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Review article

The current and future applications of microorganism in the bioremediation of cyanide contamination

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Abstract

Inorganic cyanide and nitrile compounds are distributed widely in the environment, chiefly as a result of anthropogenic activity but also through cyanide synthesis by a range of organisms including higher plants, fungi and bacteria. The major source of cyanide in soil and water is through the discharge of effluents containing a variety of inorganic cyanide and nitriles. Here the fate of cyanide compounds in soil and water is reviewed, identifying those factors that affect their persistence and which determine whether they are amenable to biological degradation. The exploitation of cyanides by a variety of taxa, as a mechanism to avoid predation or to inhibit competitors has led to the evolution in many organisms of enzymes that catalyse degradation of a range of cyanide compounds. Microorganisms expressing pathways involved in cyanide degradation are briefly reviewed and the current applications of bacteria and fungi in the biodegradation of cyanide contamination in the field are discussed. Finally, recent advances that offer an insight into the potential of microbial systems for the bioremediation of cyanide compounds under a range of environmental conditions are identified, and the future potential of these technologies for the treatment of cyanide pollution is discussed.

Introduction

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 (Commeyras et al. 2004). As a result, these compounds have had a significant presence in the environment throughout the evolution of life. However, recent concerns have focussed on the potential toxicity of such compounds, generated chiefly through anthropogenic activity, but also as a result of cyanogenic activity by a number of organisms (Dubey and Holmes 1995).

Cyanides are a group of compounds that contain a C ≡ N group, they are present in the environment in several forms including; hydrogen cyanide; simple inorganic salts including NaCN and KCN; complex metal cyanides, thiocyanates, and as nitriles where the CN binds to an organic radical (Kjeldsen 1999). The toxicity of cyanide is dependent upon the form in which it occurs; free cyanide (HCN or CN^-) is an extremely potent metabolic poison, whereas metal-cyanide complexes or nitriles vary in toxicity according to the

degree of cyanide liberation by these compounds (Figueira et al. 1995).

Cyanide containing compounds find a wide range of uses. For example, metal cyanides, chiefly potassium and sodium salts are employed in electroplating processes, where they are used in the basic degreasing and the electroplating baths to control the concentration of metal ions (Mohler 1969; Smith 2003). In the mining of precious metals, cyanide is widely used to leach gold and silver from the ore. The cyanidation process converts gold and silver to water soluble cyanide complexes (Arslan et al. 2003), each year over a billion tonnes of gold ore are treated using this process, as a result the demand for cyanide by this industry is significant (Young and Jordan 1995). Another significant application of iron cyanide is as anticaking agents both in road salt and fire retardants. In the USA 10 million tonnes of road salt representing an environmental input of 700 tonnes of iron cyanide are used each year (Mudder and Botz 2004). In addition, at the salt storage depots, runoff of precipitation may represent a significant input of cyanide into surface waters (Paschka et al. 1999). Similarly, the use of iron cyanide compounds in chemical retardants for fire fighting results each year in 400 tonnes of cyanide derivatives being applied during forest fire control in the USA (Mudder and Botz 2004).

A recent study estimated that 834,000 tonnes of HCN per annum was required to satisfy the requirements of the US steel, mining, electroplating and chemical industries (ATSDR 2004). Most HCN produced is diverted into the synthesis of adiponitrile essential for the manufacture of nylon, but organic cyanides (nitriles) have significant applications in the manufacture of a variety of man made compounds and products, for example, acrylonitrile, that is used in the manufacture of acrylic fibres and resins (Donberg et al. 1992). Cyanide is also used in plastics, electronic component production and agrochemicals (ATSDR 2004).

As a consequence of its widespread use in industry, and because many cash and food crops naturally synthesise cyanide compounds, there are large volumes of waste generated during industrial and agricultural production and processing that contain significant concentrations of cyanide. These must be treated before being discharged to prevent environment degradation. In this study the most commonly occurring forms of cyanide entering soil and water are identified, with an emphasis on those that result from anthropogenic activity. The literature on the biological degradation of these compounds both in the field and in laboratory based bioremediation studies is reviewed, with particular attention to the contribution that the microbial flora make to these processes. Finally we highlight the future potential for microbial bioremediation of cyanides in water and soil.

Sources of cyanide in the environment

Naturally occurring cyanides

Cyanide compounds are synthesised by many taxa including higher plants, arthropods, fungi and bacteria. Plants in particular are a significant source of cyanide compounds, they exploit the bitter tasting cyanogenic glucosides as a defence against herbivores and pathogens (Jones 1998). Similarly, cyanide compounds are also found within the defence secretions several arthropod taxa (Eisner et al. 1996). However, they are also deployed in other roles by some insects, for example, as pheromones to control mating behaviour (Seidelmann et al. 2003).

A number of fungi and bacteria utilise the toxicity of cyanide compounds in a more overt way by producing and secreting antibiotic cyanide compounds to inhibit competitive organisms. For example, *Chromobacterium violaceum* ATCC 53434 produces the isonitrile antibiotic aerocyanidin that is primarily active against Gram-positive bacteria (Parker et al. 1988), and the fungus *Trichoderma harzianum* produces homothallin II, a nitrile with a broad activity against fungi and Gram-positive and Gram-negative bacteria (Faull et al. 1994). In nutrient limited environments, such as soil, or where competition for resources is intense, as in the rhizosphere, these compounds presumably increase the competitive advantage of the cyanogenic organisms.

