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CHAPTER 12

APPLICATION OF MICROORGANISMS IN BIODEGRADATION OF CYANIDE FROM WASTE WATER

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ABSTRACT

Various procedures exist for treating cyanides from different industrial waste waters, and comprise several physical, chemical and biological methods. It is still widely discussed and examined due to its potential toxicity and environmental impact. Physical and chemical methods are highly expensive and also cause secondary pollution. The treatment of cyanide by said method is rarely used, owing to cost-effectiveness. Thus, there is a pressing need for the development of an alternative treatment process capable of achieving high removal efficiency without troubling the environment. Several microbial species can degrade cyanide well into less toxic products. Biodegradation of cyanide compounds may take place through various enzymatic pathways, the enzymes of which are produced by microorganisms that utilize cyanides as substrate. The biological methods for the treatment of cyanide are not only cost-effective but also do not produce any secondary pollution. The present chapter describes the mechanism and ardent approaches of proficient biological methods for the removal of cyanide compounds and their advantages over other treatment processes.

12.1   INTRODUCTION

12.1.1 GENERAL

Cyanide is an extremely toxic nitrogenous compound for almost all living creatures found in waste water. There are several bacteria, fungi, algae and plants that are able to produce cyanide as a defence mechanism against predation.[53] Cyanide is present in various foodstuffs consumed by humans such as cassava, apricots, bean sprouts, soya beans, cashews, almonds,

cherries, potatoes, lentils, olives, sorghum and bamboo shoots. However, it is a common compound crucial to nature; it is extensively considered as a highly dangerous substance. In this drive it has been formed by its use as a genocidal agent during World War II, in mass suicides, and its continued use in some parts of the world for judicial executions.[17] Cyanide is included in the Comprehensive Environmental Response, Compensation and Liability Act (CERCLA) priority list of hazardous substances and it occupies 35th position in the priority list (revised in 2015) of most hazardous substances.[5] Cyanide has also been used worldwide in the extraction of gold and silver. The demand for sodium cyanide worldwide is about 360,000 tonnes per annum of which about one-third is used in the recovery of gold and silver.[64] The process was developed to extract gold from mines in Scotland in the year 1987, and commercially it was used on a large scale by the New Zealand Crown Mines Company at Karangahake in the year 1989.[42,28] Annually production of HCN has been reported to be around 1.4 million tonnes[63] whereby 13% is converted in sodium cyanide for the mining use. Different forms of cyanide have dreadful health effects on people as well as other living organisms.[23]

12.2   CYANIDE CONTAMINATION IN WATER, ITS SOURCES AND TOXIC EFFECT ON ENVIRONMENT

Cyanide compounds are present in environmental matrices and waste streams as free, simple and complex cyanides, cyanate and nitriles.[27] It enters into the water from natural sources and anthropogenic/man-made sources (industrial activities).

12.2.1   NATURAL SOURCES

Cyanide compounds are synthesized by many taxa including higher plants, arthropods, fungi and bacteria. Plants are an important source of cyanide compounds; they exploit the bitter tasting cyanogenic glucosides as defence against herbivores and pathogens.[88] Cyanides are produced by certain fungi, bacteria, algae and plants as well as particular foods such as lima beans and almonds. In certain plant foods, including almonds, millet sprouts, lima beans, soy, spinach, bamboo shoots and cassava roots (major

source of food in tropical countries), cyanides occur naturally as part of sugars or other naturally occurring compounds. However, the edible parts of plants that are being eaten in the United States, including tapioca which is made from cassava roots, contain relatively low amounts of cyanide. Various food products may either contain cyanide naturally or it is used for their production.[59] In certain food products the cyanide concentration per kg observed is; cassava (104 mg/100 g plant tissue), lima beans (100–300 mg/100 g plant tissue), packed fruit juice (0.03–15.84 mg/l in sour cherry and orange), wild cherries (140–370 mg/100 g plant tissue), almond (297 mg/kg), almond products (50 mg/kg) and sorghum (250 mg/100 g plant tissue). Varying amounts of cyanide may be found in other vegetables and fruits such as apple, apricot, peach, cauliflower and strawberry.[51] Cyanide is also produced from forest fires and released into the atmosphere.[26] Much smaller amounts of cyanide may enter into water through storm water runoff where road salts are used that contain cyanide.

12.2.2 ANTHROPOGENIC/MAN-MADE SOURCES

Major sources of cyanide are discharged in large quantities by various industries such as electroplating and metal finishing, steel tempering, mining (extraction of metals such as gold and silver), automobile parts manufacturing, photography, pharmaceuticals and coal processing units.[3,60,72] Cyanide in landfills can contaminate underground water. However, cyanide has been detected in underground waters of a few landfills and industrial waste disposal sites. Cyanide becomes toxic to soil microorganisms at high concentrations found in some landfill leachates (water that seeps through landfill soil) and in the wastes stored in some disposal sites. Because these microorganisms can no longer change cyanide to other chemical forms, cyanide is able to pass through soil into the underground water. Generally, the concentrations of total cyanide in the effluent of such industries vary within the range of 0.01–10 mg/l.[89,33] The effluent of silver plating industry and coke plant industry containing total cyanide is around $5 \cdot 1 \pm 1 \cdot 5$ mg/l and 4–100 mg/l, respectively.[72] However, some industrial effluents from electroplating plants have been found to contain even higher cyanide levels of 100,000 mg/l.[89,47] One of the most significant cyanide complex species is thiocyanate (SCN), found in some coal conversion and coal coking effluents in the concentration range of 17–1500 mg/l SCN.[54]

Cyanide concentrations reported in the literature for several industrial waste water effluents are presented in Table 12.1.

