore seems likely that the large-scale hydrolysis of dhurin, which probably provides a defense mechanism against herbivores by liberating HCN, occurs only after tissue disruption allowing the mixing of contents of the different tissues. Cyanogenesis (in cassava) is initiated when the plant is damaged. Rupture of the vacuole releases linamarin, which is hydrolyzed by linamara, a cell wall-associated β-glycosidase (McMahon et al., 1995). Hydrolysis of linamarin yields an unstable hydroxyl-nitrile intermediate, acetone cyanohydrin. Acetone cyanohydrin spontaneously decomposes to acetone and HCN at pH >5.0 or temperatures >35°C and can be broken down enzymatically by HNL (Hasslacher et al., 1996 and Wajant and Pfizenmaier, 1996) to HCN, and an aldehyde or ketone (Poulton, 1990). The need for hydroxynitrile lyases appears puzzling, but it should be noted that, while non-enzymic decomposition proceeds rapidly at alkaline pH, it is negligible below pH 5.5. The major role of α-hydroxynitrile lyases is presumably to accelerate release of HCN (and carbonyl compounds) in plant macerates, which commonly are slightly acidic (pH 5.0-6.5). This assumption is supported by mixed enzyme incubations in which various ratios of hydroxynitrile lyase to β-glucosidase were analyzed for rapidity of HCN evolution (Selmar et al., 1989). A ratio of 2 : 4, close to the average found in seven species tested, accelerated the rate of acetone cyanohydrin dissociation 20-fold over non-enzyme rates. Noting that the efficacy of cyanogenesis as a defense mechanism against herbivory undoubtedly depends upon the rate of HCN release as well as the total amount liberated, Selmar et al., (1989) proposed categorizing cyanogenic plants according to their ability for rapid or slow cyanogenesis.

**Bacterial cyanogenesis and rhizospheric processes**

The surfaces and surroundings of plants form a nutrient-rich habitat for complex microbial populations that can positively or negatively influence plant health and growth (Francis et al., 2010). Plant growth and development are significantly influenced by the presence and activity of microorganisms and can be promoted by a diversity of mechanisms that increase nutrient accessibility, facilitate mineral and nutrient uptake, decrease soil toxicity, release growth-stimulating phytohormones, modulate hormone production by the plant, supply nitrogen and phosphate via symbioses, or enhance the effects of symbioses (Welbaum et al., 2004, Podile and Kishore, 2006). Bacteria can attack, repel, antagonize, compete or collaborate with other organisms affecting the composition of the microbial communities and plant development (Welbaum et al., 2004)

Many microorganisms in the natural environment exist in multicellular aggregates severally described as biofilms (Parsek and Faulk, 2003 and Stoodley et al., 2002). Bacterial biofilms may be defined as highly structured, surface-attached communities of cells encased within a self-produced extracellular polymeric matrix (Costerton et al., 1995). Bacterial cells adhere to surfaces and to each other through a complex matrix comprising of a variety of extracellular polymeric substances (EPS) including exopolysaccharides, proteins and DNA (Ramey et al., 2004). Most plant-bacterial associations rely upon the physical interaction between bacteria and plant tissues. Direct observations of bacteria adhered to plant surfaces have revealed multicellular assemblies variably described as microcolonies, aggregates and cell clusters (Morris and Monier, 2003, Monier and Lindow, 2004 and Bloomberg and Lugtenberg, 2004).

