

# Biological Degradation of Cyanide, Thiocyanate, and Phenolic Compounds in Wastewater

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## Abstract

Wastewater from various industries, such as coking, mining, pharmaceutical, petroleum, etc., often contains high concentrations of cyanides, thiocyanates, and phenolic compounds, individually or in combination, making them serious sources of pollution for natural watercourses. Protecting water bodies and preserving life within them requires reducing the concentrations of these compounds to legally prescribed levels. Given the complexity of such wastewater effective treatment generally involves multiple technologies, with biological treatment being both indispensable and highly effective. Therefore, exploring the potential for biological degradation of these compounds, along with the mechanisms influencing their degradation under various environmental conditions, is crucial. This paper provides an overview of the biological degradation of toxic compounds in industrial wastewater, investigating various microorganisms and degradation techniques to ensure effective and environmentally friendly water treatment.

## Keywords

Cyanide, thiocyanate, phenol, wastewater, biological degradation

## 1 Introduction

In nature, phenols and cyanides are produced as by-products of certain microbes and plants. Phenols, known for their antioxidant properties, are almost always found in plants, while cyanides are key components of legumes, almonds, and cassava. Although the presence of these compounds in small quantities may not significantly impact the environment, their mass production, widespread use, and uncontrolled release make them major pollutants. Thiocyanates, on the other hand, are considerably less toxic than their parent compound, cyanide, and therefore pose fewer ecological concerns. However, wastewater from various industries containing cyanides, thiocyanates, and/or phenolic compounds must be treated before discharge to protect natural water bodies. The concentrations of cyanide, thiocyanate, and phenolic compounds in different industrial wastewater streams are given in Table 1. From the 1970s to the 1990s, wastewater treatment mainly focused on reducing and eliminating floating and suspended solids, biochemical oxygen demand (BOD<sub>5</sub>), and pathogens. Since 1990, advancements in technical knowledge and growing scientific awareness have led to an increased focus on environmental and health risks associated with hazardous and potentially lethal substances in wastewater. This paper describes the mechanisms of toxic pollutant removal from wastewater, and the effectiveness of microbial transformations through bioremediation and biotechnological approaches.<sup>1–4</sup> Although wastewater contaminated with cyanides, thiocyanates, and phenols is among the most toxic industrial effluents, research on the biological degradation and ecotoxicity of these compounds – individually or in combination – remains limited, especially in recent years.

## 2 Cyanides

Cyanides are extremely toxic chemical compounds containing a functional group composed of one carbon atom triple-bonded to a nitrogen atom [C≡N]<sup>-</sup>. They are ubiquitous in both biotic and abiotic components of ecosystems, and have played a pivotal role in the evolution of life on Earth. However, rapid industrialisation has resulted in a significant increase in cyanide levels in the environment. Major sources of cyanide pollution include chemical, petrochemical, metallurgical, coke, automotive, pharmaceutical, and mining industries. All of these industries, their operations and facilities generate wastewater containing high concentrations of cyanide. In wastewater, cyanides occur in the form of hydrogen cyanide/free cyanide (HCN/CN<sup>-</sup>) or cyanide complexes. Free cyanide can react with 28 elements in various oxidation states, thereby forming 72 different metal cyanides of specific stability and solubility levels, most of which are anionic. On the other hand, the release of free cyanide from cyanide complexes typically requires boiling, digestion, or a combination of different degradation methods. Simple cyanide complexes, such as potassium and sodium cyanide, readily dissociate into free cyanide at neutral pH. Weak to moderately strong cyanide complexes, such as cadmium, copper, nickel, and zinc cyanide, dissociate at pH 4.5, while strong cyanide complexes, such as iron, cobalt, and gold cyanides dissociate at pH 2. The different forms of cyanide in wastewater are illustrated in Fig. 1. Cyanide compounds can exist as solids, liquids, or gases, while free cyanide is a colourless gas or liquid that is highly soluble in water and volatile in aqueous solutions. The toxicity of cyanide depends on its form, with free cyanide being the most toxic.<sup>1,4,7,9,16–18</sup>

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Table 1 – Concentrations of cyanide, thiocyanate, and phenolic compounds in industrial wastewater  
 Tablica 1 – Koncentracije cijanida, tiocijanata i fenolnih spojeva u industrijskim otpadnim vodama

Type of industry	Cyanide	Thiocyanate	Phenol	Ref.
	mg l <sup>-1</sup>			
coke manufacturing	2.8–9	301–542	414–662	5
	4.0–15	200–500	400–1200	6
	10–150	–	28–1150	7
	–	1500	–	8
	20	–	1200	9
	200	56	560	10
	–	100–500	350–1200	11
steel manufacturing	3–8	1–16	0.01–0.1	5
	–	50–400	–	12
petrochemical	–	–	3–6800	13
mining	18–22	–	6–500	7, 13
petroleum refinery	0–15	–	6–88	7
oil industry	2.25	–	10–100	7
galvanisation	4000–10000	–	–	7
pharmacy	–	–	10–100	7
paper	–	–	0.6–600	7
coal processing	–	–	9–6800	14
thermal power plant	–	986	–	15
gold mining	–	986	–	15
pesticide production	–	5–100	–	15

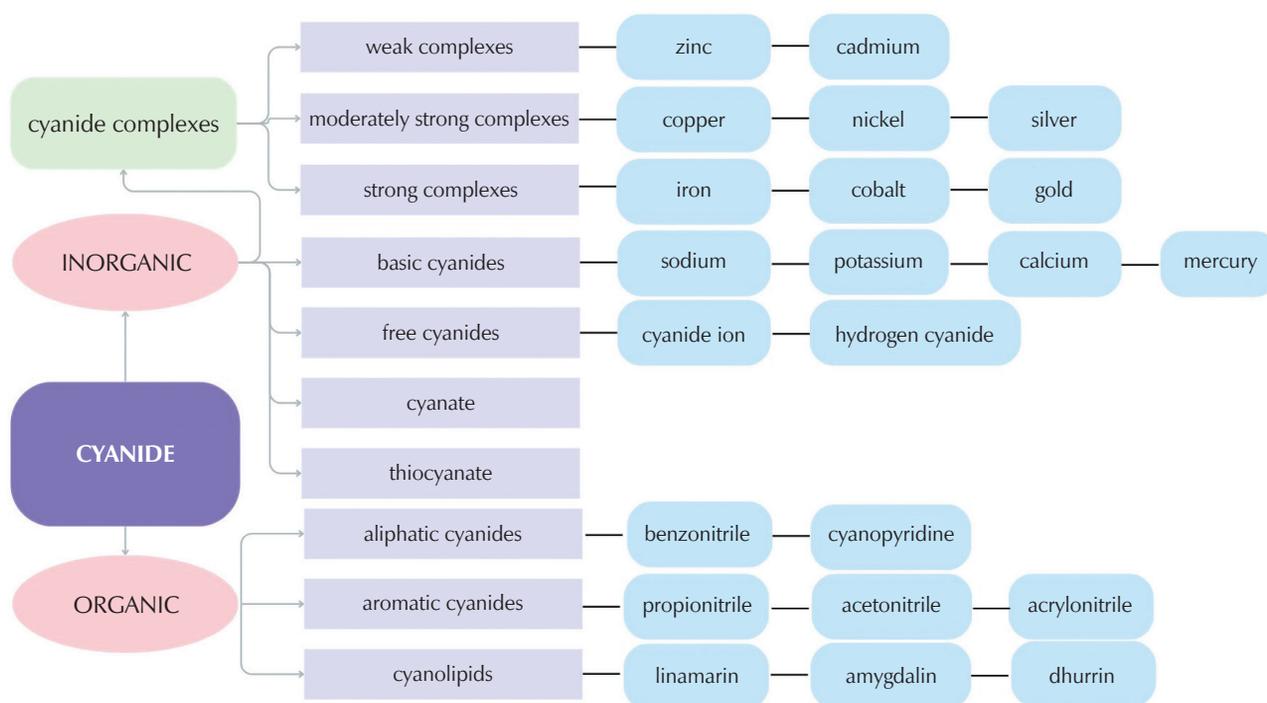


Fig. 1 – Forms of cyanide present in wastewater  
 Slika 1 – Oblici cijanida prisutni u otpadnoj vodi

### 3 Thiocyanates

Thiocyanates are inorganic pollutants found in soil, air, and water, primarily originating from wastewater in the textile, coke, metal, and mining industries, where their concentration can reach up to 1500 mg l<sup>-1</sup>. They consist of carbon, nitrogen, sulphur, and other elements, and are formed through the reaction of sulphides, thiosulfates, and cyanides, mainly existing in the form of [S-C≡N]<sup>-</sup>. Thiocyanates are good ligands, readily forming complexes with transition metals, strongly toxic, but seven times less toxic than cyanide, chemically stable, and difficult to degrade. On the other hand, thiocyanates can serve as a source of carbon, nitrogen, and sulphur for microorganisms.<sup>1,8,19–21</sup>

