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Optimization of Biodegradation Conditions for Cyanide Removal by a Volcanic Bacterial Consortium

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ABSTRACT
Cyanide is a highly toxic pollutant produced by the activities of mining, electroplating, metal finishing, and chemical manufacturing industries. Available conventional physicochemical treatment methods are often costly and can produce secondary pollutants. Bioremediation is an attractive alternative as once the introduced cyanide-degrading microorganisms have acclimatized, degradation becomes sustainable. In this study, we evaluated the ability of a bacterial consortium isolated from volcanic soil to grow and degrade cyanide as a nitrogen source under different environmental conditions. Characterizations of the effects of carbon source, pH, temperature, inoculum size, cyanide concentration, and heavy metals on bacterial growth and cyanide degradation gave a maximum growth of 7.0 log CFU/mL, and 57.26% cyanide degradation was observed with glucose as the best source of carbon. Optimum biodegradation was achieved at pH 7.2 and between 25 and 35 °C, with cyanide removal efficiencies of 70.4% and 66.46%, respectively. The consortium was found to demonstrate tolerance up to cyanide concentrations of 500 mg/L but significant inhibition was observed at concentrations above 600 mg/L. Mercury and silver had the most inhibitory effect on growth and degradation. Growth profile studies at an initial cyanide concentration of 200 mg/L indicated approximately 72.5% removal. The results indicate that microbial consortia from volcanic soil are promising candidates for bioremediation of cyanide-contaminated soils and wastewater under moderate operating conditions.

KEYWORDS: Cyanide biodegradation, microbial consortium, volcanic soil, bioremediation, cyanide detoxification
SDG Keywords: SDG 6 Clean water and sanitation; SDG 12 Responsible consumption and production; SDG 9 Industry, innovation and infrastructure

INTRODUCTION

Cyanide is a highly toxic compound widely used in industry, for example in gold mining, especially in Indonesia, where it is used in artisanal or small-scale operations. Other uses include in electroplating, metal finishing and the making of polymers. This extensive use of cyanide has resulted in large-scale environmental pollution that disrupts natural biogeochemical cycles and poses severe threats to ecosystem stability (Ebbs, 2004; Gupta *et al.*, 2025). Every year, cyanide pollution continues to be a global environmental concern due to the large amounts of cyanide-containing wastewater generated from these activities (Akcil *et al.*, 2003; Luque-Almagro *et al.*, 2018). Hence, the development of efficient and sustainable cyanide remediation technologies is greatly needed.

The current conventional cyanide treatment techniques, such as chemical oxidation and physical removal processes, are generally used for industrial wastewater management. However, in soils polluted by cyanide in small-scale gold mining activities, these methods are often accompanied by high operating costs, inadequate detoxification, and the formation of secondary pollutants (Basri *et al.*, 2017). Biological treatment is an eco-friendly and cost-effective alternative where, once the cyanide-degrading bacteria have acclimatized and the cyanide-degrading pathways have been activated, the biodegradation of cyanide in soils occurs almost without needing any amendments with external energy or carbon source (Meyers *et al.*, 1991; Figueira *et al.*, 1996; Flores-Encarnación *et al.*, 1999; Adjei and Ohta, 2000; Nakamura and Yagi, 2000; Akcil *et al.*, 2003; Ebbs, 2004; Jandhyala *et al.*, 2005). Many reports are available on cyanide and related compounds degradation by microorganisms, which convert these compounds into less toxic products and at the same time utilize cyanide as a nitrogen source for their growth (Razanamahandry *et al.*, 2019; Gupta *et al.*, 2025). These biological processes have shown promising potential for the treatment of cyanide-contaminated industrial effluents (Avcioglu and Bilkay, 2016; Razanamahandry *et al.*, 2019).