The two most extensively studied bacteria for cyanogenesis commonly found in soil are *Pseudomonas aeruginosa* and *Pseudomonas fluorescens*. *Pseudomonas aeruginosa* is renowned for its nutritional and ecological versatility. The effectiveness of this organism in causing infection is likely due to a suite of well-regulated virulence factors and defense mechanisms such as multidrug resistance pumps (Chuanchuen et al., 2001) and biofilm formation (Costerton et al., 1999). They are capable of producing various protein toxins and small molecular toxins such as cyanide that facilitates the overall virulence of this opportunistic bacterium against multiple hosts (Lyczak et al., 2000, Terada et al., 1999, Britigan et al., 1999; Olivera et al., 1999, Blumer and Haas, 2000 and Walker et al., 2004). Some *Pseudomonas* has been reportedly characterized as root colonizers of several food crops that evade pathogenesis against multiple pathogens (Bano and Mussarrat, 2003). Comparatively, *Pseudomonads* are one of the important groups of soil microorganisms playing various roles in plants growth and development. Although they have been reported to inflict both beneficial and harmful effects on plants, they act through various mechanisms. Among the various mechanisms, cyanogenesis is one of the important factors used by *Pseudomonads* to cause positive and less studied negative effects in the rhizosphere (Rudrappa and Bais, 2008). The effects of *Pseudomonad* cyanogenesis on *Bacillus subtilis* colonization and biofilm formation on Arabidopsis has been demonstrated (Rudrappa et al., 2008). Plant-associated
...between cutin layer may offer greater potential for direct signaling with a hydrophobic cutin layer. Arguably, lack of such a uptake, and present a large surface area that is not covered to leaf surfaces, roots are designed for nutrients and water Pseudomonas; (ii) root surfaces colonized by rhizosphere Pseudomonas; (iii) intercellular spaces in leaves colonized by endophytic Pseudomonas; and (iv) intercellular spaces in roots colonized by endophytic Pseudomonas. In contrast to leaf surfaces, roots are designed for nutrients and water uptake, and present a large surface area that is not covered with a hydrophobic cutin layer. Arguably, lack of such a cutin layer may offer greater potential for direct signaling between Pseudomonas and epidermal cells than on foliar surfaces. Roots are known to release substantial quantities of root exudates, which are rich in sugars, dicarboxylic acids, amino acids, and sloughed off root border cells, which support a complex microflora and microfauna of saprotrophs, symbionts and predators (Gilroy and Jones, 2000; Hawes et al., 2000). Roots also produce significant levels of secondary metabolites, many of which have antimicrobial activity. In addition to direct interactions with plant cells, root-colonizing Pseudomonas can affect plant physiology through interactions with other rhizosphere organisms, such as mycorrhizal fungi, soil-borne plant pathogens, and nitrogen-fixing and nitrogen-cycling bacteria (Lugtenberg et al., 2001).

Attachment of bacteria to root surface is by the use of lipopolysaccharide, cell surface agglutinin, and exopolysaccharide (Michiels et al., 1991; Amellal et al., 1998) and Gram-negative bacteria has been associated with the production of acylhomoserine lactones (AHLs) (Whitehead et al., 2001). AHLs are known to regulate quorum sensing (QS) behaviour and biofilm formation and its production is more frequent in fluorescent pseudomonads isolated from the rhizosphere than in isolates from the bulk soil (Elasri et al., 2001). As a complex and dynamic organ, the root controls various biochemical and physiological processes that are crucial for the survival of the plant (Mally et al., 2004), among such critical processes, regulation of microbial recruitment and dynamics are most vital. It has been reported that plants regulate microbial processes by deterring pathogenic microorganisms and selectively attracting beneficial microorganisms (Ramey et al., 2004). How these microorganisms establish themselves as communities on the root surface is a critical question because, like their plant counterpart, microorganisms are equally dynamic and employ various mechanisms to cope with changed conditions (Langer et al., 2004), such as multidrug resistance pumps (Chuanchuen et al., 2001). Apart from secretion of AHLs, plant-derived compounds also influence biofilm formation by interfering with the bacterial QS mechanism (Rasmussen et al., 2005). One system for which chemical and molecular evidence for QS inhibition has been identified is that of the primitive plant, Deliseea pulchra, a marine red alga (Yoon et al., 2006). Deliseea pulchra produces structural analogs of AHLs, halogenated furanones, which bind competitively to AHL receptors, instigating proteolytic degradation and inhibition of associated QS signals (Teplitski et al., 2004). This activation suggests that there is significant QS cross-talk between different bacterial species on plant roots, thereby regulating the outcome of root-associated biofilm formation. In addition to their associations with QS mechanisms, plants may also regulate bacterial associations by influencing the structure of biofilms attached to their root surface by varying rhizosphere nutrient status, as suggested by abiotic surface studies (Shrout et al., 2006). Generally, the root surfaces of plants are continually subjected to the two-way traffic of solutes from plants to the soil and vice versa (Lugtenberg et al., 1999; Dakora and Phillips, 2002). A broad range of environmental factors could cause fluctuations in root surface properties and this dynamic environment may therefore make it challenging for two-way communication between plants and microbial communities in the rhizosphere (Bais et al., 2002). This interaction becomes more complicated when more than one bacterium is involved, as observed in the case of multispecies microbial associations (An et al., 2006). A plant-bacteria interaction may be categorized as beneficial if the net benefit (suppression of pathogens, promotion of plant growth and disease resistance) outweighs the net cost. The potential negative effects of any single factor are strongly affected by the genetic and ecological context (Preston, 2004).

