Journal of Hazardous Materials 176 (2010) 1–13

Contents lists available at ScienceDirect

Journal of Hazardous Materials

journal homepage: www.elsevier.com/locate/jhazmat

Review

Enzymatic mechanism and biochemistry for cyanide degradation: A review

Neha Gupta*, Chandrajit Balomajumder, V.K. Agarwal

Chemical Engineering Department, Indian Institute of Technology Roorkee, Roorkee 247667, India

ARTICLE INFO

Article history:
Received 11 June 2009
Received in revised form 6 November 2009
Accepted 6 November 2009
Available online 11 November 2009

Keywords:
Cyanide degrading enzymes
Plants and microbes
Biodegradation of cyanide

ABSTRACT

Cyanides are fast-acting poisons, can be lethal if exposed in excess. In spite of fact, cyanides are discharged as effluents in large scale from industries every year. Certain bacteria, fungi, algae and plants produce cyanides. It has been observed that microbes and plant systems can degrade cyanides to less toxic compounds. There are many enzymes, which are produced by microorganisms that utilize cyanides as substrate to make alanine, glutamic acid, alfa-amino-butyric acid, beta-cyanoalanine, etc. Present paper deals with different enzymes, their mechanisms and corresponding pathways with respect to the known biochemistry of enzyme and feasibility for the use in treatment of cyanides containing industrial effluents.

Contents

1. Introduction

Cyanide plays a pivotal role in the development of amino acids, protein, nucleotides, lipids and membranes on primitive earth. Historically, cyanide has been used as chemical weapon in First World War. Hydrogen cyanide vapor has been used to fumigate buildings and ships. It is used for extraction of gold [1,2]. Some industrial processes, such as iron and steel, chemical, drugs, pesticides and plastics manufacturing, electroplating, photo developing and the process used in wastewater treatment and create cyanides products. Cyanide is a source of nitrogen in all living organisms. There are organism that synthesis cyanides, as well as that assimilate cyanide as a source of carbon and nitrogen. There are about 3000 species of plants, animals, microbes and fungi that contain cyanide.

* Corresponding author. Tel.: +91 9760211510.
E-mail address: gnehadch@iitr.ernet.in (N. Gupta).

0304-3894/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.jhazmat.2009.11.038

Higher plants produce small quality of HCN during their normal metabolism [3].

Some food crops (cassava, corn, and lima beans), forages (alfalfa, sorghum, and Sudan grasses) and horticulture plants (ornamental cherry and laurel) produce cyanogenic glucosides that are stored in plant vacuoles and during stress condition or when the plant tissues are damaged they are released as nitrogen source. In general HCN is produced by the plant as a defense mechanism against herbs. Various algae like *chloorolla Valgaris*, *cayanobacteria*, *sencedusmus* and *nostoc muscorum* [4,5] bacteria like *Chromobacterium violaceum* and certain *Pseudomonas* species [6] and fungi like *tricholoma* and *actinoycetes* produce and metabolize cyanide. In bacteria normally HCN is produce during transition stage of growth and in fungus when fruiting bodies are formed or during damaged or stress condition as a mechanism to provide nitrogen and carbon source [7]. In animals antropods also produce HCN [8].

As cyanides are produced regularly by industries in large quantity in waste water streams, it is a potent health hazard for human and ecosystem. Cyanides can be removed from industrial wastes by biodegradation, physical and chemical methods [9,10]. Chemical and physical methods deal with chemical oxidation through alkaline chlorination, ozonization in presence of UV, hydrogen peroxide, Air/SO_2 process and chlorine dioxide gas [11,12], adsorption on granulated activated carbon [13], ion exchange, membrane concentration, air stripping and evaporation, subsequently through thermal treatment by alkaline chlorination and hydrolysis at high temperature [14].

These techniques are only effective for free cyanide (HCN, CN^-) and cyanides that are weakly bonded to metals. Cyanides that are strongly bonded or complexes with metals cannot be treated with these methods. All these methods are based on cyanide recovery by acidification and/or destruction by chemical oxidation [15,16]. In many cases the process is burdened with high capital, reagent costs and royalty payments. Various reagent and chemical used in the process are toxic itself when released in environment accidentally also the end product of these technologies are also required some additional treatment prior to disposal [17].

The biodegradation method of cyanides removal is better to physical and chemical methods [18,19]. Biodegradation is more economical and faster [20]. It is more efficient and has less capital and operative cost. The sudden increase in input does not affect the process adversely [21]. Biological transformation involves cyanide degradation and assimilation in the form of amino acids, thiocyanate, β-cyanoanaline and vitamins by the microorganisms and plants [22]. Cyanide is converted to carbon and nitrogen source by various enzymes present in microorganism. The metabolic pathway for conversion of cyanide is influenced by its initial concentration, pH, temperature, availability of other energy source in the form of organic carbon required for cell maintenance and growth, presence of oxygen, ammonia, and various metals ions [23,24].

In this paper a detailed discussion of various enzymes and there corresponding cyanide degrading pathways and mechanism in microorganisms, their biochemical reactions with their potency and optimum conditions to improve the commercial applications of microbial degradation of cyanides.

2. Enzymatic pathways for cyanide degradation

The microbial degradation involves enzymatic pathways and specific conditions, like pH, temperature, concentration of cyanide, etc. Generally degradation of cyanide is induced by the presence of cyanide in the media and then conversion of cyanide into carbon and nitrogen. Various researchers have described various organisms, which use the different pathway for cyanide degradation [25,26]. Some times more than one pathway can be utilized for cyanide biodegradation in some organisms [31]. Five general pathways for the biodegradation of cyanide are listed below [27–30]. First three pathways are degradation pathways in which enzymes catalyze the conversion of cyanides into simple organic or inorganic molecules and further converted to 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 [32,33].

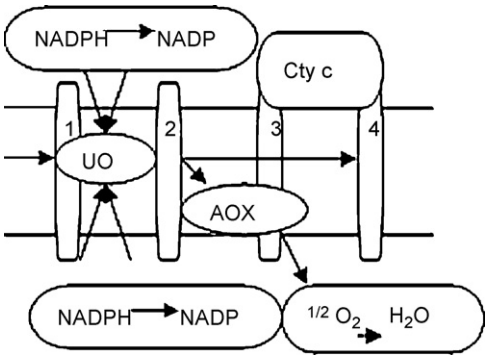


Fig. 1. This figure represents the mitochondrial electron chain in presence and absence of cyanide ion.

- Hydrolytic pathway.
- Oxidative pathway.
- Reductive pathway.
- Substitution/transfer pathway..
- Syntheses pathway.

All these pathways depend on the mechanism of tolerance of cyanide in microbes and on the process that it uses to dissociate the cyanide metal complexes or for chelating metals. Cyanide is a prominent metabolic inhibitor and is best known as respiratory inhibitor. It binds to various metal cofactors and enzymes used in various vital processes [34,35].

During respiration cyanide block electron transport chain by binding with the iron ion in terminal electron acceptor cytochrome C oxidase thus quickly decreasing respiration rates. ATP synthesis in mitochondria is inhibited. Plant and various microorganisms have resistance to cyanide poisoning since they have developed alternate pathway for ATP production. Some of them have different oxidase rather then cytochrome C oxidase [36].

Fig. 1 represents the mitochondrial electron transport chain. It shows that in presence of cyanide alternate oxidase (AOX) to transfer electron to oxygen atom in place of cytochrome C oxidase. In addition some microbe uses metal-cyano complexes as nitrogen source and for the dissociation of these complexes they form biofilm on the surface to adsorb metal ions or form siderophores. Siderophores are the iron sequestering compounds produced by microbes for iron chelating [37–39].

Mechanism of various enzymes and there cyanide degrading pathways are shown in flow diagram below in Fig. 2. This flow chart shows how the cyanide compounds are first converted to HCN then to CO_2, ammonia and formic acid which are required by the microorganisms for growth.

2.1. Hydrolytic pathway

Hydrolytic pathway of cyanide degradation is catalyzed by five enzymes present in microbial system: (i) cyanide hydratase, (ii) nitrile hydratase, (iii) carbonyl pathways (thiocyanate hydrolase), (iv) nitrilase, and (v) cyanidase. First three enzymes have specific substrate and directly hydrolysis and cleave the carbon–nitrogen

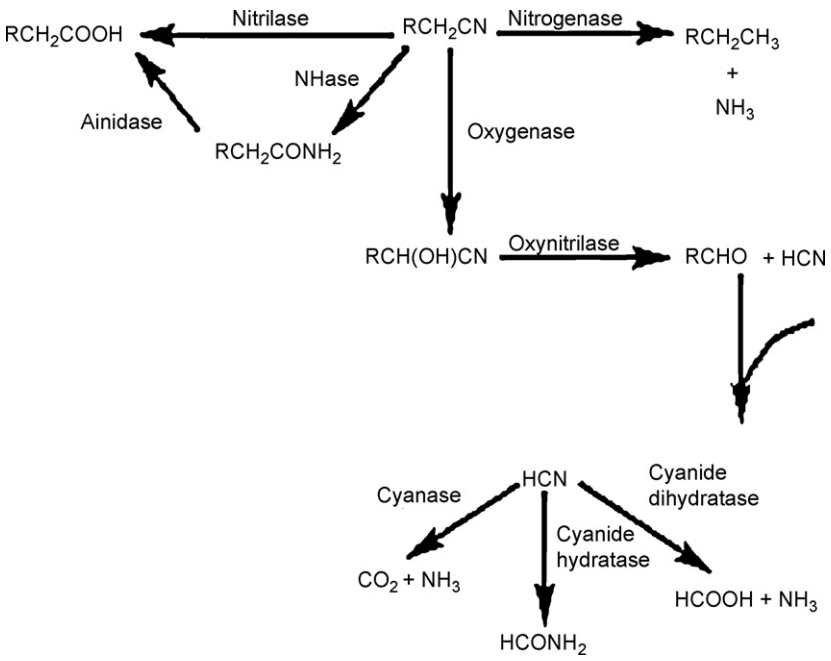


Fig. 2. This figure represents flow diagram of various cyanide degrading pathways.

triple bond to form formamide, and last two convert it to ammonia and carboxylic acid, which are utilized in there metabolism activity. Each is discussed as follows.

2.1.1. Cyanide hydratase

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

$$HCN + H_2O \leftrightarrow HCONH_2 \quad (1)$$

This enzyme belongs to the family of leases, specifically the hydrolyases, which cleave 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.

2.1.1.1. Enzyme structure. The data shows that cyanide hydratases of various microorganism have similarities to each other and represent a much more closely related group of enzymes. The subunit molecular mass for all the enzymes is approximately similar. The enzymes from *Gloeocercospora sorghi*, *Fusarium lateritium* and *Fusarium solani* have native molecular masses 300 kDa [40].

2.1.1.2. Substrate specificity. The HCN is the most effective substrate for this group of enzymes. Further, the enzymes from *F. solani* and *F. oxysporum* N-10 can hydrolyse a metal-cyano complex, tetracyanonickelate (II) (TCN). *F. oxysporum* N-10 cyanide hydratase also has some activity with a number of aliphatic cyanides compounds showing best activity with acrylonitrile, methacrylonitrile and crotononitrile [41].