It is known that the success of bioremediation is largely dependent on the metabolic flexibility and degradation capacity of the cyanide-degrading microorganisms employed. To date, the majority of the cyanide-degrading microorganisms have been isolated from mining areas, industrial effluents, or other cyanide-affected environments where long-term exposure led to adaptation to cyanide toxicity. The degradation of cyanide by several bacterial genera, including *Pseudomonas, Bacillus, Burkholderia, Serratia, Rhodococcus* and *Klebsiella* under different environmental conditions has been reported (Razanamahandry *et al.*, 2016, 2019; Karamba *et al.*, 2018; Luque-Almagro *et al.*, 2018; Moradkhani *et al.*, 2018; Ojaghi *et al.*, 2018; Singh *et al.*, 2018; Asadi *et al.*, 2019; Sáez *et al.*, 2019;

Vallenas-Arévalo *et al.*, 2019; Yakasai *et al.*, 2019; Mahendran *et al.*, 2020; Mpongwana *et al.*, 2020; Jiang *et al.*, 2021; Suharti *et al.*, 2021; Alvarado-López *et al.*, 2022; Arevalo *et al.*, 2022; Justo Arevalo *et al.*, 2022; Terada *et al.*, 2022; Banwo *et al.*, 2023; Lawal *et al.*, 2023; Ajao *et al.*, 2024; Triani *et al.*, 2024; Uribe-Ramírez *et al.*, 2024; Gupta *et al.*, 2025; Marlida *et al.*, 2025). However, extremophilic natural environments have been poorly explored for isolating novel cyanide-degrading microorganisms.

Volcanic soils are unique in that they possess specific physicochemical properties, which include high mineral content, fluctuating environmental conditions, limited nutrient content, and exposure to naturally occurring toxic compounds. These selective pressures frequently give rise to microbial communities with unique metabolic capacities, meaning that uncharacterized microorganisms capable of utilizing unusual nitrogen sources, such as cyanide, may be present in volcanic ecosystems. Hence, studies of such an environment may reveal new microbial resources with enhanced cyanide biodegradation ability (Meintanis *et al.*, 2006; Schubotz *et al.*, 2011; Djamaan *et al.*, 2016; Rakhmawati *et al.*, 2021; Rusnam and Gusmanizar, 2021, 2022).

Compared to singular microorganisms, microbial consortia generally perform better in biodegradation than individual bacterial isolates due to a synergistic interaction between community members, greater resilience to environmental toxicants, and metabolic complementarity. The cooperative metabolism action of microbial consortia can increase the efficiency of substrate utilization, the rates of detoxification and the range of compounds that can be degraded. This makes consortia particularly attractive for bioremediation applications (Alvarado-López *et al.*, 2022; Behera *et al.*, 2022; Manogaran *et al.*, 2023; Parvin *et al.*, 2023; Shebl *et al.*, 2024; Xia *et al.*, 2024).

For biological degradation of cyanide to be effective, characterization of the environmental and nutritional factors that influence the growth and degradation activity of the consortium is imperative as a preliminary step to enhance cyanide degradation before other optimization methods such as RSM or ANN are employed. Optimization of cultivation parameters can significantly enhance biodegradation performance and promote its application at industrial scale. In this work, a bacterial consortium able to use cyanide as the unique nitrogen source was isolated from volcanic soils and its potential for cyanide biodegradation was characterized.

MATERIALS AND METHODS

Bacterial strain and growth conditions

The buffer medium with the following composition in 1 L of tap water was used for this study: KH_2PO_4—7.2 g, K_2HPO_4—3.5 g, yeast extract—0.05 g. The medium was sterilized at 121 °C for 15 min. Filter-sterilized glucose—5 g/L was separately autoclaved, and filter-sterilized KCN was incorporated into the medium. Several serial transfers of growing cyanide-degrading colonies were carried out over five serial transfers. Growth on agar plates was carried out at room temperature by supplementing the liquid medium with 1.5% agar before autoclaving. Resting cells of the bacterial consortia were inoculated into a 50 mL buffer medium containing 200 mg/L filter-sterilised KCN (Karamba *et al.*, 2018). The medium was placed in a 250 mL flask. It was incubated in a Protech orbital shaker for 72 h at room temperature (28 °C). Cyanide utilization was determined using the analytical method described as follows.

Analysis of Cyanide

The residual cyanide concentration was determined by a modified pyridine-free spectrophotometric method. This method is based on the reaction of cyanide with chloramine-T and color development with p-picoline and barbituric acid reagents (Nakamura and Yagi, 2000). Aseptically, 1 mL of the culture sample was collected in Eppendorf tubes and centrifuged at 10,000 ×g for 10 min to remove the bacterial cells. Briefly, then, 100 µL of the cleared supernatant was suitably diluted for cyanide determination. For the analysis of cyanide, 500 µL of diluted sample was mixed with 250 µL of acetate buffer (pH 5.2) and then 13 µL of 1% (w/v) chloramine-T solution was added. A complete conversion of cyanide to cyanogen chloride was achieved by vortexing the reaction mixture and allowing it to stand at room temperature for 1-2 min. Then, 150 µL of p-picoline–barbituric acid reagent was added and incubated at 25°C for 5 min for colour development. Absorbance was measured at 605 nm in UV–Visible spectrophotometer. Cyanide concentrations were determined based on a calibration curve constructed from potassium cyanide (KCN) standards from 0.1 to 3.5 mg/L.