Anthropogenic sources of cyanide

There are a number of significant anthropogenic inputs of cyanide into the soil and water

environment. Perhaps the most significant is that associated with the discharge of municipal wastewater by sewage treatment plants, particularly from facilities that serve industrialised areas, where cyanide can reach 0.1 mg l^{-1} (Lordi et al. 1980). Despite its toxicity, there is a substantial demand for cyanide in many processes such as steel manufacture gold mining and electroplating as discussed earlier. Metal-cyanide complexes are formed in large volumes during the leaching of gold from the ore by the cyanidation process, as a consequence there is a large body of literature that has characterised the formation and toxicological consequences of the release of different classes of metal cyanides into the environment. In gold ore, iron, copper, zinc, silver and nickel are frequently present, as cyanide is a strong ligand it readily complexes with these metals. Iron, gold and silver form complexes that are very stable and resistant to chemical and biological treatment, these are described as strong acid dissociables (SADs), whereas copper, zinc and nickel are much less stable and are described as weakly acid dissociable (WADs), the latter are the most significant toxicologically (Akcil 2003; Young and Jordan 1995). The spent potlinings generated during aluminium manufacture are also a significant source of cyanide contamination. Much of this material is awaiting disposal via a environmentally acceptable route (Pong et al. 2000).

Those industries for whom cyanide compounds are an essential raw material or product have devised extensive guidelines for the storage, handling and distribution of cyanide compounds in order to disseminate best practice and minimise potential hazards (ECIC 2003). However, because of the large volumes of effluent generated by these industrial activities, in total, 18 billion litres of cyanide containing waste are estimated to be generated annually in the USA (ATSDR 2004), accidental spillages do occur and are responsible for the contamination of soil and water. The most significant example in recent years occurred when the tailing dam of a mining operation near Baia Mare in Romania was breached, releasing approximately 100,000 m^3 of cyanide and heavy metal contaminated liquid waste into the Tizsa river system (UNEP 2000) resulting in severe mortalities among aquatic organisms and animals living close to the contaminated rivers (Soldan et al. 2001). A follow up study has confirmed that the concentration of cyanide remains high in the sediments of the river and that biodiversity particularly among higher organisms remained substantially reduced (Cordos et al. 2003). Additional sources of cyanide pollution result from effluents of food and feed production. For example, during cassava starch production, the staple food of at least 500 million people in Africa and the tropics, large amounts of cyanogenic glycosides are released and are hydrolysed by plant-borne enzymes leading to cyanide concentrations in wastewater as high as 200 mg l^{-1} (Siller and Winter 1998).

Agriculture also contributes to the discharge of cyanide into the environment, by the application of nitrile pesticides such as bromoxynil and chlorothalonil. Bromoxynil (3,5-dibromo-4-hydroxybenzonitrile) is used as a post-emergence herbicide for the control of diseases of broad leaf crops. Repeated application and runoff from agricultural land may lead to concentrations of bromoxynil as high as 0.33 μg l^{-1} in receiving surface waters (Grover et al. 1997). In addition to its use in agriculture, chlorothalonil (2,4,5,6-tetrachloroisopthalonitrile), a broad spectrum fungicide, is used as an anti-fouling agent on boat hulls (Thomas et al. 2003). Significant concentrations have been found in marinas in the Mediterranean and in the UK coastal environment, leading to a concern that it could cause serious environmental damage impacting species other than the target fouling organisms (Sakkas et al. 2002).

In the European Union and the USA there are also thousands of sites contaminated with cyanide compounds as a result of historical industrial activity, including former coking works, steel manufacturing facilities and electroplating plants. Most common, however, are the sites of former manufactured gas plant (MGP) facilities, of which thousands are estimated to exist (Young and Thiele 1991; Thomas and Lester 1993). During the manufacturing process, contaminants, such as tars, hydrogen sulphide and cyanides were removed using bog iron ore, that when exhausted, was used as an on-site fill (Ghosh et al. 1999). The result of which are sites contaminated with a wide variety of toxic by products and residuals including polyaromatic hydrocarbons (PAH), benzene, toluene, ethylbenzene, xylene (BTEX), heavy metals and

cyanide (Dyer 2003). Soils and groundwaters at former MGPs have cyanide concentrations ranging from 10 to 5000 mg kg^{-1} and 3 to 280 mg l^{-1}, respectively (Thomas et al. 1991).

Factors affecting the environmental fate of cyanides

The success of biodegradation depends upon the presence of microbes with the physiological and metabolic capabilities to degrade the pollutants in the contaminated environment. Cyanide compounds are found relatively widely in nature and microbial mechanisms for cyanide degradation already exist, however, the concentrations of cyanides within a soil or water can have a significant impact on passive biodegradation. For example, acrylonitrile at high concentrations proved toxic to *Brevibacterium imperialis* CBS 489-74, by causing irreversible damage to the nitrile degrading enzyme hydratase and inhibiting the biodegradation of the compound by the organism (Alfani et al. 2001). In soils, the availability of nutrients may effect the biodegradation 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. A soil characterisation study at a former MGP site revealed very low levels of organic carbon in areas where there were elevated cyanide concentrations (Ferguson et al. 2003). It has also been noted that in such soils, phosphorus may be rendered unavailable by iron present in the spent oxide wastes producing additional limitations to growth (Thomas and Lester 1993). The availability of oxygen is a significant factor in the microbial mineralisation of cyanide, as oxygen is consumed during several of the cyanide degrading pathways (O'Reilly and Turner 2003). Cyanide can be toxic to anaerobic bacteria, particularly methanogens, therefore cyanide compounds may remain unmineralised in anoxic soils (Annachhatre and Amornkaew 2000). Additional pollutants present at contaminated sites may also affect biodegradation, the presence of high concentrations of co-contaminants can impact on cyanide degradation by influencing the indigenous microbial population, selecting for, or inhibiting, the growth of particular organisms (Kang and Park 1997).

