



An overview of biological cyanide elimination from tailing wastewater as a promising tool for sustainable utilization

Ainur Berkinbayeva^a, Bagdaulet Kenzhaliyev^a, Kenzhegali Smailov^a , Alan Aimagambetov^b , Bekzat Kamenov^{b,*} , Shynar Saulebekkyzy^a, Nazerke Tolegenova^a, Primandari Sri Rizki Putri^c

^a Institute of Metallurgy and Ore Beneficiation, Satbayev University, Almaty 050013, Kazakhstan

^b Sustainability of Ecology and Bioresources, Al-Farabi Kazakh National University, Almaty 050040, Kazakhstan

^c Department of Mechanical Engineering, Universitas Negeri Padang, Padang, West Sumatera, 25173, Indonesia

ARTICLE INFO

Keywords:

Cyanide
Tailing wastewater
Cyanide biodegradation
Cyanomics

ABSTRACT

Cyanide compounds, both organic and inorganic, are widely present in natural and industrial environments, especially in effluents from mining and metallurgical processes. Their high toxicity, particularly in the form of free cyanides and hydrogen cyanide, poses severe risks to ecosystems and public health by disrupting cellular respiration via inhibition of cytochrome c oxidase. Conventional chemical treatments such as alkaline chlorination are effective but can be costly, energy-intensive, and generate secondary pollutants. In contrast, microbial bioremediation has emerged as a potentially more sustainable and cost-effective alternative, particularly for on-site treatment of cyanide-laden wastewater from massive tailings dams. Microorganisms including cyanotrophs utilize cyanide as a nitrogen or carbon sources, transforming it into less toxic compounds such as ammonia and carbon dioxide through enzymatic systems like cyanide hydratase, nitrilase, and rhodanese. While bioremediation may operate more slowly than chemical methods, its advantages lie in lower energy consumption, reduced material input, simpler maintenance, and minimized toxic by-products. This review synthesizes current understanding of cyanide's chemical nature, toxicity, and environmental impact, and explores microbial cyanide degradation mechanisms. It further highlights how advances in metagenomics and synthetic biology ("cyanomics") are enabling the design of more robust biocatalytic systems. Integrating these biological approaches into environmental management frameworks could reduce long-term operational costs and improve sustainability across cyanide-intensive industries.

1. Introduction

1.1. Cyanides and their chemical nature

Cyanides are a group of organic or inorganic molecules that contain the cyano group, $\text{C}\equiv\text{N}$. Cyanides are present in various forms. There may be free cyanide ions in the form of CN^- at alkaline pH or HCN at neutral or acidic pH, simple inorganic salts of alkaline metals with hydrocyanic acid, or insoluble salts of heavy metals, complex salts with different solubilities, inorganic oxidized forms like SCN^- or CNO^- , and organic molecules with a $-\text{CN}$ group, called nitriles (Kumar et al., 2017; Roldán et al., 2021; Alvillo-Rivera et al., 2021).

Due to the emptiness of p and d orbitals, cyanide can be oxidized to form cyanates or thiocyanates. Moreover, the cyanide group is a

nucleophile in addition reactions. Anionic cyanides can share an electron pair to form a σ bond with the d orbitals and form complexes with transition metals. In addition, the cyanide group can participate in conjugation with adjacent π -systems, which affects the electron distribution and contributes to the stability and reactivity of the molecule (Vaca-Escobar et al., 2024). Complex salts made by the cyanide and Cd, Zn, Ag, Hg, Cu, or Ni cations are considered weak complexes due to their low stability, while Co, Fe, Pt, Au, and Pd establish strong complexes. The differences in binding strengths can be attributed to multiple factors, including the metal's electronic configuration, oxidation state, and the nature of metal–ligand bonding. Soft metals like Pt^{2+} and Au^+ form stronger bonds with the soft cyanide ligand due to favorable soft-soft interactions as per Hard and Soft Acids and Bases theory. Additionally, transition metals in higher oxidation states or with partially filled

* Corresponding author.

E-mail address: kamenovbekzat01@gmail.com (B. Kamenov).

<https://doi.org/10.1016/j.wroa.2025.100400>

Received 9 June 2025; Received in revised form 28 July 2025; Accepted 16 August 2025

Available online 17 August 2025

2589-9147/© 2025 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>).

d orbitals tend to form more stable π -backbonding interactions with cyanide, thereby enhancing complex stability. Conversely, metals with filled d orbitals or lower oxidation states exhibit weaker binding due to less effective orbital overlap and diminished π -acceptor ability of the cyanide ligand.

Cyanide toxicity depends on the molecule's ability to release cyanide ions. The most toxic forms of cyanides are free cyanides and HCN (Roldán et al., 2021). Cyanides induce rapid fatality at high concentrations. People with acute intoxication exhibit deep and rapid respiration. The inability to supply the organism with oxygen is followed by convulsions and suffocation. However, when cyanides are encountered in low doses, they do not tend to accumulate in body tissues, since they are efficiently metabolized to the less toxic cyanates through enzymatic oxidation and subsequently excreted from the body (Logsdon et al., 1999). The toxicity of cyanide for living organisms comes from its ability to bind to metals. The main target of cyanide in animals is the co-factor of the cytochrome c oxidase. Moreover, they form complexes with haemoglobin in blood and prevent the oxygen and carbon dioxide transfer, which leads to asphyxia and death (Roldán et al., 2021). Apart from cytochromes, cyanides form complexes with hydrolases, superoxide dismutase, and carbonic anhydrase. Thus, the electron transport chain is disturbed in animals, plants, and microorganisms. The decreased blood saturation leads to the appearance of the cherry red skin colour as a result of acute intoxication by cyanides. The animal's fetal development may be disrupted by the intoxication of cyanide. The intoxication leads to teratogenic effects like encephalocele, exencephaly, and abnormal development of costal arches (Alvillo-Rivera et al., 2021). Apart from that, cyanides cause carbamylation of proteins in the eyes and kidneys (Roldán et al., 2021).

The ceiling threshold of gaseous HCN is marked as 4.7 ppm, while more than 50 ppm may cause severe damage to the respiratory system (Secretaría de Medio Ambiente y Recursos Naturales (SEMARNAT) 1996). The lethal dose by ingestion or inhalation of free cyanides for humans is 1 or 3 mg per kg of body mass, while the deadly dose by skin absorption is about 100 mg per kg of body mass (Logsdon et al., 1999).

1.2. Natural cyanide sources

Though cyanides are very toxic, these molecules are abundant in nature and perform various functions. Cyanides are produced by bacteria (cyanogenic bacteria), plants (mango, apples, beans, almonds, cashew nuts), fungi, and even by some arthropods (centipedes, beetles, and some butterflies) (Baxter and Cummings, 2006; Dash et al., 2009). Thus, they serve many functions, like defense molecules in many plants or some insects, controllers of mating behavior in insects, and for producing antibiotics (Kumar et al., 2017; Baxter and Cummings, 2006).

So-called cyanogenic bacteria possess a special enzyme, called HCN synthase. This enzyme is encoded by the hcbABC genes and used to convert glycine, found in the rhizosphere and soil, into CO_2 and HCN (Vaca-Escobar et al., 2024).

Some plants store cyanogenic glycosides, molecules that are degraded to form organic molecules, which signal other cells in the tissue of the damage, and cyanides, which attack pathogens. Some examples of cyanogenic glycosides are amygdalin, linamarin, preunasin, dhurrin, and others (Kumar et al., 2017). Moreover, plants release cyanides during the synthesis of ethylene (Vaca-Escobar et al., 2024). Some fungi like *Chromobacterium violaceum* or *Trichoderma harzianum* produce cyanide antibiotics that attack the cell walls of bacteria in very nutrient-poor environments or the "populated" areas like rhizospheres (Baxter and Cummings, 2006).

1.3. Anthropogenic cyanide sources

Generally, cyanides played a crucial role in the early stages of life and have always been present in the environment (Baxter and Cummings, 2006). Though nowadays, a hazardous increase in the free or

inorganic cyanides is thought to be due to anthropogenic activity (Vaca-Escobar et al., 2024). The most trivial disposal of cyanides in soil is done by applying herbicides. Nitrile herbicides are used to control diseases associated with some valuable crops. But the amount of cyanides released by the agriculture sector is minuscule compared to industrial application of cyanides and their anthropogenic sources (Kumar et al., 2017). According to available literature, only a small amount of worldwide use of cyanide (approximately 6 %) is associated with mining, though the level of pollution that results from the precious metals production is higher (Mudder and Botz, 2004). 18 % of all produced cyanides are used in the production of gold.

Relatively cheap, free cyanide ions have been used for the production of gold since the 1880s (Vaca-Escobar et al., 2024; Yahya et al., 2020). Free cyanides are used to form water-soluble complexes with gold atoms, which are used for gold extraction and called the pregnant solution (Hilson and Monhemius, 2006). 52 % of all metallurgy complexes use cyanidation as a main method for gold or silver production. This is due to the absence of titrimetric or gravimetric separation techniques. As a result of this metallurgy process, there are wanted products, i.e., recovered precious metals, and unwanted mining tailings. This tailing water contains toxic metals like Hg, Sb, or Co, precious metals such as Pt, Ag, or Ru, and radionuclides like U, Ra (Alvillo-Rivera et al., 2021).

Although this method of gold extraction is highly effective, the resulting wastes are stored in artificial ponds, which usually tend to cause ecological catastrophes. For instance, in 2000, a dam burst in Baia Mare, Romania, releasing 100 tonnes of cyanides into the Somes River, consequently killing 80 % of all wildlife (Roldán et al., 2021). In 1998, 2 tonnes of cyanides were accidentally spilled into the environment from Kuntor Mine, Kyrgyzstan (Hilson and Monhemius, 2006).

Such hazardous accidents and constant release of cyanides by industrial wastes and effluents make cyanide removal vital in industrial facilities (Vaca-Escobar et al., 2024; Dash et al., 2009). As a result, various legislative acts and amendments are being developed, though legislation won't reduce the cyanide exposure to the environment. Thus, the cyanide degradation and cleaning technologies have to be employed to terminate the release of cyanides, even in smaller amounts, incompatible with the natural cyanide level, especially in water and soil (Roldán et al., 2021). This paper aims to discuss the main types of cyanide degradation, the advantages of biological cyanide degradation, and the diversity and metabolic action of bacteria from cyanide-associated environments.

The main aim of the present review is to explore the existing state of cyanide biodegradation, specifically microbial enzymatic pathways involved in cyanide bioconversion to safe end-products. The traditional physico-chemical methods are also provided to compare the effectiveness. Furthermore, the recent advancements and application of novel omics technologies have been provided to identify potential strains, genes, and proteins related to cyanide tolerance or biodegradation. A few process optimization strategies are described, such as immobilization technique, consortium construction, plant, or electro induction, that might enhance the degradation rate. However, the main set of limitations and drawbacks that interfere with the scaling up of the biological cyanide elimination from lab scale to industry have been analyzed. Biological detoxification of cyanide compounds in tailing wastewater remains one of the promising, eco-friendly approaches.

2. Types of cyanide neutralization from wastewater

As was mentioned above, several standards and legislative acts have been found to control the cyanide discharge, especially into water sources. For instance, Indian legislators have established the cyanide concentration in drinking water to be less than 0.2 ppm, while the US Environmental Protection Agency demands it to be 200-50 ppb (Dwivedi et al., 2019). To maintain minuscule amounts of cyanide, huge amounts of cyanide, found in wastewater, have to be efficiently degraded. The selection of a particular treatment method is based on

different factors (Vaca-Escobar et al., 2024):

- **Source and concentration of cyanide.** Thus, biological methods are suitable for smaller concentrations, while chemical or physical methods are generally applicable for higher concentrations.
- **Chemical composition of the effluent.** Chemical composition affects the forms of cyanide present in the effluent. For instance, heavy metals can stabilize and “hide” free cyanides, making them less susceptible to chemical reactions.
- **Applicability of method.** Not all methods that are theoretically tested in the laboratory are appropriate and applicable in practice.
- **Cost.** The cost of the method drives research on the cheapest ways of cyanide degradation. Moreover, decreased energy consumption and the utilization of cheaper reagents must be considered too.

Several methods based on biological, chemical, and physical reactions are being developed and used (Alvillo-Rivera et al., 2021; Vaca-Escobar et al., 2024; Meher et al., 2020; Kuyucak and Akcil, 2013). Chemical and physical methods are based on the oxidation and stabilization and are effectively applicable for free and weak complex cyanides (Alvillo-Rivera et al., 2021). The gold mining industry mainly develops methods for the detoxification of complex cyanides by oxidation, complexation, and stabilization (Baxter and Cummings, 2006). Stabilization prevents the spread of cyanides and their chemical activity, while oxidation changes the toxic cyanides into oxidized, non-toxic forms (Alvillo-Rivera et al., 2021).