TABLE 12.1　Concentration of Cyanide Released from Various Industries/Industrial Effluent (mg/l).

Industrial waste water source	Total cyanide	Reference
Oil refinery	2.25	[39]
Petroleum refining	0–1.5	[47]
Steel mill Coke plant liquor	7.5–396	[47]
Paint and ink formulation	0–2	[13]
Gold ore extraction	18.2–22.3	[47]
Coke plant waste	91–110	[47]
Coke oven plant	10–150	[54]
Coke plant	0.1–0.7	[54]
Coke plant	100–1000	[54]
Coke plant ammonia liquor	20–60	[47]
Explosives manufacture	0–2.6	[47]
Plating rinse	32.5	[10]
Plating industries (rising waste)	1.4–256	[47]
Electroplating plants	0.03–0.27	[89]
Electroplating plants	0.01–14.24	[89]
Chemical industry	10.4–50.9	[87]

Cyanide is a prominent metabolic inhibitor and inhibition is done by the inactivation of respiration due to its tight binding to cytochrome C oxidase. In this process, electron transport chain is blocked by the cyanide by binding with the iron ion in the terminal electron acceptor cytochrome C oxidase, consequently rapidly falling respiration rates, and ATP synthesis in mitochondria is inhibited.[36] Assimilation of cyanide can also result in either acute poisoning (including death) or chronic poisoning to human beings and animals.[24] The lowest reported oral lethal dose in humans is 0.54 mg/kg body weight; the average absorbed dose at the time of death has been estimated at 1.4 mg/kg body weight (calculated as hydrogen cyanide).[91] Aquatic organisms are very sensitive to cyanide. Fish have been shown to be the most sensitive aquatic organisms followed by invertebrates. Concentrations of free cyanide in the aquatic environment

ranging from 5.0–7.2 µg/l, it reduces the swimming performance and inhibit reproduction in many species of fish. Other adverse effects include delayed mortality, pathology, susceptibility to predation, disrupted respiration, osmoregulatory disturbances and altered growth patterns. Concentrations of 20–76 µg/l free cyanide can cause the death of many species, and concentrations in the excess of 200 µg/l are rapidly toxic to the most species of fish. Algae and macrophytes can tolerate much higher environmental concentrations of free cyanide than fish and invertebrates, and do not exhibit adverse effects at 160 µg/l or more of free cyanide (Environmental and Health Effects of Cyanide, USA). Birds seem to have varying sensitivity to cyanide. It has been reported that oral lethal dose (LD50) for birds ranges from 0.8 $mg^{-1}kg$ of body weight (American racing pigeon) to 11.1 $mg^{-1}kg$ of body weight (domestic chickens). Workers in various professions may be exposed to cyanides primarily through inhalation and less frequently by skin absorption. Exposure to cyanide can cause breathing disorders, headaches, coma, heart pains, thyroid gland enlargement and even death. All forms of the cyanide can be toxic at high levels, but the most toxic form of cyanide is hydrogen cyanide. Various microorganisms and plants have shown the resistivity against cyanide poisoning since they have developed an alternate pathway for ATP production. Some of them have a diverse oxidase instead of cytochrome C oxidase.[80]

Cyanide containing waste waters must be treated before being discharged into the environment to protect the water resources and environment. Hence, environmental regulations require reducing the cyanide concentration in waste water and setting the permissible limit of cyanide in the surface water. Indian standard has set a minimal national standard (MINAS) limit for cyanide in effluent as 0.2 mg/l.[41] US Environmental Protection Agency (USEPA) standard for drinking and aquatic biota waters regarding total cyanide is 200 and 50 ppb, respectively.[90,85]

12.3   AVAILABLE TECHNIQUES OF CYANIDE REMOVAL FROM WASTE WATER

Several treatment processes such as physical, chemical and biological oxidation have been exploited for the reduction of cyanide levels in waste solutions/slurries in compliance with environmental regulations. Physicochemical methods for cyanide treatment can be accomplished using

dilution, membranes, electrowinning and hydrolysis/distillation, chemical oxidation, alkaline chlorination,[56,4] electrochemical method,[68] capillary electrophoresis, adsorption and so forth.[1] Activated carbon has been reported to have cyanide adsorption capacity. However, in several cases the process is hampered with elevated funds, regent costs and royalty payments. In chemical process, various chemicals and reagents are used and secondary pollutants are created which need some additional treatment prior to their disposal. Adsorption is a simple and attractive method for the removal of toxic compounds from the effluents due to its high efficiency, easy handling, and economic feasibility. Adsorption systems are not affected by the toxicity of the target compound(s) and do not require hazardous chemicals. Moreover, adsorption facilitates concentrating and then recovering the adsorbed compounds if desired.[62] The biosorption of cyanide from aqueous solutions is quite a new process that has proven to be promising in the removal of contaminants from aqueous effluents.[25] Various agro-based adsorbents have been reported for cyanide removal from water and waste water due to their abundant availability and low cost.[62] The adsorption of cyanide is, however, highly dependent on solution pH and initial cyanide concentration. Adsorbent concentration, agitation period and temperature also influence the cyanide adsorption process.