The factors influencing biofilm formation are most likely diverse, including proteins, secondary metabolites, organic acids, amino acids and small peptides (Charon et al., 1997). These factors may function in a differentially selective manner to enhance the competitive ability of a particular species from a heterogenous rhizosphere microbial community when compared with bulk soil (Small et al., 2001). It has been suggested that many polysaccharides produced by bacteria modulate the chemical and physical properties of P. aeruginosa biofilms on abiotic surfaces (Friedman and Kolter, 2004) and that the plant might also secrete specific compounds, which can suppress pathogenic interactions by reducing attachment and binding (Teplitski et al., 2000). In contrast to pathogenic multistrophic interactions, biofilm formation can be beneficial for many organisms. Biofilm formation in Bradyrhizobium elkanii SEMIA 5019 and Penicillium sp. significantly increases nodulation, and nitrogen accumulation in soybeans compared with planktonic inoculant (Jayasinghearachi and Seneviratne, 2004). One beneficial rhizobacterium is B. subtilis, which is ubiquitous in soil, can promote plant growth, protect against fungal pathogen attack (Ukhiede and Smith, 1992, Asaka and Shoda, 1996, Emmert and Handelsman, 1999) and play a role in the degradation of organic polymers in the soil (Emmert and Handelsman, 1999). The site of one such
ecologically beneficial bacterial community is the rhizosphere, where a rich microflora develops around the readily available nutrients released by roots (Weller and Thoma-Show, 1994).

Biofilm formation is much more robust in wild-type B. subtilis isolates than in highly subcultured laboratory strains (Kinsinger et al., 2003), and biofilm-like structures (pellicles on liquid media or on semi-solid media) are dependent on the secretion of surfactin. This assertion was further validated by the elegant research conducted by Rudrappa and co-workers, (2008) in which they demonstrated that the biofilm depth in the root tip is less than in mature root regions. They concluded that such variations may be due to fluctuations in the composition of the root exudates and nutrient availability at the root plane or specific secretion of antimicrobials from the root tip. In addition, involvement of the point of emergence of lateral roots in secretion and subsequent chemo-attraction of bacteria leading to microcolony formation (McDougall and Rovira, 1987; Cooley et al., 2008) may have increased biofilm thickness in mature regions of the root. Concomitantly, Shroult et al., (2006) showed that variations in nutrients influence Pseudomonas aeruginosa bacterial swarming. Thus, a reduction in bacterial swarming is associated with abundant nutrient availability leading to a more ‘structured’ three-dimensional (3-D) biofilm. In contrast, lower nutrient levels influenced increased swarming of P. aeruginosa, resulting in a more ‘flat’ biofilm structures (Shroult et al., 2006).