The physical nature of soils and sediments also has 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). It has been shown that pH, redox potential and total cyanide concentration in soils and groundwater greatly affect the stability of iron cyanide complexes. Iron cyanides are largely stable in acidic soils, such as those at MGPs and have little environmental mobility (Meeussen et al. 1992b, 1995; Shifrin et al. 1996). However, at alkaline pH, the solubility of precipitated iron-cyanide complexes, such as Prussian blue, is greatly increased and allows cyanide to became mobile. It is possible, therefore, for iron-cyanide complexes to dissociate into free cyanide under these conditions and contaminate groundwater (Meeussen et al. 1992a). In reality field observations have indicated that 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. This buffering capacity can be so low, depending on the soil, that complete dissolution of Prussian blue may take decades (Meeussen et al. 1995). However, Ghosh et al. (1999) demonstrated, in the field, that metal-cyanide complexes from MGP materials in the ground act as a constant source of cyanide contamination in groundwater due to leaching. As regards the behaviour of other forms of cyanide in soils, very little literature exists (Meeussen et al. 1995).

Temperature is an important parameter in the determination of the rate of biodegradation and different soil communities 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 (Cowan et al. 1998).

Soil pH may be a particularly important factor in the bioremediation of cyanide contaminated soils. For example, at former MGP sites, soil as

low as pH 2 has been reported (Kjeldsen 1999). The pH optima for bacterial and fungal growth are typically 6–8 and 4–5, respectively, and cyanide degrading enzymes generally have pH optima between 6 and 9, therefore, extremes of pH may have a significant effect on biodegradation. However, *Fusarium solani* and mixed cultures of fungi, including *F. solani*, *F. oxysporum*, *Trichoderma polysporum*, *Scytalidium thermophilum* and *Penicillin miczynski*, were capable of degrading iron-cyanides at pH 4 (Barclay et al. 1998b).

The fate of nitriles in the environment is largely unknown and the majority of the data is based upon suggested classification schemes that predict the behaviour of these compounds in soil, water and the atmosphere, based upon their vapour pressure at 25 °C (Swann et al. 1983). According to these classifications, acrylonitrile, adiponitrile, acetonitrile and 3-cyanopyridine should degrade in the atmosphere by reaction with photochemically produced hydroxyl radicals (HSDB 2004). Moreover, if spilled on land or released in water, acrylonitrile and acetonitrile are predicted to volatilise rapidly due to their relatively high Henry's law constants and low adsorption to soil or suspended solids and sediments in aquatic systems (HSDB 2004). However, in reality, studies have shown that acetonitrile is unreactive towards photochemically generated free radicals (Arijis et al. 1983), and in a soil contaminated by accidental spillage, very little removal of acrylonitrile was observed, even after 8 months (Deshkar et al. 2003).

Far more literature exists regarding the environmental fate of the hydroxybenzonitrile pesticides and their behaviour in soil and water systems. The vast majority of pesticides, when applied to turf, were found in the top 10 cm layer of soil, where biodegradation will be the most important factor important factor in the dissipation of bromoxynil and chlorothalonil (Smith and Cullimore 1974). Heavy rain and irrigation can result in leaching of the hydroxybenzonitriles from treated agricultural land or turf and can affect the quality of receiving waters (Cessna et al. 2001). In water, direct photolysis of these compounds may occur due to sunlight. The rate of photolytic destruction is increased by the presence of iron(III), manganese(II) and fulvic acids, which are commonly found in aquatic systems (Kochany 1992, Preuß et al. 1995).

Detoxification of cyanide wastes

The extensive use of large quantities of cyanide in industries such as steel manufacture, electroplating, polymer synthesis and gold and silver mining has led to the development of several technologies to remediate the waste that is inevitably generated during these activities. There are several widely used physical and chemical treatments of cyanide wastes that have achieved acceptance in commercial applications. The gold mining industry as been in the vanguard of developing treatment processes particularly of metal complexed cyanides. The most widely used detoxification method include chemical oxidation of cyanide to less toxic compounds, complexation and stabilisation. Determining the most appropriate method of cyanide removal should be based on the nature and volume of the waste in the context of the regulatory framework that governs its discharge into the environment (Akcil 2003).

The chemical oxidation of cyanide is commonly employed, amongst the earliest methods developed was alkaline breakpoint chlorination (Equation (1a, b))

$$Cl_2 + CN^- \longrightarrow CNCl + Cl^- \qquad (1a)$$

$$CNCl + H_2O \longrightarrow OCN^- + Cl^- + 2H^+ \qquad (1b)$$

This process has been replaced by other treatments due to several problems including the lack of effect on SADs, high reagent costs to control the pH, and the high consumption of chlorine which is also present at high concentrations in the discharge (Young and Jordan 1995). One such alternative approach is the SO_2/air (INCO) process that involves combining sulphur dioxide with oxygen and cyanide compounds in the presence of a copper catalyst (Equation (2)).

$$CN^- + SO_2 + O_2 + H_2O \xrightarrow{Cu^{2+}\text{catalyst}} OCN^- + H_2SO_4 \qquad (2)$$

The technique is used primarily in the treatment of tailing slurries. The reaction generates acid, therefore, pH needs to be regulated by the addition of lime, which in turn leads to the production of metal hydroxide sludges. In addition, the reaction

is ineffective against SADs (Young and Jordan 1995; Demopoulos and Cheng 2004).

Oxidation of cyanide compounds has also been achieved using hydrogen peroxide, the Degussa process (Equation (3)).

$$CN^- + H_2O_2 = \xrightarrow{Cu^{2+}\text{catalyst}} OCN^- + H_2O \quad (3)$$

Hydrogen peroxide is cheap and a more potent oxidant than oxygen, and the process is used in mining operations in North America where it can achieve acceptable levels of effluent treatment. Unfortunately the process is ineffective against SADs and thiocyanate (Young and Jordan 1995; Akcil and Mudder 2003).

Other approaches to cyanide waste remediation have included complexation by acidification/volatilisation (Equation (4)).

$$M(CN)_x^{y-x} + xH^+ \longrightarrow x\text{HCN (g)} + M^{y+} \quad (4)$$

A recent modification of this technique has been a process called Cyanisorb that has found applications in mining operation in New Zealand, the USA and Argentina (Young 2001; Botz and Mudder 2000).

