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Biological Treatment of Cyanide Containing Wastewater

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Abstract

Cyanide compounds are released in to the water stream from a number of industrial effluent and several routes including biodegradation for their removal from the waste water are under investigation. The present review describes the mechanism and advances in the use of biological treatment for the removal of cyanide compounds and its advantages over other treatment processes. It also includes various microbial pathways for their removal. The feasibility of the concomitant biogas generation during anaerobic biodegradation of cyanide has also been described. A comparative study has been done on the efficiency of aerobic and anaerobic biological degradation of cyanide. The limitations of this process have also been discussed.

Key words: Biodegradation, cyanide, algae, simultaneous adsorption and biodegradation, waste water.

Introduction

"Cyanide is a triple-bonded molecule with a single negative charge consisting of one atom of carbon in the +2 oxidation state and one atom of nitrogen in the -3 oxidation state". Immensity of cyanide amount in environment is mainly due to metal finishing and mining industries. Although cyanide can be removed and recovered by several processes, it is still usually discussed and examined due to its potential toxicity and environmental impact[1]. The chemical speciation of cyanides depends on their sources and also in response to a variety of environmental factors. Different forms of cyanide include: free cyanide, cyanide ion, cyanide salt, metallocyanide complexes and synthetic organocyanides, also known as nitriles and total cyanide[2]. Cyanide occurs in water as hydrocyanic acid (HCN), cyanide ion (CN^-), simple cyanides, metallocyanide complexes and as simple chain and complex ring compounds. Simple cyanides include water-soluble salts of alkaline earth, alkali and heavy metals. Typical simple cyanides are NaCN, KCN, $Ca(CN)_2$, $Hg(CN)_2$, $Zn(CN)_2$, $Cd(CN)_2$, $Ni(CN)_2$ and AgCN. The simple cyanides such as potassium cyanide and sodium cyanide ionize in water to release a cation and cyanide ion[2]. Metallocyanide complexes have a wide range of stabilities. Zinc [$Zn(CN)_4^{-2}$], cadmium [$Cd(CN)_3^{-2}$] and $Cd(CN)_4^{-2}$] complexes dissociate rapidly and nearly completely in dilute solutions[3]. Nitriles are organic compounds (R-CN) containing the cyanide group (CN). Cyanide bound to carbon as nitriles (other than as cyanogenic glycosides) is comparatively innocuous in the environment, low in chemical reactivity and is biodegradable[2]. Cyanide and chemically related compounds are formed, excreted and degraded in nature by hundreds of species of bacteria, algae, fungi, plants and insects[4]. As a result, low levels of cyanide can appear in naturally occurring surface or groundwater samples which normally would not be expected to contain it. At least 1,000 species of plants and micro-organisms from 90 families have been shown to contain one or more of nearly twenty compounds capable of producing cyanide[5]. About 800 species of higher plants from 70 to 80 families, including agriculturally important species such as the cassava, flax, sorghum, alfalfa, bamboo, peach, pear, cherry, plum, corn, potato, cotton, almond and beans are cyanogenic[6]. Cyanide is fast-acting broad spectrum toxin and it affects all living organisms. Cyanide ion exerts an inhibitory action on certain metabolic enzyme systems, most notably cytochrome oxidase, the enzyme involved in the ultimate transfer of electrons to molecular oxygen. In presence of even weak acids, HCN gas is liberated from cyanide salts[7]. Cyanide poisoning can occur through inhalation, ingestion and skin or eye contact. One teaspoon of a 2% solution can kill a person. In general, fish and other aquatic life are killed by cyanide concentrations in the *microgram per liter* range (part per billion), whereas bird and mammal deaths result from cyanide concentrations in the *milligram per liter* range (part per million). The release of cyanide from industries worldwide has been estimated to be more than 14 million kg/yr[8]. Industries concerned with the production and use of cyanide compounds generate wastes which contain large amounts of cyanide. Especially paint manufacture and use, polymer production, chemical and pharmaceutical industry; steel industry, mining operations and coal manufacturing all produce wastes and waste water with high cyanide content. Electroplating industrial wastes contain 0.5% to 20% cyanide. Complex cyanide, Prussian blue is used for dyeing jeans. Since, cyanide is a toxic compound well-known as a metabolic inhibitor, cyanide-containing effluents cannot be discharged without being subjected to treatment to reduce their cyanide contents to very low levels (>0.1 mg of CN^- per liter)[7]. US-health service cites 0.01mg/L as guideline and 0.2 mg/L as permissible limit for cyanide in effluent. Indian standard has set a minimal national standard (MINAS) limit for cyanide in effluent as 0.2 mg/L[9].