**Fate and transport of cyanide in soil**

Soil as a habitat for microorganisms, is probably the most complex and diverse on the planet. It is a biomembrane and can be a source or sink for most gases. A further source of complexity in soil biological activity is the existence of exocellular enzymes, presumably derived from past populations of organisms but stabilized by sorption on mineral surfaces and retaining at least part of their activity (Burns, 1978). Soil is also used for waste disposal, so detoxification and filtering functions are important. A vast range of organic wastes are applied to soil including sewage sludge, composted municipal waste and effluents from biologically-based industries such as the processing of oil palm and cassava (Powlsion et al., 2001). Hydrogen cyanide is ubiquitous in nature. Principal natural sources of cyanides are from over 2,000 plant species, including fruits and vegetables that contain cyanogenic glycosides which are from over 2,000 plant species, including fruits and vegetables that contain cyanogenic glycosides which are decomposed to ammonia, carbon dioxide and nitrogen, or form stable nitrile derivatives (Solomonson, 1981; Knowles, 1988). Natural soil microfloras have been demonstrated to convert cyanide to carbonate and ammonia (Strobel, 1967). Cyanide present at low concentrations will be decomposed to ammonia, carbon dioxide and nitrogen, or nitrate under aerobic conditions, and to ammonium ion, nitrogen, thiocyanate and carbon dioxide under anaerobic conditions (Rouse and Pyrh, 1990). A strain of Bacillus pumilus from clay samples planted with flax was found to degrade a 0.1 ml L−1 cyanide solution to carbon dioxide and ammonia (Knowles, 1976). Cyanide is a major inhibitor of the enzyme cytochrome oxidase as well as hemoproteins and other metal-containing oxidases or oxygenases. At concentrations of about 10−4 mol L−1 or lower, cyanide is usually highly inhibitory to cytochrome oxidase while other enzymes require 10−5 to 10−7 mol L−1 of cyanide for significant inhibition (Knowles, 1976). It has been proven that unacclimatized mixed microbial populations are significant when compared with rates of volatilization and biodegradation (Callahan et al., 1979 and ATSDR, 1991). However, small amounts of cyanide in soil may be oxidized to cyanate (HCNO) (Chatwin, 1989). It has been hypothesized that cyanide must be present as hydrogen cyanide as in surface waters in order to volatilize from soils (Higgs, 1992). However, the rate of volatilization from soils is complex and depends on many factors, including pH, cyanide solubility, hydrogen cyanide vapour pressure, free cyanide concentration, soil water content, soil sorptive properties, soil porosity, organic matter content, density and clay content and atmospheric conditions such as barometric pressure, humidity, and temperature (Chatwin and Trepansowski, 1987; Chatwin, 1989). Empirical studies on the partitioning of hydrogen cyanide between gas and solution phases in unsaturated soils showed that its migration through soil occurs mainly through gas diffusion. Hydrogen cyanide volatilization from unsaturated soils could account for up to 10% of total cyanide losses (Chatwin, 1989). In acidic soils, alteration of soil 

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Table 1. Cyanide degradation pathways

<table>
<thead>
<tr>
<th>Reaction and enzyme</th>
<th>Micro-organism</th>
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<tr>
<td>Oxidative</td>
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<tr>
<td>Cyanide mono-oxygenase</td>
<td>Pseudomonas sp.</td>
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<tr>
<td>HCN + O₂ + H⁺ + NADPH → HOCN + NADP⁺ + H₂O</td>
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<tr>
<td>Cyanide dioxygenase</td>
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<tr>
<td>HCN + O₂ + H⁺ + NADPH → CO₂ + NH₃ + NADP⁺</td>
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<tr>
<td>Cyanase</td>
<td>Escherichia coli, Rhodococcus Rhodochrous Pathogenic fungi</td>
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<tr>
<td>Hydrolytic</td>
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<tr>
<td>Cyanide hydratase</td>
<td>Pseudomonas, Corynebacterium, Brevibacterium</td>
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<tr>
<td>HCN + H₂O → HCONH₂</td>
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<tr>
<td>Nitrile hydratase</td>
<td></td>
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<tr>
<td>R-CN + H₂O → RCONH₂</td>
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<tr>
<td>Cyanidase</td>
<td>Alcaligenes xylosoxidans</td>
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<tr>
<td>HCN + 2H₂O → HCOOH + NH₃</td>
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<td>Nitralase</td>
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<tr>
<td>R-CN + 2H₂O → RCOOH + NH₃</td>
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<tr>
<td>Substitution/transfer</td>
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<tr>
<td>Rhodanese</td>
<td>Thiobacillus denitrificans, Bacillus subtilis, Bacillus stea rothermophilus</td>
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<tr>
<td>HCN + S₂O₃²⁻ → HCN₅ + SO₃²⁻</td>
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<tr>
<td>Cyanooalanine synthase</td>
<td>Bacillus megar etium</td>
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<tr>
<td>Cys + HCN → β – cyanalanine + HS⁻</td>
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</table>

Courtesy: Huertas et al., 2006

adversely affected by cyanide at concentrations of 0.3 mg HCN.Kg⁻¹. In contrast, acclimatized populations in activated sewage sludge may be unaffected by concentrations as high as 60 mg total cyanides.Kg⁻¹ (Towill et al., 1978). Cyanide ions may also form complexes with heavy metals, particularly iron, and precipitate out of solution (Lagas et al., 1982; Chatwin, 1989). It has been reported that hydrogen cyanide is not susceptible to photolysis in soils (Cicerone and Zellner, 1983), but complex cyanides, such as ferrocyanides and ferricyanides, may rapidly photo-dissociate and release free cyanide when exposed to sunlight (Callahan et al., 1979; Fiksel et al., 1981; Meeussen et al., 1992).