Another alternative is stabilisation that prevents the spread of contaminants by mixing reagents such as fly ash and cement into the soil to reduce permeability. However, this technique is not appropriate for contaminated soils with high concentrations of coal tars and sulphur, that are often found as co-contaminants with cyanide compounds (Thomas and Lester 1993). Natural attenuation of free and metal cyanides occurs through a number of processes. A recent study modelled how HCN and metal cyanides concentrations were reduced in tailing impoundments by processes such as volatilisation, oxidation photolysis and precipitation (Botz and Mudder 2000). Adsorption of cyanides by minerals such as clays and feldspars that are naturally occurring in soils has also been shown to contribute to this process (Chatwin and Trepanowski 1987). As none of the physical or chemical treatments currently employed appear to be fully acceptable in the detoxification of cyanide in contaminated wastes, microbial treatment (bioremediation) may represent a potentially inexpensive, environmentally friendly alternative to conventional processes.

The economics of cyanide degradation

The cost benefit analysis of any regime to treat cyanide containing waste needs to satisfy economic, environmental and legislative requirements. The majority of cyanide treatment processes employed *in situ* are to be found in the remediation of wastewaters derived from the mining of gold and silver or that produced in steel and electroplating activity. Botz (2001) summarised the key factors that need to be considered for effective cyanide treatment of wastes in such operations. These include the assessment of water and cyanide balance under both typical and extreme climatic conditions. The articulation of the treatment objectives such that appropriate processes may be utilised, and thorough testing of the design, construction and maintenance of the water and cyanide management facilities. A recent case study investigated the cost of effluent treatment using the Cyanisorb and INCO SO_2/air process to treat effluent from a gold mill. Cyanide destruction by the INCO SO_2/air process was the most economic when the concentration of WAD cyanides were below 200 ppm. However, at higher concentrations Cyanisorb allowing cyanide recovery becomes more cost effective despite effluent treated in such a way requiring secondary treatment to meet environmental discharge criteria (Demopoulos and Cheng 2004).

Biological treatment has a number of advantages over physical and chemical treatments, although capital costs of such systems are higher the operating costs for such treatments are significantly lower (Akcil 2003). In addition, biological treatment offers the ability to couple detoxification of the cyanide with denitrification of the resulting ammonia to produce an effluent with a less deleterious environmental impact after it is discharged. The disadvantages of biological treatment processes, that can limit their economic viability, are their susceptibility to environmental conditions, for example temperature, that may inhibit the activity and as a result affect the quality of the discharged effluent. Aerobic treatments are also sensitive to high organic carbon loading that can reduce effective waste treatment (Bitton 1994). As a result detailed development of biological treatment processes from bench to full scale treatment via pilot studies are required, these in turn add cost

and delay the application of such systems *in situ* (Mosher and Figuera 1996).

Biodegradation of cyanides compounds

Biodegradation of simple and inorganic cyanides

The degradation of cyanide containing compounds has been demonstrated in a diverse range of eukaryotic and prokaryotic taxa (Dubey and Holmes 1995). The pathways involved include hydrolytic, oxidative, reductive, substitution and transfer reactions (Ebbs 2004). There is a considerable literature on the degradation of simple and free cyanide by bacteria, for example *P. fluorescens* NCIMB 11764, has two degradation systems whose induction depend on the source of the cyanide, when KCN is supplied as the sole source of nitrogen, a NAD(P)H dependent cyanide oxygenase activity is expressed. However, cyanase activity is induced when potassium cyanate is added to the growth medium. Both enzymes degrade cyanide with the production of ammonia and carbon dioxide, but the enzymes are not co-induced and are physiologically distinct (Dorr and Knowles 1989). The degradation of cyanide to ammonia and formate catalysed by a cyanide dihydratase has been observed in several bacteria including *Pseudomonas stutzeri* AK61, *Alcaligenes xylosoxidans* subsp. *denitrificans* DF3 and *Bacillus pumilus* C1 (Ingvorsen et al. 1991; Meyers et al. 1991; Watanabe et al. 1998). Other pathways utilising cyanide dioxygenase and nitrogenase have also been identified in *E. coli* and *Klebsiella oxytoca*, respectively (Figuera et al. 1996; Kao et al. 2003). The degradation of simple cyanides has also been demonstrated in fungi, for example, a purified cyanide hydratase from *Fusarium lateritium* catalyses the hydration of cyanide to formamide (Cluness et al. 1993). This metabolic pathway was clarified further in *F. solani* and it was demonstrated that fungal metabolism of cyanide proceeds by a two-step hydrolytic mechanism: conversion of cyanide to formamide by cyanide hydratase, followed by the conversion of formamide to formate by an amidase (Dumestre et al. 1997). Cyanide hydratase activity was also demonstrated by several *Trichoderma* strains, which metabolised cyanide by rhodanese activity (Ezzi and Lynch 2002).

The microbial treatment of inorganic cyanide wastes

Microbial technologies have already been applied to the detoxification of simple cyanides in industrial wastewaters. Immobilised forms of *F. lateritium* ('Cyclear' granules) have been used by ICI for cyanide decomposition, however, 'Cyclear' is sensitive to some chemicals, for example, heavy metal salts, that are often present in cyanide wastewater. The biocatalyst 'Cyanidase', developed by Novo Nordisk, utilises an immobilised preparation of *Alcaligenes denitrificans* and has been developed for optimum cyanide degradation and stability with respect to the pH, ionic strength and presence of metal ions in cyanide contaminated wastewaters (Basheer et al. 1992).

A number of laboratory studies have been conducted to identify key parameters influencing the effective bioremediation of inorganic cyanides. An investigation using aerobic biorectors in both batch and continuous flow configurations compared the roles of both sorption and biodegradation of cyanide. The results indicated that the most significant factor in the removal of free cyanide was biodegradation, sorption in contrast appeared to play very little importance (Haghighi-Podeh and Siyahati-Ardakani 2000). Petrozzi and Dunn (1994) described the continuous aerobic transformation of a synthetic cyanide wastewater in a fluidised bed reactor, derived from an almond seed extract, by soil bacteria. An additional carbon source, such as lactate, was required for growth and enabled the system to operate at cyanide concentrations of 160 ppm.