### 4 Phenolic compounds

Phenol is an organic molecule with a hydroxyl (–OH) group attached to a carbon atom in an aromatic ring, also known as monohydroxy benzene or carboic acid, with a molecular formula C<sub>6</sub>H<sub>5</sub>OH. Phenol is a crystalline substance, ranging from white to slightly pinkish in colour and has a characteristic odour. Phenolic compounds are the most common organic pollutants present in wastewater from industries such as petrochemical, coke, mining, petroleum, pharmaceutical, tannery, paper, wood, etc., constituting 40–80 % of the total chemical oxygen demand (COD). Generally, industrial wastewater is the primary source of phenolic pollutants in the environment, which along with their metabolites, cause mutagenicity and carcinogenicity in living cells. Thus, the presence of phenolic compounds in water poses a significant risk to human health and the environment. Due to their high solubility in water (phenol 8.28 g/100 ml; cresols 2.15–2.60 g/100 ml), phenolic compounds persist in high concentrations in aquatic ecosystems. Their toxicity is related to the unspecified toxicity associated with the hydrophobicity of individual compounds and the formation of free radicals. Exposure to phenol disrupts the metabolic system of microorganisms, animals, and humans, and can strongly inhibit the growth of bacteria, algae, and molluscs.<sup>9,13,22–24</sup>

### 5 Biological degradation

Among the available technologies for removing toxic and hazardous pollutants from wastewater, biological degradation is the most promising due to its numerous advantages, such as environmental acceptability, economic sustainability, and practical feasibility.<sup>13</sup> A key advantage of biological wastewater treatment is its ability to simultaneously remove multiple pollutants in one process, often at significantly lower costs compared to other methods.<sup>19,25</sup> The success of biological degradation depends on the presence and quantity of microorganisms with physiological and metabolic capabilities for degrading the target pollutant.<sup>18</sup>

Furthermore, the pH value of wastewater also affects the efficiency of biological degradation, influencing the solubility of pollutants and the physiology and enzymatic activities of microorganisms. Additionally, temperature is

directly related to the degree of solubility, biological availability, as well as the chemical and physical properties of pollutants, and it also affects the biochemical activity of microorganisms. Wastewater is typically contaminated with various compounds, so competition and inhibition can also significantly impact the growth of microorganisms and the efficiency of degrading the target pollutant, especially if the pollutants are structurally related.<sup>13</sup> In addition to the mentioned factors, other factors directly affecting the efficiency of biological degradation include the concentration of the pollutant, oxygen availability, retention time, mixing rate, and additional carbon source.<sup>9</sup>

#### 5.1 Biological degradation of cyanide

Microorganisms must be capable of overcoming the inhibitory effect of cyanide, therefore requiring higher activation energy for cellular respiration. During cellular respiration, microorganisms generate the energy needed to degrade nutrients into useful components. The energy of cellular respiration must exceed the activation energy of microbial growth, especially in the presence of a metabolic inhibitor like cyanide.<sup>26</sup> Through the process of biological degradation, cyanide is oxidised by microorganisms to a less toxic compound, typically cyanate. The organic portion is transformed into ammonia (NH<sub>4</sub><sup>+</sup>) and carbonate (HCO<sub>3</sub><sup>-</sup>), while free metals, if present, are adsorbed into the biofilm. The overall biologically mediated reaction of cyanide oxidation is shown by Eq. (1).



Approximately 0.62 g of oxygen is consumed per 1 g of oxidised cyanide, and about 0.54 g of ammonia is formed. The biomass yield per 1 g of oxidised cyanide ranges from 0.05 to 0.1 g.<sup>19</sup> The degree of cyanide biodegradability varies depending on the chemical stability of the compound. It can be carried out in one or two steps, depending on the enzymes involved and the desired end product.<sup>1,4,16,18,27,28</sup> Different members of the *Bacillus* genus, especially *Bacillus pumilus* species, have been described as good cyanide degraders. Studies have shown that a *Bacillus* consortium degrades 41 % of the initial 500 mg l<sup>-1</sup> cyanide within 96 h,<sup>29</sup> while a pure culture of *Bacillus pumilus* degrades 99.8 % of the initial 500 mg l<sup>-1</sup> free cyanide over 301 h, albeit assisted by an electric current source.<sup>30</sup> López-Ramírez et al.<sup>31</sup> found that *Bacillus* sp. and *Enterococcus* sp. degraded cyanide with efficiencies of 21 % and 14 %, respectively. Although the cyanide degradation pathway for *Enterococcus* sp. is not yet known, their degradation capability may be associated with the ability to adapt and survive in environments where cyanide and heavy metals are present, which is a common characteristic of gram-positive bacteria.<sup>31</sup> The study by Mirizadeh et al.<sup>32</sup> reported the best results from an uncategorised bacterial species isolated from wastewater of the coking industry, which degraded 96 % of the initial 200 mg l<sup>-1</sup> cyanide over 96 h. Besides bacteria, fungi are also capable of cyanide degradation. *Basidiomycetes* fungi, such as *Polyporus arcularius*, *Schizophyllum commune*, and *Ganoderma lucidum*, have shown greater cyanide degradation ability compared to other strains such as *Pleurotus eryngii*, *Ganoderma applanatum*, *Clavariadelphus truncates*,

Table 2 – Overview of studies on biological cyanide degradation  
 Tablica 2 – Pregled znanstvenih istraživanja biološke razgradnje cijanida

Pure bacterial culture	Cyanide/mg l <sup>-1</sup>	Efficiency/%	Time/h	pH/–	Temperature/°C	Ref.
<i>Bacillus</i> sp.	200	21	24	9	30	31
<i>B. pumilus</i>	500	100	301	9	30	30
<i>Enterococcus</i> sp.	200	14	24	9	30	31
Unclassified	200	96	96	10	34	32
Mixed bacterial culture						
<i>Bacillus</i> sp.	500	41	96	10	34	29
Algae						
<i>Chlorella vulgaris</i>	10	61	72	7	25	33
Fungi						
<i>Fusarium oxysporum</i>	100	77	144	11	22	26

and *Trametes*.<sup>9,27</sup> A culture of *Fusarium oxysporum* grown on *Beta vulgaris* achieved cyanide degradation efficiency of 77 % at 22 °C, while simulated winter conditions (5 °C) impaired the activity of the microorganism. *Fusarium oxysporum* is capable of producing numerous enzymes when grown on *Beta vulgaris*, orange peel, carrot peel, and pineapple peel.<sup>26</sup> While bacteria and fungi are commonly used in cyanide degradation, only a few studies have reported cyanide degradation using algae. *Liu et al.*<sup>33</sup> investigated the cyanide degradation efficiency using the algae *Chlorella vulgaris*. The cyanide removal rate increased from 38 to 61 % with the increase in cyanide concentration from 0.1 to 10 mg l<sup>-1</sup> over 72 h. However, the algae were unable to degrade cyanide at concentrations greater than 50 mg l<sup>-1</sup>. High cyanide concentrations pose a danger to the integrity of the cell membrane. Once the cell membrane is destroyed, cyanide ions easily enter the cell and damage the intracellular system.<sup>33</sup> Table 2 provides an overview of mutually comparable scientific studies on biological cyanide degradation published in the last 10 years.

### 5.1.1 Metabolic pathways of cyanide degradation

There are four metabolic pathways of cyanide degradation, depending on the type of enzyme present: hydrolytic, oxidative, reductive, and substitution/transfer. The metabolic pathway by which cyanide will degrade depends on the cyanide concentration and pH value.<sup>4,16,28</sup> However, enzymes responsible for cyanide degradation are mainly produced under mesophilic conditions but can be inhibited by metals. The relative order of inhibitory concentration is copper > nickel > zinc.<sup>16</sup> The same authors reported in 2023 that copper inhibits bacterial cyanide degradation, while iron can promote it.<sup>17</sup> Sometimes, more than one metabolic pathway can be used for cyanide degradation. Hydrolytic, oxidative, and reductive pathways involve the catalytic conversion of cyanide into simple organic or inorganic molecules, while the substitution pathway involves the assimilation of cyanide into microorganisms as a source of nitrogen and carbon.<sup>2</sup> Table 3 provides an overview of cyanide degradation metabolic pathways and their corresponding microorganisms.