The mobility of cyanide compounds in soil depends on stability and dissociation characteristics of the compound, soil type, soil permeability, soil chemistry, and the presence of aerobic and anaerobic microorganisms (Fuller, 1984; Higgs, 1992). In aerobic conditions, biodegradation is expected to be an important cyanide process. Experimental studies on the mobility of cyanide in saturated anaerobic soils have shown that aqueous simple cyanides and aqueous ferricyanides tend to be very mobile. Cyanide dissolved in leachate were found to move through soils much more slowly than those in aqueous solution as they tended to precipitate out as the relatively immobile compound (Prussian blue) (Alessi and Fuller, 1976; Fuller, 1977, 1984). It should be noted, however, that although Prussian blue tends to precipitate out in soils with pH >4, some of the compound remains in solution and may result in contamination of ground water by iron cyanide (Meeussen et al., 1992). Copper, cobalt, zinc, and nickel-cyanide complexes were found to be relatively mobile in soils compared to iron and manganese-cyanide complexes (Chatwin, 1989 and Higgs, 1992). Soils conditions that increase the mobility of cyanide include low pH, high negative soil charges, and low clay content. Neutral to alkaline pH, high clay content, high positive soil charges, and the presence of organic matter and iron or other metal oxides appear to increase the attenuation of cyanide in soils (Alessi and Fuller, 1976; Fuller, 1977, 1984). The presence of aerobic soil microbes is particularly important to the attenuation of cyanide since mobility under aerobic conditions is greatly reduced due to higher rates of biodegradation (Fuller, 1984). Thus, cyanide leaching in groundwater is enhanced under anaerobic conditions.

Biodegradation and distribution of cyanide

Cyanide-yielding organic compounds are introduced naturally into the soil, by a great number of living systems. One of the most common natural sources originates from plants. They are characterized most abundantly by the glycosides that yield hydrocyanic acid (HCN) upon hydrolysis (Fuller, 1984). One of the best known
cyanogenic (or cyanophoric) glycosides occur in members of the *Rosaceae* family and are called amygdalin. The amount of amygdalin glycogen accumulated by a single plant varies between species, depending on environmental conditions for example, plants that have wilted, frosted, or have been stunted are most suspect in the incidence of HCN rumen poisoning than unstressed plants (Fuller, 1984). It has been postulated that cyanides accumulate in soils via biological degradation of plants that produce abundant cyanogenic glycosides, such as sorghum and through the activities of mankind (Fuller, 1984). Cyanides are also generated by a great number of soil microorganisms including fungi, bacteria, actinomycetes, and algae. The cyanide from natural sources does not persist in the soil. The relatively small amounts produced are readily attacked by soil microorganisms and converted to carbonate and ammonia. Some cyanide may be released to the atmosphere and dispersed depending on the pH and redox of the soil environment. Cyanide (CN⁻), up to 200 ppm at least, is readily converted to nitrogen in the soil (Fuller, 1984). In fact, levels of many cyanides equivalent to the nitrogen requirements of cultivated crops support plant response, which is almost identical to that from other nitrogen sources such as sodium or ammonium nitrate on an equivalent N basis. According to Connemeyras et al., (2004), organic and inorganic cyanide compounds are widely distributed on earth, indeed they have been postulated to have played a key role in the prebiotic chemistry that led to the evolution of biological macromolecules and primitive life. As a result, these compounds have had a significant presence in the environment throughout the evolution of life. The toxicity of cyanide is dependent upon the form in which it occurs. However, the cyanide anion CN⁻ is the primary toxic agent, regardless of origin. Many toxic effluents and compounds that have entered the environment as a result of man’s activities are biodegradable or potentially biodegradable to less toxic compounds. Many microorganisms have an inherent capacity to degrade the toxic organic compounds that enter the environment as a result of pollution and natural activities. The success of biodegradation depends upon the presence of microbes with the physiological and metabolic capabilities to degrade the pollutants in the contaminated environment and a range of physico-chemical parameters (Cummings and Baxter, 2006; Ubalua, 2007). Although there are many natural sources of cyanide, including the plants, bacteria and fungi that synthesize and excrete it, the most significant sources of cyanide in the environment are industrial wastes. Cyanide is one of nature’s most toxic substances. The level of toxicity of the more stable cyanides depends on the metal present and on the proportion of CN⁻ groups converted to simpler alkali cyanides. The loading rate in soil is the paramount factor determining toxicity to microorganisms or hazard for movement into groundwater and food chains (Ubalua, 2007). High concentrations in the environment usually are associated with accidental spills or improper waste disposal. Some of the reactions attributed to the low levels of cyanides in soils are:

- Biological dissemination and assimilation (metabolism)
- Microbial transformation to CO₂, H₂O, NH₃, and metals (hydration and oxidation)
- Dispersion to the atmosphere as gases and/or to water sources (translocation, volatilization, and dispersion)
- Complex formations with metals (chelation)
- Chemical combination and precipitation (precipitation)
- Adsorption to surfaces (surface physical chemistry) and
- Photodegradation (Fuller, 1984)

Several methods (physico-chemical and biological) can be utilized to effectively degrade cyanide. Presently, physico-chemical treatments are more expensive and may also present some collateral effects (Huertas et al., 2006), compared to biological treatment. Arguably, since cyanide is a natural biodegradable compound, biological treatments may be more suitable and effective in the elimination of cyanide from industrial effluents (Whitlock and Mudder, 1998). Suggestively, biodegradation of cyanide may have been favoured because cyanide is a good source of nitrogen for bacterial growth (Huertas et al., 2006). In addition to existence of biodegradable pathways in some microorganisms to convert cyanide into an assimilative product (NH₃), they also contain cyanide resistance mechanism and a system for taking up Fe⁺ from the medium ( siderophores), since Fe⁺ forms very stable complexes with cyanide (Huertas et al., 2006).

The ability to degrade cyanides has been demonstrated by both eukaryotes and prokaryotes from a diverse range of taxa across a wide range of metabolic pathways (Baxter and Cummings, 2006). Microbes capable of cyanide detoxification are widely distributed in natural systems (Knowles and Wyatt, 1992). These cyanide degrading organisms have enzymatic systems that can be broadly described as oxidative, hydrolytic and substitution/transfer in nature. Oxidation of cyanide begins by the formation of CNO⁻ (cyanate) (Knowles and Wyatt, 1992) and eventually forming both carbon dioxide and ammonia. Cyanide monoxygenase converts cyanate to cyanate, with cyanase catalyzing the bicarbonate-dependent conversion of cyanate to ammonia and carbon dioxide (Table 1). Cyanases, reportedly have been variously identified in numerous bacteria, fungi, plants and animals (Guillotin et al., 2002). The presumed role of cyanase has long been as protective against cyanate poisoning (Raybuck, 1992). As cyanate is not a common metabolite, more fundamental roles for cyanases in biocarbonate/carbon dioxide and nitrogen metabolism have been proposed. Additional suggested roles for plant cyanases include ammonia assimilation following cyanate biodegradation and a role in the concentration and delivery of carbon dioxide for photosynthesis (Guillotin et al., 2002). Ebbs, 2004 considered these roles to be speculative and to rely heavily upon the assumption that cyanate arises at sufficient rate, such as through the degradation of urea and the nucleotide precursor carbamoyl phosphate. If these results are substantiated, then additional emphasis might need to be placed upon the biological role of cyanate and its biodegradation. A second oxidative pathway utilizes cyanide dioxygenase to form ammonia and carbon dioxide directly (Table 1). Recently, the requirement for a pterin cofactor in this reaction has been proposed (Kunz et al., 2001). Additionally, in *Escherichia coli* strain BCN6 and *P. fluorescens* NC1MB 11764, the formation of cyanohydrin complexes was reportedly necessary for oxygenase-mediated cyanide biodegradation (Kunz et al., 1992; Figueira et al., 1996). Whether or not complexation is obligatory for cyanide biodegradation via oxygenase activity is yet to be established. Hydrolytic reactions are mainly characterized
for the direct formation of the products; formamide or formic acid and ammonium, which are less toxic than cyanide and may also serve for growth (Huertas et al., 2006). Cyanidase (cyanide dihydrodase) is principally bacterial. Cyanide hydratase and cyanidase have recently been shown to have similarity at both the amino acid and structural levels to nitrilase and nitrile hydratase enzymes (O’Reilly and Turner, 2003). Nitrile-utilizing enzymes have been reported found in a wide variety of bacterial, fungal, and plant species. Nitrilases and nitrile hydratases convert both aliphatic and aromatic nitriles to the corresponding acid or amide, respectively, but show less substrate specificity than cyanide hydrates and cyanidase (Ebbs, 2004). Direct hydrolysis of cyanide to formic acid and ammonium has been demonstrated, and in parallel with the nitrile-hydrolysing enzyme nitrilase, both have been named cyanidase (Table 1).