Biological treatments are feasible alternatives to chemical methods without creating or adding new toxic and biological persistent chemicals.[22,72] Cyanide is converted to carbon and nitrogen source by various enzymes present in the microorganisms. The metabolic pathway for the conversion of cyanide is influenced by its initial concentration, pH, temperature, availability of other energy sources in the form of organic carbon required for cell maintenance and growth, presence of oxygen, ammonia and various metal ions.[84,73] Microorganisms have the capability to degrade and assimilate cyanide into the form of amino acids, thiocyanate, β-cyanoalanine and vitamins.[84] White et al., (1988) reported the conversion of cyanide to formate and ammonia by *Pseudomonas* which is isolated from industrial waste water. The strain of isolated *Bacillus pumilus* could survive in the solutions of potassium cyanide of up to 2.5 M and grow in the media containing 0.1 M KCN.[81] Biodegradation was performed in the presence of microbes either in mobilized or immobilized phase.[46] The immobilization of living microbial cells on a suitable adsorbent improves the removal efficiency.[61] There were some reports on the biodegradation of cyanide compounds by immobilized cells of

Pseudomonas species, *Fusarium solani.*[19,22,12] Adsorption and biodegradation are two significant methods for the treatment of cyanide-containing waste water.[24] The biological process is generally the preferred technique for treating waste water owing to its cost-effectiveness and environmental easiness.

12.3.1   MICROBIAL MECHANISM FOR CYANIDE DEGRADATION

The biodegradation of cyanide compounds may take place through various pathways. Generally, degradation of cyanide is induced by the presence of cyanide in the media which is followed by the conversion of cyanide into carbon and nitrogen. Various researchers have described various organisms that use different pathways for cyanide degradation.[21] Sometimes, more than one pathway can be applied for cyanide biodegradation in some organisms.[65] Five general pathways as reported in the literature for the biodegradation of cyanide are: hydrolytic pathway, oxidative pathway, reductive pathway, substitution/transfer pathway and syntheses pathway.[52,79] First three are degradation pathways in which enzymes catalyze the conversion of cyanides into simple organic or inorganic molecules and convert them into ammonia, methane, CO_2, formic acid and carboxylic acid. Last two pathways are for the assimilation of cyanide in the microbe as nitrogen and carbon source.[9] All these pathways depend on the mechanism of tolerance of cyanide in microbes and on the process it uses to dissociate the cyanide metal complexes or for chelating metals.[36]

12.3.1.1   HYDROLYTIC PATHWAY

The ydrolytic pathway of cyanide degradation is catalyzed by various enzymes present in microbial system, like cyanide hydratase, nitrile hydratase, cyanidase and nitrilase.[27,52,9] First two enzymes have a specific substrate and they direct hydrolyse and cleave the carbon–nitrogen triple bond to form formamide, while the last two convert it to ammonia and carboxylic acid, which are utilized in their metabolism activity.[36] Cyanide compounds are degraded by the following enzymes through different reactions as discussed below:[27]
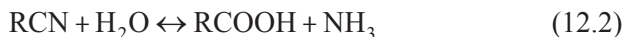
12.3.1.1.1 Cyanide hydratase

Cyanide hydratase is primarily a fungal enzyme; the most frequently encountered cyanide conversion takes place through this inducible enzyme, resulting in the formation of formamide which subsequently decomposes to carbon dioxide and ammonia by another enzyme formamide hydratase (FHL).

$$HCN + H_2O \rightarrow HCONH_2 \tag{12.1}$$

This enzyme belongs to the family of lyases, specifically the hydro-lyases which cleave the carbon–nitrogen bonds. The systematic name of this enzyme class is formamide hydro-lyase (cyanide-forming). Other names in common use include formamide dehydratase, and formamide hydro-lyase. This enzyme participates in cyanoamino acid metabolism.[36] Cyanide hydratase was first partially purified by *Stemphylium loti*, and is highly conserved between species.[8]

12.3.1.1.2 Cyanidase

Cyanidase is also known as cyanide dihydratases. It comprises a group of bacterial enzymes that are available in *Pseudomonas stutzeri* AK61, *Alcaligenes xylosoxidans* subsp. *Denitrificans* DF3 and *Bacillus pumilus* C1.[43,58,44] Cyanide dihydratases readily convert cyanide to relatively nontoxic formate directly as shown below:

$$RCN + H_2O \leftrightarrow RCOOH + NH_3 \tag{12.2}$$

12.3.1.1.3 Nitrile hydratase

Primarily aliphatic nitriles can be effectively degraded by nitrile hydratases. Nitrile hydratases convert cyanides to their corresponding amides as shown in the following reaction:

$$R–CN + H_2O \rightarrow R–CONH_2 \tag{12.3}$$

The nitrile hydratase, isolated from *Pseudonocardia thermophila* shows high activity compared to other microorganisms known for the production of nitrile hydratase, that is *Rhodococcus rhodoclous, Pseudomonas*, *Corynebacterium*, *Klebsiella* and *Rhizobium*. Some new bacterial strains, *Pseudomonas putida* MA113 and *Pseudomonas marginales* MA32, containing nitrile hydratases were isolated from soil samples by an enrichment procedure. This isolated microbe could tolerate up to 50 mM cyanide and also has broad substrate range small substrates like acrylonitrile, nitriles with longer side chains and even nitriles with quaternary alpha-carbon atoms. *P. putida* MA113 and *Pseudomonas marginales* MA32 were used as whole cell biocatalysts for the hydration of acetone cyanohydrin to a hydroxyisobutyramide, which is a precursor of methacrylamide.[67] Nitrile hydratase is composed of two types of subunits, α and β, which are not related in amino acid sequence. Nitrile hydratase exists as αβ dimers or $\alpha_2\beta_2$ tetramers and bind one metal atom per αβ unit.[34]

12.3.1.1.4   Nitrilase

Nitrilase enzymes catalyze the hydrolysis of nitriles to carboxylic acids and ammonia, without the formation of 'free' amide intermediates.