However, the use of biological oxidation is thought to be efficient (Dash et al., 2009). The most notable advantage is the absence of the addition of toxic chemicals into the original substrate. Additionally, biological treatment results in the creation of environmentally compatible CO₂ and ammonia by different microbes and their enzymes. As a result, cells accumulate cyanides not in the form of relatively toxic cyanate, but in the form of amino acids, vitamins, etc (Dwivedi et al., 2019). The next section of the present paper is dedicated to the discussion of the biological way of cyanide degradation.

3. Biological elimination of cyanide

3.1. Significance

Biological methods of cyanide removal are associated with the application of the ability of organisms to degrade or accumulate cyanides. While chemical or physical methods try to eliminate all possible concentrations of cyanide, biological methods reduce the amounts of cyanides to the naturally acceptable level and produce non-toxic by-products, and do not require additional chemicals (Logsdon et al., 1999; Nawaz et al., 1989). Biological methods are divided into 2 main types such as phytoremediation and bacterial remediation. Phytoremediation means the implementation of plants, algae, or fungi for the sequestration of cyanides and their further detoxification. Though the exact biological mechanisms are mainly unknown, it's thought that plants utilize cyanides as precursors for the synthesis of amino acids (Cobos et al., 2015). Metal cyanide complexes bind to siderophores or secondary metabolites secreted by rhizosphere microbiota and are further absorbed by roots. Then cyanides are degraded by rhizosphere fungi or chelated and stored in vacuoles. Stored cyanides are further catabolized by many enzymes, such as β -cyanoalanine synthase or rhodanese (VM et al., 2005).

Bacterial remediation suggests the application of microbial metabolism for the degradation of cyanides. Bacteria such as *Bacillus* sp., *Pseudomonas* sp., *Klebsiella* sp., and *Alkaligenes* sp. oxidize cyanides into CO₂, NH₃, amino acids, and other simple molecules (Nawaz et al., 1989; Olaya-Abril et al., 2019).

Cyanide degradation through phytoremediation is a very slow process and applicable only at cyanide concentrations less than 10 ppm, which in practice is much higher. However, compared to phytoremediation, bacterial cells are less effective in degrading complex

cyanides (Cobos et al., 2015). In general, heavy metals such as Ni, Cu, or Zn are precipitated or absorbed by biofilms, but it was found that particularly all bacteria can't metabolize iron complexes. Plants eliminate complexes more effectively by binding them to phytosiderophores or other stabilizing molecules as described above (VM et al., 2005).

Biological degradation is highly dependent on environmental factors such as pH, temperature, nutrient supply, oxygen level, and initial cyanide concentration, as the main principles of biological degradation are associated with the acclimatization of native bacterial consortia for certain conditions (Nawaz et al., 1989). Bacterial degradation acts at a broad pH range from 5.2 to 10.5, while fungal and plant-based phytoremediation techniques operate at narrow pH ranges of 6–8.5 and 12, respectively. The optimal temperature range may be identified as from 25 °C up to 50 °C, which is the range of mesophilic soil bacteria, which are typically observed to produce cyanide-degrading enzymes. Thus, bacterial remediation does not require as much energy as some physical methods to maintain this temperature range (Nawaz et al., 1989; Sáez et al., 2019). As the name of this paper suggests, the main focus is on the bacterial biodegradation of cyanides. Thus, further sections are dedicated to describing bacterial metabolism and different pathways used by bacteria to utilize cyanides as a nitrogen and sometimes carbon source.

3.2. Metabolism

Generally, 5 different pathways used for cyanide degradation are reported in bacteria (Nawaz et al., 1989). These pathways are either degradative or assimilative, as well as aerobic or anaerobic (VM et al., 2005). Table 1 and Figs. 1–5 evaluate all known bacterial metabolic pathways.

Many bacteria tend to use different pathways in different conditions. The choice of a pathway is dictated by pH, oxygen levels, temperature, metal ions, and nutrient levels. As a result of certain enzymatic reactions, different cyanides found in a medium are converted into amides like formamide, carboxylic acids like formic acid, amino acids, and ammonia (Alvillo-Rivera et al., 2021).

Generally, cyanide-degrading bacteria like *Pseudomonas* sp., *Escherichia coli*, or *Rhodococcus* sp. are alkaliphiles. Thus, they inhabit environments with a pH of more than 9. Such a high value of pH prevents free cyanides from volatilization (Alvillo-Rivera et al., 2021).

3.2.1. Biodegradation of free and inorganic cyanides

Bacterial degradation of free and simple cyanides mainly results in their conversion into CO₂ and ammonia through oxidative metabolic pathways or conversion into formic acid and ammonia via hydrolytic pathways, as presented in Table 1 (Baxter and Cummings, 2006).

Research on the growth of *P. fluorescens* NCIMB 11764 showed that both cyanase and cyanide oxygenases are activated separately and have distinct physiological activities. Their activity and induction are dependent on the source of the cyanide in the medium. Thus, the addition of KCN and NAD(P)H results in the dioxygenase and mono-oxygenase activity, whereas the addition of KCON induces the activity of cyanase (Dorr and Knowles, 1989).

In general, alkali metal cyanide salts are highly susceptible to bacterial degradation (Alcoba et al., 2025; Ibrahim et al., 2015). In 1969, Skowronski & Strobel reported on the ability of *Bacillus pumilus* to decrease the amount of KCN from 5 ppm to 2.5 ppm at a pH of 9 (Skowronski and Strobel, 1969). The most efficient strain of *Rhodococcus* UKMP-5 M showed a degradation rate of 47.78 % (Nallapan Maniyam et al., 2011). Other bacteria reported to degrade KCN are *B. megaterium*, *Pseudomonas acidovorans*, *E. coli* BCN6, *Klebsiella oxytoca*, *P. pseudoalcaligenes* CECT5344, and *Agrobacterium tumefaciens* SUTS1, while *B. stearothermophilus*, *P. putida* were grown in the presence of NaCN (Ibrahim et al., 2015). *Alcaligenes denitrificans* was used for the development of a biocatalyst called CYANIDASE to effectively treat industrial wastewater (Basheer et al., 1992).

Overall, bacterial degradation of free and inorganic cyanides is

Table 1
Main metabolic pathways found in bacteria for cyanide utilization.

Metabolic pathway	Enzyme	Description	End products	Co-factors	Strains	Ref.
Hydrolytic (Fig. 1)	Cyanide hydratase (Fig. 1a)	Mainly a fungal enzyme. Belongs to the lyases family, which cleaves the $\text{C}\equiv\text{N}$ bond. As a result, formamide is formed, which is then decomposed to formic acid and NH_3 by formamide hydratase (Roldán et al., 2021; Dwivedi et al., 2019; Sharma et al., 2019). Act mainly on HCN (Dash et al., 2009).	Formamide	-	<i>Pseudomonas fluorescens</i> <i>Stemphylium loti</i> and <i>Gloeocercospora sorghi</i> <i>Fusarium</i> sp. <i>Fusarium lateritium</i> <i>Pseudomonas</i> sp.	(Dash et al., 2009; Dwivedi et al., 2019) (Sharma et al., 2019). (Ibrahim et al., 2015) (White et al., 1988)
	Cyanidase or cyanide dehydratase (Fig. 1b)	Strictly a bacterial enzyme (Dwivedi et al., 2019). Also acts mainly on HCN (Dash et al., 2009). Directly converts cyanides into formic acid and ammonia (Alvillo-Rivera et al., 2021).	Formic acid and NH_3	-	<i>Pseudomonas stutzeri</i> <i>Alcaligenes xylosoxidans</i> subsp. Denitrificans DF3 <i>Alcaligenes xylosoxidans</i>	(Alvillo-Rivera et al., 2021) (Ingvorsen et al., 1991) (Alvillo-Rivera et al., 2021) (Meyers et al., 1993) (Dwivedi et al., 2019)
	Nitrile hydratase (Fig. 1c)	Catalyzes mainly the reaction of the conversion of primary aliphatic nitriles into their amides (Dwivedi et al., 2019; Ibrahim et al., 2015). Many bacterial isolates of <i>Pseudomonas</i> sp. showed the ability to degrade a wide range of nitriles and withstand high cyanide concentrations up to 50 mM (Nawaz et al., 1989)	Amides	Metal cofactor that binds to the enzyme dimer or tetramer (Baxter and Cummings, 2006; Dwivedi et al., 2019)	<i>Bacillus pumilus</i> C1 <i>Pseudonocardia thermophila</i> , <i>Rhodococcus rhodoclaus</i> , <i>Pseudomonas putida</i> MA113 and <i>P. marginales</i> MA32, <i>Corynebacterium</i> sp., <i>Klebsiella</i> sp., and <i>Rhizobium</i> sp.	
	Nitrilase (Fig. 1d)	Catalyzes the conversion of nitriles into corresponding acids and ammonia (Roldán et al., 2021).	Carboxylic acids and NH_3	-	<i>Rhodococcus rhodococcus</i> J1, <i>Klebsiella oxytoca</i> , <i>R. rhodochrous</i> PA-34, <i>Nocardia</i> sp., <i>Alcaligenes faecalis</i> JM3, <i>Acinetobacter</i> sp. AK226, and <i>Pseudomonas thermophila</i> JCM3095	(Sharma et al., 2019)
	Thiocyanate hydrolase (Fig. 1e)	The enzyme degrades thiocyanates to carbonyl sulfides (RCOS) and ammonia (Katayama et al., 1992; Sorokin et al., 2001; Bezudnova et al., 2007).	COS and NH_3	-	<i>Nocardia</i> sp. <i>Thiobacillus thioparus</i> <i>Methylobacterium thiocyanatum</i> <i>T. thioparus</i> , <i>T. thiocyanoxidans</i> (however, taxonomically it's identical to <i>T. thioparus</i>), and <i>T. denitrificans</i> Some <i>Paracoccus</i> sp. were reported to degrade thiocyanates; however, no actual data for growth on thiocyanates are present	(Dwivedi et al., 2019) (Katayama et al., 1992) (Wood et al., 1998) (De Kruffy et al., 1957) (Sorokin et al., 2001)
Reductive (Fig. 2)	Nitrogenase	Not very common among microorganisms. This enzyme catalyzes the conversion of cyanides into methane and ammonia.	CH_4 and NH_3	Fe or Co (Sharma et al., 2019). Mo may be required for certain strains (Koksunan, 2013)	<i>Klebsiella oxytoca</i> <i>Azotobacter chroococcum</i> N.C. I.B. 8003 <i>A. vinelandi</i>	(Liu et al., 1997) (Kelly, 1968) (Koksunan, 2013)
Oxidative (Fig. 3)	Cyanase (Cyn)	Converts cyanates into CO_2 and ammonia.	CO_2 , NH_3	HCO_3^-	<i>Methylobacterium thiocyanatum</i> <i>Pseudomonas pseudoalcaligenes</i> CECT5344	(Wood et al., 1998) (Cobos et al., 2015; VM et al., 2005; Olaya-Abril et al., 2019; Sáez et al., 2019; Cabello et al., 2018; Wibberg et al., 2014) (Sung and Fuchs, 1992) (Alvillo-Rivera et al., 2021)
	Cyanide monooxygenase (Cynm)	Converts cyanides into cyanates (Roldán et al., 2021, Alvillo-Rivera et al., 2021).	CNO^-	Reduced pterin is a cofactor and NADH or NADPH	<i>Pseudomonas fluorescens</i> NCIMB 11764 <i>P. fluorescens</i> , <i>Trametes versicolor</i> , <i>Phanerochaete</i>	(Kunz et al., 1992) (Sharma et al., 2019)

(continued on next page)

Table 1 (continued)

Metabolic pathway	Enzyme	Description	End products	Co-factors	Strains	Ref.
Substitution/Transfer (Fig. 4)	Cyanide dioxygenase (Cynd)	Directly converts cyanides into ammonia and CO ₂	CO ₂ , NH ₃	NADH or NADPH	<i>chrysosporium</i> , <i>Pleurotus sajor-caju</i> <i>Pseudomonas fluorescens</i> NCIMB 11764	(Kunz et al., 1992)
	Rhodanase (Rho)	Catalyzes the transfer of a sulfur atom from thiosulfate to cyanide (Roldán et al., 2021; Dwivedi et al., 2019).	SO ₃ ²⁻ and SCN ⁻	No cofactor, but requires phosphate, inos, and divalent anions (Dwivedi et al., 2019).	<i>Azotobacter vinelandii</i> , <i>E. coli</i> , <i>Pseudomonas aeruginosa</i> , <i>Ferrobacillus ferrooxidans</i> , <i>Thermobacillus denitrificans</i> , and <i>Desulfotomaculum nitrificans</i> Current data does not mention any bacterial strain to be able to degrade cyanides via this enzymatic reaction	(Alvillo-Rivera et al., 2021; Sharma et al., 2019)
Synthesis (Fig. 5)	Mercaptopyruvate sulfurtransferase (3-MST)	Catalyzes the transfer of the mercapto group of 3-mercapto-pyruvate to cyanide (Dwivedi et al., 2019)	SCN ⁻ and pyruvate	-		n.d.
	β-Cyanoalanine synthase (Fig. 5a)	Catalyzes the conversion of cysteine into β-cyanoalanine (Alvillo-Rivera et al., 2021). Because aspartate and asparagine may be synthesized from β-cyanoalanine, this lyase was found to play an important role in the metabolism of many cyanide-degrading bacteria (Dwivedi et al., 2019).	β-Cyanoalanine and H ₂ S	Pyridoxal phosphate (Sharma et al., 2019)	<i>E. coli</i> , <i>Chromobacterium violaceum</i> , and <i>Bacillus magaterium</i>	(Sharma et al., 2019)
	γ-cyano-α-aminobutyric acid synthase (Fig. 5b)	Catalyzes the transfer of the cyano group on the O-acetyl-L-homoserine, thus converting it into γ-Cyano-α-aminobutyric acid (Gupta 2010). This pathway is very rare and is used by some fungi and <i>C. violaceum</i> to synthesize glutamate and glutamine (Brysk and Ressler, 1970, Omura et al., 2003)	γ-Cyano-α-aminobutyric acid	Pyridoxal phosphate (Dwivedi et al., 2019)	<i>C. violaceum</i> D341 <i>Bacillus stearothermophilus</i> CN3	(Brysk and Ressler, 1970) (Omura et al., 2003)