Substitution reactions are principally mediated by two sulphur transferases: rhodanese and cyanoalanine synthase. Cyanide has a high affinity for sulphur and accordingly there are two sulphur transferases able to produce thiocyanate from cyanide (Table 1). In this context, the physiological function of the rhodanese seems to be the maintenance of the sulphane sulphur pool in organism and the incorporation of reduced sulphur for iron/sulphur proteins. Kinetic studies suggest that the enzyme works in two steps: first, thiosulphate donates a sulphur to a cysteine thiol on the protein to form an intermediate and secondly, cyanide attacks to produce thio-cyanate and regenerate the enzyme. Huertas et al., (2006), corroborated the production of nitrates or α-amino acids from cyanide by pyridoxal phosphate enzymes through substitution reactions. They proposed that the β-cyanoalanine synthase catalyses the substitution of a three-carbon amino acid with cyanide and concluded that the three-carbon substrate is often cysteine or β-acyctylserine (Table 1).

Currently, the most accepted and most feasible form of biological treatment of cyanide is through the use of bacteria and some of them that are commonly utilized are *Pseudomonas, Achromobacter, Flavobacterium, Nocardia, Bdellovibrio, Mycobacterium, Nitrosomonas* and *Nitrobacter* (Akcil, 2003). In the biological treatment process, bacteria are used to naturally biodegrade both free and metal-complexed cyanides to biocarbonate and ammonium (Akcil, 2003). The metals that are freed in the process are either absorbed by the biofilm or are precipitated out of solution and the rate at which these metal-cyanide complexes (zinc, copper, nickel and iron) degrade is directly related to their chemical stability (Akcil, 2003). In addition, the ability of a biological system to be adapted/engineered to handle large flows and high cyanide levels makes biological treatment even more valuable.

**Cyanide Reactions**

The soil as a weathered system does not contain cyanides nor does it generate cyanides, except indirectly in supporting the growth of microorganisms, plants, and other intimate soil life and of course through anthropogenic activities. In fact soil organic matter inactivates the toxic effects of cyanide as a result of its affinity for combining. Cyanides that enter the soil from the low-level natural sources are rapidly biodegraded and quickly metabolized by soil microorganisms. One of the important aspects of biodegradation is the optimization of the growth of the microorganisms involved in the process, in terms of pH, temperature, nutrient status, oxygen availability, population density and the presence of interacting inorganic and organic compounds. For example, strains of *Alcaligenes* sp. (DSM 4010 and DSM 4009) show maximum cyanide degradation at 37°C and pH 6 to 8.5 (Ingvorsen, 1990). Though most strains of *Salmonella* and *Escherichia coli* are cyanide sensitive, some natural isolates from cyanide-contaminated soil and water produce inducible enzymes. These enzymes catalyse the conversion of cyanide into ammonia and either formate or carbon dioxide. Availability of nutrients and the physical nature of soils also have wide ranging effects on bioremediation. The bioavailability of a contaminant is controlled by a number of physico-chemical processes, such as sorption, desorption, diffusion and dissolution (Boopathy, 2000). Temperature is an important parameter in the determination of the rate of biodegradation and different soil contaminants may have dissimilar temperature optima (Thomas and Lester, 1993). Populations in the upper layers of soil are exposed to varying temperatures, due to fluctuations throughout the day and seasonal changes, whereas populations in the soil subsurface are subjected to low temperatures with less fluctuation. Cyanide degrading enzymes are generally produced by mesophilic microorganisms, often isolated from soil, with temperature optima typically ranging between 20 and 40°C, reflecting the growth optima of the source organism.