Studies of HCN degradation using the fungus *F. solani* under alkaline conditions (pH 9.2–10.7) demonstrated that the cyanide was degraded via a cyanide hydratase and amidase pathway. Alkaline pH reduces the risk of cyanhydric acid volatilisation, making the use of alkaline tolerant organisms and enzymes more attractive for degradation of cyanide containing effluents (Dumestre et al. 1997).

Generally, anaerobic organisms, particularly methanogens, are sensitive to the presence of cyanide, however, successful acclimatisation of methanogenic consortia in a laboratory scale UASB (upflow anaerobic sludge blanket) reactor to cyanide influent levels as high as 125 mg l^{-1} has been demonstrated (Gijzen et al. 2000). A fixed bed methanogenic reactor was used to treat

wastewater derived during starch production from cassava. The cyanoglycosides found in cassava were rapidly hydrolysed to free cyanide, and reached levels of 200 mg l^{-1} in the wastewater. The establishment of the reactor required several months but when achieved the cyanide nitrogen was converted to biomass with 150 mg l^{-1} of cyanide being degraded during a 3 day hydraulic retention time (Siller and Winter 1998). Subsequently these workers went on to demonstrate that a methane reactor culture enriched from soil residues of cassava roots and sewage sludge was able to remove up to 4 g l^{-1} KCN at a rate of 400 mg CN l^{-1} day^{-1} with residual concentrations of 0.2–0.5 mg l^{-1} (Siller and Winter 1998).

Biodegradation of metal-cyanide complexes

The increasingly stable complexation of cyanide with Zn, Cu, Ni and Fe respectively can inhibit the application of biodegradation technologies to their remediation (Raybuck 1992). When copper complexed with cyanide it could no longer be used as a nitrogen source by one strain of *P. fluorescens* (Silvos-Avilos et al. 1990). However, in contrast several strains of *P. fluorescens* have been shown to be capable of degrading metal-cyanides to levels almost equivalent with that seen using physical and chemical methods (Akcil et al. 2003). For example, strain BKM B-5040 degraded metal-cyanide complexes, including zinc, copper, silver and iron cyano complexes (Shpak et al. 1995). Degradation of nickel cyanide by *P. fluorescens* NCIMB 17764 was originally considered to be mediated by cyanide oxygenase activity (Dorr and Knowles 1989), then, a subsequent study suggested that this enzyme acted on the free cyanide liberated as the metal-cyanide complex dissociated (Kunz et al. 1998). It was also observed that non-enzymic mechanisms were involved in cyanide utilization by this bacterium. Chen and Kunz (1997) proposed that α-keto acids, pyruvate and α-ketoglutamate reacted with free cyanide, which may be liberated from metal cyanides, to form the corresponding cyanohydrin. It was further proposed that the cyanohydrins were then metabolised to ammonia and CO_2 by the action of cyanide oxygenase and utilised for growth (Kunz et al. 2001). *Burkholderia cepacia* C-3 was demonstrated to degrade a range of metal complexed cyanide compounds at alkaline pH, representative of those found in heap leach effluents (Adjei and Ohta 2000). *E. coli* BCN6 was able to use several metal-cyanides, including iron, zinc and copper cyanide complexes, as nitrogen sources. Each metal-cyanide complex was observed to have a different effect on growth, most likely due to the differing toxicities of the metal ions that were taken up by cells through a biosorption mechanism (Figuera et al. 1995). An *Acinetobacter* sp. strain RBPI isolated from gold mine effluents was shown to be capable of degrading gold, silver, cadmium, zinc, copper, cobalt and iron cyanide complexes. This bacterium appeared to have a single, purified cyanide degrading complex that was capable of degrading metal-cyanides, simple cyanides and nitriles, in contrast to other cyanide degrading enzyme known that are specific to the particular form of cyanide (Finnegan et al. 1991).

Several metal-cyanide degrading fungal species have been isolated from contaminated sites. Pure cultures and mixed consortia of fungi isolated from a contaminated gasworks soil, including *F. solani*, *F. oxysporum*, *Trichoderma polysporum*, *Scytalidium thermophilum* and *Penicillin miczynski*, could degrade iron and nickel cyanides (Barclay et al. 1998a, b). In *F. solani* this activity was shown to be catalysed by cyanide hydratase (Barclay et al. 2002). A yeast, *Cryptococcus humicolus* MCN2, isolated from coke-plant wastewater and grown on KCN as a sole nitrogen source, degraded concentrations of potassium tetracyanonickelate up to 65 mM when supplied with sufficient carbon (Kwon et al. 2002).

Microbial treatment of metal cyanide wastes

The biological treatment of metal-cyanide contaminated wastewaters has been successfully demonstrated in laboratory scale experiments under aerobic and anaerobic conditions. A variety of reactor configurations have been examined using both bench scale and pilot studies. Relatively simple batch reactor set ups have exploited fungal biomass to degrade model metal cyanide containing wastewaters (Ezzi and Lynch 2005). A similar reactor configuration was used to demonstrate adsorption of metal cyanides to *Cladosporium cladosporioides* biomass, with a binding

efficiency to nickel and copper cyanides higher than that of activated charcoal (Patil and Paknikar 1999). This study suggested that biosorption may offer potential for the recovery of metal cyanides. The degradation of metal cyanides have been modelled in both suspended growth and fixed film bioreactors. A sequence batch reactor was used to treat mine water containing WADs, thiocyanate and cyanate. The set up allowed the system to operate both aerobically and under anoxic conditions enabling denitrification to be undertaken after aerobic treatment. As a result over 90% of the nitrogen was removed from the wastewater (Kapoor et al. 2003). The biological treatment of cyanide wastes with fixed film processes has been demonstrated using both trickling filters and rotating biological contactors. A trickling filter operating without recirculation was capable of removing over 90% of the cyanide, thiocyanate, Ni, Fe and Cu from a gold mill effluent (Evangelho et al. 2001). Rotating biological contactor reactors have also been successfully applied for laboratory based studies on the remediation of silver complexed cyanides derived from electroplating wastewaters (Patil and Paknikar 2000).