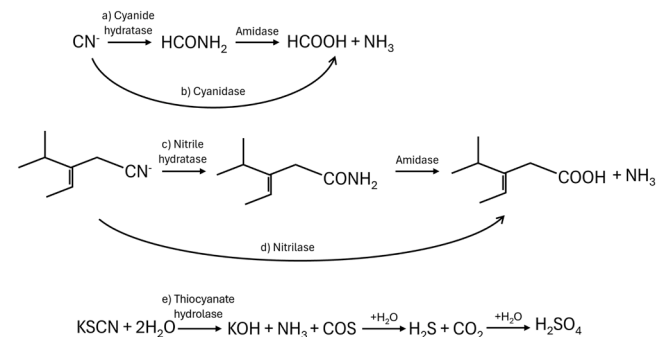


Fig. 1. The general overview of the hydrolytic pathways of microbial degradation.

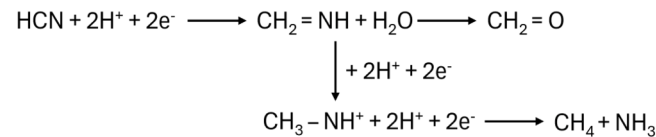


Fig. 2. The general overview of the reductive pathway of microbial degradation.

mainly associated with the action of oxygenases or hydrolases.

3.2.2. Biodegradation of metal-cyanide complexes

Auriferous minerals are made up not only of gold and iron, but also from many heavy metals such as Co, Mo, Cu, Zn, Ag, etc. Thus, the cyanidation results in the formation of several metal complexes with

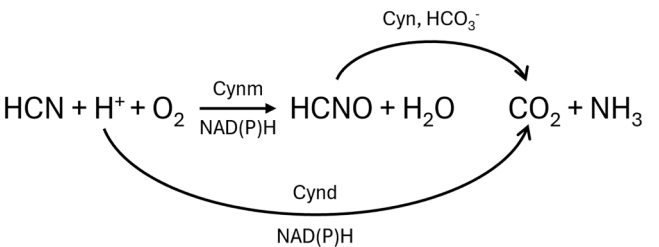


Fig. 3. The general overview of the oxidative pathway of microbial. Abbreviations: Cynm – cyanide monooxygenase; Cynd – cyanide hydratase; NAD(P)H – nicotinamide adenine dinucleotide (phosphate), reduced form.

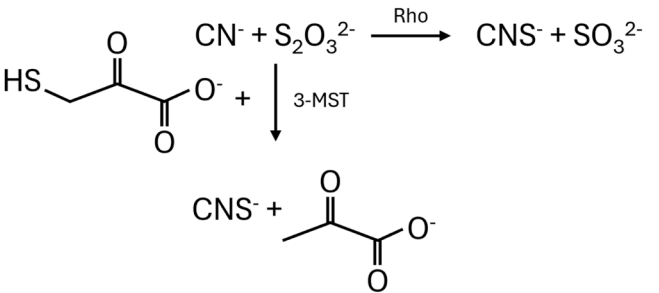


Fig. 4. The general overview of the substitution pathway of microbial degradation. Abbreviations: Rho – rhodanese; 3-MST – 3-mercaptopyruvate sulfur transferase.

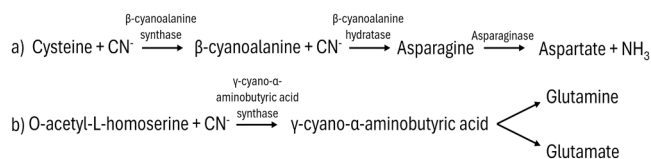


Fig. 5. The general overview of the synthesis pathways of microbial degradation.

cyanides, as well as the creation of cyanates, thiocyanates, and simple cyanide salts, which were mentioned above. Alvillo-Rivera et al. (2021) mention the formation of 72 different complex ions from 28 different d-elements (Alvillo-Rivera et al., 2021). Relative strengths of those complexes decrease in the following trend: Fe < Ni < Cu < Zn.

The degradation of these complexes is limited by the degree of tolerance for the heavy metal ions of microorganisms. Usually, bacteria adapt 3 main mechanisms to tolerate heavy metals (Alvillo-Rivera et al., 2021):

- Cellular compartments that capture and neutralize metal ions.
- Express enzymes that modify the redox state of metal ions.
- Membrane transporters that maintain the outflow of metal ions from the cytoplasm.

Moreover, heavy metals that were extracted from cyanide complexes may be precipitated in the biofilms (Lova-soa et al., 2017). However, bacterial degradation of complex cyanides is a controversial topic since the degradation of complexes varies within the species. For instance, one strain of *Pseudomonas fluorescens* cannot degrade complex cyanides, whereas the majority of strains possess this ability (Baxter and Cummings, 2006). Chen and Kunz (2006) found that cyanide oxygenase of *P. fluorescens* NCIMB 11764 does not act directly on cyanide complex. Instead bacterium releases iron chelating agents, which liberate cyanides, and the oxidation of cyanide ions takes place (Chen and Kunz, 2006). *E. coli*, *Acinetobacter* sp. RBPI and *Burkholderia cepacia* C-3 have been found to degrade several heavy metal complexes (Baxter and Cummings, 2006).

3.2.3. Nitriles biodegradation

Nitriles are catalyzed via 2 main pathways, indicated in Table 1. Bacteria catalyze the degradation of both aromatic and aliphatic nitriles, though they show certain specificity. For instance, *Klebsiella pneumoniae* subsp. *ozaenae* is specific for bromoxynil, benzonitrile, and ioxynil; the nitrile hydratase of *Rhodococcus (Nocardia) rhodochrous* LL100-21 is induced for acetonitrile, while benzonitrile is degraded by nitrilase; *Bacillus pallidus* Dac521 was reported to degrade both acetonitrile and benzonitrile as a sole carbon and nitrogen source (Baxter and Cummings, 2006).

4. 'Cyanomic' analysis of cyanide-containing environments

Recently, the 'omics' technology in biological research has garnered great attention because of its novelty and capacity to provide a holistic and comprehensive overview of biological systems at different molecular levels. 'Omics' technology can be divided into several sectors, such as genomics, metagenomics, proteomics, transcriptomics, and metabolomics, each of which is used to investigate specific organic compounds and interactions in living organisms.

Currently, the microbial diversity and abundance within cyanide-contaminated environments are being explored under *in situ* conditions, and the related information from research was displayed in table form (Table 2). Indeed, metagenomics technology possesses great interest in ecological studies to explore the bioremediative potential of resident microbial associations in contaminated environments, as well as the investigation of microbial abundance and key enzymes involved

in the main biochemical pathways. Thus, the metagenomics analysis is not only a high-throughput approach to understanding the prevalent taxonomical group in environmental samples, some of which are undetectable and unculturable under lab conditions, but also identifies the hypothetical strains in a wide range of biotechnological applications (Offiong et al., 2023; Chettri et al., 2024).

In the current paper, the microbial diversity of cyanide-associated polluted environments sheds light on the optimal taxon group involved in neutralization of cyanidic compounds under *in situ* conditions and reveals the most stress-tolerant and cyanotrophic microorganism groups. It is well established that the whole microbial community before and after cyanide biodegradation may be changed due to the intermediate and end-products' effect on community abundance released throughout biodegradation. For example, shock loading of CN⁻ has shown an inhibitory effect on the biodegradation of SCN⁻ and the nitrification process, as well as on the nitrite-oxidizing bacteria (NOB) and ammonia-oxidizing bacteria (AOB) balance in the composition of activated sludge (Kim et al., 2011).

In the aspect of the present study, cyanomics technology facilitates a comprehensive approach to addressing cyanide biodegradation, which integrates omics and metaomics methodologies to provide a systems-level understanding of the biological processes involved in cyanide microcosm (VM et al., 2016; Roldán et al., 2021). Since the whole genome sequence of *Pseudomonas pseudoalcaligenes* CECT5344, a strain capable of degrading cyanide into simpler compounds, was elucidated, key enzymes, gene clusters, and proteins involved in cyanide transformation and metal tolerance have been identified, providing critical insights into the molecular mechanisms underlying these processes (Wibberg et al., 2014; Cabello et al., 2018). The interplay and correlation among omics technologies related to cyanide degradation, including transcriptomics, genomics, and metabolomics, offer a more detailed and sophisticated framework for research, facilitating their integration into real-world large-scale applications. Thus, the implementation of metagenomic technology to identify effective, predominantly unculturable microbial strains from cyanide-contaminated environments represents a crucial advancement in bioremediation strategies.

During cyanide biodegradation by *Klebsiella oxytoca*, proteomic analysis has been applied to examine the main proteins involved in cyanide biodegradation (Tang et al., 2010). Furthermore, it has been assumed that the 27 proteins found and identified in catalyzing reactions related to nitrogen assimilation pathways (glutamine synthetase), oxidative stress repair (catalase), and protection (neutral trehalase and glycosyltransferase). The proteomic research that are devoted to alkaline bacterial strain *Pseudomonas pseudoalcaligenes* CECT 5344 during cyanide detoxification and heavy metal adsorption has revealed the presence of mercury resistance gene clusters coupled with nitrilase and oxidase enzymes responsible for cyanide conversion, which highlights the comprehensive and multifaceted functions within one microorganism (Bieho et al., 2023). Proteomics in the context of pollutant remediation might be a 'bridge' for uncovering the most potent active enzymes. To better understand the proteins involved in cyanide compounds' breakdown, bioinformatic tools should be integrated together.

5. Optimization of bacterial cyanide elimination

The detoxification of cyanide compounds from tailing wastewaters strictly depends on several physico-chemical and biological factors that collectively may influence the outcome of cyanide bioelimination. The Fig. 6 represents the main set of modulated parameters that may be altered in the biodegradation process to optimize the final yield. In this section, we define the 6 parameters and try to unravel their impact on cyanide removal. Other factors, such as agitation rate, microbiological medium, initial biomass concentration, duration of treatment, application of wild or mutant strain, etc., are not considered in this paper. The chemical forms of cyanide and their impacts on degradation rate have

Table 2

Dominant taxa distribution in cyanide-associated ecosystems and mining areas based on microbial diversity.