#### 5.1.1.1 Hydrolytic pathway

The hydrolytic pathway of cyanide degradation is catalysed by enzymes such as cyanide hydratase, cyanidase, nitrile hydratase, and nitrilase. Nitrile hydratase and nitrilase degrade nitriles (RC≡N), while cyanide hydratase and cyanidase degrade hydrogen cyanide.<sup>2</sup>

##### Cyanide hydratase

Cyanide hydratase belongs to the lyase family, specifically hydrolases, which cleave covalent bonds between carbon and nitrogen. The most common degradation of cyanide occurs through this enzyme, resulting in the formation of formamide (HCONH<sub>2</sub>), which is subsequently degraded into carbon dioxide and ammonia by another enzyme, formamidase. Most fungal species utilize cyanide hydratase.<sup>2,4,16,18</sup>

##### Cyanidase (Cyanide dihydratase)

Cyanidase is also known as cyanide dihydratase. It is a group of bacterial enzymes present in *Alcaligenes xylosoxidans*, *Bacillus pumilus*, and *Pseudomonas stutzeri*. Cyanidases directly convert cyanide into relatively nontoxic formate.<sup>2</sup>

##### Nitrile hydratase

Nitrile hydratase converts aliphatic nitriles into corresponding amides (R–CONH<sub>2</sub>) and is isolated from *Pseudonocardia thermophila* shows high activity compared to other microorganisms such as *Rhodococcus rhodochrous*, *Pseudomonas*, *Corynebacterium*, *Klebsiella*, and *Rhizobium*.<sup>2</sup>

##### Nitrilase

Nitrilase catalyses the hydrolysis of nitriles into carboxylic acids (R–COOH) and ammonia without the formation of free amide intermediates.<sup>2</sup> Nitrilase was first discovered in

*Arthrobacter* sp., which was later identified as *Rhodococcus rhodochrous*. To date, about 40 microorganisms have been isolated and characterised as possessing nitrilase, while more than 60 have been characterised as possessing nitrile hydratase.<sup>34</sup>

#### 5.1.1.2. Oxidative pathway

The oxidative pathway produces ammonia and carbon dioxide through the oxygenolytic conversion of cyanide: 1) directly, using the enzyme cyanide dioxygenase; or 2) in two steps, using the enzyme cyanide monooxygenase, which converts cyanide to cyanate, which is then catalysed by cyanide oxygenase to yield ammonia and carbon dioxide, consuming 1 mole of oxygen per 1 mole of cyanide. This pathway requires nicotinamide adenine dinucleotide phosphate (NADPH) and an additional carbon source. Immobilised cells of *Pseudomonas putida* efficiently produce ammonia and carbon dioxide via the oxidative pathway, and cyanide degradation by this pathway has also been observed in three white rot fungi, *Trametes versicolor*, *Phanerochaete chrysosporium*, and *Pleurotus sajor-caju*.<sup>2,4,16,18</sup>

##### Cyanide dioxygenase

Cyanide dioxygenase catalyses the direct formation of ammonia and carbon dioxide from cyanide.<sup>2</sup>

##### Cyanide monooxygenase and cyanide oxygenase

Cyanide monooxygenase converts cyanide to cyanate, which is further catalysed by cyanide oxygenase, resulting in the overall conversion of cyanate to ammonia and carbon dioxide.<sup>2</sup>

#### 5.1.1.3 Reductive pathway

The reductive pathway is relatively uncommon as it is believed to occur primarily in anaerobic conditions. The enzyme nitrogenase, responsible for this process, has been found in only a few rare species.<sup>2,4,16,18</sup>

##### Nitrogenase

Nitrogenase is an oxygen-sensitive enzyme that utilizes various substrates containing triple bonds between carbon and nitrogen, such as hydrogen cyanide, nitriles, and isonitriles. Both molybdenum and vanadium nitrogenases catalyse a two-step reaction and convert hydrogen cyanide into methane and ammonia.<sup>35</sup> *Klebsiella oxytoca* can degrade cyanide compounds through this pathway.<sup>2,4,16,18</sup>

#### 5.1.1.4 Substitution pathway

The substitution pathway produces  $\beta$ -cyanoalanine or  $\gamma$ -cyano- $\alpha$ -aminobutyrate from amino acids as precursors reacting with cyanide compounds, followed by the release of ammonia and acids by hydrolysis, with enzymes  $\beta$ -cyanoalanine synthase or  $\gamma$ -cyano- $\alpha$ -aminobutyrate synthase. The activity of this pathway involves cyanide assimilation, which typically leads to increased microbial growth or biomass increment. There are two types of enzymes that catalyse cyanide assimilation, rhodanase and sulphurtransferase. Both enzymes are widely distributed in living organisms and catalyse the formation of pyruvate and thiocyanate from mercaptopyruvic acid. If the reaction is mediated by the enzyme sulphurtransferase, cyanide is converted to less toxic thiocyanate, which can then be degraded by the carbonyl or oxidative pathway.<sup>2,4,16,18,35</sup>

##### $\beta$ -Cyanoalanine synthase

$\beta$ -Cyanoalanine synthase is believed to play an important role in removing endogenous cyanide and is produced during very active microbial growth periods.  $\beta$ -Cyanoalanine synthase is induced by various amino acids such as serine, cysteine, asparagine, etc. During this process, there is no direct requirement for oxygen or NAD(P)H, nor is carbon dioxide released.  $\beta$ -Cyanoalanine can be further hydrolysed into ammonia and aspartate in a simple reaction with asparagine as an intermediate.<sup>2</sup>

##### $\gamma$ -Cyano- $\alpha$ -aminobutyrate synthase

$\gamma$ -Cyano- $\alpha$ -aminobutyrate synthase catalyses an alternative pathway for cyanide assimilation. This pathway requires pyridoxal phosphate and is induced by amino acids glutamine or glycine. Once  $\gamma$ -cyano- $\alpha$ -aminobutyrate is synthesised, it slowly converts to amino acid glutamate. A cyanide ion-tolerant thermophilic bacterium, *Bacillus stearothermophilus*, isolated from a hot spring in Japan, has been shown to produce thermostable  $\gamma$ -cyano- $\alpha$ -aminobutyrate synthase.<sup>2</sup>

##### Rhodanase

Rhodanases are common enzymes currently considered to be among the enzymes evolved for cyanide detoxification. They catalyse the irreversible transfer of sulphur atoms from a suitable donor (e.g., thiosulfate) to cyanide, resulting in the formation of less toxic sulphate and thiocyanate. Enzyme activity is regulated by phosphate ions and divalent anions. Rhodanases have been identified in various bacterial species, including *Escherichia coli*, *Azotobacter vinelandii*, and several species of *Thiobacillus*.<sup>2</sup>

##### Sulphurtransferase

Sulphurtransferase belongs to the transferase family that transfers sulphur-containing groups. This enzyme is involved in cysteine metabolism.<sup>2</sup>

Table 3 – Metabolic pathways of cyanide degradation  
 Tablica 3 – Metabolički putevi razgradnje cijanida