The cyanide hydratase was first partially purified by *Sternphylium loti*. According to its kinetic study it was observed that, maximal activity occurred in the pH range of 7.0–9.0. The K_m for cyanide biodegradation was estimated at 15–20 mM. EDTA, ammonium chloride, potassium chloride, magnesium chloride or calcium chloride did not inhibit the enzyme activity [44]. Later on it was shown that there is *Stemphylium loti* when immobilized and used in a continuous reactor system to degrade cyanide wastes to formamide, K_m increased to 43 mM and V_{max} was about 600 μmoles h^{-1}(mg protein). But the activity was totally lost by incubation of the mycelia for 16 h at 22–24 °C and the storage at 4 °C resulted in loss of 50% activity after 4 days up to 100 mM of cyanide is degraded in 2 h [43].

A high level of specific activity was observed in *Gloeocercospora sorghi* and *Helminthosporium turcicum* [42]. It was observed that *Gloeocercospora sorghi* fungus when immobilized after induction of cyanide hydratase with exposure to 0.5 mM KCN at 10–12 h before harvesting [45]. K_m for cyanide doubled to 40–70 mM and also enzyme stability was dramatically increased to 30 days. Addition of 1 mM glucose in the feed stocks gave a further increase to 40 days.

Also in non-phytopathogenic strain of *Fusariums* [46] it was absorbed that the immobilization increase the enzyme activity and microorganism can tanning this enzyme can tolerate higher cyanide concentrations up to 200–10,000 mg/L, as a consequence of the reasonably high K_m value of 50 mM. However there is one limitation that it does not degrade metal cyanide in complexes wastes [47].

Cyanide hydratase enzyme was also purified from other fungi *Fusarium lateritium*. The enzyme obtained has subunit molecular mass of 43 kDa, while the native protein appeared to form aggregates of up to 1217 kDa and its maximal activity was observed at 12–18 h after exposure to hydrogen cyanide. Another strain of *Fusarium oxysporum* CCMI 876, isolated from a high-cyanide containing industrial effluent, was capable of limited growth in the presence of 10 mM cyanide concentration and survived at maximum 20 mM cyanide. This enzyme is not substrate specific, i.e. it can be used for broad range of cyano compounds and nitriles as substrates [48].

The synthesis of this enzyme in *F. oxysporum* CCMI 876 is dependent on the energy provided by an alternative cyanide resistant respiration. This ensures the organism survival in presence of cyanide compound.

Cyanides biodegradation at alkaline pH are scarce but still there are several cyanide-tolerant microorganisms selected from alkaline wastes and soils contaminated with cyanide. Among them, a fungus identified as *Fusarium solani IHEM 8026* shows a good potential for

Table 1
Characterstics of cyanide hydratase from different microorganisms.

Microorganism	Properties of nitrile hydratases						References
	Optimum			Utilise CN as nitrogen source	K_m	Substrate specificity	
	pH	Concentration	T (°C)				
Sternphyliumloti	7.0–9.0	2 mM	25	Nitrogen	15–20 mM	NaCN	[36]
Sternphyliumloti (at immobilized phase)	6.5–7.5	100 mM	22–24	Nitrogen	43 mM	NaCN	[37]
Fusarium solani IHEM 8026	9–10.7	0.8 mM	30	Nitrogen	–	–	[38]
Fusarium oxysporum	4–7	20 mM	25	Nitrogen	50 mM	KCN	[40]
Gloeocercospora sorghi	5.3–5.7		35	Nitrogen	40–70 mM	KCN	[42]

cyanide biodegradation under alkaline conditions (pH 9.2–10.7). Fungal metabolism seems to proceed by a two-step hydrolytic mechanism: (i) the first reaction involves the conversion of cyanide to formamide by a cyanide-hydrolyzing enzyme, cyanide hydratase and (ii) the second reaction consists of the conversion of formamide to formate, which is associated with fungal growth. No growth occurred during the first step of cyanide degradation. The presence of organic nutrients in the medium has a major influence on the occurrence of the second step [49]. The microorganisms containing this enzyme can be used to degrade metal cyanide and can tolerate high concentration cyanide and some other examples are mentioned in Table 1.

2.1.2. Nitrile hydratases

Nitrile hydratases can effectively degrade a wide variety of structurally diverse nitriles, primarily aliphatic nitriles. These enzymes are iron or cobalt can tanning enzymes that catalyze the hydration of various cyanides to their corresponding amides as shown in following reaction:

$$R–C{\equiv}N + H_2O \rightarrow R–C(O)NH_2 \quad (2)$$

Metal cofactor

Nitrile hydratase is one of the rare enzymes that use cobalt directly as a cofactor. The mechanism by which the cobalt is transported to nitrile hydratase without causing toxicity is unclear, although a cobalt permease has been identified, which transports cobalt across the cell membrane [50].Metabolic pathway

In some bacteria and fungi, nitrile hydratase and amidase are present together and are responsible for the sequential metabolism of nitriles. These microbes are capable of utilizing aliphatic nitriles as the sole source of nitrogen and carbon. First nitrile hyratase converts cyanides to amide intermediate, which is then converted to corresponding acid and ammonia by amidase.Industrial applications

Nitrile hydratase have been efficiently used for the industrial production of acrylamide from acrylonitrile and for removal of nitriles from wastewater.Structure

It was observed that photosensitive nitrile hydratase intrinsically possess nitric oxide (NO) bound to the iron centre and its photo dissociation activates the enzyme.

Nitrile hydratase are composed of two types of subunits, α and β, which are not related in amino acid sequence. Nitrile hydratase exist as αβ dimers or $\alpha_2\beta_2$ tetramers and bind one metal atom per αβ unit. The 3D structures of a number of nitrile hydratase have been determined. The α subunit consists of a long extended N-terminal "arm", containing two α-helices, and a C-terminal domain with an unusual four-layered structure (α–β–β–α). The β subunit consists of a long N-terminal loop that wraps around the α subunit; a helical domain that packs with N-terminal domain of the α subunit; and a C-terminal domain consisting of a β-roll and one short helix [51].Enzymatic mechanism

The active site, contains non-heme iron or non-corrin cobalt, is buried in the protein core at the interface of two domains, alpha and beta. The metal ion is octahedrally coordinated, with the protein ligands at the five vertices of an octahedron. The sixth position, accessible to the active site cleft, is occupied either by NO or by hydroxide. Hydrogen bonds between betaArg56 and alphaCys114 sulfenic acid are important to maintain the enzymatic activity. The enzyme may be inactivated by endogenous nitric oxide (NO) and activated by absorption of photons of wavelength of 630 nm [52].

In *Rhodococcus rhodochrous* M8 the enzyme responsible for cyanides utilization are nitrile hydratase and amidase [53]. The activity of nitrile hydratase mainly depended on carbon and nitrogen supply to cells. The activity of nitrile hydratase was high in the presence of glucose and ammonium at medium concentrations and decreased at concentrations of glucose more than 0.3%. Saturated unsubstituted aliphatic nitriles and amides were found to be a good source of nitrogen and carbon. However, the presence of nitriles and amides in the medium was not absolutely necessary for the expression of the activity of nitrile hydratase isolated from the *Rhodococcus sp*. The optimal temperature and pH for the catalytic reaction of the enzyme was 25 °C and pH 7.6. The enzyme activity of the purified nitrile hydratase was strongly inhibited by some oxidizing agents and heavy metals [54].

The nitrile hydratase, isolated by *Pseudonocardia thermophila* show high activity compared to other microorganism, known for the production of nitrile hydratase, i.e. *Corynebacterium*, *Pseudomonas*, *Rhodococcus rhodoclous*, *Rhizobium* and *Klebsiella* [55].

Two other new bacterial strains, *Pseudomonas marginales* MA32 and *Pseudomonas putida* MA113, containing nitrile hydratases resistant to cyanide was 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 quarternary alpha-carbon atoms. *Pseudomonas marginales* MA32 and *Pseudomonas putida* MA113 were used as a whole cell biocatalyst for the hydration of acetone cyanohydrin to a hydroxyisobutyramide, which is a precursor of methacrylamide. After optimization of the process conditions a maximum amide concentration of more than 1.6 M could be reached within 5 h with 5 g/L biocatalyst (referred to cell dry weight). This microorganism requires some concentration of cyanide for induction of enzyme.

Another bacterium *Pseudomonas putida* contains this enzyme and is capable of utilizing high concentrations of acetonitrile as the sole source of carbon and nitrogen was isolated from soil. This bacterium utilizes butyronitrile, glutaronitrile, isobutyronitrile, methacrylonitrile, propionitrile, succinonitrile, valeronitrile, and some of their corresponding amides, such as acetamide, butyramide, isobutyramide, methacrylamide, propionamide, and succinamide as growth substrates. Acetonitrile-grown cells oxidized acetonitrile with a K_m of 40.61 mM. It was observed that 66% of carbon of acetonitrile was released as CO_2 and 14% was associated with the biomass. This enzyme is intracellular, inducible and hydrolyzed a wide range of substrates [55].

Klebsiella pneumoniae use aliphatic nitriles as the sole source of nitrogen was adapted to benzonitrile as the sole source of carbon and nitrogen. Gas chromatographic and mass spectral analyses of culture filtrates indicated that *K. pneumoniae* metabolized 8.4 mM

Table 2
Characteristics of nitrile hydratases from different microorganisms.

Microorganisms	Molecular mass (kDa)		pH optima	Temp. optima	Substrate	Reference
	Native	No Of subunit and its Mwt (kDa)				
Myrothecium	170	6 (27.7)	7.7	55 °C	Cyanamide	[56]
Bacillus sp.	78	4 (α: 28) (β: 29)	7.0	50 °C	Alkylnitrile	[57]
Corynebacterium sp. C5	61.5	2	8.5	55 °C	Aliphatic dinitrile	[58]
Brevibacterium imperalis CBS489–74	–	–	7.5	–	Acrylonitrile	[59]
Pseudomonas putida	54	α: 23	9.2–11.4	30 °C	Aliphatic nitrile	[60]
NRRL-18668	95	(β: 25)				
Pseudomonas chlororaphis B 23	100	4 (α: 25) (β: 25)	7.5	20 °C	Aliphatic nitrile	[61]
Arthrobacter sp. J1	420	2 (24)	7.2	35 °C	Aliphatic nitriles	[62]
Pseudonocardia thermophila JCM3095	–	2 (α: 29) (β: 25)	9.0	55 °C	Acrylonitrile Acetonitrile Benzonitrile	[63,65]
Rhodococcus sp. N 774	70	2 (α: 28.5) (β: 29)	8.5	35 °C	Aliphatic nitriles	[64,66]

benzonitrile to 4.0 mM benzoic acid and 2.7 mM ammonia. In addition, butyronitrile was metabolized to butyramide and ammonia. The isolate also degraded mixtures of benzonitrile and aliphatic nitriles. Cell extracts contained nitrile hydratase and amidase activities. *K. pneumoniae* appears to be a promising microbe for the bioremediation of sites contaminated with aliphatic and aromatic nitriles. Degradation of aliphatic nitriles in the presence of benzonitrile proceeded without glucose supplementation. Nitrilases of *Fusarium* sp., *Rhodococcus* sp., and *Arthrobacter* sp. are responsible for the metabolism of benzonitrile. None of these nitrilases catabolizes aromatic amides and aliphatic nitriles. This can be an alternate degradative pathway that involves a nitrile hydratase and an amidase responsible for the metabolism of aliphatic as well as aromatic nitriles [56].