Bacterial Growth Measurement

Bacterial growth was monitored using the viable plate count method, which was expressed as colony forming units per millilitre (CFU mL^{-1}). Serial dilutions of the samples of the culture were prepared and plated on nutrient agar. Colonies were counted after incubation and bacterial population was calculated as CFU mL^{-1}. The resultant values were log transformed and presented as log CFU mL^{-1}.

Statistical analysis

All the experiments were conducted in triplicate. Experimental errors were depicted as error bars of standard deviation of triplicates determinations. Further statistical analyses were carried out using GraphPad Prism.

Cyanide tolerance

The maximum cyanide concentration that Isolate can tolerate was tested. The same buffer medium was tested for a series of cyanide concentrations from 100 to 1000 mg/L for a period of 72 h. The remaining cyanide concentrations were measured as described above.

RESULTS AND DISCUSSION

Microorganisms capable of utilizing or transforming cyanide into less toxic compounds or achieving complete biomineralization have been studied extensively due to their potential applications in the bioremediation of cyanide-contaminated industrial effluents (Meyers *et al.*, 1991; Jandhyala *et al.*, 2005; Mekuto *et al.*, 2016a, 2016b; Razanamahandry *et al.*, 2016; Karamba *et al.*, 2018; Luque-Almagro *et al.*, 2018; Moradkhani *et al.*, 2018; Ojaghi *et al.*, 2018; Singh *et al.*, 2018; Mahendran *et al.*, 2020; Ajao *et al.*, 2024; Triani *et al.*, 2024; Uribe-Ramírez *et al.*, 2024; Gupta *et al.*, 2025; Marlida *et al.*, 2025). Although many bacterial species have been reported to degrade cyanide, few studies have examined microbial consortia isolated from volcanic soils. This environment presents unique physicochemical conditions and diverse microbial communities. In this study, we investigated the ability of an isolated bacterial consortium from volcanic soil to grow and degrade cyanide under different environmental and nutritional conditions. Parameters studied include the effects of carbon source, pH, temperature, inoculum size, initial cyanide concentration, and selected heavy metals on bacterial growth and cyanide degradation.

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The biodegradation of cyanide is known to be significantly affected by environmental factors influencing the microbial metabolism and enzyme activity. Microorganisms that can degrade cyanide overwhelmingly use cyanide as a nitrogen source, and less frequently as the only source of carbon and nitrogen (Karamba *et al.*, 2018; Luque-Almagro *et al.*, 2018; Sáez *et al.*, 2019). Numerous bacteria that degrade cyanide need additional carbon sources for better growth and degradation efficiency. In this study, five serial transfer growth on cyanide as a nitrogen source gave a stable cyanide-degrading bacterial consortium consisting of one Gram-positive and one Gram-negative bacterium. When the consortium was separated into individual colonies, the cyanide degradation was significantly reduced, suggesting a synergistic interaction between the two microorganisms. Therefore, further experiments were performed with the mixed consortium rather than individual isolates. Hence, the culture conditions need to be optimized for maximum cyanide removal and to understand the physiological responses of the microbial consortium. The effects of the main environmental and nutritional parameters on bacterial growth and cyanide biodegradation by the volcanic soil consortium are detailed in this study.

Effect of carbon source

The results show that the type of carbon source markedly influenced both bacterial growth and cyanide degradation ability, where glucose supported the highest cell density (7.06 log CFU/mL) and cyanide degradation efficiency (57.26%), among the substrates tested. This is anticipated since glucose is one of the most readily assimilable carbon sources for cellular metabolic functions. This is followed by fructose and sucrose, which also promoted substantial growth (5.97–6.64 log CFU/mL) and degradation (45.92–46.74%), respectively. These hexose sugars and their derivatives are a source of reducing power, such as NADH, and energy required for cyanide detoxification. On the other hand, pentoses and less preferred carbohydrates such as ribose, arabinose, and galactose resulted in less efficient bacterial growth (1.78–2.30 log CFU/mL) and a reduced cyanide removal efficiency (16.94–32.37%) (**Figure 1**). The positive correlation between cell abundance and cyanide degradation suggests that cyanide biotransformation is a process that is growth-associated with higher biomass production, enhancing overall biodegradation capacity (Karamba *et al.*, 2018; Luque-Almagro *et al.*, 2018; Sáez *et al.*, 2019).