Hydrolytic pathway	Microorganism
cyanide hydratase $\text{HCN} + \text{H}_2\text{O} \rightarrow \text{HCONH}_2$	<i>Fusarium</i> sp., <i>P. fluorescens</i>
cyanide dihydratase/cyanidase $\text{R-CN} + 2\text{H}_2\text{O} \rightarrow \text{R-COOH} + \text{NH}_3$	<i>Alcaligenes xylosooxidans</i> , <i>B. pumilus</i> , <i>P. stutzeri</i>
nitrile hydratase $\text{R-CN} + \text{H}_2\text{O} \rightarrow \text{R-CONH}_2$	<i>Brevibacterium</i> sp., <i>Brevibacterium</i> , <i>Corynebacterium</i> , <i>K. oxytoca</i> , <i>Rhodococcus</i> sp., <i>Pseudomonas (chlororaphis, marginales, putida)</i> , <i>Pseudonocardia thermophila</i> , <i>Rhizobium</i>
nitrilase $\text{R-CN} + 2\text{H}_2\text{O} \rightarrow \text{R-COOH} + \text{NH}_3$	<i>Acinetobacter</i> sp., <i>Alcaligenes faecalis</i> , <i>Arthrobacter</i> sp., <i>F. oxysporum</i> , <i>F. solani</i> , <i>K. ozaenae</i> , <i>Nocardia</i> , <i>Pseudomonas</i> , <i>Rhodococcus rhodochrous</i>
Oxidative pathway	Microorganism
cyanide dioxygenase $\text{HCN} + \text{O}_2 + 2\text{H}^+ + \text{NADPH} \rightarrow \text{NADP}^+ + \text{CO}_2 + \text{NH}_3$	<i>B. pumilus</i> , <i>P. cereus</i> , <i>P. fluorescens</i> , <i>E. coli</i>
cyanide monooxygenase $\text{HCN} + \text{O}_2 + \text{NADPH} + \text{H}^+ \rightarrow \text{CNO}^- + \text{NADP}^+ + \text{H}_2\text{O}$	<i>Pseudomonas</i> sp.
cyanide oxygenase $\text{CNO}^- + 3\text{H}^+ + \text{HCO}_3^- \rightarrow \text{NH}_4^+ + 2\text{CO}_2$	<i>E. coli</i> , <i>Flavobacterium</i> , <i>Rhodococcus</i> , <i>P. pseudoalcaligenes</i>
Reductive pathway	Microorganism
nitrogenase $\text{HCN} + 2\text{H}^+ + 2\text{e}^- \rightarrow \text{CH}_2\text{NH}$ $\text{CH}_2\text{NH} + 2\text{H}^+ + 2\text{e}^- \rightarrow \text{CH}_3\text{NH}_2$ $\text{CH}_3\text{NH}_2 + 2\text{H}^+ + 2\text{e}^- \rightarrow \text{CH}_4 + \text{NH}_3$	<i>Azospirillum</i> sp., <i>Azotobacter</i> sp., <i>Herbaspirillum seropedicae</i> , <i>K. oxytoca</i> , <i>Rhodopseudomonas gelatinosa</i> , <i>Rhodospirillum rubrum</i> , <i>Streptomyces thermoautotrophicus</i>
Substitution pathway	Microorganism
$\beta$ -cyanoalanine synthase $\text{HCN} + \text{HS-CH}_2\text{CH}(\text{NH}_2)\text{COOH}$ (L-cistein) $\rightarrow$ $\rightarrow \text{H}_2\text{S} + \text{NC-CH}_2\text{CH}(\text{NH}_2)\text{COOH}$ ( $\beta$ -cyanoalanine)	<i>B. megaterium</i> , <i>Chromobacterium violaceum</i> , <i>Enterobacter</i> sp., <i>E. coli</i> , <i>Pseudomonas</i> sp.
$\gamma$ -cyano- $\alpha$ -aminobutyrate synthase	<i>B. stearothermophilus</i>
sulphurtransferase and rhodanase	<i>Azotobacter vinelandii</i> , <i>Desulfotomaculum nitrificans</i> , <i>E. coli</i> , <i>Ferrobacillus ferrooxidans</i> , <i>Fusarium</i> sp., <i>P. aeruginosa</i> , <i>Thermobacillus denitrificans</i>

### 5.1.1.2 Anaerobic cyanide degradation

Although most microorganisms degrade cyanide aerobically, anaerobic degradation is also possible, albeit slower and more sensitive than aerobic degradation. Generally, the process of anaerobic cyanide degradation is poorly understood, and little is known about the involved microbial communities. The chemical nature of cyanide biodegradation reactions explains the fact that under anaerobic conditions, cyanide can only be degraded through reduction or hydrolysis pathways. The mechanism of cyanide degradation via these two pathways in anaerobes is analogous to aerobes.<sup>9,27,35</sup> Maciel et al.<sup>36</sup> argue that anaerobic degradation of cyanide and cyanate occurs only in the presence of hydrogen sulphide, with the process being slower than aerobic degradation. Anaerobic microorganisms, especially methanogens, are more sensitive than aerobes because they contain metalloproteins that are inhibited in the presence of cyanide. For comparison, the cyanide toxicity threshold is around 200 mg l<sup>-1</sup> for most aerobic microorganisms and 2 mg l<sup>-1</sup> for some anaerobes.<sup>28,35</sup>

### 5.2 Biological degradation of thiocyanate

Thiocyanate can serve as a source of energy, carbon, sulphur, or nitrogen for various bacteria, with some utilising nitrogen from ammonia liberated from thiocyanate.<sup>15,35</sup> Most microorganisms degrade thiocyanate autotrophically (including *Paracoccus* sp., *Thiobacillus denitrificans*, *Thiobacillus thioparus*, and *Thiohalophilus thiocyanoxidans*) and use thiocyanate as the sole energy source through sulphur oxidation, while other microorganisms use thiocyanate as a carbon source (including *Acinetobacter* sp., *Bacillus* sp., *Klebsiella* sp., *Proteus* sp., *Pseudomonas* sp., *Thiobacillus* sp., and some fungi) or as a nitrogen source (including *Arthrobacter* sp., *Klebsiella* sp., *Methylobacter* sp., *Pseudomonas* sp., *Ralstonia* sp., and *Sphingomonas* sp.). The latter derive their energy mainly from organic carbon. As an inorganic salt, thiocyanate is less readily utilised by microorganisms compared to organic carbon sources. Besides the mentioned bacteria, *Acremonium strictum*, a fungus isolated from coke plant wastewater, can degrade thiocyanate at neutral pH and high concentrations of nitrate and phenol. However, there have been no reports of

the metabolic pathway of thiocyanate degradation by fungi. Although microorganisms utilise thiocyanate as a growth factor, converting it into non-toxic and harmless substances, incomplete oxidation of thiocyanate can result in cyanide production. The advantage of mixed cultures over pure cultures is that a mixed culture can simultaneously degrade both cyanide and thiocyanate.<sup>1,8,19–21</sup> Compared to the biological degradation of cyanide and phenol, the biological degradation of thiocyanate is the slowest and most sensitive process, determining the hydraulic retention time (HRT) of wastewater in real systems. Aerobic degradation of thiocyanate produces sulphate ion, ammonium ion, and carbon dioxide.<sup>20,21</sup> Generated ammonia is removed by subsequent nitrification.<sup>37</sup> The biologically mediated oxidation reaction of thiocyanate is described by Eq. (2).<sup>38</sup>



Stoichiometrically, the oxidation of 1 g of thiocyanate requires 1.1 g of oxygen, resulting in the release of 0.24 g of ammonia, of which 10 % is utilised by microorganisms as a nitrogen source, while the remaining portion persists in the water body as ammonium ion. A mixed culture in the study conducted by Raper et al.<sup>12</sup> produced 0.26 mol of ammonia for every mol of thiocyanate degraded, indicating that ammonia production under controlled conditions is consistent with the expected theoretical production. Biomass yield is approximately 0.08 g per 1 g of oxidised thiocyanate.<sup>12</sup> Ammonia is produced during the biological degradation of thiocyanate via all known degradation pathways. It has been observed that ammonia has an inhibitory effect on thiocyanate degradation. The efficiency of thiocyanate removal decreases by 24, 19, 22, and 43 % with the addition of 250, 500, 1000, and 1500 mg l<sup>-1</sup> of ammonia, respectively. Sulphate, on the other hand, at concentrations between 80 and 2000 mg l<sup>-1</sup>, has a negligible effect on thiocyanate degradation, while phenol inhibits biological thiocyanate degradation.<sup>11,12</sup> Thiocyanate degradation rates depend not only on oxygen concentrations but also on nutrient concentrations. Phosphate concentrations > 45 mg l<sup>-1</sup> have been shown to stimulate biological thiocyanate degradation. The presence of cyanide significantly inhibits biological thiocyanate degradation.<sup>15,39</sup> In natural water systems, thiocyanate degradation can be carried out by individual organisms as well as microbial consortia.<sup>15</sup> Thiocyanate-degrading bacteria have been isolated and identified from various sources, including the genera *Acinetobacter*, *Arthrobacter*, *Bacillus*, *Burkholderia*, *Chryseobacterium*, *Escherichia*, *Klebsiella*, *Methylobacterium*, *Pseudomonas*, *Ralstonia*, *Thioalkalivibrio*, and *Thiobacillus*.<sup>12,20,21</sup> Multiple bacterial species of these genera are responsible for aerobic degradation of aromatic compounds and thiocyanate, using thiocyanate as a carbon and nitrogen source.<sup>39,40</sup> Pan et al.<sup>37</sup> investigated the efficiency of biological thiocyanate degradation depending on pH value. Increasing the pH value from 6 to 7 drastically increased thiocyanate biodegradation efficiency from 5.6 to 56.4 %. With a gradual increase in pH value to 8, the efficiency of thiocyanate biodegradation reached a maximum of 66.4 %, then significantly decreased to 48.4 % as the pH further increased to 9. In addition to pH control, maintaining temperature within the mesophilic range (25–35 °C) favours biological thiocyanate degradation. On the other hand, if the temperature is not controlled, bio-

logical degradation is significantly affected, and degradation efficiency decreases.<sup>12</sup> In the study by Raper et al.,<sup>12</sup> a mixed culture was dominated by *Thiobacillus* (26 %), but active sludge also contained significant representation of the genera *Mizugakiibacter* (13 %), *Comamonas* (12 %), and *Rhodanobacter* (11 %). It has been documented that *Thiobacillus thioparus* and *Thiobacillus denitrificans* are capable of using thiocyanate as the sole energy source. After 120 h, the removal of 110 mg l<sup>-1</sup> thiocyanate was complete. As initial thiocyanate concentrations increased to 610 mg l<sup>-1</sup>, degradation efficiency decreased.<sup>12</sup> On the other hand, Li et al.<sup>20</sup> demonstrated that high concentrations of thiocyanate in wastewater (1818 mg l<sup>-1</sup>) can be effectively biologically degraded (99 %) provided that a sufficient amount of dissolved oxygen (3–6 mg l<sup>-1</sup>) and sufficient retention time (46 h) are available.<sup>20</sup> Multiple pathways for biological thiocyanate degradation have been identified, including the action of autotrophic and heterotrophic bacteria. Autotrophic bacteria utilise inorganic carbon from thiocyanate as a carbon source, while heterotrophic degraders use thiocyanate as a nitrogen source and organic carbon as an energy source. Autotrophic pathways are the most common, while heterotrophic pathways are mainly associated with tests in synthetic wastewater.<sup>12</sup> There are two metabolic pathways for thiocyanate degradation, carbonyl and cyanate, both of which are essentially aerobic.<sup>8</sup>