There are several examples of biological treatments finding applications in the treatment of industrial effluents at metal-mining sites in the USA (Dictor et al. 1997; Patil and Paknikar 2000). One aerobic biological treatment process that has been successfully applied in the detoxification of industrial effluents at a Homestake Mining Co. operation in South Dakota, USA utilises a strain of *P. paucimobilis* specifically acclimatised to the waste. Wastewater was first dosed with phosphoric acid as a nutrient source, and was fed into a set of rotating biological contactors (RBCs), which metabolised the cyanide to ammonia (Equation (5)) (Whitlock 1990).

$$M(CN)_x^{y-x} + 3xH_2O + x/2O_2\ (aq) \rightarrow M^{y+} + xNH_4^+ + xHCO_{3-} + OH^- \quad (5)$$

The system also remediated thiocyanate (Equation (6)) (Akcil 2003)

$$SCN^- + 3H_2O + 2O_2\ (aq) \rightarrow SO_4^{2-} + NH_4^+ + HCO_{3-} + H^+ \quad (6)$$

Subsequently, a second set of RBCs converted the ammonia to nitrate. All forms of cyanide were removed from the wastewater during this process, and the resulting effluent was rendered safe enough to be disposed of into a receiving stream. Removal rates of cyanide varied between 91 and 99.5% of the total cyanide depending on the plant operation, reducing influent levels from 10 to 0.3 ppm in the effluent (Mudder and Whitlock 1984). In addition to WADs cyanide compounds, SADs, particularly iron complexes were degraded to some extent as well as adsorbed to the biomass (Akcil 2003). The Homestake company went on to develop other biological treatments including a passive biological treatment system at the Nickel Plate mine in Canada. It was designed to treat tailings impoundment seepage. The process was based on a suspended sludge system incorporating both aerobic and anaerobic treatment processes. It was effective in removing cyanide, thiocyanate, cyanate and metals, as well as undertaking nitrification and denitrification to remove ammoniacal nitrogen (Botz 2001). Another system developed by Homestake was the Biopass process, used in a mining operation in Santa Fe, USA. It was implemented to treat draindown during the decommissioning of a heap leach operation. The Biopass process is suitable for the removal of cyanide, thiocyanate, cyanate, ammonia, nitrate and metals at solution flows of less than about 10 $m^3\ h^{-1}$ for (Botz 2001).Other biological treatment of cyanide wastes have been implemented *in situ*, for example, at the USMX Green Springs gold mine in Nevada, USA. In this process, a *P. pseudoalcaligenes* strain was isolated from a tailings pond and introduced into carbon adsorption tanks at the site, that were used to recirculate the cyanide solution through the ore heaps to the tailings pond. The tanks were thus transformed into bioreactors in which the concentration of cyanide was reduced from 20 to 8.5 mg l^{-1} over a period of 15 weeks (USEPA 1994).

Pintail System Inc. developed an approach for the treatment of ore heap cyanide solutions using bacteria native to the project environment (USEPA 1994). The process involved isolating and enhancing native bacteria with the ability to degrade cyanide and then growing them to concentrations capable of supporting effective treatment. This system was utilised at a cyanide detoxification project at the Hecla Mining Company's Yellow Pine Mine in Idaho, USA. Bacteria used for the treatment were isolated from the Yellow Pines

area, then 10,000 gallons of treatment bacteria solution were added to the tailings pond and to the spent ore (USEPA 1994). Cyanide solutions with an average weak acid dissociable (WAD) cyanide concentration of 46.6 ppm were reduced to 0.2 ppm by the end of the project.

Biodegradation of nitriles

Biodegradation of nitriles can occur by two pathways; either a nitrilase catalysed conversion of the nitrile to the corresponding acid and ammonia, with very little formation of the free amide (Thompson et al. 1988) or; via a two step pathway in which the nitrile is initially converted to the corresponding amide by a nitrile hydratase, and then to the acid and ammonia by an amidase. The enzymes of the two pathways have little or no sequence and structural homology. Nitrilases are generally homomultimers, consisting of up to 16 subunits, whereas nitrile hydratases are metalloenzymes, incorporating Fe^{3+} or Co^{2+} ions in the catalytic centre and typically exist as α and β subunit dimers or tetramers (Komeda et al. 1996).

The substrate specificities of nitrile degrading enzymes have been recently reviewed (O'Reilly and Turner 2003) and a summary is presented in Table 1. From this it is clear that many of these enzymes can catalyse the degradation of both aliphatic and aromatic nitriles. In contrast, the nitrilase of *Klebsiella pneumoniae* subsp. *ozaenae* was highly specific for bromoxynil, and the structurally related compounds benzonitrile and ioxynil (Stalker et al. 1988). A number of microorganisms have multiple nitrile degrading pathways. Characterisation of nitrile utilisation by *Rhodococcus (Nocardia) rhodochrous* LL100-21 identified the induction of nitrile hydratase and amidase activities by acetonitrile, however, the degradation of benzonitrile was found to proceed via nitrilase activity (Collins and Knowles 1983; Thompson et al. 1988). Similarly, the thermophilic bacterium *Bacillus pallidus* Dac521 was found to grow on acetonitrile and benzonitrile as a sole source of nitrogen and carbon and it was suggested that acetonitrile degradation occurred via the nitrile hydratase pathway, whereas, benzonitrile degradation was via a nitrilase pathway. Molecular characterisation of the nitrile hydratase from the bacterium showed activity against a narrow range of aliphatic nitrile substrates and not against any of the cyclic, hydroxy-, di- or aromatic nitriles tested. However, nitrile hydratase activity was irreversibly inhibited by the aromatic nitrile, benzonitrile, strongly suggesting that benzonitrile degrading activity occurred via the nitrilase pathway (Cramp and Cowan 1999).