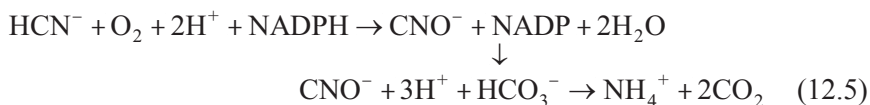$$R\text{–}CN + 2H_2O \rightarrow R\text{–}COOH + NH_3 \qquad (12.4)$$

Nitrilases are involved in the biosynthesis of proteins and their post-translational modifications in plants, animals, fungi and certain prokaryotes. The structure of nitrilases is usually inducible enzymes composed of one or two types of subunits of different size and number, and these subunits of nitrilase self-associate to convert the enzyme to the active form. *Nocardia* sp. nitrilase was reported to be induced by enzonitrile.[35] Acetonitrile has been used as an inducer for the formation of nitrilase in *F. oxysporum*.[15]

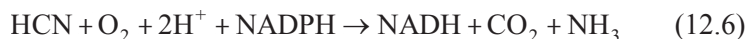### 12.3.1.2   OXIDATIVE PATHWAY

In oxidative pathway, cyanide conversion involves oxygenolytic conversion to carbon dioxide and ammonia. This pathway requires NADPH to catalyze this degradation pathway. Microorganisms that opt for cyanide conversion by this pathway also require additional carbon source with

cyanide. There are two types of oxidative mechanisms involving three different enzymes:

i) Cyanide monooxygenase and cyanase: Cyanide monoxy-genase[27,75] converts cyanide to cyanate which is further cata-lyzed by cyanase, resulting in the overall conversion of cyanate to ammonia and carbon dioxide as given in the following reaction:
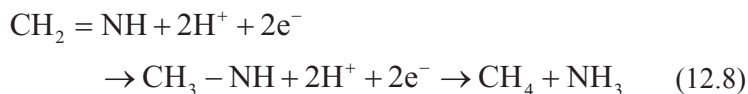
$$HCN^- + O_2 + 2H^+ + NADPH \rightarrow CNO^- + NADP + 2H_2O$$
$$\downarrow$$
$$CNO^- + 3H^+ + HCO_3^- \rightarrow NH_4^+ + 2CO_2 \quad (12.5)$$

ii) Cyanide dioxygenase: This oxidative pathway utilizes cyanide dioxygenase to form ammonia and carbon dioxide directly.[27]

$$HCN + O_2 + 2H^+ + NADPH \rightarrow NADH + CO_2 + NH_3 \quad (12.6)$$

Immobilized cells of *P. putida* can effectively use oxidative pathway to produce ammonia and carbon dioxide.[12] The cyanide degradation in three white rot fungi, *Trametes Versicolor* ATCC 200801, *Phanerochaete chrys-osporium* ME 496 and *Pleurotus sajor-caju,* was achieved by an oxidative reaction whose end products were ammonia and CO_2. In *T. versicolor,* this reaction was the most effective with 0.35 g dry cell/100ml degrading 2 mM KCN (130 mg/l) over 42 h, at 300 °C, pH 10.5 with stirring at 150 rpm.[50]

12.3.1.3   REDUCTIVE PATHWAY

The reductive pathways of degradation of cyanide are generally consid-ered to occur under anaerobic conditions. This pathway is mediated by an enzyme nitrogenase. The enzyme utilizes HCN and produces methane and ammonia as the end products.

$$HCN + 2H^+ + 2e^- \rightarrow CH_2 = NH + H_2O \rightarrow CH_2 = O \quad (12.7)$$

$$CH_2 = NH + 2H^+ + 2e^-$$
$$\rightarrow CH_3 - NH + 2H^+ + 2e^- \rightarrow CH_4 + NH_3 \quad (12.8)$$

Klebsiella oxytoca is able to degrade cyanide compounds to methane and ammonia by this path.[45]

12.3.1.4   SUBSTITUTION/TRANSFER PATHWAY

The activity of this pathway involves cyanide assimilation and useably this tends to increase the growth of the microorganism by providing extra nitrogen source and preventing it from cyanide toxicity. There are two types of enzymes that catalyze cyanide assimilation through this pathway such as rhodanese and mercaptopyruvate sulfurtransferase. Both the enzymes are widely distributed in living organisms and catalyze the formation of pyruvate and thiocyanate from mercaptopyruvate.[78] Rhodaneses are extremely conserved and the prevalent enzymes are presently regarded as one of the mechanisms evolved for cyanide detoxification. In vitro rhodaneses catalyze the irreversible transfer of a sulphur atom from a suitable donor (that is thiosulfate) to cyanide, leading to the formation of less toxic sulfite and thiocyanate. The enzyme activity is modulated by phosphate ions and divalent anions are found to interact with the active site.[11]

$$\text{Thiosulfate} + \text{cyanide} \rightarrow \text{sulphite} + \text{thiocyanate} \qquad (12.9)$$

Rhodaneses have also been recognized in a variety of bacterial species including *Escherichia coli*[14] *Azotobacter vinelandii*[30] and several species of *Thiobacillus*.