Material and Methods

Cyanide and its related compounds can be removed from industrial effluent through various physical, chemical and biological/biodegradation treatment methods. Physical methods for cyanide treatment can be accomplished using dilution, adsorption, membranes, electrowinning and hydrolysis/distillation. Adsorption is commonly used in physical treatment methods for the removal of cyanide. In chemical methods, some of the more common processes used to degrade cyanide are the hydrogen peroxide process, alkaline chlorination, sulphur dioxide-air oxidation, electrolytic oxidation etc. These methods are used to purify effluents for discharge by concentrating and recovering the cyanide for recycling. On the other hand, oxidation processes are used to destroy the cyanide and include various biological, catalytic, electrolytic, chemical and photolytic methods[10]. These aforesaid methods are really effective removal methods but have a few drawbacks over biodegradation method. These are much expensive, produce secondary pollution also and suitable for low concentration of cyanide, some time pretreatment is also required.

Biological Degradation of Cyanide: Mechanism of biological degradation: Biological degradation is the process of breaking and transforming hazardous materials into simple nontoxic substances by a biological treatment. Cyanide can be broken into simpler substances by microorganisms in the wastewater, piles and in the soil. Some bacteria, fungus, algae and plants can decompose cyanide and cyanide compounds. The bacterial detoxification would be safer, quicker and cheaper than the chemical treatment to handle cyanide wastes. The process that demands oxygen is an aerobic biodegradation and the process that occurs in the absence of oxygen is an anaerobic biodegradation.

Cyanide-oxidizing bacteria normally break it down into harmless compound. Under aerobic conditions, biological process may consume hydrogen cyanide and generate hydrogen cyanate as shown in the equation 1. The hydrogen cyanate is in turn hydrolyzed into ammonia and carbon dioxide as shown in equation 2.

$$2HCN + O_2 + \text{Enzyme} \longrightarrow 2HCNO \quad (1)$$

$$HCNO + H_2O \longrightarrow NH_2 + CO_2 \quad (2)$$

Anaerobic biodegradation of cyanide and hydrogen cyanide is restricted to the moderately to strongly reduced portions of the heap environment and can only occur if HS_2 or H_2S are present. The Sulphur species present depends on pH. At a pH value greater than 7, HS_2 is the dominant species. At a lower pH, H_2S present predominantly.

$$CN_2 + H_2S \longrightarrow HCNS + H_2 \quad (3)$$

$$HCN + HS_2 \longrightarrow HCNS + H_2 \quad (4)$$

The HCNS then hydrolyze, to form NH_2, H_2S and CO_2. Anaerobic biodegradation is much slower than aerobic biodegradation of cyanide, and anaerobic bacteria have a cyanide toxicity threshold of only 2 mg/l compared to 200 mg/l for aerobic bacteria. Consequently, anaerobic biodegradation is a much less effective cyanide removal mechanism[12].

In the waste streams and environmental matrices, cyanide can be present as a form of simple cyanides (HCN, CN^-, NaCN), metal cyanide complexes, cyanates and nitriles. Degradation pathways are susceptible to the concentration and form of the cyanide compounds, the physicochemical conditions of the media and the presence of interfering and inhibitory compounds[8]. The development of biodegradation strategies for these varied surroundings requires a comprehensive understanding of the biological pathways.