The availability of easily metabolizable carbon sources that support increased microbial growth and stimulate cyanide degradation is consistent with previous studies on cyanide-degrading bacteria. For instance, Karamba et al. (2018) reported that cyanide degradation and enhanced substrate utilization lead to improved detoxification efficiency by *Serratia marcescens* AQ07. Similarly, several studies demonstrated that cyanide removal by microorganisms was strongly dependent on metabolic activity and biomass development (Terada *et al.*, 2022; Banwo *et al.*, 2023; Lawal *et al.*, 2023; Ajao *et al.*, 2024; Triani *et al.*, 2024; Uribe-Ramírez *et al.*, 2024; Gupta *et al.*, 2025; Marlida *et al.*, 2025). It is known that efficient simple carbon utilization supplies ATP and reducing equivalents such as NADH that are necessary for cyanide-metabolizing enzymes. These enzymes include cyanide dihydratase and nitrilase systems (Jandhyala *et al.*, 2005; Asadi *et al.*, 2019; Sáez *et al.*, 2019; Arevalo *et al.*, 2022; Justo Arevalo *et al.*, 2022; Terada *et al.*, 2022; Gupta *et al.*, 2025). These enzymes accelerate cyanide conversion to less toxic metabolites. Glucose has a rapid uptake and forms a central role in supporting both bacterial proliferation and cyanide biodegradation.

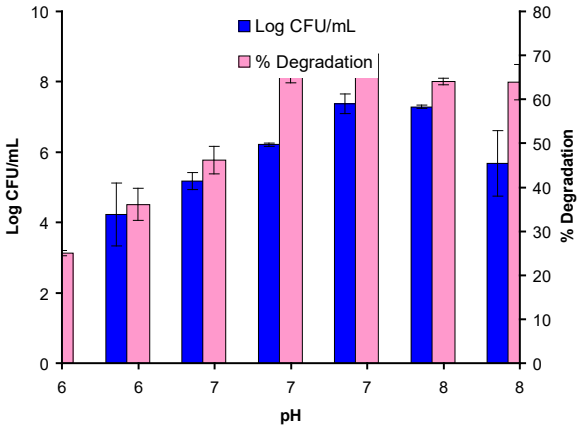


Figure 1. Effect of different carbon sources on bacterial growth and cyanide degradation. Bacterial growth (log CFU/mL) and cyanide degradation (%) were evaluated in media supplemented with various carbon sources. Error bars represent mean ± standard deviation (SD) of triplicate determinations (n = 3).

Effect of pH

The pH of the growth medium had a strong influence on both bacterial growth and cyanide degradation, where bacterial growth increased gradually from 3.74 log CFU/mL at pH 5.8 to a maximum of 7.37 log CFU/mL at pH 7.2. The results support the requirement of near-neutral pH for the optimum pH for cellular metabolism. The cyanide degradation followed a similar trend with an increase from 25.08% at pH 5.8 to a maximum of 70.37% at pH 7.2. Above this optimum, slight reductions in growth and degradation were recorded. The cell density was reduced to 5.68 log CFU/mL and the degradation was maintained at around 64% at pH 7.8 (**Figure 2**). The good correlation between biomass production and degradation efficiency indicated that the cyanide removal was mainly growth-associated and dependent on active cellular metabolism.