The second enzyme mercaptopyruvate sulphurtransferase belongs to the family of transferases, specifically the sulphurtransferases, which transfer sulphur-containing groups. The systematic name of this enzyme class is 3-mercaptopyruvate: cyanide sulphurtransferase. This enzyme participates in cysteine metabolism. It catalyzes the following chemical reactions:

$$HSCH_2COCOO^- + E \leftrightarrow CH_2COCOO^- + ES\,(\text{step1}) \qquad (12.10)$$

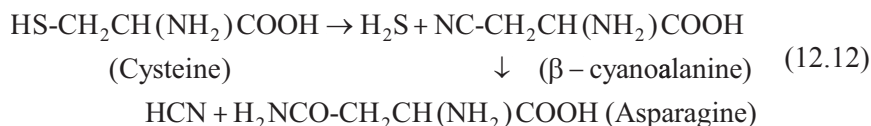$$ES + CN^- \leftrightarrow E + SCN^-\,(\text{step2}) \qquad (12.11)$$

3-Mercaptopyruvate is converted to thiosulfate by the two-step reaction as mentioned above. In step 1, the enzyme-sulphur (ES) intermediate

is formed, and in step 2, intermediate reacts with cyanide to E (mercapto-pyruvate sulfurtransferase) and thiocyanide.[36]

12.3.1.5   SYNTHESES PATHWAY

This pathway is also a cyanide assimilation pathway by using enzyme β-cyanoalanine synthase and γ-cyano-α-aminobutyric acid synthase. It involves the synthesis of amino acid, β-cyanoalanine and γ-cyano-α-aminobutyric acid by using amino acid residues as precursors that react with cyanide compounds.[66] β-Cyanoalanine synthase is believed to play an important role in the removal of endogenous cyanide and is produced during a highly active growth period in the microbe.[66] This enzyme belongs to the family of lyases, specifically the class of carbon–sulphur lyases. The systematic name of this enzyme class is L-cysteine hydrogen-sulphide-lyase. *Bacillus magaterium* grows by converting cyanide to β-cyanoalanine and then to asparagines. β-Cyanoalanine synthase is induced by various amino acids such as serine, cysteine, asparagine and so forth. Cyanide produced by *C. violaceum* can first be converted to β-cyanoalanine then into asparagines as shown in the reaction below.[32]

$$\text{HS-CH}_2\text{CH(NH}_2)\text{COOH} \rightarrow \text{H}_2\text{S} + \text{NC-CH}_2\text{CH(NH}_2)\text{COOH}$$
$$\text{(Cysteine)} \qquad\qquad\qquad \downarrow \quad (\beta - \text{cyanoalanine}) \quad (12.12)$$
$$\text{HCN} + \text{H}_2\text{NCO-CH}_2\text{CH(NH}_2)\text{COOH (Asparagine)}$$

γ-Cyano-α-aminobutyric acid synthase is an alternative pathway for cyanide assimilation. This pathway requires pyrodoxal phosphate for function, and is induced by glutamate or glycine. Once γ-cyano-α-aminobutyric acid is synthesized, it is slowly converted to glutamate. A thermophilic and cyanide ion-tolerant bacterium, *Bacillus stearothermophilus* CN3, isolated from a hot spring in Japan, was found to produce thermostable γ-cyano-α-aminobutyric acid synthase.[69]

12.4   FACTORS RESPONSIBLE FOR THE BIODEGRADATION OF CYANIDES

Physiological and metabolic capabilities of microbes play a very essential role for the successful degradation of cyanide. Various parameters

that play a major role in the degradation of cyanide include temperature, oxygen, pH, nutrients and so forth. Temperature is a significant factor for the determination of biodegradation rate. Cyanide degrading enzymes are generally produced by mesophilic microorganisms, often isolated from soil, with temperature optima typically ranging between 20 and 40°C.[9,45,2] pH of the solution is also a predominantly important factor in the bioremediation of cyanide. The optimum pH for bacterial growth is 6–8, and for fungal growth it is normally 4–5. Cyanide degrading enzymes usually have pH optima between 6 and 9; therefore, extremes of pH may have a significant effect on biodegradation. However, *F. solani* and mixed cultures of fungi including *F. solani*, *F. oxysporum*, *Trichoderma polysporum*, *Scytalidium thermophilum* and *Penicillin miczynski* have been found to be capable of degrading iron cyanides at pH 4.[7] Biodegradation rate of cyanide compounds may also be affected by the availability of nutrients. Carbon has been identified as a limiting factor in the microbial degradation of metal-cyanides, which may prevent the bioremediation of industrially contaminated soils.[9] Availability of oxygen is an important factor in the microbial mineralization of cyanide, as oxygen is consumed during several of the cyanide degrading pathways.[9] Physical and chemical processes employed to degrade cyanide and its related compounds are often high-priced and difficult to operate. A verified substitute to these processes is biological treatment, which typically relies upon the acclimation and enhancement of indigenous microorganisms. Biological degradation of cyanide has often been offered as a potentially economical and environmental-friendly alternative over conventional processes.[19]