The nitrile hydratase and nitrilase pathways are the most common mechanisms for nitrile degradation, however, enzymes involved in inorganic cyanide degradation such as the cyanide hydratase from *F. laterium*, when overexpressed in *E. coli* showed a low but significant nitrilase activity towards benzonitrile, propionitrile and acetonitrile. Site directed mutation of the cyanide hydratase gene indicated that a loss of cyanide hydratase activity also led to a loss of nitrilase activity, suggesting that the active site for cyanide hydratase and nitrilase activity in the protein was the same (Nolan et al. 2003). The PCP-hydrolase from *Flavobacterium* sp. ATCC 39723 degraded bromoxynil with the production of cyanide, which proved to be inhibitory to this organism (Topp et al. 1992).

Microbial treatment of nitrile wastes

Nitriles are used in many manufacturing and engineering activities and are, therefore, common contaminants in industrial wastes and effluents. Due to their high toxicity, it is important that nitriles are removed from wastes before they are released into the environment. Several studies have addressed the microbial detoxification of acrylonitrile bearing wastes, however, efforts to develop microbial treatment methods have been hampered by the fact that such wastes often contain high levels of acrylonitrile and other toxic components that are inhibitory to microbial growth (Wyatt and Knowles 1995; Battistel et al. 1997; Cheng et al. 2004).

Wyatt and Knowles (1995) developed a stable mixed microbial population capable of degrading a highly toxic effluent generated by acrylonitrile production, that contained acrylonitrile, fumaronitrile, succinonitrile, acrylic acid, acrylamide, acrolein, cyanopyridine and maleimide. The members of the microbial consortia were isolated on the individual components of the effluent and were then acclimatised by growth in a synthetic

Table 1. A summary of the diversity of cyanide degrading microbial taxa with their respective enzymic activities and specificities.

Microorganism	Enzyme pathway and associated metal ions	Substrate specificity	Reference
Bacteria			
Rhodococcus rhodochrous J1	L-NHase[a] (Co)	Aromatic nitriles	Komeda et al. (1996)
	Nitrilase	Aliphatic and aromatic nitriles	Kobayashi et al. (1989)
Rhodococcus sp. AJ270	NHase[a] (Fe)	Aliphatic and aromatic nitriles and dinitriles	Blakey et al. (1995)
Bacillus pallidus Dac521	NHase[a] (Co)	Aliphatic nitriles	Cramp et al. (1997)
	Nitrilase	Aromatic nitriles	Almatawah et al. (1999)
Arthrobacter sp. J1	NHase[a]	Aliphatic nitriles	Asano et al. (1982)
	Nitrilase A	Aromatic nitriles	
	Nitrilase B	Aromatic nitriles	
Brevibacterium imperialis CBS489-74	NHase[a]	Acrylonitrile	Alfani et al. (2001)
Corynebacterium sp. C5	NHase[a] (Fe)	Aliphatic dinitriles	Yamamoto et al. (1992)
Pseudomonas chlororaphis B23	NHase[a] (Fe)	Aliphatic nitriles	Nagasawa et al. (1987)
P. putida	NHase[a] (Co)	Aliphatic nitriles	Payne et al. (1997)
	Isonitrile hydratase	Isonitriles	Goda et al. (2001)
Pseudomonas sp. S1	Nitrilase	Aliphatic, aromatic and arakyl nitriles	Dhillon and Shivaraman (1999)
P. fluorescens NCIMB 11764	Cyanide oxygenase	KCN	Kunz et al. (2001)
P. stutzeri AK61	Cyanidase	KCN	Watanabe et al. (1998)
P. aeruginosa	Rhodanese	Cyanide	Cipollone et al. (2004)
Escherichia coli	Cyanide dioxygenase	KCN	Figuera et al. (1996)
Klebsiella oxytoca	Nitrogenase	KCN	Kao et al. (2003)
K. pneumoniae subsp. *ozaenae*	Nitrilase	Bromoxynil	McBride et al. (1986)
Agrobacterium tumefaciens strain d3	NHase[a]	Aromatic nitriles	Bauer et al. (1998)
Alcaligenes xylosoxidans subsp. denitrificans DF3	Cyanidase	HCN	Ingvorsen et al. (1991)
Flavobacterium sp. strain ATCC 39723	PCP-hydrolase	Bromoxynil	Topp et al. (1992)
Fungi			
Myrothecium verrucaria	NHase (Zn)	Cyanamide	Maier-Greiner et al. (1992)
Fusarium solani	Nitrilase	Aromatic nitriles	Harper (1977)
	Cyanide hydratase	KCN, HCN, $K_2Ni(CN)_4$, $K_4Fe(CN)_6$	Dumestre et al. (1997), Barclay et al. (2002)
F. oxysporum f sp. *melonis*	Nitrilase	Aliphatic and aromatic nitriles	Goldlust and Bohzak (1989)
F. laterium	Cyanide hydratase	Benzonitrile, HCN	Nolan et al. (2003), Cluness et al. (1993)
Aspergillus niger	NHase	Aliphatic and aromatic nitriles	Šnajdrová et al. (2004)
Gloeocercospora sorghi	Cyanide hydratase	HCN	Wang et al. (1992)
Trichoderma harzianum	Cyanide hydratase	KCN	Ezzi and Lynch (2002)

[a]Nitrile hydratase.

effluent in which the concentration of toxic waste components was gradually increased. This consortium was capable of degrading acrylonitrile effluents from an acrylonitrile manufacturing plant in Texas, USA. In contrast, an effluent containing high levels of cyanide, maleimide and fumaronitrile, with a chemical oxidation demand (COD) range between 25,000 and 40,000 ppm, required dilution prior to degradation, to prevent poisoning the consortia. Acclimation of microorganisms was also found to be a significant factor in the degradation of toxic and refractory chemicals in acrylonitrile waste waters and the addition of biodegradable substrate, such as glucose or peptone, and the presence of a higher microbial concentration was found to promote acclimation in fluidised bed reactors and aerobic biofilters (Hu et al. 1998; Cheng et al. 2004).