Arthrobacter cell extract benzonitrilases, grown on benzonitrile as a sole carbon and nitrogen source. These enzymes hydrolyzed benzonitrile to benzoic acid and ammonia without forming benzamide as an intermediate. The molecular weight of benzonitrilases was found to be 30,000. The optimum temperature and pH were 40 °C and 8.5, respectively. The K_m value for benzonitrilases was 6.7 mM, respectively [57]. Table 2 shows some more detail about the enzyme nitrile hydratase.

2.1.3. Cyanidase (cyanide dihydratases)

Cyanide dihydratases, comprises a group of bacterial enzymes that include those from *Alcaligenes xylosoxidans* subsp. *denitrificans* DF3, *Bacillus pumilus* C1, and *Pseudomonasstutzeri* AK61 [67–69]. Cyanide dihydratases readily convert cyanide to relatively nontoxic formate directly as shown below.

$$RCN + H_2O \leftrightarrow RCOOH + NH_3 \quad (3)$$

It does not require any cofactor, and do not have any known ancillary substrate. Electron micrographs of the cyanide dihydratase at pH 8.0 showed a variety of shapes. It consists of a tightly packed spiral that sometimes associated end to end to form rods of up to 50 nm in length. The pitch of the spiral is approximately 8 nm, and the particle has an overall length of 18.5 nm and an outer diameter of 9.5 nm [69].

Alcaligenes xylosoxidans and *Bacillus pumilus* C1 were also shown to degrade cyanide by the same pathway. A cyanide dihydratase enzyme from *Bacilus pumius* Cl consisted of three polypeptides of 45.6, 44.6, and 41.2 kDa; molecular mass was 417 kDa. Cyanide was rapidly degraded to formate and ammonia. Enzyme activity was optimal at 37 °C and pH 7.8–8.0. Activity was enhanced by the presence of Sc^{3+}, Cr^{+}, Fe^{3+}, and Th^{3+} ions. Enhancement was independent of metal ion concentration. K_m is 2.56 ± 0.48 mM for cyanide and a V_m of 88.03 + 4.67 mmol of cyanide per min/(mg liter). The K_m for cyanide degradation, increased approximately twofold in the presence of 10 mM Cr^{+} to 5.28 + 0.38 mM for cyanide, and the V_{max}, increased to 197.11 ± 8.51 mmol of cyanide per min/(mg liter) [70]. The gene encoding cyanide dihydratase in *B. pumilus* C1 has been cloned and sequenced. The putative amino acid sequence is about 80% identical to that of the cyanide dihydratase of *P. stutzeri* AK61 and it is not significantly more related to cyanide hydratases than it is to other nitrilases, thereby supporting its classification as a cyanide specific nitrilase. But, induction of the cyanide degrading activity of *B. pumilus* C1 was inhibited by addition more cyanide into the medium. The cyanide degrading activity of this enzyme was observed in only late exponential or early-stationary-phase cells culture.

This enzyme is not inducible enzyme as observed in *P. stutzeri* AK61. The degradation of cyanide by the cells cultured without cyanide was similar to that of the cells cultured with cyanide. *Pseudomonas stutzeri* whole cells were able to degrade cyanide rapidly in a 1 mM solution containing no organic substances, and produced ammonia as a product. The cyanide degrading enzyme was purified approximately 49-fold from a cell extract of *P. stutzeri* AK61. The enzyme had a K_m of 1.7 mM for cyanide. The activity of the enzyme was optimal at 30 °C and pH range of 6–10. The activity was strongly inhibited by addition of Hg at a concentration of 0.1 mM [71]. *Pseudomonas fluorescens* NCIMB11764 is a unique strain that degrades cyanide by three pathways depending on oxygen availability and cyanide initial concentration. Table 3 shows some more microbes containing this enzyme.

2.1.4. Nitrilase

Nitrilase enzymes catalyze the hydrolysis of nitriles to carboxylic acids and ammonia, without the formation of "free" amide intermediates. Nitrilases are involved in biosynthesis of proteins and there post translational modifications in plants, animals, fungi

Table 3
Characteristics of cyanidase from different microorganisms.

Microorganism	Properties of cyanidase					References
	Optimum		Utilise CN as source	K_m	Substrate	
	pH	T (°C)				
Bacillus pumilus C1	7.8–8.0	37	Nitrogen	2.56 mm	NaCN	[68]
P. stutzeri AK61	6–10	30	Nitrogen	40–70 mm	KCN	[69]

Table 4
Characteristics of nitrilase from different microorganisms.

Microorganism	Molecular mass (kDa)		pH optima	Temp. optima	Substrate	References
	Native	No Of subunit and its Mwt (kDa)				
Nocardia sp.	560	45	8	30 °C	Aromatic nitrile	[79]
Rhodococcus rhodochrous J1	78	2 (41.5)	7.5	45 °C	Benzonitrile, 2-furanocarbonitrile	[80]
R. rhodochrous K22	650	15–16 (41)	5.5	50 °C	Crotononitrile, acrylonitrile	[81]
Rhodococcus rhodochrous PA-34	45	45	7.5	35 °C	Benzonitrile 100%, acrylonitrile 22AE4, crotononitrile 20AE9%	[82]
Arthrobacter sp. J1	30	1	8.5	40 °C	Aromatic nitriles	[83]
Alcaligenes faecalis JM3	260	6 (44)	7.5	45 °C	Mandelonitrile, p-aminobenzyl cyanide	[84]
Acinetobacter sp. AK226	580		8.0	50 °C	Aliphatic and aromatic nitriles	[85]
Pseudomonas thermophila JCM3095	41	2 (40,38)	9.0	55 °C	Acrylonitrile, acetonitrile, benzonitrile	[86]
Klebsiella ozaenae	74	2 (37)	9.2	35 °C	Bromoxynil	[87]
Fusarium solani	620	8 (76)	7.8–9.1		Aromatic nitriles	[88]
Fusarium oxysporum	550	Oligomeric (27)	6.0–11.0	40 °C	Aliphatic and aromatic	[89,90]

and certain prokaryotes. Nitrilases can also be used as catalysts in preparative organic chemistry. Among others, nitrilases has been used for the resolution of racemic mixtures. Nitrilase should not be confused with nitrile hydratase which hydrolyses nitriles to amides. Nitrile hydratases are almost invariably co-expressed with an amidase, which converts the amide to the carboxylic acid, consequently it can sometimes be difficult to distinguish nitrilase activity from nitrile hydratase plus amidase activity.

2.1.4.1. Enzyme structure. Nitrilases are generally inducible enzymes composed of one or two types of subunits of different size and number. *Nocardia* sp. nitrilase was reported to be induced by enzonitrile [72]. Acetonitrile has been used as an inducer for the formation of nitrilase in *Fusarium oxysporum*. The use of isobutyronitrile or isovaleronitrile enhanced the production of benzonitrilase in *R. rhodococcus* J1 [73].

It has been observed in most cases that different subunits of nitrilase self-associate to convert the enzyme to the active form. This association is accelerated by temperature and enzyme concentration. It has been reported that a nitrilase from *R. rhodochrous* cells is converted to the active form by subunit association when incubated with substrate or in the presence of higher concentration of enzyme, salt or organic solvent [74]. The hydrophobic effect resulting from the presence of the above-mentioned conditions might change the conformation of the enzyme, exposing hydrophobic sites thereby enabling subunit assembly and enzyme activation. Nitrilases, unlike nitrile hydratases, do not show the presence of any metal cofactor or prosthetic group. They are reported to have catalytically essential cysteine residues at, or near, the active site [75].

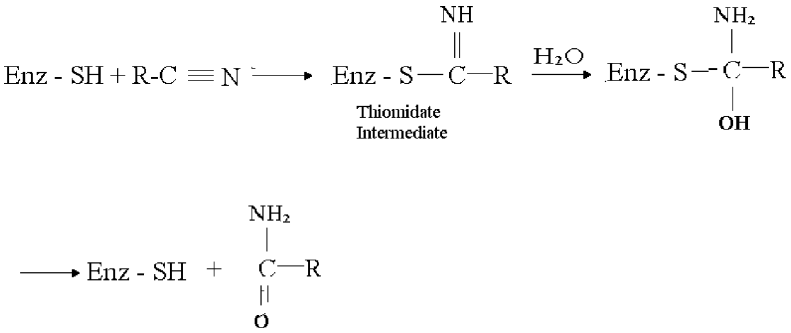
2.1.4.2. Mechanism. A possible mechanism for the nitrilase-catalyzed reaction indicates a nucleophilic attack by a thiol group on the carbon atom of the nitrile. Subsequent steps involve attack by two water molecules and protonation of the nitrogen atom, which is lost as ammonia. In some cases the tetrahedral intermediate formed which break down anomalously to produce amide instead of the normal acid product [76,77].

Nitrilase from *Rhodococcus*, neither reacts with nor produces a significant amount of the corresponding acid amide. Lack of acid amide production is due to the covalent mechanism: the enzyme does not add water to a nitrile, but rather forms an enzyme-linked thioimidate intermediate [78] as thiocyanate.

Klebsiella oxytoca, isolated from cyanide-containing industrial wastewater, was shown to be able to biodegrade cyanide to non-toxic endproducts ammonia and methane. The nrilase activity is inactivated (an oxygen-labial enzyme) by the oxygen exposure [79]. The cyanide consumption by resting cells of *Klebsiella oxytoca* was induced by the pretreatment of these cells with cyanide. But, the cyanide degrading capability of resting cells pretreated with ammonia was inhibited [80]. The free suspension systems reveal that the cell viability was highly affected by initial cyanide concentration and pH. It was observed that it degrade cyanide at pH 7 [81]. It could tolerate a higher level of KCN concentration. In the batch experiments, the maximum KCN removal efficiencies using alginate and cellulose triacetate immobilized beads were 0.108 and 0.101 mM h^{-1} at pH 7, respectively. The maximum KCN removal rates using alginate and cellulose triacetate immobilized beads in continuous-column system were 0.224 and 0.192 mM h^{-1} with initial KCN concentration of 3 mM, respectively. This indicates that immobilized system can support a higher biomass concentration [91,92]. Also some other microorganism containing this enzyme and there corresponding optimum parameter for cyanide degradation are mentioned in Table 4.

2.1.5. Carbonyl pathway (thiocyanate hydrolase)

A thiocyanate hydrolase catalyzes thiocyanate degradation and convert thiocyanate to sulfata and its corresponding amide is shown in reaction (4):



(4)

This enzyme belongs to the family of hydrolases, those acting on carbon–nitrogen bonds other than peptide bonds, specifically in thiocyanate. The systematic name of this enzyme class is thiocyanate aminohydrolase.