12.5   ADVANTAGES OF THE BIOREMOVAL OF CYANIDE

The constant generation of enormous amount of cyanide-bearing wastes from various industries has resulted in the contamination of soil and water which recommends that novel processes are required to alleviate the serious problem of cyanide pollution in environment. The utilization of various chemical and physical treatment processes to rectify the cyanide problem from environment may be disagreeable because of the economic view point, less efficiency and secondary pollution. The biological treatment method is an eco-sociable and substitutional approach for removal of cyanide from waste water. It can be less expensive than

chemical and physical methods, and much faster than natural oxidation with efficiency equal or exceeding that of chemical or physical methods. Lower plant construction and operating cost, that is no chemical handling equipment or expensive control instruments are needed. Costs are relatively uniform and greater volumes of waste do not necessarily increase the costs proportionately. Efficiency wise, biodegradation provides superior resistance to shock loading, as well as recovery from such upsets and results in lower production of total dissolved solids and sludge, making it environment-friendly.

12.6   REVIEW ON THE TOPIC

The biodegradation of cyanide compounds by various microorganisms have been reported by a number of researchers. Table 12.2 presents the summary of literature on the biodegradation of various cyanide compounds.

Potivichayanon and Kitleartpornpairoat (2010)[74] isolated and identified cyanide-degrading bacteria *Agrobacterium tumefaciens* SUTS 1 and studied cyanide removal efficiency. The maximum growth rate of SUTS 1 obtained was 4.7×108 CFU/ml within 4 days. The cyanide removal efficiency was studied at 25, 50, and 150 mg/l cyanide. The cyanide removal efficiency at 25 and 50 mg/l cyanide, was approximately 87.50%. At 150 mg/l cyanide, SUTS 1 enhanced the cyanide removal efficiency up to 97.90%. Cell count of SUTS 1 increased when the cyanide concentration was set lower. Ammonia increased when the removal efficiency increased.

Ozel et al. (2010)[70] investigated to determine cyanide degradation characteristics in some basidiomycetes strains including *Polyporus arcularius* (T438), *Schizophyllum commune* (T 701), *Clavariadelphus truncatus* (T 192), *Pleurotus eryngii* (M 102), *Ganoderma applanatum* (M 105), *T. versicolor* (D 22), *Cerrena unicolor* (D 30), *S. commune* (D 35), and *Ganoderma lucidum* (D 33). The cyanide degradation activities of *P. arcularius*, *S. commune* and *G. lucidum* were found to be more than that of the other fungi examined. Maximum cyanide degradation was obtained after 48 h of incubation at 30°C by *P. arcularius* (T 438). The optimum pH and agitation rate were measured as 10.5 and 150 rev/min, respectively. The amount of biomass was found as 3.0 g for the maximum cyanide biodegradation with an initial cyanide concentration of 100 mg/l.

TABLE 12.2 Details of Different Microbial Species Involved in Biological Processes for Cyanide Removal.

Microorganisms	CN⁻ compound used	pH	Temp. (°C)	Time (h)	Initial conc.	% Removal of CN⁻	Remarks	Reference
Polyporus arcularius	KCN	10.5	30	48	100 mg/l	72.08	–	[70]
Pseudomonas pseudoalcaligenes	NaCN	9.5–10	30	57.6	45 mg/l	~60	–	[40]
Scenedesmus obliquus	NaCN	10.3	-	77	77.9	91	–	[37]
Bacillus sp. (Consortium)	KCN	9.5	37	192	200	65	–	[57]
Pseudomonas fluorenscens	ferrous (II) cyanide (ferrocyanide)	5	25	-	100 mg/l	78.9	–	[29]
Escherichia coli	KCN	6–8	20–40	-	50 mg/l	90	–	[13]
Pseudomonas fluorescens NCIMB 11764	KCN	7	30	6.3	50 mM KCN	80	in 6 h	[49]
Klebsiella oxytoca	KCN	7	30	80	0.58 mM	91.4	–	[45]
Rhodococcus species	KCN	7	30	10	12 mM	50	–	[55]
Pseudomonas fluorescens	FeCN	6	26	60	50 mg/l	99.9	–	[19]
Pseudomonas sp. (CM5, CMN2)	–	9.2–11.4	30	70	–	100	Bacteria remove cyanide between 45–70 h without high concentration of acclimatization	[2]
Fusarium solani	KCN	7.5	–	–	–	50	–	[7]
Pseudomonas putida	FeCN	7	30	–	100 mg/l	78.2	–	[20]

TABLE 12.2 *(Continued)*

Microorganisms	CN⁻ compound used	pH	Temp. (°C)	Time (h)	Initial conc.	% Removal of CN⁻	Remarks	Reference
Pseudomonas sp. *Citrobacter* sp.	K, Zn, Cu-cyanide	7.5	35	15	0.5 mM	68–93% 88–93%	Biodegradation in a rotating biological contactor	[72]
Mixed culture of *Fusarium solani* and *T. polysporum*	$K_2Ni(CN)_4$	7.0	–	–	0.75 mM	90%	–	[7]
Rhizopus oryzae	CN⁻	5.6	25	120	150 mg/l	83%	–	[18]
Stemphylium loti	CN⁻	7.2			150 mg/l	90%	–	
R. oryzae S. loti	CN⁻	5.6 7.2	25	120	150 mg/l	95.3% 98.6%	Removal efficiency increased by using SAB process at same parameters	[18]
Mixed (Anaerobic)	CN⁻	7.5	–	48	>100 mg/L	>70%	Methanogenesis	[31]

'–' Not reported

Gupta et al. (2010)[36] reported that there are many enzymes, which are produced by microorganisms that utilize cyanides as substrate to make alanine, glutamic acid, alpha-amino-butyric acid, beta-cyanoalanine and so forth. Five types of enzymatic pathways in various microorganisms have been reported, which are involved in cyanide degradation. All of them have their advantages and disadvantages. Cyanide hydratase is very substrate specific. The microorganisms containing this enzyme can degrade cyanide up to 200 ppm.