Common biochemical pathways responsible for cyanide degradation: There are four common pathways for the biodegradation of cyanide. These are; hydrolytic, oxidative, reductive and substitution/transfer, described in figure 1. Various reviews have already explained these pathways and the organisms in which they are found[11-12]. More than one pathway can be utilized for cyanide biodegradation in some organisms[13]. Hydrolytic reactions are catalyzed by cyanide hydratase, forming formamide, or cyanidase, which produces formate and ammonia figure 1. Cyanide hydratase is mainly a fungal enzyme and is highly conserved between species[14]. Cyanidase (cyanide dihydratase) is mainly bacterial enzyme. Cyanide hydratase and cyanidase have recently been shown to have similarity at both the amino acid and structural levels to nitrilase and nitrile hydratase enzymes[15]. The oxidative reactions for the biodegradation of cyanide form ammonia and carbon dioxide figure 1. Cyanide monoxygenase converts cyanide to cyanate, with cyanase then catalyzing the bicarbonate-dependent conversion of cyanate to ammonia and carbon dioxide[8]. A second oxidative pathway utilizes cyanide dioxygenase to form directly carbon dioxide and ammonia figure 1. The biodegradation of thiocyanate (cyanide compound) has also been studied figure 1. Thiocyanate is used in a variety of chemical processes and can be produced from the reaction of cyanide with pyretic materials in waste streams. Thiocyanate is produced in vivo by the action of thiosulfate-cyanide sulfurtransferase enzymes figure 1. Biodegradation of thiocyanate can take place by two pathways[16-17]. According to the first pathway, Cyanate is converted in to ammonia and carbon dioxide in the presence of high concentration of Cyanase[17]. In a second pathway, thiocyanate is converted to ammonia and carbonyl sulfide.

Hydrolytic Reactions
Cyanide hydratase
$HCN + H_2O \rightarrow HCONH_2$
Cyanidase
$HCN + 2H_2O \rightarrow HCOOH$

Reductive Reactions
$HCN + 2H^+ + 2e^- \rightarrow CH_2{=}NH + H_2O \rightarrow CH_2{=}O$
$\downarrow$
$CH_2 = NH + 2H^+ + 2e^- \rightarrow CH_3 - NH + 2H^+ + 2e^- \rightarrow CH_4 + NH_3$

Oxidative Reactions
Cyanide monoxygenase
$HCN + O_2 + H^+ + NAD(P)H \rightarrow HOCN + NAD(P)^+ + H_2O$
Cyanide dioxygenase
$HCN + O_2 + 2H^+ + NAD(P)H \rightarrow CO_2 + NH_3 + NAD(P)^+$

Substitution/Transfer Reaction
Thiosulfate:cyanide sulfurtransferase
$CN^- + S_2O_3^{2-} \rightarrow SCN^- + SO_3^{2-}$

Thiocyanate biodegradation
Carbonyl pathway {Thiocyanate hydrolase}
$SCN^- + 2H_2O \rightarrow COS + NH_3 + OH^-$
Cyanate pathway {cyanase}
$SCN^- + 3H_2O + 2O_2 \rightarrow CNO^- + HS^- \rightarrow HS^- + 2O_2 \rightarrow SO_4^{2-} + H^+$
$\downarrow$
$CNO^- + 3H^+ + HCO_3^-$
$\downarrow$
$NH_4^+ + 2CO_2$