The enzyme had a molecular mass of 126 kDa and was composed of three different subunits: alpha (19 kDa), beta (23 kDa), and gamma (32 kDa). Thiocyanate hydrolase of *Thiobacillus thioparus THI115* is a cobalt(III)-containing enzyme catalyzing the degradation of thiocyanate to carbonyl sulfide and ammonia. Thiocyanate hydrolase in both the apo- and native forms have three alphabetagamma hetero-dodecameric structure at a resolution of 2.0 A. All three alphabetagamma hetero-dodecamerics are structurally equivalent. One alphabetagamma hetero-dodecameric was composed of the core domain and the betaN domain, core domain is located at the center of the molecule and linked the heterotrimers with novel quaternary interfaces. In both the apo- and native thiocyanate hydrolase, the core domain is structurally conserved between both of iron- and cobalt-types of nitrilehydratase.

Thiocyanate hydrolase have significant homologies to bacterial nitrile hydratases and convert nitrile to the corresponding amide, which is further hydrolyzed by amidase to form acid and ammonia. The two enzymes were homologous over regions corresponding to almost the entire coding regions of the genes: the beta and alpha subunits of thiocyanate hydrolase were homologous to the amino- and carboxyl-terminal halves of the beta subunit of nitrile hydratase, and the gamma subunit of thiocyanate hydrolase was homologous to the alpha subunit of nitrile hydratase [93]. Inspite of the similarities, the size as well as the electrostatic properties of the substrate-binding pocket is totally different from nitrile hydratase. The charge distribution and the substrate accessibility, explains the differences in the substrate preference between thiocyanate hydrolase and nitrile hydratase [94].

The enzyme exhibited optimal activities at pH 7.5–8.0 and at temperatures ranging from 30 to 40 °C. The K_m value for thiocyanate degradation was observed to be approximately 11 mM. Immunoblot analysis with polyclonal antibodies against the purified enzyme suggested that it was induced in *T. thioparus* cells when the cells were grown with thiocyanate [95].

2.2. Oxidative pathway

A second route of cyanide conversion involves oxygenolytic conversion to carbon dioxide and ammonia. This pathway requires NADPH to catalyze this degradation pathway. Microorganisms that exhibit this pathway also require an extra carbon source with cyanide.

There are two types of oxidative pathways involving three different enzymes:

(i) Cyanide monooxygenase and cyanase.
(ii) Cyanide dioxygenase.

2.2.1. Cyanide dioxygenase

The growth on cyanide requires that cyanide should be enzymatic ally converted to ammonia, which is then assimilated by well-established pathways [96]. In this case, cyanide decomposition initiates oxygenatively by an enzyme described as a cyanide dioxygenase [97]:

$$HCN + O_2 + 2H^+ + NADPH \rightarrow NADH + CO_2 + NH_3 \quad (5)$$

2.2.1.1. Mechanism. It was shown that in first step a single atom of molecular oxygen is incorporated during substrate conversion by crude cell extracts. The cyanide oxygenase functions as a cyanide monooxygenase. Then second atom of oxygen derived from water is incorporated. It is the way this mechanism works to give unidentified monooxygenated intermediate (X-OH), which eventually rises NH_3 and CO_2 following hydrolysis [98].

$$\begin{aligned} HCN + O_2 + NADPH &\rightarrow NADH + H_3[X\text{–}OH] \\ &\rightarrow H_2O + NAD + +[X\text{–}OH] + H_2O \\ &\rightarrow 3NH_3 + CO_2 \end{aligned} \quad (6)$$

It was usually observed that cyanide-grown cells contain elevated levels of both cyanide oxygenase and formate dehydrogenase (FDH) [99]. It was recently reported that cyanide oxygenase is a pterin-dependent hydroxylase that means this enzyme requires pterin as cofactor [100].

The cyanide degradation in three white rot fungi, *Trametes Versicolor* ATCC 200801, *Phanerochaete chrysosporium* ME 496 and *Pleurotus sajor-caju*, were achieved by an oxidative reaction that results in the end products of ammonia and CO_2. In *T. versicolor* this reaction was the most effective with 0.35 g dry cell/100 mL degrading 2 mM KCN (130 mg/L) over 42 h, at 300 °C, pH 10.5 with stirring at 150 rpm [101].

Pseudomonas fluorescens NCIMB11764 is one such organism that grows at concentrations of CN equivalent to 300 ppm resulting in oxidatively conversion of cyanide to carbon dioxide and ammonia, where ammonia satisfying the nitrogen requirement. Substrate attack is initiated by cyanide oxygenase (CNO), which has been found to have properties of a pterin-dependent hydroxylase, catalyzes the conversion of cyanide supplied at micromolar concentrations (10–50 μM) to formate and ammonia [102]. The specific activity of the partially purified enzyme was approximately 500 mU/mg of protein. Substrate conversion was accompanied by the consumption of 1 and 2 molar equivalents of molecular oxygen and NADH, respectively. When coupled with formate dehydrogenase, the complete enzymatic system for cyanide oxidation to carbon dioxide and ammonia was reconstituted and displayed an overall reaction stoichiometry of 1:1:1 for cyanide, O_2, and NADH consumed. Cyanide was also attacked by CNO at a higher concentration (1 mM), but in this case formamide accumulated as the major reaction product (formamide/formate ratio, 0.6:0.3) and was not further degraded [103].

2.2.2. Cyanate pathway (cyanase)

Cyanase is an inducible enzyme present in various microorganisms. It catalyzes cyanide conversion to cyanate, which in turn convert to ammonia and bicarbonate as shown in above equation. Cyanide is converted to cyanate by cyanide monooxygenase as shown below in reaction.

$$\begin{aligned} &HCN^- + O_2 + 2\,H^+ + NADPH \rightarrow CNO^- + NADP + 2H_2\,O \\ &\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad \downarrow \\ &CNO^- + 3H^+ + HCO_3^- \rightarrow NH_4^+ + 2CO_2 \end{aligned} \quad (7)$$

The enzyme is composed of 8–10 identical subunits of molecular weight of 17,008 and which has a ά-helix and sheet structure [104].

Pseudomonas pseudoalcaligenes bacterial strain contains this enzyme, and use cyanide, as the sole nitrogen source under alkaline conditions. Cyanide consumption is an assimilative process. It was observed that (i) the bacterial growth was proportional to cyanide degradation and (ii) the bacterium stoichiometrically converted cyanide into ammonium in the presence of L-methionine-D,L-sulfoximine, and a glutamine synthetase inhibitor. The bacterium can be used in cyanide biodegradation and bioremediation processes because it grows at pH 9.5 with 2 mM cyanide, and it is even able to survive at pH 11.5 in the presence of 30 mM cyanide, makes it a good candidate for the biological treatment of cyanide contaminated residues. Both acetate and D,L-malate had

been used as suitable carbon sources for cyanotrophic growth, but no growth was detected in media with cyanide as the sole carbon source. This bacterium also uses other nitrogen sources, namely ammonium, nitrate, cyanate, cyanoacetamide, nitroferricyanide (nitroprusside), and a variety of cyanide–metal complexes. Cyanide and ammonium was assimilated simultaneously. Cyanase activity was induced during growth with cyanide or cyanate, but not with ammonium or nitrate as the nitrogen source [105].

In addition, this strain uses metal-cyano complexes from the jewellery industry. Cyanate, β-cyanoalanine, cyanacetamide and nitroferricyanide were used as nitrogen sources. Cyanide biodegradation involves three processes: a cyanide assimilation pathway, a mechanism for cyanide tolerance and the production of siderophores, which are required for breaking down cyanide complexes and chelating iron from the media [106].

Pseudomonas putida utilizes cyanide as the sole source of carbon and nitrogen. The end products of biodegradation of cyanide were identified as ammonia (NH_3) and carbon dioxide (CO_2). *P. putida* degraded cyanides, cyanates and thiocyanates to NH_3 and CO_2. The substrate-dependent kinetics indicated that the K_m and V_{max} values of *P. putida* for the substrate, NaCN were 14 mM and 29 nmol of oxygen consumed in mg protein^{-1} min^{-1}, respectively [107].

2.3. Reductive pathway

The reductive pathways of degradation of cyanide are generally considered to occur under anaerobic conditions. This pathway is mediated by an enzyme nitrogenase. The enzyme utilizes HCN and produce methane and ammonia as end product.

2.3.1. Nitrogenase

Nitrogenase is the enzyme used by some organisms to fix atmospheric nitrogen gas (N_2) according to the reaction (8).

The reaction that this enzyme performs is:

$$N_2 + 8H^+ + 8e^- + 16ATP \rightarrow 2NH_3 + H_2 + 16ADP + 16Pi \quad (8)$$

Nitrogenase breaks the triple bond by getting electron donors for each of the three bonds, and then bonds the nitrogen to hydrogen atoms. The process is complex because each bond is broken individually. Nitrogenase requires both the Mo Fe protein and ATP, which supplies the energy. Nitrogenase bonds each atom of nitrogen to three atoms of hydrogen to form ammonia, and then ammonia is bonded to glutamate and becomes glutamine [108].

The enzyme therefore requires a great deal of chemical energy, released from the hydrolysis of ATP, and reducing agents, such as dithionite *in vitro* or ferredoxin *in vivo*. The enzyme is composed of the heterotetrameric MoFe protein that is transiently associated with the homodimeric Fe protein. Nitrogenase is supplied reducing power when it associates with the reduced, nucleotide-bound homodimeric Fe protein. The heterocomplex undergoes cycles of association and disassociation to transfer one electron, which is the limiting step in the process. ATP supplies the reducing power [109].

Nitrogenase is able to bind acetylene and carbon monoxide, which are non-competitive substrates and inhibitors, respectively. Dinitrogen, however, is a competitive substrate for acetylene. This is because binding of dinitrogen prevents acetylene binding, and acetylene requires only one electron to be reduced, and it does not inhibit.

All nitrogenases have an iron- and sulfur-containing cofactor that includes heterometal atom in the active site (e.g. FeMoCo). In most, this hetero metal is molybdenum, though in some species it is replaced by vanadium or iron.

Due to the oxidiative properties of oxygen, most nitrogenases are irreversibly inhibited by dioxygen, which degradatively oxidizes the Fe–S cofactors. This requires mechanisms for nitrogen fixers to avoid oxygen *in vivo*. Despite this problem, many use oxygen as a terminal electron acceptor for respiration. One known exception, a recently discovered nitrogenase of *Streptomyces thermoautotrophicus*, is unaffected by the presence of oxygen. The Azotobacteraceae are unique in their ability to employ an oxygen-labile nitrogenase under aerobic conditions [110].

Nitrogenase is also inhibited by ammonium ion, and amino acids in higher concentration. It was founded that nitrogenase activity in the root-associated N_2-fixing bacterium *Herbaspirillum seropedicae*, *Azospirillum* spp. and *Rhodospirillum rubrum* was inhibited by the production of ammonium, a product of the nitrogenase activity. However, the inhibition of nitrogenase activity was only partial even with concentrations of ammonium chloride as high as 20 mM. Amides such as glutamine and asparagine partially inhibited nitrogenase activity, but glutamate did not [111].

2.4. 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.

2.4.1. Rhodanese

In enzymology, a thiosulfate sulfurtransferase is an enzyme that catalyzes the following chemical reaction (9):

$$\text{Thiosulfate} + \text{cyanide} \rightarrow \text{sulfite} + \text{thiocyanate} \quad (9)$$

This enzyme belongs to the family of transferases, specifically the sulfurtransferases, which transfer sulfur-containing groups. The systematic name of this enzyme class is thiosulfate: cyanide sulfurtransferase. Other names in common use include thiosulfate cyanide transsulfurase, thiosulfate thiotransferase, rhodanese, and rhodanase.Thiosulfate sulfurtransferase (rhodanese), also known as TST, is a human gene.