Gurbuza et al. (2009)[39] reported that the biological degradation of cyanide by *Scenedesmus obliquus* has shown a viable and healthy process for degrading cyanide in the mining process waste waters. Gold mill effluents containing weak acid dissociable (WAD) cyanide concentration of 77.9 mg/l was fed into a batch unit, to examine the ability of *S. obliquus* for degrading cyanide. Cyanide was reduced to 6 mg/l in 77 h. Microbial growth and metal uptake of Zn, Fe and Cu was examined during cyanide degradation. The bio-treatment process was considered to be successful in degrading cyanide in the mine process water.

Kao et al. (2003)[45] reported cyanide degradation by *K. oxytoca*. They observed that resting cells could degrade, but cell-free extract was not able to degrade which happened as a result of inactivation of nitrogenase (an oxygen-labile enzyme) caused by the oxygen exposure after cell disruption. *K. oxytoca* also possessed enzymatic mechanisms to degrade nitriles. Nitriles can also be degraded by *Nocardia rhodochrous*, *Arthrobacter, Brevibacterium, P. putida, Pseudomonas marginalis*, *Pseudomonas aeruginosa, Rhodococcus erythropolis, Rhodococcus rhodochrous*.

Luque V. M. (2005)[53] studied the alkaline cyanide biodegradation by *Pseudomonas pseudoalcaligenes* CECT5344 at the pH of lower than 9.5, and found that cyanide compounds were used as the sole source of nitrogen under alkaline conditions, which prevents the volatile HCN formation. The cyanide consumed by this strain is stoichiometrically converted to ammonium. In addition, this bacterium grows with the heavy metal cyanide-containing waste water generated by the jewellery industry. The strain CECT5344 was able to grow with the residue generated in the jewellery industry. This residue contains free cyanide and heavy metals such as Fe, Cu and Au complexed with cyanide, thus making the residue highly toxic.

Timur et al. (2005)[83] investigated a process for recovering gold from aqueous cyanide solutions using naturally occurring soil microorganisms.

In this research, microbes successfully liberated gold cations from auro-cyanide complexes. The gold was subsequently captured on iron and zinc electron donors. After cyanide degrading microorganisms populated microcosm reactors, the biomass was fed with a gold cyanide solution. Microorganisms consumed the CN^- moiety of aurocyanide complexes, releasing gold cations. A portion of the gold was recovered on steel wool or zinc which served as cathodes. Although gold recovery rates were low, methods for improving efficiency were investigated.

Akcil et al. (2003)[2] used two strains of *Pseudomonas sp.* isolated from a copper mine for biodegradation of cyanides at a concentration range of 100–400 mg/l. They compared their studies with chemical treatment methods and concluded that biological treatment methods are less expensive and environment-friendly but are as effective as chemical method.

Kwon et al. (2002)[52] investigated yeast, *Cryptococcus humicolus* MCN2, which was isolated from coke plant waste water and grown on KCN as a sole nitrogen source, degraded concentrations of potassium tetracyanonickelate up to 65 mM when supplied with sufficient carbon.

Patil and Paknikar (2000)[72] used a mixed consortium of three *Pseudomonas species* and a *Citrobacter species* and obtained very high cyanide degradation capability as compared to the microbes used alone. Silver, nickel and zinc cyanide complexes were degraded successfully by the consortium.

Dursun et al. (1999)[23] investigated degradation of ferrous (II) cyanide complex (ferrocyanide) ions by free cells of *P. fluorescens*, in the presence of glucose and dissolved oxygen, as a function of initial pH, initial ferrocyanide, and glucose concentrations, and aeration rate in a batch fermenter. The microorganism used the ferrocyanide ions as the sole source of nitrogen. They found that 79% cyanide removal efficiency was achieved with maximum biodegradation rate at pH 5, and glucose concentration at 0.465 g/l.

Cowan et al. (1998)[16] reported that the cyanide degrading enzymes are generally produced by mesophilic microorganisms, often isolated from soil, with temperature optima usually ranging between 20 and 40°C.

Suh et al. (1994)[82] also used cyanide degrading bacteria, *Pseudomonas fluorescens* NCIB 11764 for the treatment of cyanide containing waste water. After successive subculture for two months in cyanide containing medium, culture was completely adapted to grow in a medium containing up to 260 mg/l of cyanide.

Apart from the degradation, biosorption process also occurs, in which microorganisms adsorb the toxic compounds instead of degrading it.[71] Various fungal species such as *Aspergillus niger, Aureobasidium pullulans, Aspergillus fumigatus, Fusarium moniliforme, Fusarium oxysporum, Cladosporiumsp* and so forth, can act as biosorbents for removal of cyanide compounds.