### 5.2.1 Carbonyl pathway of thiocyanate degradation

The carbonyl pathway of thiocyanate degradation (CAS) occurs under the influence of thiocyanate hydrolase in two steps. The first step is the hydrolytic cleavage of the C≡N bond, generating ammonia and carbonyl sulphide, which readily diffuses out of the cells and can be detected in the gas phase. The second step involves the breaking of the carbon-sulphur bond within O=C=S into carbon dioxide and hydrogen sulphide, which is further oxidised to sulphate.<sup>8,35,41</sup>

### 5.2.2 Cyanate pathway of thiocyanate degradation

In the cyanate pathway of thiocyanate degradation (CNO), the carbon-sulphur bond within N≡C-S- is hydrolysed to cyanate by thiocyanate dehydrogenase, which is then hydrolysed to ammonia and carbon dioxide by cyanase. The sulphide ion is further oxidised to sulphide and sulphate.<sup>8</sup> Cyanase has been discovered in *Escherichia coli* and *Flavobacterium* sp.<sup>18,20</sup> Li et al.<sup>20</sup> reported that *Thiobacillus* isolated from a coke wastewater treatment plant degrades thiocyanate via the carbonyl pathway, while *Pseudomonas putida* and *Pseudomonas stutzeri*, isolated from soil contaminated with wastewater from gold mining, belong to the cyanate degradation pathway. The mechanisms of thiocyanate degradation are illustrated in Fig. 2.

### 5.2.3 Anaerobic degradation of thiocyanate

Anaerobic degradation of thiocyanate has been observed, and the relatively low rate of anaerobic degradation can be explained by the lower yield of metabolic energy as-

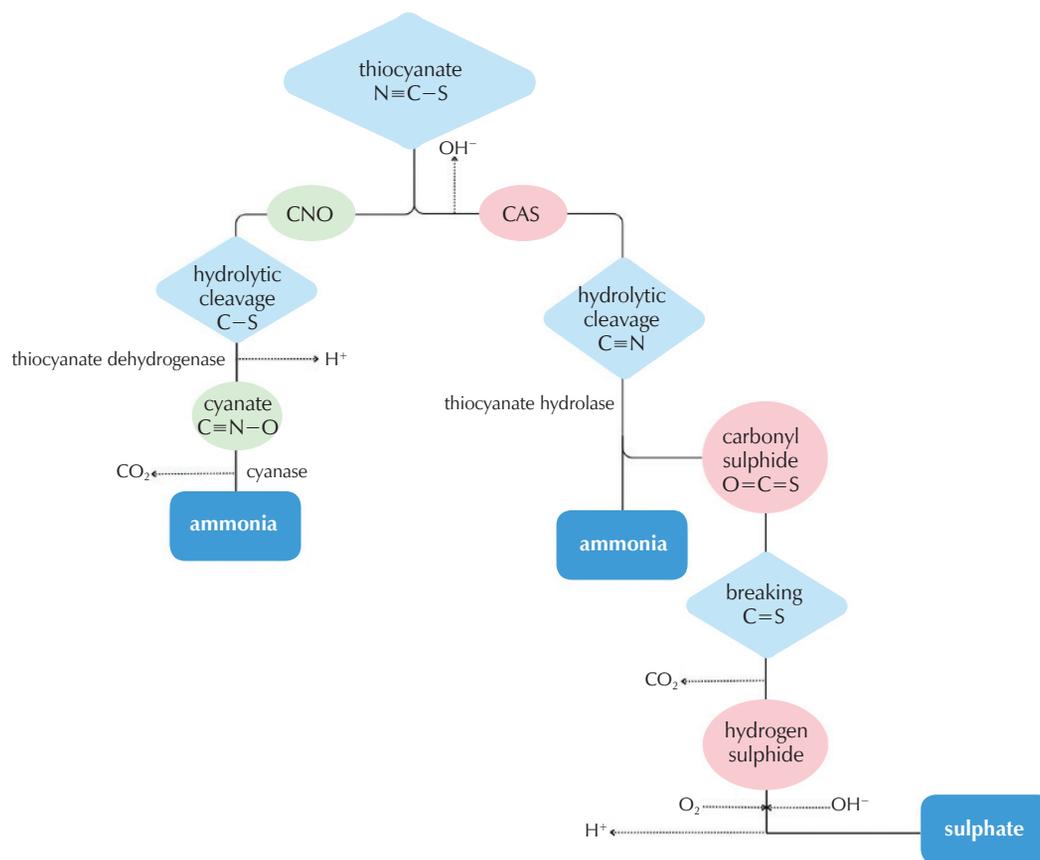


Fig. 2 – Mechanisms of thiocyanate degradation  
Slika 2 – Mehanizmi razgradnje tiocijanata

sociated with the use of electron acceptors weaker than oxygen. Since the only electron acceptor that has been demonstrated to support bacterial thiocyanate oxidation in the absence of oxygen is nitrate, degradation under such conditions is limited by nitrate. Anaerobic degradation is generally not detected in industrial systems.<sup>15</sup> However, several end products are produced by the biological degradation of thiocyanate, including ammonia, sulphate, carbonyl sulphide, and trithionate. Ammonia and sulphate are produced under aerobic and non-toxic conditions, carbonyl sulphide only under aerobic conditions, and trithionate only under anoxic conditions. Additionally, several intermediates have been observed, including thiosulfate, tetrathionate, and cyanate.<sup>12</sup> Kurashova et al.<sup>15</sup> reported that *Thiobacillus* isolated from wastewater from steel production is capable of degrading 300 mg l<sup>-1</sup> of thiocyanate over 8 days under anaerobic denitrifying conditions.

### 5.3 Biological degradation of phenolic compounds

Biological degradation of phenolic compounds involves the complete mineralisation of phenolic pollutants into carbon dioxide, water, and harmless end products, a process facilitated by a diverse group of microorganisms including bacteria, microalgae, yeasts, and fungi. The presence or absence of molecular oxygen plays a crucial role in the biological degradation of phenol. Generally, phenol can be biologically degraded both under aerobic and anaerobic

conditions. Anaerobic biological degradation is less promising due to the incomplete mineralisation of phenolic compounds, which often requires an external carbon source, and generates toxic by-products.<sup>42–44</sup> Phenol serves as the sole carbon and energy source for microorganisms in both aerobic and anaerobic conditions. However, aromatic pollutants such as phenols resist microbial degradation due to the stability of the phenyl ring, with substituted phenols, particularly halogenated phenols, being even more resistant to biological degradation. Although recent literature on the biological degradation of phenol in wastewater using bacteria is limited, research conducted over the past decade has provided valuable insights into the effectiveness and potential application of various bacterial strains. For instance, Sarwade and Gawai<sup>45</sup> investigated the efficiency of biological phenol degradation using the bacterium *Bacillus badius* at different phenol concentrations (420–1680 mg l<sup>-1</sup>). They found that *Bacillus badius* was able to degrade 98 % of the phenol at the lowest concentration, while the degradation efficiency at higher concentrations was around 70 %. Environmental conditions play a crucial role in phenol degradation. In this study, the effects of pH, temperature, salinity, and additional carbon and nitrogen sources were investigated over a 48-hour period. The highest phenol degradation was observed at pH 9, while moderate but significant degradation occurred at pH 7, 10, and 11. At a sodium chloride concentration of 0.5 %, 82 % of phenol was degraded, with a slight decrease in degradation efficiency as salinity increased up to 2.5 %. The opti-