Rhodaneses are highly conserved and widespread enzymes, currently regarded as one of the mechanism evolved for cyanide detoxification. In vitro rhodaneses catalyze the irreversible transfer of a sulfur atom from a suitable donor (i.e. thiosulfate) to cyanide, leading to formation of less toxic sulfite and thiocyanate. The enzyme activity is modulated by phosphate ions and divalent anions that found to interact with the active site [112].

Rhodaneses had been also identified in a variety of bacterial species including *Escherichia coli* [113], *Azotobacter vinelandii* [114] and several species of *Thiobacillus* but in prokaryotes the physiological function of the enzyme had not been known. Constitutive rhodanese activity had been also found in the cyanide producing bacterium *Pseudomonas aeruginosa* [115].

The rhodanese enzyme is also present in *Trichoderma*. The kinetics of rhodanese in certain *Fusarium* strains, had been reported to degrade cyanides, was also analyzed and compared to the enzyme from the *Trichoderma*. The rhodanese enzyme in all the *Trichoderma* strains demonstrated a broad pH optimum generally in the range from 8.5 to 11.5, along with a wide temperature optimum of 35–55 °C. The K_m of CN degradation and V_{max} values ranged from 7 to 16 mM and from 0.069 to 0.093 mole ml^{-1} min^{-1} mg protein^{-1}, respectively, between the strains of *Trichoderma*. Although the enzyme rhodanese is found ubiquitously in nature and a number of bacterial and mammalian sources exist, there are very few reports on the characterization of rhodanese in fungi. Rhodanese catalyses the conversion of cyanide into a relatively less toxic product, thiocyanate that assimilates in the cytoplasm of microbe, in presence of thiosulphate. Alternatively, cyanide in the presence of the other enzyme, cyanide hydratase, is hydrolyzed to form formamide

Table 5
Characteristics of rhodanese from different microorganisms.

Microorganism	Properties of rhodanese				References
	Optimum		Utilise CN as source	K_m	
	pH	T (°C)			
Ferrobacillus ferroxidans	9	25 °C	Nitrogen	10 mM	[112]
Thermobacillus denitrificans	9	–	Nitrogen	–	[113]
E. coli	–	–	Nitrogen	17 mM	[114]
Desulforomaculum nitrificans	8.6	–	–	120 mM	[115]

that eventually gets converted to carbon dioxide and ammonia. The rhodanese enzyme was found to be predominantly presented extracellular in all the fungal strains [116]. Also some other examples are mentioned in Table 5.

2.4.2. Mercaptopyruvate sulfurtransferase

A 3-mercaptopyruvate sulfurtransferase is an enzyme that catalyzes the chemical reactions (10) and (11). This enzyme belongs to the family of transferases, specifically the sulfurtransferases, which transfer sulfur-containing groups. The systematic name of this enzyme class is 3-mercaptopyruvate: cyanide sulfurtransferase. This enzyme is also called beta-mercaptopyruvate sulfurtransferase. This enzyme participates in cysteine metabolism.

$$HSCH_2COCOO^- + E \leftrightarrow CH_2COCOO^- + ES \quad (step1) \tag{10}$$

$$ES + CN^- \leftrightarrow E + SCN^- \quad (step2) \tag{11}$$

3-Mercaptopyruvate is converted to thiosulfate by the two-step reaction as mentioned above. In step 1 (*ES*) the enzyme-sulfur intermediate is formed and in step 2 intermediate reacts with cyanide to *E* (mercaptopyruvate sulfurtransferase) and thiocyanide.

Mercaptopyruvate sulfurtransferase and rhodanese are widely distributed in living organisms. Mercaptopyruvate sulfurtransferase and rhodanese catalyze the formation of pyruvate and thiocyanate from mercaptopyruvate [117,118].

It was first identified and characterized in a parasite trypanosomatid *Leishmania major* [119]. Expression of this enzyme is increased during oxidative stress conditions. This enzyme is also present in mammals [120].

Leishmania major 3-mercaptopyruvate sulfurtransferase is a crescent-shaped molecule comprising N-terminal, central domains and C-terminal domain. The N-terminal and central domains are similar to the thiosulfate sulfurtransferase rhodanese and the active site containing a persulfurated catalytic cysteine (Cys-253) and an inhibitory sulfite are coordinated by Arg-74 and Arg-185. A serine protease-like triad, comprising Asp-61, His-75, and Ser-255, is near Cys-253 and represents a conserved feature that distinguishes 3-mercaptopyruvate sulfurtransferases from thiosulfate sulfurtransferases. The *L. major* 3-mercaptopyruvate sulfurtransferase is unusual with an 80-amino acid C-terminal domain, like *cis*/*trans*-isomerase protein. This domain involves in protein folding and sulfurtransferase-protein interactions [121].

Sulfurtransferases involves in the formation and maintenance of iron–sulfur clusters in protein [122,123] detoxification of cyanide [123], biosynthesis of the molybdopterin cofactor of xanthine oxidase [124], selenium metabolism [125,126] and thiamine and 4-thiouridine biosynthesis. The expression of specific sulfurtransferases is up regulated under conditions of peroxide or hypo-sulfur stress, osmotic shock, and phage infection [127] suggesting that such enzyme activity is protective of the cell and/or involved in repair processes.

2.5. Synthases pathways

This pathway is also a cyanide assimilation pathway. It involves the synthesis of amino acid, β-cyanoalanine and γ-cyano-α-aminobutyric acid by using amino acid residues as precursor that react with cyanide compounds.

2.5.1. β-Cyanoalanine synthase

β-Cyanoalanine synthase is believed to play the prominent role in the removal of endogenous cyanide and is produced at highly active growth period in the microbe. The enzyme catalyzes the following chemical reaction (12) [128]:

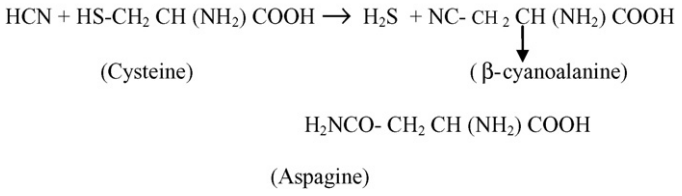
$$\text{L-cysteine} + \text{hydrogencyanide} \rightarrow \text{L-}\beta\text{-cyanoalanine} + \text{hydrogensulfide} \tag{12}$$

The two substrates of this enzyme are L-cysteine and hydrogen cyanide, whereas its two products are L-β-cyanoalanine and hydrogen-sulfide [129].

This enzyme belongs to the family of lyases, specifically the class of carbon–sulfur lyases. The systematic name of this enzyme class is L-cysteine hydrogen-sulfide-lyase. Other names in common use include beta-cyanoalanine synthase, beta-cyanoalanine synthetase, beta-cyano-L-alanine synthase, and L-cysteine hydrogen-sulfide-lyase [129].

The enzyme has a molecular weight of 52,000 and contains 1 mole of pyridoxal–phosphate per mole of protein; its isoelectric point is situated at pH 4.7. L-Cysteine is the natural primary (amino acid) substrate and cyanide as co substrates for cyanoalanine synthase [130].

β-Cyanoalanine synthase is synthesized in plants from serine and cyanide [131]. *E. coli* is able to convert cyanide to β-cyanoalanine [132]. Cyanide produced by *C. violaceum* can first convert cyanide to β-cyanoalanine than it into asparagines as shown in reaction (13) below [133].



(13)

β-Cyanoalanine synthase is induced by various amino acids as cysteine, serine, asparagines, etc.

Bacillus magaterium is a organism that grow by converting cyanide to β-cyanoalanine and then to asparagines [134]. β-Cyanoalanine synthesis has been found in more species and may be potentially more useful. Therefore this pathway for metabolism of the resulting nitriles must be considered for use in the detoxication of cyanide. *B. megaterium*, *E. coli*, and *C. violaceum* convert β-cyanoalanine via hydrolysis into asparagine or aspartate. Therefore

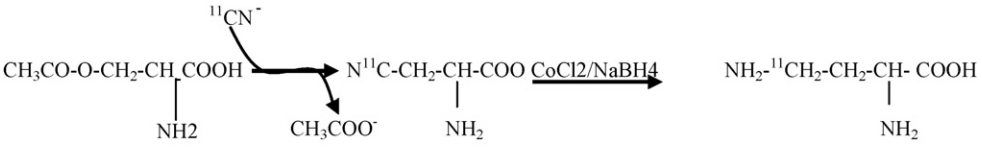
this enzyme is of current interest for the use in the biodegradation of cyanide [135] and the degradation of nitrile wastes. Since cyanide toxicity at initial stage is main hurdle for biodegradation of cyanide, use of microorganism containing this enzyme, along with other nitralase or cyanide hydrolase enzyme containing microorganisms can be a better option for biodegradation process.

2.5.2. γ-Cyano-α-aminobutyric acid synthase

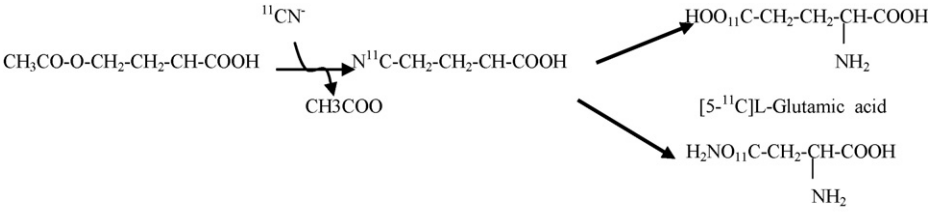
γ-Cyano-α-aminobutyric acid synthase is alternative pathway for cyanide assimilation. This pathway also requires pyrodoxal phosphate for function and induced by glutamate or glysine. Ones this γ-cyano-α-aminobutyric acid is synthesized it is slowly converted to amino acid glutamate.

This γ-cyano-α-aminobutyric acid is a homologue of β-cyanoalanine. It was interesting that γ-cyano-α-aminobutyric acid had recently been reported as the product of cyanide fixation in an unidentified psychrophilic basidiomycete. The latter amino acid nitrile and γ-cyano-α-aminobutyric acid are structural isomers both of which form glutamic acid on acid hydrolysis. Therefore, biosynthesis of γ-cyano-α-aminobutyric acid from inorganic cyanide and aspartic acid can be an alternative method to degrade cyanide [136].

A thermophilic and cyanide ion-tolerant bacterium, *Bacillus stearothermophilus* CN_3 isolated from a hot spring in Japan, was found to produce thermostable γ-cyano-α-aminobutyric acid synthase. The enzyme was purified and characterized. The purified enzyme has a molecular mass of approximately 180 kDa and consists of four identical subunits. It was stable in the pH range of 6.0–10.5 and up to 60 °C. The enzyme catalyzed the γ-replacement reaction of O-acetyl-L-homoserine with cyanide ions as shown in reactions (14) and (15) below [137]:



O- Acetyl-L-Serine [4-^{11}C] β-cyano-α-alanine [4-^{11}C]L-2-4-Damino butyric acid (14)



O- Acetyl-L-Homoserine [5-^{11}C]β-cyano-α-alaninebutyric acid [5-^{11}C]L-Glutamine (15)

The apparent K_m values for O-acetyl-L-homoserine and L-homocystine were 1.87 and 4.17 mM, respectively.