12.7   ADVANCES ON BIODEGRADATION OF CYANIDE

Immobilization of cells improves the degradation rate[48,22,6,82] by preventing washing of cells, and it also increases the cell density. The immobilization technique was used by Kowalska et al. (1998). They used ultrafiltration membranes made of polyacronitrile[48] and carried out simultaneous degradation of cyanides and phenol using *Agrobacterium radiobacter, Staphylococcus sciuri,* and *Pseudomonas diminuta*. The efficiency of phenol and cyanide biodegradation was dependent on transmembrane pressure. The immobilization technique was earlier studied by Babu et al., (1992) where immobilized cells of *P. putida* were able to degrade sodium cyanide as a sole source of carbon and nitrogen. Various immobilization matrixes are available like granular activated carbon (GAC), alginate beads, zeolite, which have shown very high efficiency, and improved degradation rate.[6,12,23]

Biodegradation and biosorption processes can be used in combination for removal of cyanides as they can be very proficient as reported by Patil and Paknikar (1999). Simultaneous adsorption and biodegradation (SAB) is the latest development in cyanide removal.[19] The major mechanisms that are concerned with simultaneous adsorption and biodegradation are:

- Less biodegradable organics can be adsorbed on carbon at first, and are then slowly degraded by microorganisms[86,76]
- Adsorbent can be partially regenerated by microorganisms while the carbon bed is in operation[77,76]
- Biological reaction rate becomes higher on adsorbent due to an enrichment of the organics by carbon adsorption[86]

Dash et al. (2008)[19] reported high removal efficiency using *P. fluorescens* immobilized on granular activated carbon in SAB process and Dwivedi et al. (2016) reported cyanide removal using *Bacillus cereus*

immobilized on natural bioadsorbent almond shell in SAB process. The degradation of cyanides has also been observed in several fungal strains. *F. solani*, *Trichoderma polysporum*, *Fusarium oxysporum*, *S. thermophilum*, *Penicillium miczynski* are able to grow on metallo-cyanide complex as a source of nitrogen.[19] A strain, *P. pseudoalcaligenes* CECT5344,[82] isolated from the Guadalquivir river (Cordoba), was found to use several nitrogen sources including cyanide, cyanate, b-cyanoalanine, cyanacetamide, and nitroferricyanide under alkaline conditions, which prevents volatile HCN (p*K*a 9.2) formation. This bacterium was also grown on heavy metal from cyanide containing waste water generated by the jewellery industry.[40]

Besides bacteria and fungi, algae such as *Arthrospira maxima*, *Chlorella* sp. and *S. obliquus* could be used for the degradation of cyanides.[37] The detoxification of cyanide by algae was examined by exposing cultured suspensions of *A. maxima*, *Chlorella* sp. and *S. obliquus* at the pH of 10.3. The removal efficiency was 99%. This study has explored the use of algae in this field under extreme conditions with high removal efficiency.

Biosorption process is also used in cyanide removal. In biosorption processes, the microorganisms adsorb the toxic compounds instead of degrading it.[72] Several live fungal species (*A. fumigatus*, *A. niger*, *A. pullulans*, *Cladosporium cladosporioides*, *F. moniliforme*, *F. oxysporum*, *Mucor hiemalis*), can act as biosorbents for cyanide compounds. Biodegradation and biosorption process can be used in combination for removal of cyanides as they can be very efficient as shown by Patil and Paknikar (2000). Iron (III) cyanide complex is known to get adsorbed on *Rhizopus arrhizus* which is a filamentous fungus.[3] This fungus can adsorb the complex at very high alkaline conditions of pH 13 with very high loading capacity of 612.2 mg/g of cyanide.

One novel study has proposed a transcription-based assay for monitoring the biodegradation of both simple and metal cyanides.[8] The gene encoding cyanide hydratase (chy) in *F. solani* has been sequenced and primers utilized in reverse transcription-polymerase chain reaction (RT–PCR) to demonstrate transcription of this gene. The *chy* gene from *F. solani* displays significant homology to the corresponding cyanide hydratase gene from *Gloeocercospora sorghi, F. lateritium,* and *Leptosphaeria maculans*. This observation implies that the assay could be utilized in different contexts, provided that expression of the *chy* gene can be conclusively linked with the activity of cyanide hydratase. This assay could be an important tool to site managers, regulators and industries that generate cyanide wastes.[27]

12.8   CONCLUSION

From the above discussions the following conclusions are made:

- The development of microbial processes to be competent in severe environmental conditions, such as low pH, and contaminant toxicity, is required to ensure a technology that is competitive with the current physiochemical remediation strategies now practiced to reduce cyanide pollution.
- New microorganisms must be identified with respect to their degrading capabilities.
- Cyanide hydrolysis has an optimal pH range of 7–9, whereas thiocyanate biodegradation can occur at pH 10.
- The drawback of hydrolytic pathway is that, the microorganisms using cyanide hydratase to degrade cyanide do not use the carbon atom of cyanide as nutrient source; they require an additional carbon source. Cyanide hydratase is a really substrate specific enzyme.
- Microorganisms containing nitrile hydratase use cyanide as both nitrogen and carbon source.
- Biological processes have significant advantages over the physicochemical ones, and are attractive alternative methods for the cyanide removal.
- Biological treatment can be less expensive than chemical and physical methods, and much faster than natural oxidation with efficiency equal or exceeding that of chemical or physical methods.

KEYWORDS

- **Bacteria**
- **biodegradation**
- **cyanide**
- **fungus**
- **waste water**
- **SAB**

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