Figure 1
Chemical reactions for the biodegradation of cyanide and thiocyanate

Microorganisms responsible for cyanide degradation: The microorganisms primarily responsible for degradation of cyanide include a diverse group of anaerobic and aerobic bacteria. These single-celled microorganisms exhibit a wide range of metabolic functions and are capable of degrading a wide range of chemical structures and concentrations. Bacteria utilize a combination of extracellular and intracellular adaptive and constitutive enzymes in the biochemical breakdown and assimilation of the organic and inorganic compounds present in liquid wastes. These compounds, along with various nutrients (i.e. nitrogen, phosphorous and trace metals) are degraded and/ or assimilated for the purposes of energy production and cell synthesis. The extracellular enzymes found on the cell wall or in the capsule layer surrounding the cell wall aid in the breakdown of larger and more complex molecules, converting these compounds to smaller, more soluble by-products. The breakdown products are then transported into the bacterial cell for conversion to cellular material and energy through the use of intracellular enzymes[18]. Whilst bacteria and fungi have often been identified as cyanide detoxifying microorganisms, cyanide detoxification by algae has been shown in only a few studies[18]. Several algal cultures can effectively degrade cyanide as carbon and/or nitrogen source for their growth. Cyanide effluent degradation by *Scenedesmus obliquus* has been examined[19]. Microbial detoxification of cyanide by algae species was significantly influenced by the various factors, including initial concentration of cyanide, initial cell density and time. There are few microbes such as *Klebsiella oxytoca,* a bacterial strain, which can utilize these compounds as a source of nitrogen and carbon for their own growth. Several microorganisms such as *Pseudomonas fluorenscens, Escherichia coli, Klebsiella oxytoca, Fusarium solani, Stemphylium loti, R. oryzae* etc. are responsible for cyanide degradation. The bacterium *Pseudomonas fluorescens* utilizes KCN under nitrogen-limiting conditions in fed batch culture[20-21]. Metabolism of cyanides by strains of *Escherichia coli, Acinetobacter, Alcaligenes,* and *Bacillus, Stemphilium loti* has also been studied. Many other bacteria including *P.*

putida, *Pseudomonas paucimobilis* and *P. fluorescens* utilize moderately strong metal complexed cyanides at neutral pH[22]. Some microorganisms are listed to having cyanide removal capability from contaminated water shown in table 1.

Table 1
Microorganisms having cyanide removal capabilities

Bacteria	**Enrichment Source**
Pseudomonas putida	Sewage sludge, Soil, Creek water
Pseudomonas pickettii	Soil
Pseudomonas paucimobilis	Mining waste water
Klebsiella pneumoniae	Soil, Creek water
Klebsiella sp.	Creek water
Alcaligenes xylosoxidans	Soil
Fungi	
Fusarium solani	Soil (contaminated)
F. oxysporum	-
F. lateritum	-
Rhizopus oryzae	-
Cryptococcus humicolus (Yeast)	Cock-plant waste water
Algae	
Arthrospira maxima	-
Scenedesmus obliquus	Lake
Chlorella sp	seawater

'-' not reported

Simultaneous adsorption and biodegradation (SAB) process: The application of simultaneous adsorption and biodegradation processes in the same reactor is known to be effective in the removal of biodegradable contaminants in various kinds of wastewater. The use of simultaneous adsorption and biodegradation is effective in treatment of organic toxic pollutants, present in industrial wastewaters. Recently simultaneous adsorption and biodegradation process has been found to be much effective over the individual processes by using microbes *Rhizopus oryzae* and *Stemphylium loti* with granular activated carbon (GAC) as adsorbent[9]. Maximum removal efficiency of cyanide achieved by biodegradation alone as 83% by *Rhizopus oryzae* while 90% by *Stemphylium loti* with the initial cyanide concentration of 150 mg/L at initial pH of 5.6 and 7.2 respectively[9] as shown figure 2. In the SAB process the efficiency of cyanide removal of *Rhizopus oryzae* and *Stemphylium loti* were 95.3% and 98.6% respectively as shown figure 3[9]. It has been observed that SAB process is suitable methodology for the removal of toxic material with higher efficiency and effectively. The results are encouraged with SAB; therefore, it can be better treatment option when compared to adsorption or biodegradation alone. The removal percentage and specific uptake have been studied in the both cases of adsorption and simultaneous adsorption-biodegradation (SAB) and have been compared.