γ-Cyano-α-aminobutyric acid formation was also been reported in strain D341 of *C. violaceum*. It is able to form γ-cyano-α-aminobutyric acid [138]. This compound was not detected during the growth phase of this microorganism on a glutamate containing minimal salts medium.

3. Conclusion

Five types of enzymatic pathways have been discussed in this review. 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, but they have one drawback that it does not use the carbon atom of cyanide as nutrient source, it requires additional carbon source. In contrast to that, microorganisms containing nitrile hydratase use cyanide as nitrogen and carbon source both. Its activity increased by the absorption of photon but inhibited by the presence of heavy metal cyanide complex and oxidizing agent. This enzyme could not degrade aliphatic nitriles. Cyanide dihydratases activity increases in the presence of metal ions and it does not require any cofactor. Cyanide dihydratases present in *Pseudomonas stutzeri* have no effect of cyanide concentration on it in the medium. Microorganism containing nitrilase along with amidase degrade cyanide at highly alkaline pH 11.2. Thiocyanide cannot be degraded easily and it is present in most of the industrial effluents. Thiocyanate hydrolase is one such enzyme that degrades thiocyanate. The microorganisms that use oxidative pathway to degrade cyanide, can degrade cyanide at very high concentration, usually they use metal cyanide complex as source of nutrition at alkaline pH but side by side require pterin cofactor for its activity. One primitive type of enzyme nitrogenase mostly breaks nitrogen–nitrogen triple bond and can be a good alternative for degradation of cyanide. Rhodonase and other enzymes that follow substitution transfer pathway are secreted extracellular, thus is an advantage to be used in the degradation of cyanides. All these enzymatic pathways that are discussed here will be of great help in development of variety of biotreatment and bioremediation technologies for cyanide.

References

[1] T. Mudder, M. Botz, A. Smith, Chemistry and Treatment of Cyanidation Wastes, 2nd ed., Mining Journal Books Ltd., London, UK, 2001, ISBN 0-900117-51-6.
[2] R. Zajtchuk, R. Bellamy, Medical Aspects of Chemical and Biologic Warfare, Textbook of Military Medicine, Office of the Surgeon General, Department of the Army, Borden Institute, Walter Reed Medical Center, Washington, 1997.
[3] C.J. Knowles, A.W. Bunch, Microbial cyanide metabolism, Adv. Microb. Physiol. 27 (1986) 73–111.
[4] F. Gurbuz, H. Ciftci, A. Akcil, A.G. Karahan, Microbial detoxification of cyanide solutions: a new biotechnological approach using algae, Hydrometallurgy 72 (2004) 167–176.
[5] F. Gurbuza, H. Ciftci, A. Akcilb, Biodegradation of cyanide containing effluents by *Scenedesmus obliquus*, J. Hazard. Mater. 162 (2009) 74–79.

[6] L. Fairbrother, J. Shapter, J. Brugger, G. Southam, A. Pring, F. Reith, Effect of the cyanide-producing bacterium *Chromobacterium violaceum* on ultraflat Au surfaces, Chem. Geol. 265 (2009) 3–4.
[7] M.I. Ezzi, J.M. Lynch, Biodegradation of cyanide by *Trichoderma* spp. and *Fusarium* spp., Enzyme Microb. Technol. 36 (2005) 849–854.
[8] M. Barclay, V.A. Tett, C.J. Knowles, Metabolism and enzymology of cyanide/metallocyanide biodegradation by *Fusarium solani* under neutral and acidic conditions, Enzyme Microb. Technol. 23 (1998) 321–330.
[9] Y.B. Patil, K.M. Paknikar, Development of a process for biodetoxification of metal cyanides from wastewater, Process Biochem. 35 (2000) 1139–1151.
[10] S. Ebbs, Biological degradation of cyanide compounds, Curr. Opin. Biotechnol. 15 (2004) 1–6.
[11] T. Mudder, M. Botz, Cyanide and society: a critical review, Eur. J. Miner. Process. Environ. Prot. 4 (2004) 62–74.
[12] D.A. Dzombak, C.L. Dobbs, C.J. Culleiton, J.R. Smith, D. Krause, Removal of cyanide from spent potlining leachate by iron cyanide precipitation, in: Proceedings of the WEFTEC 69th Annual Conference & Exposition, Dallas, TX, USA, 1996.
[13] R.R. Dasha, A. Gaur, C. Balomajumder, Cyanide in industrial wastewaters and its removal: a review on biotreatment, J. Hazard. Mater. 163 (2009) 1–11.
[14] F. Xie, D. Dreisinger, Studies on solvent extraction of copper and cyanide from waste cyanide solution, J. Hazard. Mater. 169 (2009) 1–3.
[15] A. Akcil, Destruction of cyanide in gold mill effluents: biological versus chemical treatments, Biotechnol. Adv. 21 (2003) 501–511.
[16] M.M. Botz, T.I. Mudder, A.U. Akcil, Cyanide treatment: physical, chemical and biological processes, Dev. Miner. Process. 15 (2005) 672–702.
[17] A. Behnamfard, M.M. Salarirad, Equilibrium and kinetic studies on free cyanide adsorption from aqueous solution by activated carbon, J. Hazard. Mater. 170 (2009) 127–133.
[18] A. Akcil, A.G. Karahan, H. Ciftci, O. Sagdic, Biological treatment of cyanide bynatural isolated bacteria (*Pseudomonas* sp.), Miner. Eng. 16 (2003) 643–649.
[19] A. Akcil, T. Mudder, Microbial destruction of cyanide wastes in gold mining: process review, Biotechnol. Lett. 25 (2003) 445–450.
[20] J.D. Desai, C. Ramakrishna, Microbial degradation of cyanides and its commercial application, J. Sci. Ind. Res. India 57 (1998) 441–453.
[21] C.A. Young, T.S. Jordan, Cyanide remediation: current and past technologies, in: Proceedings of the 10th Annual Conference on Hazardous Waste Res., 1995, pp. 104–129.
[22] S. Trapp, M. Larsen, A. Pirandello, J. Danquah-Boakye, Feasibility of cyanide elimination using plants, Eur. J. Miner. Process. Environ. Prot. 3 (2003) 128–137.
[23] P.T. Pereira, J.D. Arrabaca, A.M.T. Maral-Collaco, Isolation, selection, and characterisation of a cyanide-degrading fungus from an industrial effluent. Isolation, selection, and characterisation of a cyanide-degrading fungus from an industrial effluent, Int. Biodeter. Biodegr. 37 (1996) 45–52.
[24] M.D Adjei, Y. Ohta, Factors affecting the biodegradation of cyanide by Burkholderia cepacia strain C-3, J. Biosci. Bioeng. 89 (2000) 274–277.
[25] S.A. Raybuck, Microbes and microbial enzymes for cyanidedegradation, Biodegradation 3 (1992) 3–18.
[26] S.K. Dubey, D.S. Holmes, Biological cyanide destruction mediated by microorganisms, World J. Microbiol. Biotechnol. 11 (1995) 257.
[27] H.K. Kwon, S.H. Woo, J.M. Park, Degradation of tetracyanonickelate (II) by *Cryptococcus humicolus MCN2*, FEMS. Microbiol. Lett. 214 (2002) 211–216.
[28] A.C. Sexton, B.J. Howlett, Characterisation of a cyanide hydratasegene in the phytopathenogenic fungus *Leptosphaeria maculans*, Mol. Gen. Genet. 263 (2000) 463–470.
[29] H. Yanese, A. Sakamoto, K. Okamoto, K. Kita, Y. Sato, Degradation of the metal-cyano complex tetracyanonickelate(II) by *Fusarium oxysporum N-10*, Appl. Biochem. Microbiol. 53 (2000) 328–334.
[30] M.D. Adjei, Y. Ohta, Isolation and characterization of a cyanide utilizing *Burkholderia cepacia* strain, World J. Microbiol. Biotechnol. 15 (1999) 699–704.
[31] I. Ezzi-Mufaddal, M. Lynch James, Cyanide catabolizing enzymes in *Trichoderma* spp, Enzyme Microb. Technol. 31 (2002) 1042–1047.
[32] A. Urbanska, B. Leszczynshi, H. Matok, A.F.G. Dixon, Cyanide detoxifying enzymes of bird cherry oat aphid, Elec. J. Polish Agric. Univ. 5 (2002).
[33] J. Baxter, S.P. Cummings, The current and future applications of microorganism in the bioremediation of cyanide contamination, Antonie Van Leeuwenhoek in place of contamination 1 (2006) 1–17.
[34] A.M. Wagner, K. Krab, The alternative respiration pathway in plants: role and regulation, Phys. Plant. 95 (1995) 318.
[35] M. Barclay, A. Hart, C.J. Knowles, J.C.L. Meeussen, V.A. Tett, Biodegradation of metal cyanides by mixed and pure cultures of fungi, Enzyme Microb. Technol. 22 (1998) 223–231.
[36] J.N. Siedow, A.L. Umbach, Plant mitochondrial electron transfer and molecular biology, Plant Cell 7 (1995) 821.
[37] M.J. Huertas, V.M. Luque-Almagro, M. Martínez-Luque, R. Blasco, C. Moreno-Vivia, F. Castillo, M.-D. Rolda, Cyanide metabolism of *Pseudomonas pseudoalcaligenes* CECT5344: role of siderophores, Biochem. Soc. Trans. 34 (2006) 152–155.
[38] V.M. Luque-Almagro, R. Blasco, M.J. Huertas, M. Martínez-Luque, C. Moreno-Vivia, F. Castillo, M.D. Rolda, Alkaline cyanide biodegradation by *Pseudomonas pseudoalcaligenes* CECT5344, Biochem. Soc. Trans. 33 (2005) 168–169.
[39] H. Figueiredo, I.C. Neves, C. Quintelas, T. Tavares, M. Taralunga, J. Mijoin, P. Magnoux, Oxidation catalysts prepared from biosorbents supported on zeolites, Appl. Catal. B: Environ. 66 (2006) 274–280.
[40] H. Yanase, A. Sakamoto, K. Okamoto, K. Kita, Y. Sato, Degradation of the metal-cyano complex tetracyanonickelate(II) by *Fusarium oxysporum N-10*, Appl. Microbiol. Biotechnol. 53 (2000) 328–334.
[41] A. Dumestre, T. Chone, J.M. Portal, M. Gerard, J. Berthelin, Cyanide degradation under alkaline conditions by a strain of *Fusarium solani* isolated from contaminated soils, Appl. Environ. Microbiol. 63 (1997) 2729–2734.
[42] W.E. Fry, R.L. Millar, Cyanide degradation by an enzyme from *Sternphylium loti*, Arch. Biochem. Biophys. 151 (1972) 468–474.
[43] N. Nazly, C. Knowles, Cyanide degradation by immobilized fungi, Biotechnol. Lett. 3 (1981) 363–368.
[44] D.A. Kunz, C.S. Wang, J.L. Chen, Alternative routes of enzymic cyanide metabolism in *Pseudomonas fluorescens NCIMB 11764*, Microbiology 140 (1994) 1705–1712.
[45] H. Nazly, C.J. Knowles, A.J. Beardsmore, W.T. Naylor, E.G. Corcoran, Detoxification of cyanide by immobilised fungi, J. Chem. Technol. Biotechnol. 33B (1983) 119–126.
[46] K.R. Richardson, Production of cyanide hydratase, European Patent Application EP 233719 (1987).
[47] P. Wang, H.D. VanEtten, Cloning and properties of a cyanide hydratase gene from the phytopathogenic fungus *Gloeocercospora sorghi*, Biochem. Biophys. Res. Commun. 187 (1992) 1048–1054.
[48] M.J. Cluness, P.D. Turner, P. Clements, D.T. Brown, D. O'Reilly, Purification and properties of cyanide hydratase from *Fusarium lateritium* and analysis of the corresponding chy1 gene, J. Gen. Microbiol. 139 (1993) 1807–1815.
[49] P.T. Pereira, J.D. Arrabaca, M.T. Amaral–Collaco, Isolation, selection and characterization of a cyanide-degrading fungus from an industrial effluent, Int. Biodeter. Biodegr 37 (1996) 45–52.
[50] K. Kubiak, W.A. Nowak, Molecular dynamics simulations of the photoactive protein nitrile hydratase, Biophysic. J. 94 (2008) 3824–3838.
[51] T. Gerasimova, A. Novikov, S. Osswald, A. Yanenko, Screening, characterization and application of cyanide-resistant nitrile hydratases, Eng. Life Sci. 4 (2004) 543–546.
[52] O.B Astaurova, T.E. Leonova, I.N. Poliakova, I.V. Sineokaia, V.K. Gordeev, A.S. Ianenko, Adaptation of acrylamide producer *Rhodococcus rhodochrous M8* to change in ammonium concentration in medium, Prikl. Biokhim. Mikrobiol. 36 (2000) 21–25.
[53] L. Song, M. Wang, X. Yang, S. Qian, Purification and characterization of the enantioselective nitrile hydratase from *Rhodococcus sp. AJ270*, Biotechnol. J. 2 (2007) 717–724.
[54] A.I.u. Maksimov, M.V. Kuznetsova, G.V. Ovechkina, S.V. Kozlov, I.u.G. Maksimova, V.A. Demakov, Effects of nitriles and amides on the growth and the nitrile hydratase activity of the *Rhodococcus sp*. strain gt1, Prikl. Biokhim. Mikrobiol. 39 (2003) 63–68.
[55] S.M. Nawaz, D.K. Chapatwala, H.J. Wolfram, Degradation of acetonitrile by *pseudomonas putida*, Appl. Environ. Microbiol. 55 (1989) 2267–2274.
[56] S.M. Nawaz, M. Thomas, H. Carl e, Metabolism of benzonitrile and butyronitrile by klebsiella pneumoniae, Appl. Environ. Microbiol. 58 (1992) 27–31.
[57] A.k. Bandyopadhyay, T.T. Nagasawa, Y. Asano, K. Fujishiro, Y. tani, H. Yamada, Purification and characterization of benzonitrilases from *arthrobacter sp. strain j-l*, Appl. Environ. Microbiol. 51 (1986) 302–306.
[58] U.H. Maier-Greiner, B.M. Obermaier-Skrobranek, L.M. Estermaier, W. Kammerloher, C. Freund, C. Wülfing, U.L. Burkert, D.H. Matern, M. Breuer, M. Eulitz, *Myrothecium verrucaria* that is highly specific for the fertilizer cyanamide and cloning of its gene, Proc. Natl. Acad. Sci. U.S.A. 88 (1991) 4260–4264.
[59] R. Pereira, D. Graham, F. Rainey, D. Cowan, A novel thermostable nitrile hydratase, Extremophiles 2 (1998) 347–357.
[60] K. Yamamoto, Y. Ueno, K. Otsubo, H. Yamane, K. Komatsu, Y. Tani, Efficient conversion of dinitrile to mononitrile-monocarboxylic acid by *Corynebacterium*, sp. C5 cells during tranexamic acid synthesis, J. Ferment. Bioeng. 73 (1992) 125–129.
[61] F. Alfani, M. Cantarella, A. Spera, P. Viparelli, Operational stability of *Brevibacterium imperialis*, CBS 489-74 nitrile hydratase, J. Mol. Catal. 11 (2001) 687–697.
[62] R. Fallon, B. Stieglitz, I. Turner, A *Pseudomonas putida*, capable of stereoselective hydrolysis of nitriles, Appl. Microbiol. Biotechnol. 47 (1997) 156–161.
[63] T. Nagasawa, H. Nanba, K. Ryuno, K. Takeuchi, H. Yamada, Nitrile hydratase of *Pseudomonas chlororaphis*, B23: purification and characterization, Eur. J. Biochem. 162 (1987) 691–698.
[64] Y. Asano, M. Tashibana, Y. Tani, H. Yamada, Aliphatic nitrile hydratase from *Arthrobacter*, J1: purification and characterization, Agric. Biol. Chem. 46 (1982) 1165–1174.
[65] T. Yamaki, T. Olikawa, K. Ito, T. Nakamura, Cloning and sequencing of a nitrile hydratase gene from *Pseudonocardia thermophila*, JCM3095, J. Ferment. Bioeng. 83 (1997) 474–477.
[66] I. Endo, I. Watanabe, Nitrile hydratase of *Rhodococcus*, N-774 purification and characterization, FEBS Lett. 243 (1989) 61–64.
[67] K. Ingvorsen, B. Højer-Pedersen, S.E. Godtfredsen, Novel cyanide-hydrolyzing enzyme from *Alcaligenes xylosoxidans subsp* denitrificans, Appl. Environ. Microbiol. 57 (1991) 1783–1789.
[68] P.R. Meyers, D.E. Rawlings, D.R. Woods, G.G. Lindsey, Isolation and characterization of a cyanide dihydratase for *Bacillus pumilus C1*, J. Bacteriol. 175 (1993) 6105–6112.
[69] K. Ingvorsen, S.E. Godtfredsen, B. Højer-Pedersen, N. Nordisk, May microbial cyanide converting enzymes, their production and use, US Patent 5,116,744 (1992).