Cynide source location	Technique	Prevalence taxa	Key findings	References
Sediment samples collected from the tailing dam (Free State, South Africa).	Illumina NextSeq	<i>Alicyclobacillus</i> , <i>Sulfobacillus</i> , <i>Acidophilum</i> , <i>Paenibacillus</i>	Uncovering the main genomic machinery, key enzymes, and metabolic pathways of cyanide-tolerant microorganisms.	(Welman-Purchase et al., 2024)
Gold mining wastewater sample (Stawell Gold Mine, Western Victoria, Australia)	Illumina HiSeq-2500	<i>Thiobacillus</i> and <i>Gammaproteobacteria</i> dominance in the changing conditions in a lab-scale bioreactor	The discovery of assimilation of different microorganism types in terms of SCN ⁻ biodegradation. Moreover, COSase and CS2 hydrolase genes transfer within the consortium were revealed.	(Watts et al., 2019)
Arsenic-rich polluted wastewater (West Azerbaijan Province, Iran)	Illumina MiSeq	<i>Proteobacteria</i> (8.06-45.49 %), <i>Bacteroidetes</i> (1.85-50.32 %), <i>Firmicutes</i> (1.00–6.2 %), and <i>Actinobacteria</i> (0.86-5.09 %)	Investigation of the main taxonomic groups occurred in the gold mining waste influent.	(Parsania et al., 2024)
Mine wastewater (Consort Plant, South Africa).	Illumina	<i>Thiobacillus</i> spp dominance with SCN ⁻ degradation, sulfide oxidation, C fixation, nitrite denitrification pathways	The detoxification of the microbial community composition of activated sludge in the context of cyanide-related compounds was achieved.	(Huddy et al., 2015)
Cyanide-affected soil samples (La Libertad Region, Peru)	Next-generation Sequencing (NGS)	<i>Alcaligenes</i> , <i>Alkalibacterium</i> , <i>Bacillus</i> , <i>Paenibacillus</i> and <i>Serratia</i>	Comparison of non-contaminated soil and cyanide-influenced soil was conducted.	(Cornejo et al., 2015)
Electroplating wastewater (Cape Town, South Africa)	Illumina MiSeq-2000	<i>Myroides odoratimimus</i> and <i>Proteus</i> sp. distribution in both cyanide and thiocyanate-related microorganisms.	The dominant species among cyanide and thiocyanate-degrading microbiological taxa were observed.	(Mekuto et al., 2017)
Coking plant wastewater (Shanghai, China)	Illumina HiSeq 2000, Roche 454 pyrosequencing	<i>Proteobacteria</i> and <i>Xanthomonadaceae</i>	Putative gene clusters responsible for cyanide tolerance and bioconversion were determined.	(Wang et al., 2015)
Soil samples from the defunct mine (Durban Deep Gold Mine, South Africa)	Illumina MiSeq	<i>Proteobacteria</i> and <i>Actinobacteria</i>	Exploration of microbial diversity in abandoned mines and discovery of functional genes responsible for PAH and toluene biodegradation	(Sibanda et al., 2019)
Soil samples affected gold mining activities (Beijing, China)	Illumina Sequence	<i>Firmicutes</i> , <i>Proteobacteria</i> , and <i>Actinobacteria</i>	Characterization of microbial community in gold mining tailings, as well as mercury-tolerant strains	(Ji et al., 2018)
Vanadium and gold mining wastewater samples (Mpumalanga and Gauteng, South Africa)	Illumina MiSeq	<i>Gammaproteobacteria</i> , <i>Alphaproteobacteria</i> , and <i>Bacteroides</i>	Determination of the most abundant phylum in 2 different mines.	(Keshri et al., 2015)
Tailing sediments from the tailing dam (Madhya Pradesh, India)	16S rRNA sequencing technology	<i>Proteobacteria</i> , <i>Firmicutes</i> , and <i>Actinobacteria</i>	Investigation of the copper mine tailings microbiological community and pH influence on structure and composition.	(Gupta et al., 2017)
Water sample from the tailing area (Shuimuchong copper tailing, China)	Roche 454 pyrosequencing technology	<i>Proteobacteria</i> , <i>Actinobacteria</i> , <i>Euryarchaeota</i> , <i>Firmicutes</i> , and <i>Nitrospirae</i>	The pH-dependent microbial diversity structure exploration in mine tailings	(Liu et al., 2014)
Enriched media for cyanide and thiocyanide degrading microorganisms, amended with water samples from the electroplating point (Western Cape, South Africa)	Illumina MiSeq-2000	<i>Proteobacteria</i> and <i>Bacteroides</i> (<i>Myroides odoratimimus</i> , <i>Proteus vulgaris</i>)	Co-occurrence of an increased biodegradation efficacy of CN ⁻ and SCN ⁻ was reached under simultaneous growth conditions by a mixed consortium.	(Mekuto et al., 2018)
Water slurry samples (Stawell Gold Mine in Victoria, Australia)	Illumina MiSeq	<i>Gammaproteobacteria</i> , <i>Alphaproteobacteria</i> , and <i>Betaproteobacteria</i> <i>Chlamydomonas</i> from eukaryotic taxa	Investigation of SCN ⁻ removal efficiency by the <i>in situ</i> growth enriched with phosphate.	(Watts et al., 2017)
Mineral solid samples from mines (Gold Fields, South Africa)	Illumina technology	<i>Sphingobacteriales</i> , <i>Bacteroidetes</i> and <i>Flavobacteriia</i> <i>Thiobacillus</i> as a main thiocyanate consumer	Activation and exploration of microbial diversity, abundance in solid samples to uncover the thiocyanate detoxification.	(Rahman et al., 2017)
Water samples from the operating gold mine heap leach (Yakutia Republic, Russia)	Pyrosequencing 454	<i>Proteobacteria</i> , <i>Actinobacteria</i> , <i>Firmicutes</i> , and <i>Bacteroidetes</i> <i>Comamonadaceae</i> (25.6 %), <i>Alcaligenaceae</i> (24.2 %) and genera <i>Serratia</i> (43.5 %), <i>Malikia</i> (1.8 %), <i>Bordetella</i> (1.6 %), and <i>Silanimonas</i> (1.4 %)	<i>Serratia</i> , <i>Comamonadaceae</i> , and <i>Alcaligenaceae</i> are presumed for the cyanide degradation process in gold heap leach.	(Belykh et al., 2017)
Operating heap leach point (the Krasnoyarsk region, Russia)	16 s rRNA-based technology	<i>Gammaproteobacteria</i> (<i>Serratia</i> sp., 64.9 % and <i>Achromobacter</i> sp., 21.6 %)	Research on microbial diversity in gold heap resistant to CN ⁻ and SCN ⁻ was conducted.	(Belykh et al., 2015)
Roadside soils treated with deicing salt containing ferrocyanide (North Rhine-Westphalia, Germany)	454 pyrosequencing technology	<i>Actinomycetales</i> (<i>Kineosporia</i> , <i>Mycobacterium</i> , <i>Micromonosporaceae</i>) dominance over other taxonomic groups in treated soil	High dissimilation of ¹³ C-labeled molecules integrated in ferrocyanide leads to microbial shift and structural composition, as evidence of biodegradation of ferrocyanide in contaminated soil.	(Gschwendtner et al., 2016)

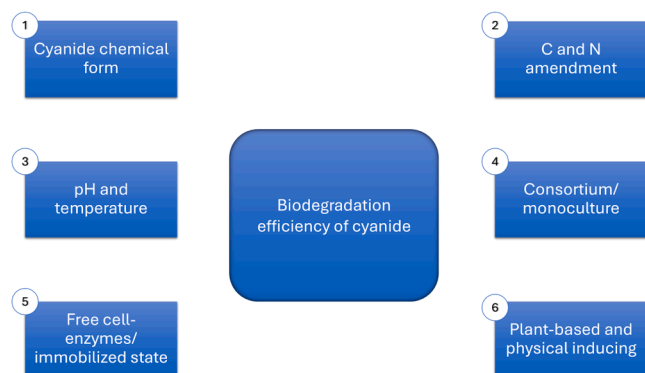


Fig. 6. A set of different parameters that impact cyanide biodegradation by microbial approaches in the framework of the present review.

already been discussed in the 1.1 and 3.2 sections.

5.1. C and N amendment/pH and temperature

Even for strains isolated from cyanide-polluted environments, low concentrations of cyanide can be inhibitory or lethal. To overcome this obstacle, most research applies different forms of organic carbon and nitrogen as stimulants. Organic carbon and nitrogen sources in the medium containing toxic cyanide can promote microorganisms' growth at the initial lag phase, consequently enhancing the biodegradation efficiency.

It is well established that the pH level of the environment directly affects the enzyme activity as well as the viability of living cells. Moreover, the kinetics of cyanide degradation strictly depend on physical factors, which can be observed in versatile products formation (Oulego et al., 2013). The amendment of different sources of N and C in conjunction with pH and temperature alteration in the framework of specific strain growth conditions may lead to combinations that are mostly applied to construct the RSM model (Jiang et al., 2022). RSM model provides various combinations of factors included in cyanide biodegradation: N and C sources with different concentrations, aeration rate, temperature, pH, initial concentration of cyanide, etc. Then the hypothetical biodegradation rate might be compared to experimental (Alvillo-Rivera et al., 2023). Taken together, the optimal pH varied 7–10, temperature 28–30 °C for cyanide degrading microorganisms. Among carbon sources, 1 % glucose is more favourable; however, the elevated concentration might be a competitive substrate for cyanide, decreasing the cyanide removal rate. Similarly, yeast extract is applied from an organic nitrogen source for growth activation at the lag phase. However, not all exogenous organic supplements could enhance the degradation rate. *Azotobacter vinelandii* nitrogen-fixing bacterium, is tested for tetracyanonickelate removal, and results showed ammonium and nitrite decreased at a rate compared to glucose (Kao et al., 2005). Watts and his colleagues (2017) have demonstrated the possibility of thiocyanate biodegradation under *in situ* conditions by phosphate addition (Watts et al., 2017). Another case where the addition of methanol as an organic carbon source in a batch bioreactor scale experiment for complete bioconversion of nitrates and ammonia nitrogen by the nitrification-denitrification reaction chain is presented (Tanabene et al., 2018). The study has indicated that activated carbon could enhance phenol and cyanide removal in bioreactor systems (Papadimitriou et al., 2009).

5.2. Consortium/monoculture

Constructing a consortium comprising biocompatible strains of microorganisms for biotechnological purposes has gained great attention due to its efficacy and rapid growth dynamics during co-culturing

compared to monoculture. In the case of cyanide biodegradation occurring in tailing wastewater systems, numerous research studies have been conducted specifically on the application of consortia to achieve more optimal results for biodegradation. Cyanide effluents can contain toxicants with different origins beyond free cyanide, such as cyanide-metal complexes, phenolic compounds, thiocyanates, etc. Ferrocyanide and free cyanide have been utilized during co-fermentation of *Exiguobacterium acetylicum* and *Bacillus marisflavi* strains, and results showed that complete degradation of the 2 toxicants can occur only in the presence of the consortium at 99 % for both contaminants. In research, *Exiguobacterium acetylicum* is responsible for SCN^- degradation but not for CN^- , while CN^- is converted by *Bacillus marisflavi*, but SCN^- concentration remains unchanged, highlighting the necessity the integration of integrating the bacterial 2 strains for a multi-remediation strategy in wastewater (Mekuto et al., 2016). Another research of these scientists has been conducted on the consortia consisting of 13 strains of the *Bacillus* genus to investigate cyanide biotransformation, followed by nitrification of by-products, basically ammonium (Mekuto et al., 2015). The co-occurrence of mixed culture growth stimulated by milk whey underscores the 99 % biodecomposition of 500 mg/l free cyanide. Approving the need for a consortium in the framework to degrade the phenol and cyanide substances simultaneously was observed in Combarros et al. (2016) research (Combarros et al., 2016). Synergistic relationships between *Paracoccus thiocyanatus* and *Pseudomonas putida* strains were analyzed in a salicylic acid and thiocyanate biodegradation study, and according to the results, each strain is capable of hydrolyzing specific compounds at a specific concentration. Such studies mostly prove the unattainability of either strain alone to degrade 2 or more complex pollutants within effluents.

The symbiosis interaction between bacterial strains is not only studied consortium example in the cyanide biodegradation process, but also in other taxonomical groups and domains, constructed between diverse strains may be a key factor in optimal cyanide and cyanide-associated compounds detoxification. Hetero-domain mixed culture, including both bacterial and algal species, has also displayed an alternative technique in cyanide biodegradation. For instance, *Bacillus licheniformis* MMT1 and *Chlorella* spp. have demonstrated the concurrent neutralisation of KCN, sodium salicylate, benzonitrile, and KSCN in a continuous stirred photobioreactor (AbdelMageed and ElRakaiby, 2025). Furthermore, the final biological treated wastewater led to the growth of garden cress, revealing no inhibitory effects on seeds compared to the untreated one in the phytotoxicity bioassay analysis, showing the complete neutralisation of pollutants by the heterogeneous consortium. Thus, the researchers are not only proving the importance of biocompatible mixed culture application in water remediation but also have suggested that the biomass after post-treatment may be harvested to biotechnological purposes. Although *C. vulgaris* is sensitive to KCN itself, which has emerged as a necessity to construct a consortium (Liu et al., 2018).

Simultaneous implementation of cyanotrophic microorganisms in the mixed form, multiple biotechnological directions have been documented in several studies. For example, *Enterobacter* sp., *Yersinia* sp., and *Serratia* sp., salt-tolerant strains, were applied in the denitrification of ammonium under cyanogenic conditions, possessing a universal tool to achieve the following aims: 1) reclamation of saline environments; 2) denitrification and benification of soil for plants; 3) cyanide detoxification (Mpongwana et al., 2016) reflecting a potential tool in a bioremediation strategy. A complex of symbiotic strains capable of biotransforming diverse pollutants simultaneously is a novel biotechnological strategy in multifaceted bioremediations. Same research conducted by Stott et al. where *Halomonas* and *Thiobacillus* strains are able to degrade thiocyanate, arsenopyrite, and pyrite in consecutive batch systems under a saline environment (Stott et al., 2001).

Activated sludge derived from polluted points is applied to degrade cyanide and other toxicants after industrial activities, including cyanide.

The naturally activated sludge is rich in miscellaneous microorganisms with complex metabolic activity, which is fit to transform several compounds simultaneously. Kubsad et al. (2011) have highlighted the potency of activated sludge to degrade cyanide, acrylonitrile, and acetonitrile with a shock loading study (Kubsad et al., 2011), whereas Kaewkannetra et al. (2009) have demonstrated the potential of *Azotobacter vinelandii* TISTR 1094 strain to neutralize cyanide in the activated sludge with resident microbiota (Kaewkannetra et al., 2009).