Figure 2
Cyanide Removal efficiency through biodegradation by R. oryzae and S. loti

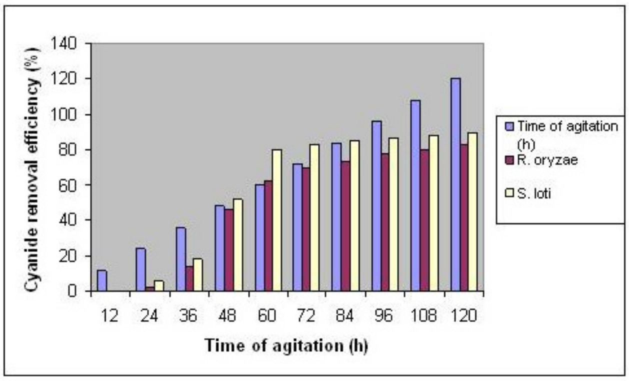
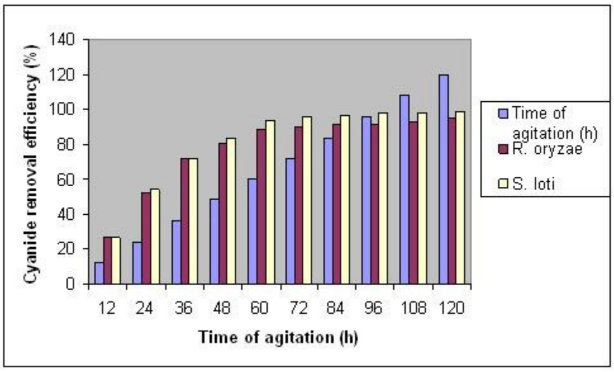


Figure 3
Cyanide removal efficiency through SAB process by R. oryzae and S. loti immobilized on granular activated carbon (GAC)



Effects of Various Physio-Chemical Parameters on Cyanide Removal Efficiency: Aerobic and anaerobic biological processes have been successfully employed in the treatment of a wide variety of industrial and hazardous liquid wastes. The optimized physical and chemical parameters like temperature, pH, flow rate, pressure, hydraulic retention time and agitation are very essential parameters for cyanide degradation from contaminated water. If any alteration is made on these parameters, the cyanide removal efficiency of microorganism can be directly affected. Table 2 shows that the efficiency of aerobic treatment of cyanide removal is much better than the anaerobic treatment method. The yeast strain *Cryptococcus humicolus,* which is isolated from coke-plant waste water, has been reported to have better cyanide removal efficiency in aerobic treatment, (approximately 100%) than in anaerobic treatment method removed 90%. The pH has also played a leading role in cyanide removal from waste water.

Table-2
Various physio-chemical parameters of aerobic and anaerobic biodegradation with their CN^- removal efficiency

Reference	**Treatment Methods / Conditions**	**Microorganism**	**pH**	**Temperature (c^o)**	**HRT (hrs)**	**Rpm**	**Reactor /column and diameter**	**Adsorbent material**	**Initial conc. Of cyanide (mg/lit)**	**Efficiency (%)**
R. D. Fallon et al. 1991	Anaerobic (methanogenesis)	Mixed	7.5	35-40	<48	-	Up flow, anaerobic fixed bed	Activated charcoal	>100	>70
Alain Dumestre et al. 1997	Aerobic (alkaline)	*Fusarium solani*	9.2-10.7	30	72	120	Rotatory shaker	-	>100	>80
R. Roshan Dash et al. 2006	Aerobic (SAB)	*Rhizopus oryzae*	5.6	25	120	140	Rotatory incubator shaker	Granular activated carbon	150	83
R. Roshan Dash et al. 2006	Aerobic ((SAB)	*Stemphylium loti*	7.2	25	120	140	Rotatory incubator shaker	Granular activated carbon	150	90
C.J. Gantzer et al. 1990	Nitrogen fixing	*anabaena*	High	-	-	-	Batch reactor	-	3	<25
Hyouk Kee Kwona et al.	Aerobic	*Cryptococcus humicolus*	7.5.	-	24	-	fixed-bed biofilm reactors	-	50 mg $CN\ dm^{-3}$	100
	Anaerobic		5.0							90
Hyouk Kee Kwona et al.	Aerobic	*Cryptococcus humicolus*	7.5.	-	9	-	fixed-bed biofilm reactors	-	50 mg $CN\ dm^{-3}$	83
	Anaerobic		5.0							61