[70] H.C. Pace, C. Brenner, The nitrilase superfamily: classification, structure and function, nitrilases than to the metal-containing nitrile hydratases, Genome. Biol. 2 (2001) 1.1–1.8.
[71] A. Watanabe, K. Yano, K. Ikebukuro, I. Karube, Cyanide hydrolysis in a cyanide-degrading bacterium, *Pseudomonas stutzeri AK61*, by cyanidase, Microbiology 144 (1998) 1677–1682.
[72] A Goldhust, Z. Bohak, Induction, purification and characterization of the nitrilase of *Fusarium oxysporum*, Biotechnol. Appl. Biochem. 11 (1989) 581–601.
[73] P. Collins, C. Knowles, The utilization of nitriles and amides by *Nocardia rhodochrous*, J. Gen. Microbiol. 129 (1983) 248–254.
[74] T. Nagasawa, M. Wieser, T. Nakamura, H. Iwahara, T. Yoshida, K. Gekko, Nitrilase of *Rhodococcus rhodochrous* J1, conversion into the active form by subunit association, Eur J. Biochem. 267 (2000) 138–144.
[75] M. Kobayashi, H. Komeda, N. Yanaka, T. Nagasawa, H. Yamada, Nitrilase from *Rhodococcus rhodochrous* J1: sequencing and overexpression of the gene and identification of an essential cysteine residue, J. Biol. Chem. 267 (1992) 20746–20751.
[76] D. Stevenson, R. Feng, F. Dumas, D. Groleau, A. Mihoc, A. Storer, Mechanistic and structural studies on *Rhodococcus*ATCC 39484 nitrilase, Biotechnol. Appl. Biochem. 15 (1992) 283–302.
[77] M. Piotrowski, S. Schonfelder, E.W. Weiler, The *Arabidopsis thalians* isogene NIT4 and its orthologs in tobacco encode beta-cyano-L-alanine hydratase/nitrilase, J. Biol. Chem. 276 (2001) 2616–2621.
[78] D.B. Harper, Characterization of a nitrilase from *Nocardia* sp. (*Rhodochrous* group) N.C.I.B. 11215, using p-hydroxybenzonitrile as sole carbon source, Int. J. Biochem. 17 (1985) 677–683.
[79] C.Y. Chen, C.M. Kao, S.C. Chen, Application of *Klebsiella oxytoca* immobilized cells on the treatment of cyanide wastewater, Chemosphere 71 (2008) 133–139.
[80] J.K. Liu, Y.W. Wu, C.H. Hsu, Physiological adaptability of a cyanide-utilizing *Klebsiella oxytoca* strain, Proc. Natl. Sci. Council. 16 (1992) 188–193.
[81] C.M. Kao, J.K. Liu, H.R. Lou, C.S. Lin, S.C. Chen, Biotransformation of cyanide to methane and ammonia by *Klebsiella oxytoca*, Chemosphere 50 (2003) 1055–1061.
[82] D. Harper, Microbial metabolism of aromatic nitriles:enzymology of C–N cleavage by *Nocardia* sp. NCIB 11216, J. Biochem. 165 (1977) 309–319.
[83] M. Kobayashi, N. Yanaka, T. Nagasawa, H. Yamada, Purification and characterization of a novel nitrilase of *Rhodococcus rhodochrous* K22 that acts on aliphatic nitriles, J. Bacteriol. 172 (1990) 4807–4815.
[84] T.C. Bhalla, A. Miura, A. Wakamoto, Y. Ohba, K. Furuhashi, Symmetric hydrolysis of a-aminonitriles to optically active amino acids by a nitrilase of *Rhodococcus rhodochrous* PA-34, Appl. Microbiol. Biotechnol. 37 (1992) 184–190.
[85] A. Bandopadhayay, T. Nagasawa, Y. Asano, K. Fujishiro, Y. Tani, H. Yamada, Purification and characterization of enzonitrilase from *Arthrobacter* sp. J1, Appl. Environ. Microbiol. 51 (1986) 302–306.
[86] T. Nagasawa, J. Mauger, H. Yamada, Novel nitrilase, arylacetonitrilase, of *Alcaligenes faecalis* JM3, purification and characterization, Biochemistry 194 (1990) 765–772.
[87] K Yamamoto, K. Komatsu, Purification and characterizationof nitrilase responsible for the hydrolysis from *Acinetobacte* sp. AK 226, Agric. Biol. Chem. 55 (1991) 1459–1466.
[88] N. Layh, J. Parratt, A. Willetts, Characterization and partialpurification of an enantioselective arylacetonitrilase from*Pseudomonas fluorescens* DSM 7155, J. Mol. Catal. B 5 (1998) 467–474.
[89] D. Stalker, L. Malyj, K. McBride, Purification and propertiesof a nitrilase specific for the herbicide bromoxynil andcorresponding nucleotide sequence analysis of the *bxn* gene, J. Biol. Chem. 263 (1988) 6310–6314.
[90] D. Harper, Fungal degradation of aromatic nitriles: enzymology of C–N cleavage by *Fusarium solani*, Biochem. J. 167 (1977) 685–692.
[91] A. Goldhust, Z. Bohak, Induction, purification and characterizationof the nitrilase of *Fusarium oxysporum*, Biotechnol. Appl. Biochem. 11 (1989) 581–601.
[92] V.K. Nigam, K. Khandelwal, R.K. Gothwal, M.K. Mohan, B. Choudhury, A.S. Vidyarthi, P. Ghosh, Nitrilase-catalysed conversion of acrylonitrile by free and immobilized cells of *streptomyces* sp., J. Biosci. 34 (2009) 21–26.
[93] Y. Katayama, Y. Narahara, Y. Inoue, F. Amano, T. Kanagawa, H. Kuraishi, A thiocyanate hydrolase of *Thiobacillus thioparus*. A novel enzyme catalyzing the formation of carbonyl sulfide from thiocyanate, J. Biol. Chem. 267 (1992) 9170–9175.
[94] T. Arakawa, Y. Kawano, S. Kataoka, Y. Katayama, N. Kamiya, M. Yohda, M. Odaka, Structure of thiocyanate hydrolase: a new nitrile hydratase family protein with a novel five-coordinate cobalt (III) center, J. Mol. Biol. 366 (2007) 1497–1509.
[95] P.R. Meyers, D.E. Rawlings, D.R. Woods, G.G. Lindsey, Isolation and characterization of a cyanide dihydratase from *Bacillus pumilus* C1, J. Bacteriol. 175 (1993) 6105–6112.
[96] A. Watanabe, K. Yano, K. Ikebukuro, I. Karube, Cyanide hydrolysisin a cyanide-degrading bacterium, *Pseudomonas stutzeri* AK61 by cyanidase, Microbiology 144 (1998) 1677–1682.
[97] S. Raybuck, Microbes and microbial enzymes for cyanide degradation, Biodegradation 3 (1992) 3–18.
[98] R. Harris, C.J. Knowles, Isolation and growth of a *Pseudomonas* species that utilizes cyanide as a source of nitrogen, J. Gen. Microbiol. 129 (1983) 1005–1011.
[99] D.A. Kunz, O. Nagappan, J. Silva-Avalos, G.T. Delong, Utilization of cyanide as a nitrogenous substrate by *Pseudomonas fluorescens* NCIMB 11764: evidence for multiple pathways of metabolic conversion, Appl. Environ. Microbiol. 58 (1992) 2022–2029.
[100] A. Cabuk, A. Taspinar, U.N. Kolankaya, Biodegradation of cyanide by a white rot fungus, Trametes versicolor, Biotechnol. Lett. 28 (2006) 1313–1317.
[101] D.A. Kunz, C.S. Wang, J.L. Che, Alternative routes of enzymic cyanide metabolism in *Pseudomonas fluorescens* NCIMB 11764, Microbiology 140 (1994) 1705–1712.
[102] D.A. Kunz, R. Fernandez, P. Parab, Evidence that bacterial cyanide oxygenase is a pterin-dependent hydroxylase, Biochem. Biophys. Res. Commun. 287 (2000) 514–518.
[103] C.S. Wang, D.A. Kunz, B.J. Venables, Incorporation of molecular oxygen and water during enzymatic oxidation of cyanide by *Pseudomonas fluorescens* NCIMB 11764, Appl. Environ. Microbiol. 62 (1996) 2195–2197.
[104] R.M. Little, P.M. Anderson, Structural properties of cyanase, J. Biol. Chem. 262 (1987) 10120–10126.
[105] P.A. Castric, G.A. Strobel, Cyanide metabolism by *Bacillus megaterium*, J. Biol. Chem. 244 (1969) 4089–4094.
[106] K.D. Chapatwala1, G.R.V. Babu, O.K. Vijaya, K.P. Kumar, J.H. Wolfram, Biodegradation of cyanides, cyanates and thiocyanates to ammonia and carbon dioxide by immobilized cells of *Pseudomonas putida*, J. Ind. Microbial. Biotechnol. 20 (1998) 28–33.
[107] V.M Luque-Almagro, M.J. Huertas, M. Martínez-Luque, C. Moreno-Vivián, M. Dolores Roldán, L. Jesús Garcıa-Gil, F. Castillo, R. Blasco, Bacterial degradation of cyanide and its metal complexes under alkaline conditions, Appl. Environ. Microbiol. 71 (2005) 940–947.
[108] L.C. Seefeldt, I.G. Dance, D.R. Dean, Substrate interactions with nitrogenase: Fe versus Mo, Biochemistry 43 (2004) 1401–1409.
[109] F.U. Haian, R.H. Burris, Ammonium inhibition of nitrogenase activity in *Herbaspirillum seropedicaet*, J. Bacteriol. 171 (1989) 3168–3175.
[110] C. Sicking, M. Brusch, A. Lindackers, K.U. Riedel, B. Schubert, N. Isakovic, C. Krall, W. Klipp, T. Drepper, K. Schneider, B. Masepohl, Identification of two new genes involved in diazotrophic growth via the alternative Fe-only nitrogenase in the phototrophic purple bacterium *Rhodobacter capsulatus*, J. Bacteriol. 187 (2005) 92–98.
[111] K. Alexander, M. Volini, Properties of an Escherichia coli rhodanese, J. Biol. Chem. 262 (1987) 6595–6604.
[112] D. Bordo, F. Forlani, A. Spallarossa, R. Colnaghi, A. Carpen, M. Bolognesi, S. Pagani, A persulfurated cysteine promotes active site reactivity in Azotobacter vinelandii Rhodanese, Biol. Chem. 382 (2001) 1245–1252.
[113] R. Cipollone, M.G. Bigotti, E. Frangipani, P. Ascenzi, P. Visca, Characterization of a rhodanese from the cyanogenic bacterium *Pseudomonas aeruginosa*, Biochem. Biophys. Res. Commun. 325 (2004) 85–90.
[114] I.M. Ezzi, A.J. Pascual, J.B. Gould, M.J. Lynch, Characterisation of the rhodanese enzyme in, *Trichoderma* spp., Enzyme Microb. Technol. 32 (2003) 629–634.
[115] R. Tabita, M. Silver, D.G. Lundgren, The rhodanese enzyme of *Ferrobacillus ferrooxidans* (*Thiobacillus ferroxidans*), J. Biochem. 47 (1969) 1141–1145.
[116] T.J. Bowen, P.J. Butler, F.C. Happold, Some properties of the rhodanese system of *Thiobacillus denitrificans*, J. Biochem. 97 (1965) 651–657.
[117] C.P. Burton, J.M. Akagi, Observations of the rhodanese activity of *Desulfotomaculum nigrificans*, J. Bacteriol. 107 (1971) 375–376.
[118] A.B. Roy, P.A. Trudinger, The biochemistry of inorganic compounds of sulphur, Microbial. Ecol. 4 (1977) 9–25.
[119] R.A.M. Williams, S.M. Kelly, J.C. Mottram, G.H. Coombs, 3-Mercaptopyruvate sulfur transferase of *Leishmania* contains an unusual C-terminal extension and is involved in thioredoxin and antioxidant metabolism, J. Biol. Chem. 278 (2003) 1480–1486.
[120] D.L. Nandi, J. Westley, Reduced thioredoxin as a sulfur-acceptor substrate for rhodanese, Int. J. Biochem. Cell Biol. 30 (1998) 973–977.
[121] D.L. Nandi, P.M. Horowitz, J. Westley, Rhodanese as a thioredoxin oxidase, Int. J. Biochem. Cell Biol. 32 (2000) 465–473.
[122] M.S. Alphey, R.A.M. Williams, J.C. Mottram, G.H. Coombs, W.N. Hunter, The crystal structure of *Leishmania major* 3-mercaptopyruvate sulfurtransferasel, J. Biol. Chem. 278 (2003) 4821–4822.
[123] K. Ogata, M. Volini, Mitochondrial rhodanese: membrane-bound and complexed activity, J. Biol. Chem. 265 (1990) 8087–8093.
[124] B.H. Sorbo, Enzymic transfer of sulfur from mercaptopyruvate to sulfite or sulfinates, Biochim. Biophys. Acta 24 (1957) 324–329.
[125] U. Hannestad, J. Martensson, R. Sjodahl, B. Sorbo, Plant mercaptopyruvate sulfurtransferases, Biochem. Med. 26 (1981) 106–114.
[126] T. Nishino, C. Usami, K. Tsushima, Reversible interconversion between sulfo and desulfo xanthine oxidase in a system containing rhodanese, thiosulfate, and sulfhydryl reagent, Proc. Natl. Acad. Sci. U.S.A. 80 (1983) 1826–1829.
[127] S. Pagani, F. Bonomi, P. Cerletti, Pro108 is important for folding and stabilization of adrenal ferredoxin, but does not influence the functional properties of the protein, Eur. J. Biochem. 142 (1984) 361–366.
[128] N. Nagahara, T. Ito, M. Minami, Mercaptopyruvate sulfurtransferase as a defense against cyanide toxication: molecular properties and mode of detoxification, Histol. Histopathol. 14 (1999) 1277–1286.
[129] E.W. Miles, Pyridoxal phosphate enzymes catalyzing [3-elimination and [3-replacement reactions, in: D. Dolphin, R. Poulson, O.A. Vramovic (Eds.), Vitamin B6 Pyridoxal Phosphate Chemical, Biochemical, and Medical Aspects, John Wiley & Sons, New York, 1986, pp. 253–310.