Microbes capable of cyanide detoxification are widely distributed in natural systems (Knowles and Wyatt, 1992). Oxidation of cyanide begins by the formation of CNO (cyanate) (Knowles and Wyatt, 1992), eventually forming both carbon dioxide and ammonia. Ammonia oxidizing organisms are also widely distributed in aquatic and soil systems (Ward, 1996); with available oxygen, nitrate formation inevitably occurs. For both oxidative and hydrolytic cyanide degradation, the carbon in cyanide ends up as bicarbonate or formate, and the nitrogen is reduced to ammonia. Anaerobic ammonia oxidation has recently been recognized to be performed by many species of nitrogen-transforming bacteria, including ammonia oxidizers and nitrate-reducing or denitrifying organisms. The end product of this ammonia oxidation is the formation of nitrogen gas. Anaerobic cyanide degradation thus has the potential of treating cyanide and additionally removing the nitrogen as a gas. In contrast, aerobic cyanide degradation inevitably exchanges cyanide contamination with nitrate contamination. It has been suggested that in heap solutions where natural or stimulated cyanide degradation has occurred in the presence of oxygen, nitrate is a common degradation product. Nitrate is produced from the oxidation of ammonia (nitrification), which is formed from cyanide degradation. Because nitrate is not readily attenuated in most soils, heap solutions containing nitrate must be treated to remove nitrate before attenuation can be considered for removal of other trace constituents. Furthermore, denitrification of nitrate by adding organic carbon can be performed in the heap materials directly, in the attenuation field during heap effluent disposal, or in ponds adjacent to the heap. Thus, Harrington and Levy, (1999), demonstrated and documented a minimal residence time of 10 days for
nitrate reduction once cyanide has been mostly degraded if sufficient amounts of organic carbon are available, and after natural microbes have been acclimated.

Conclusions

Cyanide and cyanide compounds are widely distributed in the environment, mainly as a result of anthropogenic activities and through cyanide synthesis by a range of microorganisms including higher plants, fungi and bacteria. Low levels of free cyanides in nature do not persist in soils due to many highly reactive indigenous chemical and enzymatic transformations and degradation processes. Many wastewaters are problematic for biological degradation because of the hostile environmental conditions they present to microorganisms. For example, wastewaters can often have extremes of pH or contain a variety of pollutants other than cyanide compounds. Similarly, contaminated soils present a range of physico-chemical conditions that may inhibit microbial growth (Ubalua, 2007).

Prospects for cyanide biodegradation are limited primarily by physical and economical factors. Economic considerations make biological technologies especially attractive in wastes with high organic content, in which concentrations of organics and cyanide can be reduced simultaneously by the microbial consortia (Towill et al., 1978 and White et al., 1988). Most microorganisms capable of biodegrading cyanide are sensitive to cyanide concentrations of cyanide. First, cyanobacteria are phototrophic; as such do not require aeration to obtain oxygen concentrations of cyanide. First, cyanobacteria are phototrophic; as such do not require aeration to obtain oxygen. Cyanobacteria in the biological treatment of small environmentally friendly effluent. Several potential advantages are associated with the use of nitrogen-fixing cyanobacteria in the biological treatment of small concentrations of cyanide. First, cyanobacteria are photosynthetic; as such do not require aeration to obtain oxygen and secondly they do not require the presence of organic substrates to maintain biomass (Gantzzer and Maier, 1990).

Thus, the use of cyanobacteria in the biological treatment of small amounts of cyanide should have lower operating costs than the use of heterotrophic bacteria. However, despite the promising potentials of biological treatment over physico-chemical treatments, one major disadvantage to biological treatment is its susceptibility to climatic conditions. The microorganisms that drive the process requires an operating temperature of at least 50°F. It has been suggested that cold conditions during winter periods could impose an additional thermal requirement on the plant to maintain an acceptable temperature for biological activity (Akcil et al., 2003). Another recent development in the field of cyanide biodegradation is the possible use of plants (phytoremediation) (Baxter and Cummings, 2006). In contrast, chemical treatment methods can only treat the cyanide portion of the waste and leave behind ammonia, another potentially toxic compound at high concentrations while biological treatment systems can treat not only cyanide, thiocyanate and cyanate, but also ammonia and nitrate through a biologically run nitrification and denitrification process in conjunction with the cyanide biodegradation process. Recent discoveries of new microorganisms and perfecion of the biological treatment methods to make it even more economically beneficial may have out-competed chemical treatment method in producing more environmentally friendly effluents.

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