New Biotechnological Approach Using Algae for the Detoxification of Cyanide: The detoxification of cyanide by algae was examined by exposing cultured suspensions of *Arthrospira maxima*, *Chlorella* sp. and *Scenedesmus obliquus* in growth media to varying concentrations in short-time batch tests. In each experiment, the pH was adjusted to 10.3. The effect of pH, initial concentration of algal cells, temperature and cyanide concentration on microbial detoxification were examined. Under the experimental conditions, initial microbial detoxification rates of 50 and 100 mg/L free cyanide were observed for 25 h[18]. *A. maxima* did not survive due to its sensitivity to the higher cyanide concentrations in the solutions. *S. obliquus* removed the cyanide to a greater extent than did *Chlorella* sp. *S. obliquus* detoxified 99% of the cyanide, while *Chlorella* sp. removed about 86% in the same time period. For the increase in cyanide concentrations between 100 and 400 mg/L, *S. obliquus* was the only microorganism tested for 67 h. [18]. It has been observed that *Scenedesmus obliquus* was competent algal organism in trio. It removed 99% of the cyanide from cyanide containing solution. The use of algae reported in this work offers a process for removing cyanide without pH adjustment at minimal cost as only trace amounts of nutrients are required to be added. Algae can be easily obtained and cultivated and require less nutrients than bacteria. Thus, the process has the potential of becoming an economical and an eco-friendly alternative to the conventional chemical processes.

Results and Discussion

Biodegradation of cyanides has been studied with a number of microorganisms, including bacteria and fungi. Most of the microorganisms have been investigated in aerobic conditions to apply for conventional activated sludge processes. On the contrary, anaerobic treatment of cyanides has not been intensively studied except for the cases of some methanogenic mixed cultures. Anaerobic wastewater treatment could be more advantageous, the biodegradation of cyanide under anaerobic conditions has also recently demonstrated the feasibility for concomitant biogas generation, a possible economic benefit of the process due to its low energy requirement compared with aerobic treatment. Algae can be simply obtained and cultivated; it requires fewer nutrients than other organisms. Thus, the process has the potential of becoming an economical and an eco-friendly alternative to the conventional chemical processes. Simultaneous adsorption and biodegradation process is also effective in treatment of organic toxic pollutants over individual process. Due to the delayed growth of microbes in the culture medium adsorption occurred in the first phase. It was observed that the removal efficiency of SAB process is much efficient to remove cyanide as compared to biodegradation alone. A new yeast strain, *Cryptococcus humicolus*, showed facultative property on TCN (tetracyanonickelate (II)) degradation, which is able to grow in both aerobic and anaerobic conditions. This facultative ability can be beneficial for practical applications since oxygen limitation in aerobic operation or the presence of oxygen in anaerobic operation is not detrimental to cell activity.

Conclusion

It has been concluded that, biological treatment method is an ecosociable substitutional approach for removal of cyanide. It can be less expensive than chemical and physical methods, and much faster than natural oxidation with efficiency equal or exceed those of chemical or physical methods. Lower plant construction and operating cost i.e. no chemical handling equipment or expensive control instrument 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 environmental friendly.

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