mal temperature for phenol degradation ranged between 30 and 35 °C. The addition of various carbon sources enhanced phenol degradation, with starch proving to be the most effective, while potassium nitrate as a nitrogen source further increased the degradation rate. Phenol biotransformation into catechol occurred within 12 h. The bacterium utilised both the *ortho* and *meta* degradation pathways, which will be discussed in detail later. Furthermore, *Shahryari et al.*<sup>14</sup> demonstrated the ability of a bacterial isolate, *Acinetobacter* sp., obtained from a river in southern Tehran contaminated with farmland pesticides and oil refinery pollutants, to degrade 1000 mg l<sup>-1</sup> phenol over 60 h at an optimal pH of 7 and a temperature of 30 °C. Additional investigations showed no positive effect of increasing the number of microbial cells from 10<sup>6</sup> to 10<sup>8</sup> CFU/ml on the degradation of higher phenol concentrations. On the contrary, concentrations of phenol ranging from 3000 to 4000 mg l<sup>-1</sup> caused strong toxicity in living cells, indicating that phenol concentrations exceeding 1000 mg l<sup>-1</sup> have destructive effects on the integrity of *Acinetobacter* sp. cell membranes. The results also showed that *Acinetobacter* sp. could completely degrade 400 mg l<sup>-1</sup> phenol in less than 48 h. In comparison, it took *Pseudomonas* sp. five days to completely degrade the same concentration of phenol, while *Klebsiella* sp. failed to fully degrade phenol even after two weeks.<sup>14</sup> Moreover, *Lin and Gu*<sup>46</sup> reported complete degradation of 400 mg l<sup>-1</sup> phenol by *Pseudomonas putida* within 48 h.<sup>46</sup> *Acinetobacter calcoaceticus*, isolated from phenol-contaminated wastewater, exhibited high efficiency in phenol degradation (92 %), but also inhibition by phenol at concentrations above 2000 mg l<sup>-1</sup>.<sup>47</sup> *Wen et al.*<sup>48</sup> demonstrated incomplete degradation (67 %) of 500 mg l<sup>-1</sup> phenol by the species *Rhodococcus* sp. over 42 h.<sup>48</sup> Additionally, the cold-adapted bacterium *R. erythropolis*, isolated from alpine soil, was capable of degrading large quantities of phenol at low temperatures, highlighting its potential for phenol biodegradation in cold environments.<sup>49</sup> Pure bacterial cultures often accumulate toxic by-products due to the lack of secretion of relevant enzymes, thus mixed-culture phenol degradation is a promising technique with several advantages, including high tolerance to toxicity and synergy in enzyme secretion without the accumulation of harmful by-products.<sup>4,44</sup> For example, a bacterial consortium consisting of *Pseudomonas* and *Alcaligenes* has shown promising results in degrading phenolic compounds from industrial streams.<sup>50</sup> Additionally, a community of *Pseudomonas aeruginosa* and *Klebsiella variicola* isolated from sewage sludge degraded 1000 mg l<sup>-1</sup> phenol at a pH of 7.5 and a temperature of 30 °C.<sup>51</sup> A mixed culture consisting of *Alcaligenes*, *Bacillus*, and *Pseudomonas* in the study by *Poi et al.*<sup>52</sup> degraded 90 % of the initial phenol concentration (407 mg l<sup>-1</sup>) after 96 h.<sup>52</sup> Microalgae, due to their photosynthetic activity, have the ability to absorb and metabolise various toxic compounds, including phenols, while bacteria can contribute to the degradation of these compounds through biological processes. The interaction between microalgae and bacteria in phenol removal systems may represent an innovative approach, especially in the context of wastewater treatment. For example, *Tao et al.*<sup>53</sup> demonstrated that a community of the microalga *Chlorella* sp. and the bacterium *Cupriavidus necator* degraded 1200 mg l<sup>-1</sup> phenol

within 60 h.<sup>53</sup> Additionally, *Zhang et al.*<sup>54</sup> highlighted the complete removal of pyridine (C<sub>5</sub>H<sub>5</sub>N) at an initial concentration of 125 mg l<sup>-1</sup> within 108 h in a symbiotic microalgae-bacteria system *Chlorella-Paracoccus*.<sup>54</sup> Recent but also limited research has provided insights into yeast-mediated phenol degradation. For example, *Filipowicz et al.*<sup>55</sup> identified yeast strains designated A011, B021, and L012 as members of the species *Candida subhashii*, *Candida oregonensis*, and *Schizoblastosporion starkeyi-henricii*, respectively, and demonstrated their potential in degrading 500, 750, and 1000 mg l<sup>-1</sup> phenol. Furthermore, *Mahgoub et al.*<sup>56</sup> indicated the potential of *Candida* strains for phenol degradation, suggesting their suitability for further research. In recent studies, fungi have emerged as potentially powerful tools for efficient phenol degradation due to their enzymatic mechanisms and bioremediation capabilities. For example, *Legorreta-Castañeda et al.*<sup>57</sup> highlighted the ability of fungal cells to reduce harmful water pollutants, including phenol. Furthermore, *Bernats and Juhna*<sup>58</sup> proposed biological treatment by white rot fungi such as *Trametes versicolor* and *Phanerochaete* as an effective method (93 %) for removing phenol (420 mg l<sup>-1</sup>) before conventional wastewater treatment. Additionally, *Ibrahim and Al-Chamdi*<sup>59</sup> demonstrated successful degradation of 100 mg l<sup>-1</sup> phenol by mutated and immobilised strains of *Aspergillus* (28 %) and *Penicillium* (13 %), indicating the potential for increasing phenol removal efficiency through genetic and immobilisation techniques. The mentioned research highlights the diverse metabolic capabilities and environmental adaptability of various bacteria, algae, yeasts, and fungi, making them promising candidates for phenol degradation. Table 4 presents comparable studies conducted on the effectiveness of biological phenol degradation, not older than from 2014.

### 5.3.1 Mechanism of aerobic phenol degradation

The first step in aerobic phenol degradation is the formation of the universal metabolite, catechol, which is generated by the enzymatic system of hydroxylase prior to the *ortho* or *meta* pathway of aromatic ring degradation. Under aerobic conditions, phenol degradation begins with the hydroxylation of the aromatic ring, in other words, the attachment of a hydroxyl group to the *ortho* position of the benzene ring using phenol hydroxylase monooxygenase to form catechol. Depending on the responsible microorganism, catechol undergoes ring cleavage at the *ortho* or *meta* position. The *ortho* pathway of aromatic ring degradation is catalysed by the enzyme catechol 1,2-dioxygenase, which contains Fe<sup>3+</sup> as a prosthetic group, generating *cis*, *cis*-muconate. Later, the metabolites enter the tricarboxylic acid cycle for complete phenol degradation. On the other hand, the *meta* pathway of degradation is catalysed by the enzyme catechol 2,3-dioxygenase, which contains Fe<sup>2+</sup> as a prosthetic group, generating 2-hydroxymuconate semi-aldehyde, which is further metabolised in the tricarboxylic acid cycle. Unsubstituted aromatic compounds are degraded *via* the *meta* pathway, while halogenated ones are degraded *via* the *ortho* pathway.<sup>2,42,43,63</sup> The mechanism of aerobic phenol degradation is depicted in Fig. 3.

Table 4 – Review of scientific research on the biological degradation of phenolic compounds  
 Tablica 4 – Pregled znanstvenih istraživanja biološke razgradnje fenola

Pure bacterial culture	Phenol/mg l <sup>-1</sup>	Efficiency/%	Time/h	pH/–	Temperature/°C	Ref.
<i>Acinetobacter</i> sp.	1000	100	60	7	30	14
<i>A. calcoaceticus</i>	800	92	48	8	30	47
<i>A. calcoaceticus</i>	1700	46	48	8	30	47
<i>B. badius</i>	420	98	48	9	37	45
<i>B. badius</i>	1500	82	48	9	37	45
<i>B. pumilus</i>	50	84	48	7	37	60
<i>B. subtilis</i>	500	100	36	7	35	43
<i>Pseudomonas</i> sp.	700	100	24	7	35	43
<i>Pseudomonas</i> sp.	5	98	36	7	30	61
<i>P. aeruginosa</i>	400	100	60	7	35	43
<i>P. putida</i>	400	98	47	7	30	46
<i>Rhodococcus</i> sp.	500	67	42	7	30	48
<i>Rhodococcus</i> sp.	500	89	21	7.4	30	62
Mixed bacterial culture						
<i>P. aeruginosa</i> , <i>B. subtilis</i>	250	100	36	7	37	25
<i>Alcaligenes</i> , <i>Bacillus</i> , <i>Pseudomonas</i>	300	90	96	7	30	52
<i>P. aeruginosa</i> , <i>K. variicola</i> , <i>K. pneumoniae</i>	1000	76	96	7.5	35	51
Algae						
<i>U. prolifera</i>	0.1	94	24	7	30	54
<i>Chlorella</i> , <i>C. necator</i>	1200	100	60	7.5	32	53
Yeast						
<i>Candida subhashii</i>	1000	100	48	6	18	55
<i>Candida oregonensis</i>	750	100	48	6	18	55
<i>Schizoblastosporion starkeyi-henricii</i>	1000	100	48	6	18	55
Fungi						
<i>T. versicolor</i>	420	93	168	6	25	58
<i>Aspergillus niger</i>	100	28	–	–	–	59
<i>Penicillium griseofulvum</i>	100	13	–	–	–	59
<i>Aspergillus terreus</i>	100	17	–	–	–	59