Optimum pH was found to be around 7.2, which was comparable with the previous studies on cyanide-degrading microorganisms. A previous study demonstrated similar physiological conditions promoting bacteria that influenced the metabolic activity and substrate utilization for cyanide degradation by *Serratia marcescens* AQ07 at around neutral pH (Karamba *et al.*, 2018; Luque-Almagro *et al.*, 2018; Sáez *et al.*, 2019). Likewise, several other studies have reported that near-neutral conditions are needed for efficient cyanide biodegradation under conditions that supported cellular viability and enzyme activity (Lawal *et al.*, 2023; Ajao *et al.*, 2024). Generally speaking, a low pH can impair membrane transport processes and enzyme function, which may lead to reduced growth and degradation. As HCN is produced under acidic conditions, this makes cyanide easily cross cell membranes and causes more toxic effects to microbial cells. Also, changes in enzyme conformation and efficiency of nutrient transport at alkaline pH values above the optimum can reduce microbial activity. In contrast, other cyanide-degrading microorganisms, such as *Pseudomonas pseudoalcaligenes* CECT534 (Huertas *et al.*, 2010), *Bacillus safensis* PER-URP-08 (Justo Arevalo *et al.*, 2022), and *Aspergillus welwitschiae* LOT1 (Lawal *et al.*, 2023) require alkaline conditions for optimum degradation of cyanide. Thus, the best performance obtained at pH 7.2 is ascribed to the optimal balance between bacterial growth, enzyme stability and

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cyanide bioavailability, resulting in the highest degradation efficiency.

Table 1. Some optimal pH and temperature data for cyanide-degrading microorganisms.

Microorganism	Optimum pH	Optimum Temp (°C)	Notes	Reference
Serratia marcescens AQ07	6.0–7.0	30	Cyanide degradation and growth optimized near neutrality	(Karamba *et al.*, 2018)
Immobilized *Serratia marcescens* AQ07	6.5–7.0	30	Immobilized cells retained highest degradation near neutral pH	(Karamba *et al.*, 2015)
Bacillus sp. EBE-2	~7.0	30–37	Isolated from cyanide-contaminated soil; strong cyanide degradation activity	(Terada *et al.*, 2022)
Pedobacter sp. EBE-1	~7.0	30–37	Cyanide-degrading isolate from contaminated soil	(Terada *et al.*, 2022)
Bacillus subtilis (cassava effluent isolate)	6.5–7.5	30–37	Effective cyanide degradation in cassava mill effluent	(Ajao *et al.*, 2024)
Pseudomonas putida	6.5–7.5	25–35	Common cyanide degrader under mesophilic conditions	(Ajao *et al.*, 2024)
Alcaligenes faecalis	7.0–8.0	30–35	Cyanide degradation in cassava mill effluent	(Ajao *et al.*, 2024)
Bacillus licheniformis	8.0–9.0	30	Cyanide utilized as nitrogen source; slightly alkaline optimum	(Uribe-Ramírez *et al.*, 2024)

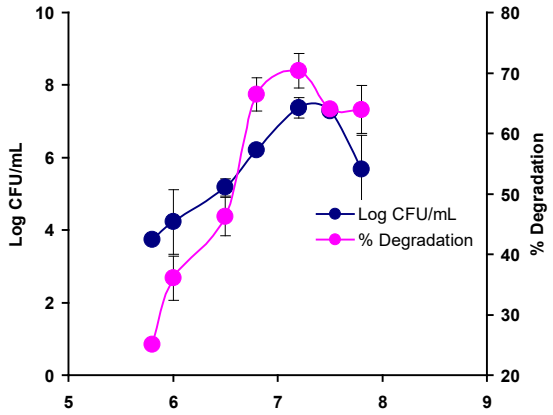


Figure 2. Effect of pH on bacterial growth and cyanide degradation. Bacterial growth (log CFU/mL) and cyanide degradation (%) were determined at different initial pH values. Error bars represent mean ± standard deviation (SD) of triplicate determinations (n = 3).

Effect of inoculum size

The effect of inoculum size on cyanide degradation and growth exerted a substantial influence on bacterial growth and cyanide degradation. A marked increase in cell density was observed when the inoculum concentration was increased from 1 to 7% (v/v). The bacterial growth rose from 3.81 log CFU/mL at 1% inoculum to a maximum of 8.57 log CFU/mL at 6% inoculum before a plateau was reached. A greater number of viable cells introduced into the system was responsible for the enhanced growth observed at the higher inoculum sizes. This increase inherently shortened the lag phase and accelerated the accumulation of biomass, of which similar observations have been reported for cyanide-degrading bacteria (Ojaghi *et al.*, 2018; Singh *et al.*, 2018; Asadi *et al.*, 2019; Razanamahandry *et al.*, 2019; Sáez *et al.*, 2019; Vallenas-Arévalo *et al.*, 2019; Yakasai *et al.*, 2019; Mahendran *et al.*, 2020; Mpongwana *et al.*, 2020; Jiang *et al.*, 2021; Nasution *et al.*, 2021; Suharti *et al.*, 2021; Alvarado-López *et al.*, 2022; Arevalo *et al.*, 2022; Justo Arevalo *et al.*, 2022; Terada *et al.*, 2022; Banwo *et al.*, 2023; Lawal *et al.*, 2023; Ajao *et al.*, 2024; Triani *et al.*, 2024; Uribe-Ramírez *et al.*, 2024; Gupta *et al.*, 2025).