5.3. Free cell-enzymes/immobilized state

Recently, immobilization of living microorganisms has been recognized as an innovative technique in a wide range of biotechnology aspects. Up to now, in the cyanide removal issue, the fixation of living microorganism cells into carriers can be an effective tool, prolonging their vitality and effectiveness compared to free cell culture (Fig. 7). During the cyanide removal process, fixed microorganism cells on sorbents have a prolonged viability and stability because of the mechanical protection from secondary metabolites of microorganisms or intermediate, end-products occurring in simultaneous biochemical processes. Additionally, the reusability of immobilized systems makes this technology more attractive from a practical angle than a free cell system.

However, even with the optimization of sorbents for cells to degrade, it should be highlighted that some polymerization agents in the immobilization process may inhibit enzymatic activity or kill cells at elevated concentrations (Kowalska et al., 1998). The proper ratio of living microorganism cells and immobilization sorbent is crucial in terms of a biotechnological approach, specifically in cyanide degradation, because excessive cells can interfere to immobilization due to agglomeration formation (Zhou et al., 2007). Immobilization of active enzymes or free cells is biotechnologically relevant in the tailing wastewater bioremediation, however, it is urgent to select appropriate carriers that are chemically inert and at the same time compatible with biological objects (Bouabidi et al., 2019). Another aspect that must be taken into account during immobilized cells construction is related to defining the initial cell dosage; otherwise, the overcrowding of cells and uncontrolled growth can interfere with the efficiency.

Except for the immobilization of whole cells of microorganisms, the immobilization of enzymes responsible for cyanide removal has been documented (Ademakinwa et al., 2021; Itakorode et al., 2023; Bodzek et al., 1996). Crude extract fixed on gellan gum of *Rhodococcus* UKMP-5M strain has shown 361 % efficiency compared to the free extract (Maniyam et al., 2019). In a one and two-column system, immobilized *Methylobacterium* sp. RXM CCM 908 and *Fusarium oxysporum* CCM 876 cells have demonstrated consecutive cyanide and intermediate products degradation (Campos et al., 2006). Besides the cyanide detoxification, fixed enzymes are also applied in biosensor systems for the detection of cyanide in the environment (Kumar et al., 2018; Mak et al., 2005).

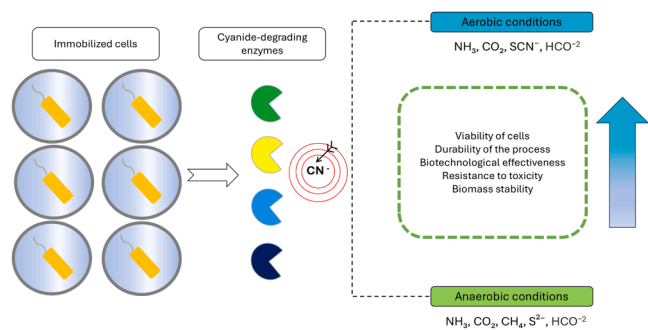


Fig. 7. Cyanide biodegradation by immobilized cells and consequent terminal products formation from aerobic and anaerobic conditions. Different colors of enzymes represent the presence of putative biochemical pathways.

Immobilization of cyanide-degrading agents on the sorbents to enhance the cyanide degradation rate is not a new technology, but challenges and obstacles, including operational cost, lack of standardization, complex wastewater composition, to scaling up to an industrial level, require further investigation.

5.4. Plant-based and physical inducing

The electro-induction, coupled with the microorganisms in the context of cyanide neutralization, provides an alternative modification compared to biological treatment due to the low operational cost and efficacy (Ojaghi et al., 2018). In their study, the *Bacillus pumilis* ATCC 7061 bacterial strain has shown a 99 % cyanide degradation rate with a 500 mg/l initial concentration in the electro-biodegradation cell, whereas a 200 mg/l initial concentration was toxic and had an inhibitory effect on the growth of the bacterial culture in traditional biodegradation. Acceleration of the biodegradation rate is accompanied by rapid bacterial growth over time. Taking into account that microbial fuel cells are a universal approach for versatile purposes, it is pivotal to broaden the investigation related to cyanide bioconversion coupled with energy production (Mathuriya, 2014). Unfortunately, the information about physical inducing is scarce and limited, underscoring the need to explore the current research area.

Plant-microorganism interaction within cyanide biodegradation is a unique and underexplored topic that should be investigated. Despite the sophisticated mechanisms underlying interactions between microorganisms and plants, specifically during exposure to xenobiotics, we identified 3 types of relationships in the plant-microorganisms system that occur in cyanide detoxification from the present literature review: 1) plant growth enhancement, 2) plant waste management, and 3) functional food production.

The microcosm experiment containing *Trichoderma* and *Fusarium* strains was conducted under cyanide conditions to germinate pea and wheat seeds (Ezzi and Lynch, 2005). Results have shown that 50 mg/l and 100 mg/l of cyanide significantly inhibit plant growth, but the addition of fungal species can enhance growth parameters compared to the control, assuming the growth induction by cyanide breakdown in fungi/plant association. Therefore, cyanide-polluted soils might be remediated via microorganisms, and the terminal products provide plant growth stimulation.

Recently, high efficiency in cyanide-containing wastewater biodegradation has been achieved by applying plant-based waste materials supplemented by microorganisms. Plants and their agrochemical wastes have been widely used in cyanide elimination from wastewater by the simple chemisorption principle because of an eco-friendly and low-cost approach (Moussavi and Khosravi, 2010). However, the absorbed cyanide and other toxic compounds remain in the plants after post-treatment, threatening the environment and creating secondary pollution. Under these circumstances, combining plant waste and cyanide-removing microorganisms in a cyanide degradation system can be an interesting field for investigation, despite the scarce information and understanding related to a combination of cyanide-degrading microorganisms with plant leftovers. Usually, the cassava waste threatens the health and environment due to toxic compounds (Mendes et al., 2024). In the given context, Ogunyemi et al. (2019) have reported the cassava wastewater and solid waste bioconversion upon isolated bacterial strains using cassava wastes as a natural growth substrate. During the process, glutaronitrile and linamarin, present in cassava, undergo hydrolysis and minimizing the hazardous toxic compounds in the discharge (Ogunyemi et al., 2019).

It must be noted that the researchers have constructed a biopolymer involving *F. oxysporum* and beetroot leftovers, remaining interesting for cyanide degradation, even coupled with the addition of highly toxic heavy metals to living cells, such as Cu, As, and Pb (Akinpelu et al., 2016). Indeed, the inhibitory effect of cyanide in the co-fermentation of the strain with the plant may be decreased due to the adsorption on the

plant surface, leading to the formation of a conducive state for bioconversion. Thus, the described approach of cyanide detoxification can be a promising tool for biotechnological applications, agricultural, and gold mining industries' waste management, accompanied by low operational cost and an eco-friendly approach. Briefly, the hypothetical interplay and synergy between microorganisms and plant agrowaste upon cyanide biodegradation in wastewater have been illustrated in Fig. 8.

Moreover, plants, besides enhancing biodegradation rate coupled with microorganisms, can be applied to synthesize some food products during the co-fermentation of specific strains, accompanied by cyanogenic compound biodegradation, specifically cyanogenic glucosides. For instance, Banwo et al., (2023) have proved the credibility of the application of *Pediococcus pentosaceus* OG08 and *Pichia exigua* OG4 consortium in probiotic production as a starter culture and as a cyanide-processing strain in the simultaneous bioelimination of cyanide-containing cassava effluent concurrently (Banwo et al., 2023). In the production of sugar syrup obtained from mume fruit fermentation, cyanogenic glucoside amygdalin from mume fruit has been converted into a sugar syrup by the *Zygosaccharomyces rouxii* fungi strain presented in the starter culture (Ramalingam et al., 2022). Therefore, the hydrolysis of cyanogenic compounds in plants by microorganisms capable of synthesizing specific enzymes is responsible for food functional processing.

6. Limitations

Over the past few decades, microbiological biodegradation of cyanide in tailing wastewater has been studied after industrial activities. Cyanide removal technology based on xenobiotic-resistant and degrading strains with the ability to use cyanide as a nitrogen and carbon source possesses an eco-friendly and cost-effective approach. Nevertheless, there are distinct drawbacks to cyanide biodegradation by microorganisms.

- 1) **Optimal substitution for cyanide in metallurgy:** Researchers have recommended using less toxic substitutes instead of cyanide in gold recovery, such as thiourea, thiocyanate, thiosulphate, coal-oil agglomeration, halides, etc (Hilson and Monhemius, 2006).
- 2) **Toxicity of produced terminal products:** The terminal end-products of cyanide biodegradation might inhibit the microorganisms' growth and decrease the efficiency, especially when a microbial consortium was used, where interspecies interaction is highly sensitive to environmental factors. Moreover, not all forms of cyanide are degraded completely by microbial treatment.
- 3) **Prolonged treatment time:** Compared to traditional physico-chemical methods, biological degradation is time-consuming. Especially, if the process occurred in batch bioreactors, where growth kinetics and

phases are slower. Furthermore, the formation of a clog from a thick biomass layer is problematic for bioreactor maintenance.

- 4) **Operational sensitivity:** Applied operational parameters such as pH, temperature, agitation rate, end-products concentration, etc., might be altered and lead to a decrease in biodegradation efficiency, highlighting the constant monitoring.
- 5) **Strain-specific limitation:** An integrated strain's proliferation in natural conditions can be inhibited by wild types or physical factors. Additionally, competition between microorganisms might negatively influence biodegradation efficiency.
- 6) **Scaling and cost issues:** While research reaches the ideal outcomes in the lab-scale, in the industrial field, scaling up may be challenging and costly. Also, there is no common standardization and monitoring system in field-scale cyanide elimination.

7. Future prospects

The obstacles in the cyanide biodegradation should be resolved by optimization or a hybridization technique. Overall, the future direction of the current research area has potential and interest despite drawbacks. During oxidative pathways, with the participation of cyanide monooxygenase cofactor named pterin is produced. Pterin substances have therapeutic and medical properties, they are widely found in living organisms in the form of folic acid, biopterin, and neopterin, etc. (Murugesan et al., 2018; Mahendran et al., 2020). Nevertheless, the direct isolation of pterin from cyanide detoxification has not been documented yet, opening new frameworks for further investigation.

Cyanide-associated microorganisms could be used as a cyanide producer for gold recovery in the biometallurgy area, except for biodegradation (Aitimbetov et al., 2005; Campbell et al., 2001; Vakili-chap et al., 2020). Additionally, gold recovery can be reached based on the e-waste treatment by *Chromobacterium violaceum* up to 22.5 % (Natarajan and Ting, 2014). Combined methods can enhance gold recovery using *Corynebacterium glutamicum* on decarboxylated biosorbent (DCB), removing carboxyl groups that interfere with the binding sites for bioabsorption (Kwak and Yun, 2010). Besides, the valuable metals capture and following utilization of mining wastewater can be processed by 2 groups of microorganisms. The bioleaching may be done by cyanide producing strains whereas mining wastewater should be treated with xenobiotic compounds degrader strains comprising cyanide, phenolic compounds and heavy metals.

Mostly, cyanide-tolerant strains are capable of converting other pollutants, such as heavy metals or phenols, into less toxic products (Nermin and Isil, 2016). In this context, some strains are described as metal sequestrators during cyanide degradation. *Aspergillus fumigatus* fungi is noted for Ag, Cu, and Ni resistance and bioadsorption (Sabatini et al., 2012). Similar 2 strains of *Fusarium* were detected as a metal-cyanide degrader (Barclay et al., 1998). Some cyanogenic strains have been described as metal solubilizers during cyanide production with glycine addition (Faramarzi et al., 2004). Currently, co-biodegradation mechanisms of several heavy metals with cyanide in mining wastewaters by microorganisms is under investigation due to the practicability in large-scale (Olaya-Abril et al., 2024).