### 5.3.2 Mechanism of anaerobic phenol degradation

Anaerobic microorganisms, although more sensitive to phenolic loads compared to their aerobic counterparts and exhibiting slower growth rates, possess significant advantages such as reduced sludge production and easier reactor setup. A wide range of different phyla has been found within anaerobic wastewater treatment bioreactors contaminated with phenol, both on a laboratory and industrial scale. These phyla include *Bacteroidetes*, *Chloroflexi*, *Cloacimonetes*, *Euryarchaeota*, *Firmicutes*, *Proteobacteria*, *Synergistetes*, and *Thermotogae*.<sup>64</sup> The anaerobic process is less stable than the aerobic process, starting with the carboxylation of phenol in the *para* position by the action of the enzyme 4-hydroxybenzoate carboxylase. Carboxylation of phenol occurs in two steps. In the first

step, a phosphate group is added to phenol from an unknown phosphorus donor, catalysed by the ATP-dependent enzyme phenyl-phosphate synthase, resulting in phenyl phosphate. The second step involves the formation of 4-hydroxybenzoate through the carboxylation of phenyl phosphate, catalysed by the enzyme phenyl-phosphate carboxylase, which requires the presence of Mn<sup>2+</sup> ion. This two-step pathway is present in bacterial genera such as *Azoarcus*, *Geobacter*, *Pseudomonas*, and *Thauera*, while in other bacteria, mainly strict anaerobes such as *Clostridium hydroxybenzoicum*, in the presence of high concentrations of phenol and carbon dioxide, phenol is directly converted to 4-hydroxybenzoate by 4-hydroxybenzoate decarboxylase.<sup>2,4,9,64</sup> 4-hydroxybenzoyl-CoA ligase then transfers CoA to 4-hydroxybenzoate, forming 4-hydroxybenzoyl-CoA. This is reduced by dehydroxylation to benzoyl-CoA, which

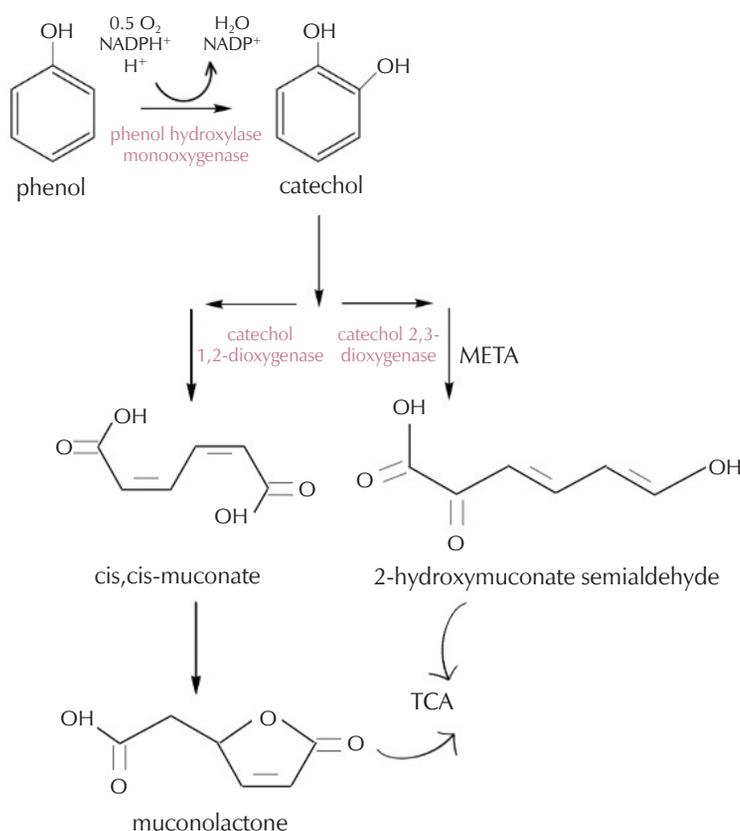


Fig. 3 – Aerobic phenol degradation  
Slika 3 – Aerobna razgradnja fenola

is a common intermediate in the metabolism of many aromatic compounds, with the help of 4-hydroxybenzoyl-CoA reductase. Benzoyl-CoA is then reductively dearomatized to cyclohexa-1,5-dien-1-carbonyl-CoA. This is catalysed by benzoyl-CoA reductase. The gene sequences for benzoyl-CoA reductase have been found in most facultative anaerobes that degrade aromatic compounds (e.g., genera *Azoarcus*, *Magnetospirillum*, and *Thauera*). Finally, cyclohexa-1,5-dien-1-carbonyl-CoA can undergo ring cleavage in two ways. In the first, e.g., *T. aromatica* hydrates cyclohexa-1,5-dien-1-carbonyl-CoA to 6-hydroxycyclohexa-1-ene-1-carbonyl-CoA and reduces it to 6-oxocyclohexa-1-ene-1-carbonyl-CoA. The ring then opens by hydrolase to form 3-hydroxypimeloyl-CoA. In the second, e.g., *R. palustris*, cyclohex-1-ene-1-carbonyl-CoA is hydrated and then reduced to 2-ketocyclohexa-1-carbonyl-CoA. The ring is then hydrolysed to form pimeloyl-CoA, which is then reduced and hydrated to 3-hydroxypimeloyl-CoA. Both pathways continue degradation to acetyl-CoA and carbon dioxide.<sup>2,4,9,64</sup> There is also an alternative pathway for anaerobic phenol degradation, from caproate to acetate. The anaerobic degradation pathway of phenol via caproate is still a relatively unknown process due to the accumulation of intermediates being hindered by rapid degradation rates, preventing detailed studies. It is known that this pathway occurs under thermophilic conditions (55 °C), whereas the previously mentioned pathway main-

ly occurs under mesophilic conditions, but is also possible under thermophilic conditions. Within this pathway, phenol is first reduced to cyclohexanone in the presence of nitrate, and then to *n*-caproate. *N*-caproate is then β-oxidised to fatty acids. Microbial communities involved in thermophilic degradation have yet to be fully characterised and identified. *Azoarcus* sp. is a rare case of bacteria found to use both phenol and caproate as substrates under denitrification conditions.<sup>64</sup> Fig. 4 illustrates the mechanism of anaerobic phenol degradation.

## 6 Simultaneous biological degradation of cyanide, thiocyanate, and phenolic compounds

*Arutchelvan et al.*<sup>42</sup> investigated the capacity for phenol degradation in the presence of thiocyanate and cyanide, as these compounds are commonly found together with phenol in most industrial wastewater. The effect of thiocyanate and cyanide on phenol degradation was studied using microorganisms *Pseudomonas cepacia* and *Bacillus brevis* at an initial phenol concentration of 1500 mg l<sup>-1</sup>. The microorganisms utilised phenol as a carbon source even in the presence of thiocyanate and cyanide. Since