[130] T.P. Chirpich, V. Zappia, R.N. Costilow, H.A. Barker, Purification and properties of a pyridoxal phosphate and s-adenosylmethionineactivated enzyme, J. Biochem. 245 (1970) 1778–1789.
[131] T.N. Akopyan, A.E. Braunstein, E.V. Goryachenkova, Beta-cyanoalanine synthase: purification and characterization, Proc. Natl. Acad. Sci. U.S.A. 72 (1975) 1617–1621.
[132] B. Goldschmidt, S.G.W. Butler, E.E. Conn, Incorporation of hydrocyanic acid labeled with C14 into asparagines in seedling, Nature 197 (1963) 718–719.
[133] L. Fowden, E.A. Bell, Cyanide metabolism by seedling, Nature 206 (1965) 110–112.
[134] P.M. Dunnil, L. Fowden, Enzymatic formation of β-cyanoalanine from cyanide by *E. coli* extract, Nature 208 (1965) 1206–1207.
[135] M.M. Brysk, W.A. Corpe, L.V. Hankes, β-Cyanoalanine formation by *Chromobactarium violaceum*, J. Bacteriol. 97 (1969) 322–327.
[136] P.A. Castric, E.E. Conn, Cyanide metabolism by *Bacillus magaterium*, J. Biochem. 244 (1969) 4088–4094.
[137] H. Omura, M. Ikemot, M. Kobayash, S. Shimizq, T. Yoshida, T. Nagasawa, Purification, characterization and gene cloning of thermostable O-acetyl-L-homoserine sulfhydrylase forming γ-cyano-α-aminobutyric acid, J. Biosci. Bioeng. 96 (2003) 53–58.
[138] M.M. Brysk, C. Ressler, γ-Cyanoa-L-aminobutyric acid, a new product of cyanide fixation in Chromobacterium violaceum, J. Biol. Chem. 245 (1970) 1156–1160.