Cyanobacterial anaerobic fermentation has garnered great attention because of biogas and diverse metabolite production. Motsoeneng et al. have reported the synthesis of biogas as a value-added compound for medical purposes (Motsoeneng et al., 2023). The anaerobic fermentation of brewery wastewater supplemented with 5 mg/l of cyanide led to the formation of biogas in an upflow anaerobic sludge blanket, at the same time solving 3 problems at once: waste management, cyanide degradation, and biogas synthesis. Interestingly, 8 mg/l of cyanide has an inhibitory effect on biogas formation, indicating that 5 mg/l is more favorable for diverse microorganisms to convert cyanide into methane (Novak et al., 2013). Present research highlights the applicability and practicality of applying microbial communities in terms of cyanide degradation under anaerobic conditions. Not only can resident whole

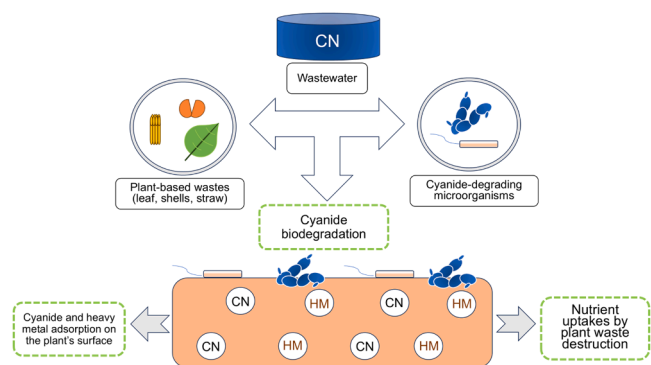


Fig. 8. Schematic representation of a heterogeneous biopolymer's performance based on plant waste and microorganisms in the cyanide biodegradation process. CN – cyanide, HM – heavy metal.

community be involved in biogas production during cyanide biodegradation, but also some isolated strains have the potential to neutralize cyanide, followed by gas production as an end-product. For instance, the *Azotobacter chroococcum* MTCC 446 strain is capable of transforming cyanide into methane via the reductive pathway, as well as phenol degradation occurs with the meta-pathway (Mondal and Balomajumder, 2013). Thus, cyanide microbiological breakdown serves in different ways in biotechnology, providing a promising tool for optimal tailing wastewater treatment.

Combination of physical/chemical methods with biological to resolve the cyanide contamination have applied, albeit knowledge and case studies are scarce. Mostly, researchers have constructed a heterogeneous biopolymer based on the plant with chemical adsorption and active microorganisms strains (Dash et al., 2009; Pathak et al., 2019), which can not be classified as a combination of biological with physical methods. Unfortunately, combination oftenly has found within physical and chemical methods rather than biological (Vashishtha et al., 2024; Morillo Esparza et al., 2019).

To further implementation of biological approach to cyanide biodegradation in large-scale definitely following obstacles should be overcome:

- Low degradation rates under high cyanide loads in industrial settings.
- Strain stability and survivability under fluctuating environmental conditions.
- Scalability of lab-scale microbial systems to industrial bioreactors.
- Integration issues with existing physico-chemical treatment infrastructure.
- Economic feasibility, including operational cost-benefit analysis compared to chemical methods.

8. Conclusion

To sum up, tailing wastewater – especially from gold mining industries – poses a considerable threat to both environment and public health, underlining the urgent need for effective treatment before discharge. Among toxic pollutants, cyanide and its derivatives are of particular concern. Although physico-chemical methods such as alkaline chlorination or ozonation are widely applied, their operational costs, energy requirements, and potential generation of secondary pollutants limit their sustainability. In contrast, microbial biodegradation emerges as a promising alternative due to its ecological compatibility and relatively lower cost. Various microorganisms can metabolize cyanide through four primary enzymatic pathways – hydrolytic, oxidative, reductive, and substitution – leading to its transformation into less harmful compounds like formamide, ammonium, and carbon dioxide. Advances in omics technologies, collectively referred to as “cyanomics”, have enhanced our understanding of the underlying genetic, enzymatic, and metabolic mechanisms of cyanide degradation, including insights into unculturable microbial populations. Moreover, recent innovations in bioprocess optimization – such as microbial consortium engineering, immobilization systems, and plant-based co-treatment strategies – have expanded the feasibility of applying this approach in field conditions. However, industrial-scale application remains limited by challenges such as long degradation times, sensitivity to environmental fluctuations, and scalability concerns. Therefore, further research should focus on validating the economic feasibility of these systems through techno-economic analyses, evaluating operational performance under real-world conditions, and dimensional scaling of bioreactors. While not yet universally applicable at large scales, microbial cyanide biodegradation continues to be a viable and evolving approach that merits deeper investigation and investment.

CRedit authorship contribution statement

Ainur Berkinbayeva: Writing – review & editing, Writing – original draft, Visualization, Investigation, Conceptualization. **Bagdaulet Kenzhaliyev:** Writing – review & editing, Writing – original draft, Investigation, Data curation. **Kenzhegali Smailov:** Writing – review & editing, Writing – original draft, Visualization, Data curation. **Alan Aimagambetov:** Writing – review & editing, Writing – original draft, Project administration, Conceptualization. **Bekzat Kamenov:** Writing – review & editing, Project administration, Data curation, Conceptualization. **Shynar Saulebekkyzy:** Writing – review & editing, Project administration, Conceptualization. **Nazerke Tolegenova:** Writing – review & editing, Data curation, Conceptualization. **Primandari Sri Rizki Putri:** Investigation, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

This research was funded by the Science Committee of the Ministry of Science and Higher Education of the Republic of Kazakhstan (Grant No. AP23488663).

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at [doi:10.1016/j.wroa.2025.100400](https://doi.org/10.1016/j.wroa.2025.100400).

Data availability

Data will be made available on request.

References

- AbdelMageed, M.F., ElRakaiby, M.T., 2025. Algal–bacterial bioremediation of cyanide-containing wastewater in a continuous stirred photobioreactor. *World J. Microbiol. Biotechnol.* 41 (2), 26. Feb 9.
- Ademakinwa, A.N., Agunbiade, M.O., Fagbohun, O., 2021. Biodegradation of cyanide in cassava wastewater using a novel thermodynamically-stable immobilized rhodanese. *Prep. Biochem. Biotechnol.* 51 (6), 607–617.
- Aitimbetov, T., White, D.M., Seth, I., 2005. Biological gold recovery from gold-cyanide solutions. *Int. J. Miner. Process.* 76 (1–2), 33–42. Apr 4.
- Akinpelu, E.A., Karabo, S., Ntwampe, O., Mpongwana, N., Nchu, F., Ojumu, T.V., 2016. Biodegradation Kinetics of Free Cyanide in *Fusarium oxysporum*-Beta vulgaris Waste-metal (As, Cu, Fe, Pb, Zn) Cultures under Alkaline Conditions, 11. *BioResources*.
- Alcoba, Y.Y.D., Ferreira, M.L., Vullo, D.L., 2025. Effective thiocyanate biodegradation mediated by bacteria associated to Argentinean mining areas potentially applicable to wastewater biotreatment. *Clean Technol. Environ. Policy* 27 (4), 1693–1705. Apr 13.
- Alvillo-Rivera, A., Garrido-Hoyos, S., Buitrón, G., Thangarasu-Sarasvathi, P., Rosano-Ortega, G., 2021. Biological treatment for the degradation of cyanide: a review. *J. Mater. Res. Technol.* 12, 1418–1433. Elsevier Editora Ltda.
- Alvillo-Rivera, A.J., Garrido-Hoyos, S.E., Rosano-Ortega, G., 2023. Optimization of factors for the biological treatment of free and complexed cyanide. *Processes* 11 (7), 2063. Jul 11.
- Banwo, K., Tosin Ojetunde, J., Falade, T., 2023. Probiotic and cyanide degrading potentials of *Pediococcus pentosaceus* and *pichia exigua* isolated from Cassava products effluent. *Food Biotechnol.* 37 (1), 1–24. Jan 2.
- Barclay, M., Hart, A., Knowles, C.J., Meeussen, J.C.L., Tetdl, V.A., 1998. Biodegradation of metal cyanides mixed and pure cultures of fungi.
- Basheer, S., Kut, O.M., Prenosil, J.E., Bourne, J.R., 1992. Kinetics of enzymatic degradation of cyanide. *Biotechnol. Bioeng.* 39 (6), 629–634. Mar 15.
- Baxter, J., Cummings, S.P., 2006. The current and future applications of microorganism in the bioremediation of cyanide contamination. Vol. 90, *Antonie van Leeuwenhoek. Int. J. General Mol. Microbiol.* 1–17.
- Belykh, M.P., Petrov, S.V., Chikin, A.Y., Voiloshnikov, G.I., Belkova, N.L., 2017. Detoxification of heap after gold leaching using biodegradation. *Solid State Phenomena. Trans Tech Publications Ltd*, pp. 587–590.