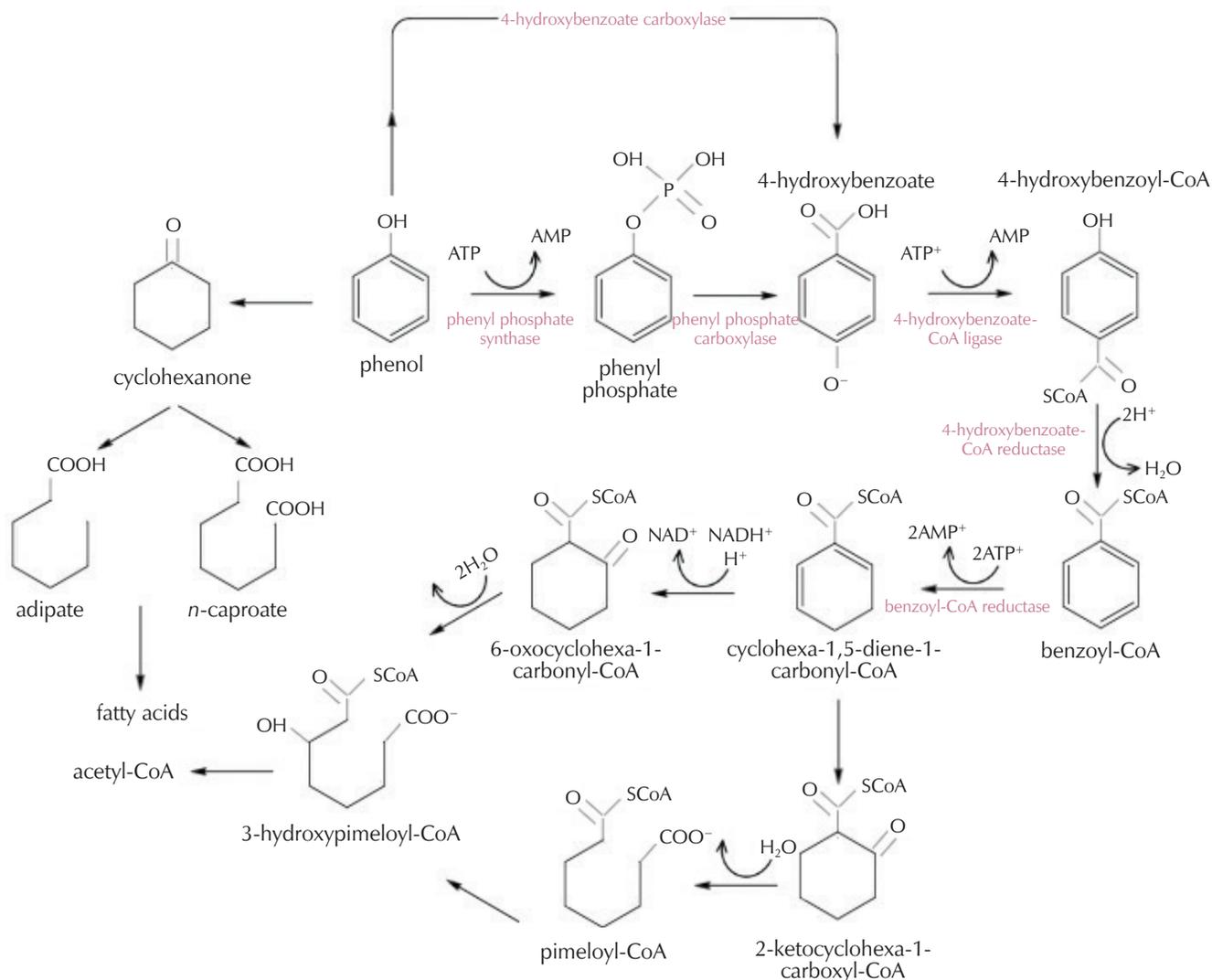


Fig. 4 – Anaerobic phenol degradation  
Slika 4 – Anaerobna razgradnja fenola

thiocyanate is biodegradable, it did not inhibit phenol degradation in *Pseudomonas cepacia* until a concentration of 1000 mg l<sup>-1</sup>, while at 1500 mg l<sup>-1</sup>, there was a significant reduction in degradation rate. Similarly, with *Bacillus brevis*, at a thiocyanate concentration of 1500 mg l<sup>-1</sup>, the efficiency of phenol removal was even lower than with *Pseudomonas cepacia*. Furthermore, *Pseudomonas cepacia* degraded 1500 mg l<sup>-1</sup> phenol in the presence of 15 mg l<sup>-1</sup> cyanide, while the degradation rate decreased at a cyanide concentration of 30 mg l<sup>-1</sup>, and there was no biomass growth or phenol degradation at 75 mg l<sup>-1</sup> cyanide. *Bacillus brevis* was also shown to degrade phenol in the presence of cyanide at initial concentrations of 15, 30, and 75 mg l<sup>-1</sup>, with approximately 86.4 % phenol removal observed at 15 mg l<sup>-1</sup> cyanide. However, at higher cyanide concentrations, the degradation efficiency of the organism was completely reduced. Although there are many reports on the individual biological degradation of phenol and cyanide, there is little research on simultaneous degradation of both compounds at high concentrations. Agarwal et al.<sup>65</sup> demonstrated the effectiveness of biological cyanide

degradation, which decreased with increasing phenol concentrations. Singh and Mishra<sup>10</sup> conducted a study on the simultaneous degradation of phenol and cyanide by microorganisms *Pseudomonas putida* and *Pseudomonas stutzeri*. *Pseudomonas putida* tolerated phenol up to 1500 mg l<sup>-1</sup> and cyanide up to 340 mg l<sup>-1</sup>, while *Pseudomonas stutzeri* tolerated phenol up to 1800 mg l<sup>-1</sup> and cyanide up to 300 mg l<sup>-1</sup>. The isolates showed the ability to degrade phenol up to 80.5 % and cyanide up to 80.6 %, but also exhibited the ability to reduce BOD<sub>5</sub>, COD, and pH values. The biological degradation of this mixture is considered extremely challenging due to the presence of two toxic compounds. However, both bacterial species not only showed the ability to grow in the presence of phenol and cyanide but also simultaneously degraded them, which is considered a newly discovered finding of this study. In combined biological degradation of phenol and thiocyanate, phenol plays an inhibitory role in the biological degradation of thiocyanate, with the addition of 80 to 180 mg l<sup>-1</sup> phenol resulting in a reduction in thiocyanate degradation by 29 to 41 %. Increasing phenol concentration results in greater

availability of organic carbon, favouring the growth of heterotrophic bacteria, which compete with slower-growing autotrophs for dissolved oxygen.<sup>11,12</sup>

## 7 Conclusion

Wastewater originating from various industries containing cyanides, thiocyanates, and/or phenolic compounds must be treated before discharge into the environment to protect water bodies. Among the available technologies for removing toxic and hazardous pollutants from wastewater, biological degradation is the most promising due to its numerous advantages, such as environmental friendliness, economic sustainability, and practical feasibility. The key advantage of biological wastewater treatment is its ability to simultaneously remove several compounds in one process, often at significantly lower costs compared to other methods. There are four metabolic pathways for cyanide degradation, depending on the type of enzyme present: hydrolytic, oxidative, reductive, and substitutional/transfer. On the other hand, two known metabolic pathways for thiocyanate degradation, carbonyl and cyanate, are both essentially aerobic. In aerobic phenol degradation, the first step is the formation of the universal metabolite, catechol, which is generated by the enzymatic hydroxylase system prior to the *ortho* or *meta* pathway of aromatic ring degradation. While most microorganisms degrade these compounds aerobically, anaerobic degradation is also possible, albeit slower and more sensitive than aerobic degradation. Overall, the process of anaerobic degradation of cyanide, thiocyanate, and phenol is not well understood, and little is known about the microbial communities involved. This paper summarises reviewed research on the biological degradation of cyanide, thiocyanate, and phenol with associated microorganisms. Due to the high rates of biological degradation of these compounds, identified genera could contribute to reducing the toxicity of industrial wastewater containing these compounds.

### List of abbreviations

#### Popis kratica

AMP	– adenosine monophosphate – adenosin monofosfat
ATP	– adenosine triphosphate – adenosin trifosfat
BOD <sub>5</sub>	– biochemical oxygen demand – biokemijska potrošnja kisika
CAS	– carbonyl pathway of thiocyanate degradation – karbonilni put razgradnje tiocijanata
CNO	– cyanate pathway of thiocyanate degradation – cijanatni put razgradnje tiocijanata
CoA	– coenzyme A – koenzim A
COD	– chemical oxygen demand – kemijska potrošnja kisika
HCN	– hydrogen cyanide – cijanovodik

HRT	– hydraulic retention time – hidrauličko vrijeme zadržavanja
NADH	– nicotinamide adenine dinucleotide (reduced) – nikotinamid adenin dinukleotid (reducirani)
NADPH	– nicotinamide adenine dinucleotide phosphate – nikotinamid adenin dinukleotid fosfat

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## SAŽETAK

### Biološka razgradnja cijanida, tiocijanata i fenolnih spojeva u otpadnim vodama

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Otpadne vode iz različitih industrija, poput koksne, rudarske, farmaceutske, naftne i slično, često sadrže visoke koncentracije toksičnih spojeva kao što su cijanidi, tiocijanati i fenolni spojevi, kako pojedinačno tako i u kombinaciji. Njihova prisutnost značajan je izazov za očuvanje prirodnih vodenih ekosustava. Radi zaštite vodenih resursa i očuvanja biodiverziteta ključno je smanjiti koncentracije tih toksičnih spojeva na razine propisane zakonom. Obrada industrijskih otpadnih voda obično uključuje niz tehnoloških postupaka, a biološka obrada ističe se kao jedan od najvažnijih i najučinkovitijih. Stoga je nužno istražiti potencijal biološke razgradnje navedenih toksičnih spojeva te razumjeti mehanizme njihove transformacije u različitim okolišnim uvjetima. Ovaj rad pruža sveobuhvatan pregled biološke razgradnje navedenih toksičnih spojeva iz industrijskih otpadnih voda, istražujući različite mikroorganizme i tehnike razgradnje s ciljem osiguranja učinkovite i ekološki prihvatljive obrade vode.

#### Ključne riječi

Cijanid, tiocijanat, fenol, otpadna voda, biološka razgradnja

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