Immobilisation of nitrile degrading organisms has also been shown to improve the microbial detoxification of acrylonitrile. Acrylonitrile–butadiene co-polymer emulsions contain contaminants such as detergents, salts and hydroxylamine, a potent inhibitor of nitrile hydratase. In the conditions of the emulsion, a significant reduction in the rate of detoxification of acrylonitrile with whole cells and lysates of *Brevibacterium imperiale* CBS 49874 and *Corynebacterium nitrophilus* ATCC 21419 was observed over time, due to the inactivation of nitrile degrading enzymes (Battistel et al. 1997). However, the immobilisation of the bacteria or enzyme prevented this inactivation, enabling the immobilised enzyme to be recycled, with good retention of overall activity. The detoxification of acrylonitrile vapour in a trickle-bed air biofilter (TBAB), utilising immobilised *Rhodococcus ruber*, was investigated by Roach et al. (2003). An elimination capacity of over 7.2 kg m^{-3} h^{-1} was achieved, which was ten times greater than that attained using a conventional TBAB system and immobilisation also removed the requirement for acclimation of the organism.

Future applications of biological cyanide degrading systems

The continuing use of cyanide in a range of industrial processes will ensure that there will be an ongoing requirement for effective and economic remediation technologies. Work is continuing to identify microorganisms that have the ability to detoxify a variety of cyanide compounds from waste. Many wastewaters are problematic for biological degradation because of the hostile environmental conditions they present to micro-organisms. 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. Therefore, in searching for effective candidates for bioremediation of contaminated soils or wastes, 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 (Cummings and Andrews 2003). Recent studies have identified *P. pseudoalcaligenes* CECT5344 that is capable of degrading cyanide and metal cyanide complexes in wastewater containing up to 30 mM free cyanide at pH 11.5, moreover, the organism is also active in jewellery industry wastewater containing heavy metals (Luque-Almagro et al. 2005). High strength acrylonitrile wastewater from resin manufacture yielded a strain of *Comamonas testosteroni* that could remove up to 1900 mg l^{-1} acrylonitrile completely within 67 h (Wang et al. 2004). The exploitation of immobilised bacterial systems has received considerable attention because bacteria in biofilms are more resilient to environmental insults they therefore offer an opportunity to treat more concentrated wastes streams. A biofilm containing a novel *Comamonas* species in a packed bed reactor demonstrated maximum acetonitrile degradation of 1 g $l^{-1}d^{-1}$ (Manolov et al. 2005). Immobilised systems also offer the potential to treat gaseous waste, *R. ruber* immobilised in a synthetic silicon polymer was used to treat gaseous acrylonitrile. The system demonstrated the ability to eliminate an order of magnitude more acrylonitrile at 90% efficiency than any previously published system (Roach et al. 2004).

A recent development has been the use of plants in the bioremediation of cyanide containing waste. A grass *Sorghum bicolour* was tested for its ability to degrade cyanide in irrigation water during a lab scale study. Cyanide concentrations up to 125 mg l^{-1} were readily degraded by the plant. The authors speculated that eventually his technology may offer a cost effective mechanism for cyanide treatment in gold mining effluents. Additionally bioaccumulation of gold by the plants may enhance the gold recovery from the mining operation (Trapp et al. 2003).

The use of cell free systems may also have some application, although enzyme only systems often lack the robustness, and have a narrower substrate range compared to whole cell technologies (Brady et al. 2004). Their future applications may be in the production and transformation of nitrile compounds used in the production of fine chemicals or pharmaceuticals, for example, *R. rhodochrous* J1 is used to produce acrylonitrile from acetonitrile (Ryuno and Nakamura 2003). Immobilised nitrilases from *Alcaligenes faecalis* ATCC 8750 have been demonstrated to be effective and robust catalysts in the hydrolysis of mandelonitrile (Rey et al. 2004).

Eukaryotic microbial systems also have potential to treat cyanide contaminated environments, whole cell fungal systems using *Aspergillus niger* K10 degraded a range of aromatic nitriles over a range of pH from 3 to 11 (Šnajdrová et al. 2004). In contrast, *Rhizopus arrhizus* could remove iron(III)-cyanide complexes from aqueous solutions by active uptake and intracellular bioaccumulation without degradation occurring (Aksu et al. 1999). Algae have also demonstrated high activity to degrade free cyanides at alkaline pH in concentrations as high as 400 ml^{-1} (Gurbuz et al. 2004).

A recent study using the cyanogenic bacterium *Chromobacterium violaceum* demonstrated a novel application for the bioremediation of electronic waste. When cultivated under cyanogenic conditions with shredded printed circuit boards a water soluble cyanide complexed copper compound was detected. The HCN generated by the bacterium allowed the mobilisation of the metal and this observation may have potential to be exploited in the microbially mediated recovery of metals from waste (Faramarzi et al. 2004).

Summary

The continuing generation of a variety of cyanide containing wastes, in tandem with historically derived contamination of soils and water suggests that novel processes are required to alleviate the serious environmental consequences of cyanide pollution. Microorganisms have been shown to possess a variety of catabolic activities that can be harnessed to remediate inorganic cyanide and nitriles. Further work is required to optimise the use of such biological systems both in wastewater and soil. In particular, the development of microbial processes that are effective and robust in the face of extreme environmental conditions, such as low pH and co-contaminant toxicity, is required to ensure a technology that is competitive with the current chemical and physical remediation strategies currently practised to combat cyanide pollution.

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