- Belykh, M.P., Petrov, S.V., Petrov, V.F., Chikin, A.Y.u., Belkova, N.L., 2015. Screening and isolation of bacterial strains with the biotechnological potential to destruct cyanide-bearing compounds under the conditions of extreme continental climate. *Adv. Mat. Res.* 1130, 19–22. Nov.
- Bezudnova, E.Y.u., Sorokin, D.Y.u., Tikhonova, T.V., Popov, V.O., 2007. Thiocyanate hydrolase, the primary enzyme initiating thiocyanate degradation in the novel obligately chemolithoautotrophic halophilic sulfur-oxidizing bacterium *thiohalophilus thiocyanoxidans*. *Biochim. Biophys. Acta (BBA) - Prot. Proteom.* 1774 (12), 1563–1570. Dec.
- Biello, K.A., Olaya-Abril, A., Cabello, P., Rodríguez-Caballero, G., Sáez, L.P., Moreno-Vivián, C., et al., 2023. Quantitative proteomic analysis of cyanide and mercury detoxification by *Pseudomonas pseudoalcaligenes* CECT 5344. *Microbiol. Spectr.* 11 (4), Aug 17.
- Bodzek, M., Bohdziewicz, J., Gorzata Kowalska, M., 1996. Immobilized enzyme membranes for phenol and cyanide decomposition. *J. Memb. Sci.* 113.
- Bouabidi, Z.B., El-Naas, M.H., Zhang, Z., 2019. Immobilization of bacterial cells for the biotreatment of wastewater: a review. In: *Environmental Chemistry Letters*, 17. Springer Verlag, pp. 241–257.
- Brysk, M.M., Ressler, C., 1970. γ -cyano- α -l-aminobutyric acid. *J. Biol. Chem.* 245 (5), 1156–1160. Mar.
- Cabello, P., Luque-Almagro, V.M., Olaya-Abril, A., Sáez, L.P., Moreno-Vivián, C., Dolores Roldán, M., 2018. Assimilation of cyanide and cyano-derivatives by *Pseudomonas pseudoalcaligenes* CECT5344: from omic approaches to biotechnological applications. In: *FEMS Microbiology Letters*, 365. Oxford University Press.
- Cabello, P., Luque-Almagro, V.M., Olaya-Abril, A., Sáez, L.P., Moreno-Vivián, C., Dolores Roldán, M., 2018. Assimilation of cyanide and cyano-derivatives by *Pseudomonas pseudoalcaligenes* CECT5344: from omic approaches to biotechnological applications. In: *FEMS Microbiology Letters*, 365. Oxford University Press.
- Campbell, S.C., Olson, G.J., Clark, T.R., Mcfeters, G., 2001. Biogenic production of cyanide and its application to gold recovery [Internet]. *J. Ind. Microbiol. Biotechnol.* 26.
- Campos, M.G., Pereira, P., Roseiro, J.C., 2006. Packed-bed reactor for the integrated biodegradation of cyanide and formamide by immobilised *fusarium oxysporum* CCMi 876 and *methylobacterium* sp. RXM CCMi 908. *Enzyme Microb. Technol.* 38 (6), 848–854. Apr 1.
- Chen, J.L., Kunz, D.A., 2006. Cyanide utilization in *Pseudomonas fluorescens* NCIMB 11764 involves a putative siderophore. *FEMS Microbiol. Lett.* 156 (1), 61–67. Jan 17.
- Chettri, D., Verma, A.K., Chirania, M., Verma, A.K., 2024. Metagenomic approaches in bioremediation of environmental pollutants. *Environ. Pollut.* 363, 125297. Dec.
- Cobos, I.M., Ibáñez García, M.I., Moreno, F.P., Sáez Melero, L.P., Luque-Almagro, V.M., Rodríguez, F.C., et al., 2015. *Pseudomonas pseudoalcaligenes* CECT5344, a cyanide-degrading bacterium with by-product (polyhydroxyalkanoates) formation capacity. *Microb. Cell Fact.* 14 (1).
- Combarros, R.G., Collado, S., Laca, A., Díaz, M., 2016. Understanding the simultaneous biodegradation of thiocyanate and salicylic acid by *Paracoccus thiocyanatus* and *Pseudomonas putida*. *Int. J. Environ. Sci. Technol.* 13 (2), 649–662. Feb 1.
- Cornejo, M., Pretell, K., Arévalo, A., Sernaqué, Y., Mialhe, E., 2015. Culture/Co-Culture dependent and independent identification of bacterial communities along a chronosequence of spontaneous reclamation on gold mine spoils in Peru. *Adv. Mat. Res.* 1130, 589–593. Nov.
- Dash, R.R., Balomajunder, C., Kumar, A., 2009. Removal of metal cyanides from aqueous solutions by suspended and immobilized cells of *Rhizopus oryzae* (MTCC 2541). *Eng. Life Sci.* 9 (1), 53–59.
- Dash, R.R., Gaur, A., Balomajunder, C., 2009. Cyanide in industrial wastewaters and its removal: a review on biotreatment. *J. Hazard. Mater.* 163, 1–11.
- De Kruyff, C.D., van der Walt, J.P., Schwartz, H.M., 1957. The utilisation of thiocyanate and nitrate by thiobacilli. In: *Antonie Van Leeuwenhoek*, 23, pp. 305–316. Dec.
- Dorr, P.K., Knowles, C.J., 1989. Cyanide oxygenase and cyanase activities of *Pseudomonas fluorescens* NCIMB 11764. *FEMS Microbiol. Lett.* 60 (3), 289–294. Aug.
- Dwivedi, N., Balomajunder, C., Mondal, P., 2019. Applications of microorganisms in biodegradation of cyanide from wastewater. *Advances in Microbial Biotechnology*. Apple Academic Press, pp. 301–328.
- Ezzi, M.I., Lynch, J.M., 2005. Plant microcosm studies demonstrating bioremediation of cyanide toxicity by *Trichoderma* and *Fusarium* spp. *Biol. Fertil. Soils* 42 (1), 40–44. Nov.
- Faramarzi, M.A., Stagars, M., Pensini, E., Krebs, W., Brandl, H., 2004. Metal solubilization from metal-containing solid materials by cyanogenic *chromobacterium violaceum*. *J. Biotechnol.* 321–326.
- Gschwendtner, S., Mansfeldt, T., Kublik, S., Toulari, E., Buegger, F., Schlöter, M., 2016. Long-term ferrocyanide application via deicing salts promotes the establishment of actinomycetales assimilating ferrocyanide-derived carbon in soil. *Microb. Biotechnol.* 9 (4), 502–513. Jul 1.
- Gupta, A., Dutta, A., Sarkar, J., Paul, D., Panigrahi, M.K., Sar, P., 2017. Metagenomic exploration of microbial community in mine tailings of Malanjkhand copper project, India. *Genom. Data* 12, 11–13. Jun 1.
- Hilson, G., Monhemius, A.J., 2006. Alternatives to cyanide in the gold mining industry: what prospects for the future? *J. Clean. Prod.* 14, 1158–1167.
- Hilson, G., Monhemius, A.J., 2006. Alternatives to cyanide in the gold mining industry: what prospects for the future? *J. Clean. Prod.* 14 (12–13), 1158–1167. Jan.
- Huddy, R., Kantor, R., Van Zyl, W., Van Hille, R.P., Banfield, J., Harrison, S.T.L., 2015. Analysis of the microbial community associated with a bioprocess system for bioremediation of thiocyanate and cyanide-laden mine water effluents..
- Ibrahim, K.K., Syed, M.A., Shukor, M.Y., Ahmad, S.A., 2015. Biological remediation of cyanide: a review. *Biotropia* 22 (2), 151–163.
- Ingvorsen, K., Højer-Pedersen, B., Godtfredsen, S.E., 1991. Novel cyanide-hydrolyzing enzyme from *Alcaligenes xylosoxidans* subsp. *Denitrificans*. *Appl. Environ. Microbiol.* 57 (6), 1783–1789. Jun.
- Itakorode, B.O., Okonji, R.E., Torimiro, N., 2023. Cyanide bioremediation potential of *Klebsiella oxytoca* JCM 1665 rhodanese immobilized on alginate-glutaraldehyde beads. *Biocatal. Biotransform.* 41 (4), 299–308. Jul 4.
- Ji, H., Zhang, Y., Bararunyeretse, P., Li, H., 2018. Characterization of microbial communities of soils from gold mine tailings and identification of mercury-resistant strain. *Ecotoxicol. Environ. Saf.* 165, 182–193. Dec 15.
- Jiang, W., Lu, Y., Feng, Z., Yu, H., Ma, P., Zhu, J., et al., 2022. Biodegradation of cyanide by a new isolated *aerococcus viridans* and optimization of degradation conditions by response surface methodology. *Sustainability* 14 (23), Dec 1.
- Kaewkannetra, P., Imai, T., Garcia-Garcia, F.J., Chiu, T.Y., 2009. Cyanide removal from cassava mill wastewater using *Azotobacter vinelandii* TISTR 1094 with mixed microorganisms in activated sludge treatment system. *J. Hazard. Mater.* 172 (1), 224–228. Dec 15.
- Kao, C.M., Li, S.H., Chen, Y.L., Chen, S.C., 2005. Utilization of the metal-cyano complex tetracyanonickelate (II) by *Azotobacter vinelandii*. *Lett. Appl. Microbiol.* 41 (2), 216–220.
- Katayama, Y., Narahara, Y., Inoue, Y., Amano, F., Kanagawa, T., Kuraishi, H., 1992. A thiocyanate hydrolase of *thiobacillus thioparus*. A novel enzyme catalyzing the formation of carbonyl sulfide from thiocyanate. *J. Biol. Chem.* 267 (13), 9170–9175. May.
- Kelly, M., 1968. The kinetics of the reduction of isocyanides, acetylenes and the cyanide ion by nitrogenase preparation from *Azotobacter chroococcum* and the effects of inhibitors. *Biochem. J.* 107 (1), 1–6. Mar 1.
- Keshri, J., Mankazana, B.B.J., Momba, M.N.B., 2015. Profile of bacterial communities in South African mine-water samples using Illumina next-generation sequencing platform. *Appl. Microbiol. Biotechnol.* 99 (7), 3233–3242. Mar 19.
- Kim, Y.M., Lee, D.S., Park, C., Park, D., Park, J.M., 2011. Effects of free cyanide on microbial communities and biological carbon and nitrogen removal performance in the industrial activated sludge process. *Water Res.* 45 (3), 1267–1279.
- Koksan, S., 2013. Growth and cyanide degradation of *azotobacter vinelandii* in cyanide-containing wastewater system. *J. Microbiol. Biotechnol.* 23 (4), 572–578.
- Kowalska, M., Bodzek, M., Bohdziewicz, J., 1998. Biodegradation of Phenols and Cyanides using Membranes with Immobilized Microorganisms, 33. *Process Biochemistry*.
- Kubsad, V., Gupta, S.K., Chaudhari, S., 2011. Biodegradation of wastewater containing cyanide, acetonitrile, and acrylonitrile using RBC and shock loading study. *Can. J. Chem. Eng.* 89 (6), 1536–1544. Dec.
- Kumar, V., Kumar, V., Singh, A.K., Verma, N., Bhalla, T.C., 2018. A potentiometric biosensor for cyanide detection using immobilized whole cell cyanide dihydratase of *Flavobacterium indicum* MTCC 6936. *J. Anal. Chem.* 73 (10), 1014–1019. Oct 1.
- Kunz, D.A., Nagappan, O., Silva-Avalos, J., Delong, G.T., 1992. Utilization of cyanide as nitrogenous substrate by *Pseudomonas fluorescens* NCIMB 11764: evidence for multiple pathways of metabolic conversion. *Appl. Environ. Microbiol.* 58 (6), 2022–2029. Jun.
- Kuyucak, N., Akcil, A., 2013. Cyanide and removal options from effluents in gold mining and metallurgical processes. *Miner. Eng.* 50–51, 13–29. Sep.
- Kwak, I.S., Yun, Y.S., 2010. Recovery of zero-valent gold from cyanide solution by a combined method of biosorption and incineration. *Bioresour. Technol.* 101 (22), 8587–8592. Nov.
- Liu, J., Hua, Z.S., Chen, L.X., Kuang, J.L., Li, S.J., Shu, W.S., et al., 2014. Correlating microbial diversity patterns with geochemistry in an extreme and heterogeneous environment of mine tailings. *Appl. Environ. Microbiol.* 80 (12), 3677–3686.
- Liu, J., Li, C., Lin, C., 1997. The role of nitrogenase in a cyanide-degrading *Klebsiella oxytoca* strain. In: *Proceedings of the National Science Council, Republic of China Part B*, 21, pp. 37–42.
- Liu, Q., Zhang, G., Ding, J., Zou, H., Shi, H., Huang, C., 2018. Evaluation of the removal of potassium cyanide and its toxicity in green algae (*Chlorella vulgaris*). *Bull. Environ. Contam. Toxicol.* 100 (2), 228–233. Feb 1.
- Logsdon, M.J., Hagelstein, K., Mudder, T., 1999. The Management of Cyanide in Gold Extraction. *International Council on Metals and the Environment*.
- Lovaso, C.R., Hela, K., Harinaivo, A.A., Hamma, Y., 2017. Bioremediation of soil and water polluted by cyanide: a review. *Afr. J. Environ. Sci. Tech.* 11 (6), 272–291. Jun 30.
- Mahendran, R., Bs, S., Thandeeswaran, M., K.G., K., Vijayarath, M., Angayarkanni, J., et al., 2020. Microbial (Enzymatic) degradation of cyanide to produce pterins as cofactors. In: *Current Microbiology*, 77. Springer, pp. 578–587.
- Mak, K.K.W., Law, A.W.C., Tokuda, S., Yanase, H., Renneberg, R., 2005. Application of cyanide hydrolase from *Klebsiella* sp. in a biosensor system for the detection of low-level cyanide. *Appl. Microbiol. Biotechnol.* 67 (5), 631–636. Jun 4.
- Maniyam, M.N., Ibrahim, A.L., Cass, A.E.G., 2019. Enhanced cyanide biodegradation by immobilized crude extract of *Rhodococcus UKMP-5M*. *Environ. Technol.* 40 (3), 386–398. Jan 28.
- Mathuriya, A.S., 2014. Eco-affectionate face of microbial fuel cells. *Crit. Rev. Environ. Sci. Technol.* 44 (2), 97–153. Jan 1.
- Meher, A.K., Labhshetwar, N., Pratap Reddy, M., Bansiwal, A., 2020. Methods for cyanide removal from wastewater: a review. *J. Environ. Sci. Eng.* 59 (1), 299–306. Sep.
- Mekuto, L., Aleghbeyeva, O.O., Ntwampe, S.K.O., Ngongang, M.M., Mudumbi, J.B., Akinpelu, E.A., 2016. Co-metabolism of thiocyanate and free cyanide by *exiguobacterium acetylicum* and *Bacillus marisflavi* under alkaline conditions. 3 *Biotech* 6 (2), Dec 1.
- Mekuto, L., Ntwampe, S.K.O., Jackson, V.A., 2015. Biodegradation of free cyanide and subsequent utilisation of biodegradation by-products by *Bacillus consortia*:

- optimisation using response surface methodology. *Environ. Sci. Pollut. Res.* 22 (14), 10434–10443. Jul 1.
- Mekuto, L., Ntwampe, S.K.O., Mudumbi, J.B.N., 2018. Microbial communities associated with the co-metabolism of free cyanide and thiocyanate under alkaline conditions. 3 *Biotech* 8 (2). Feb 1.
- Mekuto, L., Ntwampe, S.K.O., Mudumbi, J.B.N., Akinpelu, E.A., Mewa-Ngongang, M., 2017. Metagenomic data of free cyanide and thiocyanate degrading bacterial communities. *Data Brief* 13, 738–741. Aug 1.
- Mendes, H.C., Kelbert, M., Michels, C., Soares, H.M., 2024. Exploring sustainable pathways for cyanide biodegradation in cassava waste: insights into methodologies, microbial degradation, and emerging technologies. *J. Water Process Eng.* 64. Jul 1.
- Meyers, P.R., Rawlings, D.E., Woods, D.R., Lindsey, G.G., 1993. Isolation and characterization of a cyanide dihydratase from *Bacillus pumilus* Cl. *J. Bacteriol.* 175 (19), 6105–6112. Oct.
- Kumar, V., Chand Bhalla, T., Kumar, V., 2017. Microbial remediation of cyanides. Morillo Esparza, J., Cevallos Cueva, N., Sandoval Pauker, C., Vargas Jentzsch, P., Muñoz Bisesti, F., 2019. Combined treatment using ozone for cyanide removal from wastewater: a comparison. *Rev. Int. Contam. Ambient.* 35 (2), 459–467.
- Motsoneng, L.G., Naidoo, V., Mekuto, L., 2023. Biogas production and metabolite profiling from anaerobic biodegradation of free cyanide using municipal waste activated sludge. *Bioresour. Technol. Rep.* 22, 101442. Jun.
- Moussavi, G., Khosravi, R., 2010. Removal of cyanide from wastewater by adsorption onto pistachio hull wastes: parametric experiments, kinetics and equilibrium analysis. *J. Hazard. Mater.* 183 (1–3), 724–730. Nov.
- Mpongwana, N., Ntwampe, S.K.O., Mekuto, L., Akinpelu, E.A., Dyantyi, S., Mpentshu, Y., 2016. Isolation of high-salinity-tolerant bacterial strains, *Enterobacter* sp., *Serratia* sp., *Yersinia* sp., for nitrification and aerobic denitrification under cyanogenic conditions. *Water Sci. Technol.* 73 (9), 2168–2175. May 5.
- Mudder, T., Botz, M., 2004. Cyanide and society: a critical review. *Eur. J. Min. Process. Environ. Protection* 4 (1), 62–74.
- Murugesan, T., Durairaj, N., Ramasamy, M., Jayaraman, K., Palaniswamy, M., Jayaraman, A., 2018. Analeptic agent from microbes upon cyanide degradation. In: *Applied Microbiology and Biotechnology*, 102. Springer Verlag, pp. 1557–1565.
- Mondal, S., Balomajumder, C., 2013. Study on the mechanism of azotobacter chroococcum MTCC 446 after degradation of phenol and cyanide.
- Nallapan Maniyam M., Sjahri F., Latif A., Alam S., Ehsan D., 2019. Biodegradation of cyanide by rhodococcus strains isolated in Malaysia. 2011.
- Natarajan, G., Ting, Y.P., 2014. Pretreatment of e-waste and mutation of alkali-tolerant cyanogenic bacteria promote gold biorecovery. *Bioresour. Technol.* 152, 80–85.
- Nawaz, M.S., Chapatwala, K.D., Wolfram, J.H., 1989. Degradation of Acetonitrile by *Pseudomonas putida*. *Appl. Environ. Microbiol.* 55 (9), 2267–2274. Sep.
- Nermin, H.A., Isil, S.B., 2016. Biological treatment of cyanide by using *Klebsiella pneumoniae* species. *J. Ind. Microbiol. Biotechnol.* 20.
- Novak, D., Franke-Whittle, I.H., Pirc, E.T., Jerman, V., Insam, H., Logar, R.M.S., et al., 2013. Biotic and abiotic processes contribute to successful anaerobic degradation of cyanide by UASB reactor biomass treating brewery waste water. *Water Res.* 47 (11), 3644–3653. Jul 1.
- Offiong, N.A.O., Edet, J.B., Shaibu, S.E., Akan, N.E., Atakpa, E.O., Sanganyado, E., et al., 2023. Metagenomics: an emerging tool for the chemistry of environmental remediation. *Front. Environ. Chem.* 4. May 9.
- Ogunyemi, A.K., Samuel, T.A., Ilori, M.O., Amund, O.O., 2019. Cassava wastewater and solid waste leachate as cyanogenic substrates for the growth of nitrile and linamarin-utilizing bacteria. *J. Trop. Life Sci.* 9 (1), 79–87.
- Ojaghi, A., Tonkaboni, S.Z.S., Shariati, P., Doulati Ardejani, F., 2018. Novel cyanide electro-biodegradation using *Bacillus pumilus* ATCC 7061 in aqueous solution. *J. Environ. Health Sci. Eng.* 16 (2), 99–108. May 21.
- Olaya-Abril, A., Bello, K., Rodríguez-Caballero, G., Cabello, P., Sáez, L.P., Moreno-Vivián, C., et al., 2024. Bacterial tolerance and detoxification of cyanide, arsenic and heavy metals: holistic approaches applied to bioremediation of industrial complex wastes. In: *Microbial Biotechnology*, 17. John Wiley and Sons Ltd.
- Olaya-Abril, A., VM, L.-A., Perez, M.D., Lopez, C.M., Amil, F., Cabello, P., et al., 2019. Putative small RNAs controlling detoxification of industrial cyanide-containing wastewaters by *Pseudomonas pseudoalcaligenes* CECT5344. *PLoS One* 14 (2).
- Omura, H., Ikemoto, M., Kobayashi, M., Shimizu, S., Yoshida, T., Nagasawa, T., 2003. Purification, characterization and gene cloning of thermostable O-acetyl-l-homoserine sulphydrylase forming γ -cyano- α -aminobutyric acid. *J. Biosci. Bioeng.* 96 (1), 53–58. Jan.
- Oulego, P., Laca, A., Diaz, M., 2013. Kinetics and pathways of cyanide degradation at high temperatures and pressures. *Environ. Sci. Technol.* 47 (3), 1542–1549. Feb 5.
- Papadimitriou, C.A., Samaras, P., Sakellariopoulos, G.P., 2009. Comparative study of phenol and cyanide containing wastewater in CSTR and SBR activated sludge reactors. *Bioresour. Technol.* 100 (1), 31–37. Jan 1.
- Parsania, S., Mohammadi, P., Soudi, M.R., 2024. A metagenomics investigation of bacterial communities in gold mine tailings. *J. Microb. Biol. E* 13 (52), 13 [Internet].
- Pathak, U., Roy, A., Mandal, D.D., Das, P., Kumar, T., Mandal, T., 2019. Bioattenuation of phenol and cyanide involving immobilised spent tea activated carbon with *Alcaligenes faecalis* JF339228: critical assessment of the degraded intermediates. *Asia-Pacific J. Chem. Eng.* 14 (1). Jan 1.
- Rahman, S.F., Kantor, R.S., Huddy, R., Thomas, B.C., van Zyl, A.W., Harrison, S.T.L., et al., 2017. Genome-resolved metagenomics of a bioremediation system for degradation of thiocyanate in mine water containing suspended solid tailings. *Microbiologyopen* 6 (3). Jun 1.
- Ramalingam, S., Bahuguna, A., Al-Ansari, M.M., Shanmugam, G., Al-Humaid, L., Lee, J. S., et al., 2022. Whole-genome analysis guided molecular mechanism of cyanogenic glucoside degradation by yeast isolated from *Prunus mume* fruit syrup. *Chemosphere* 307, 136061. Nov.
- Roldán, M.D., Olaya-Abril, A., Sáez, L.P., Cabello, P., Luque-Almagro, V.M., Moreno-Vivián, C., 2021. Bioremediation of cyanide-containing wastes. *EMBO Rep.* 22 (11). Nov 4.
- Roldán, M.D., Olaya-Abril, A., Sáez, L.P., Cabello, P., Luque-Almagro, V.M., Moreno-Vivián, C., 2021. Bioremediation of cyanide-containing wastes. *EMBO Rep.* 22 (11).
- Sabatini, L., Ferrini, C., Micheloni, M., Pianetti, A., Citterio, B., Parlani, C., et al., 2012. Isolation of a strain of *Aspergillus fumigatus* able to grow in minimal medium added with an industrial cyanide waste. *World J. Microbiol. Biotechnol.* 28 (1), 165–173. Jan.
- Sáez, L.P., Cabello, P., Ibáñez, M.I., VM, L.-A., Roldán, M.D., Moreno-Vivián, C., 2019. Cyanate assimilation by the alkaliphilic cyanide-degrading bacterium *pseudomonas pseudoalcaligenes* CECT5344: mutational analysis of the *cyn* gene cluster. *Int. J. Mol. Sci.* 20 (12).
- Secretaría de Medio Ambiente y Recursos Naturales (SEMARNAT), 1996. NOM-001-SEMARNAT-1996. Norma Oficial Mexicana que Establece los Límites máximos permisibles de Contaminantes en las Descargas de Aguas Residuales en Agua y Bienes Nacionales. Diario Oficial de la Federación.
- Sharma, M., Akhter, Y., Chatterjee, S., 2019. A review on remediation of cyanide containing industrial wastes using biological systems with special reference to enzymatic degradation. In: *World Journal of Microbiology and Biotechnology*, 35. Springer Netherlands.
- Sibanda, T., Selvarajan, R., Msagati, T., Venkatachalam, S., Meddows-Taylor, S., 2019. Defunct gold mine tailings are natural reservoir for unique bacterial communities revealed by high-throughput sequencing analysis. *Sci. Total Environ.* 650, 2199–2209. Feb 10.
- Skowronski, B., Strobel, G.A., 1969. Cyanide resistance and cyanide utilization by a strain of *Bacillus pumilus*. *Can. J. Microbiol.* 15 (1), 93–98. Jan 1.
- Sorokin, D.Y., Tourova, T.P., Lysenko, A.M., Kuenen, J.G., 2001. Microbial thiocyanate utilization under highly alkaline conditions. *Appl. Environ. Microbiol.* 67 (2), 528–538. Feb.
- Stott, M.B., Franzmann, P.D., Zappia, L.R., Watling, H.R., Quan, L.P., Clark, B.J., et al., 2001. Thiocyanate Removal from Saline Cip Process Water by A Rotating Biological Contactor, With Reuse of the Water for Bioleaching [Internet], 62. Hydrometallurgy.
- Sung, Y.C., Fuchs, J.A., 1992. The *Escherichia coli* K-12 *cyn* operon is positively regulated by a member of the *lysR* family. *J. Bacteriol.* 174 (11), 3645–3650. Jun.
- Tanabene, R., Genty, T., Gonzalez-Merchan, C., Bussière, B., Potvin, R., Neculita, C.M., 2018. Nitrification-denitrification of thiocyanate, ammonia, and nitrates in highly contaminated gold mine effluents using methanol as energy source. *J. Environ. Eng.* 144 (5). May.
- Tang, P., Hseu, Y.C., Chou, H.H., Huang, K.Y., Chen, S.C., 2010. Proteomic analysis of the effect of cyanide on *klebsiella oxytoca*. *Curr. Microbiol.* 60 (3), 224–228. Mar.
- Vaca-Escobar, K., Arregui-Almeida, D., Espinoza-Montero, P., 2024. Chemical, ecotoxicological characteristics, environmental fate, and treatment methods applied to cyanide-containing wastewater. *NPJ Clean Water* 7 (1), 103. Oct 5.
- Vakilchap, F., Mousavi, S.M., Baniasadi, M., Farnaud, S., 2020. Development and evolution of bioremediation in metal recovery from solid waste: a review. In: *Reviews in Environmental Science and Biotechnology*, 19. Springer, pp. 509–530.
- Vashishtha, L., Bagal, M.V., Iyer, A.M., Gogate, P.R., Sharma, A., Mishra, B.R., et al., 2024. Ultrasound-assisted catalytic ozonation-based treatment of rare-earth processing effluent containing ammonium thiocyanate (NH₄ SCN). *Ind. Eng. Chem. Res.* 63 (7), 2969–2979. Feb 21.
- VM, L.-A., Huertas, M.J., Martínez-Luque, M., Moreno-Vivián, C., Roldán, M.D., García-Gil, L.J., et al., 2005. Bacterial degradation of cyanide and its metal complexes under alkaline conditions. *Appl. Environ. Microbiol.* 71 (2), 940–947.
- VM, L.-A., Moreno-Vivián, C., Roldán, M.D., 2016. Biodegradation of cyanide wastes from mining and jewellery industries. In: *Current Opinion in Biotechnology*, 38. Elsevier Ltd, pp. 9–13.
- Wang, Z., Liu, L., Guo, F., Zhang, T., 2015. Deciphering cyanide-degrading potential of bacterial community associated with the Coking Wastewater treatment plant with a novel draft genome. *Microb. Ecol.* 70 (3), 701–709. Oct 1.
- Watts, M.P., Gan, H.M., Peng, L.Y., Lê Cao, K.A., Moreau, J.W., 2017. In situ stimulation of thiocyanate biodegradation through phosphate amendment in gold mine tailings water. *Environ. Sci. Technol.* 51 (22), 13353–13362. Nov 21.
- Watts, M.P., Spurr, L.P., Lê Cao, K.A., Wick, R., Banfield, J.F., Moreau, J.W., 2019. Genome-resolved metagenomics of an autotrophic thiocyanate-remediating microbial bioreactor consortium. *Water Res.* 158, 106–117. Jul 1.
- Welman-Purchase, M.D., Castillo, J., Gomez-Arias, A., Matu, A., Hansen, R.N., 2024. First insight into the natural biodegradation of cyanide in a gold tailings environment enriched in cyanide compounds. *Sci. Total Environ.* 906. Jan 1.
- White, J.M., Jones, D.D., Huang, D., Gauthier, J.J., 1988. Conversion of cyanide to formate and ammonia by a pseudomonad obtained from industrial wastewater. *J. Ind. Microbiol.* 3 (5), 263–272. Aug.
- Wibberg, D., Luque-Almagro, V.M., Igeño, M.I., Bremges, A., Roldán, M.D., Merchán, F., et al., 2014. Complete genome sequence of the cyanide-degrading bacterium *Pseudomonas pseudoalcaligenes* CECT5344. *J. Biotechnol.* 175 (1), 67–68. Apr 10.
- Wibberg, D., Luque-Almagro, V.M., Igeño, M.I., Bremges, A., Roldán, M.D., Merchán, F., et al., 2014. Complete genome sequence of the cyanide-degrading bacterium *Pseudomonas pseudoalcaligenes* CECT5344. *J. Biotechnol.* 175 (1), 67–68.
- Wood, A.P., Kelly, D.P., McDonald, I.R., Jordan, S.L., Morgan, T.D., Khan, S., et al., 1998. A novel pink-pigmented facultative methylotroph, *methylobacterium thiocyanatum*

- sp. nov., capable of growth on thiocyanate or cyanate as sole nitrogen sources. Arch. Microbiol. 169 (2), 148–158. Feb 5.
- Yahya, F.N., Ibrahim, W.H.W., Aziz, B.A., Suli, L.N.M., 2020. Simulation of leaching process of gold by cyanidation. In: IOP Conference Series: Materials Science and Engineering. Institute of Physics Publishing.
- Zhou, X., Liu, L., Chen, Y., Xu, S., Chen, J., 2007. Efficient biodegradation of cyanide and ferrocyanide by Na-alginate beads immobilized with fungal cells of *Trichoderma koningii*. Can. J. Microbiol. 53 (9), 1033–1037. Sep.