Inhibition of degradation at lower bacterial inoculum concentration is likely due to the toxicity of cyanide by inhibiting the terminal oxidases involved in oxygen reduction, where in some bacteria, a reduction in respiratory activity by more than 50% at relatively low cyanide concentrations was observed (Sáez *et al.*, 2019). Another extreme cyanide-tolerant bacterium, like *Pseudomonas pseudoalcaligenes* CECT5344, possesses cyanide-resistant cytochrome bd oxidase, allowing the bacterium to continue respiration in the presence of cyanide (Luque-Almagro *et al.*, 2011) (**Figure 3**).

Hence, the physiological state and metabolic efficiency of microbial growth on cyanide and cyanide degradation are not solely dependent on biomass concentration but also on the cyanide concentration. Cyanide degradation by immobilized Serratia marcescens AQ07 was governed by a balance between active biomass and substrate availability, as shown by Karamba et al. (Karamba *et al.*, 2015). On the other hand, Wu et al. (Wu *et al.*, 2014) showed that excessive cyanide concentration inhibits cyanide degradation kinetics through substrate inhibition effects.

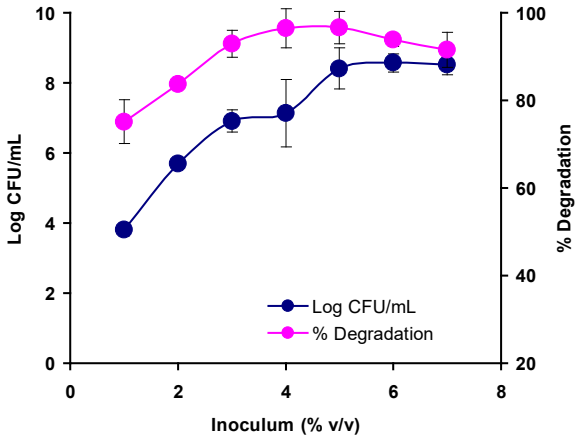


Figure 3. Effect of inoculum size on bacterial growth and cyanide degradation. Bacterial growth (log CFU/mL) and cyanide degradation (%) were assessed at different inoculum concentrations (% v/v). Error bars represent mean ± standard deviation (SD) of triplicate determinations (n = 3).

Effect of temperature

Temperature had a significant effect on both bacterial growth and cyanide degradation. This effect indicated a very temperature-dependent microbial activity. The bacterial growth ranged from 3.74 log CFU/mL to 7.50 log CFU/mL from 20 to 30°C, respectively, then gradually decreased to 2.22 log CFU/mL at 50 °C. The temperature optimum ranges from 25 to 35°C when analysed using ANOVA. Equally, cyanide degradation was found to increase to a maximum value of 66.46% at 35°C. At higher temperatures, degradation falls drastically. The results (**Figure 4**) show that the optimum temperature for biomass

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production (30 °C) was found not to be the same as that for optimum cyanide degradation (35 °C). This difference indicates that the cyanide-degrading enzymes were highly active over a wider temperature range than that supporting maximum cellular growth.

The increased growth and degradation from 20 to 35 °C can be attributed to the effect of temperature on rates of chemical or biochemical reactions, where a rate is doubled for a 10 °C increase in temperature. This increase enhances metabolic activity, nutrient uptake, membrane fluidity and enzyme catalyzed reactions including cyanide degradation (Mekuto *et al.*, 2016a; Razanamahandry *et al.*, 2016, 2019; Karamba *et al.*, 2018; Luque-Almagro *et al.*, 2018; Moradkhani *et al.*, 2018; Ojaghi *et al.*, 2018; Singh *et al.*, 2018; Asadi *et al.*, 2019; Sáez *et al.*, 2019). In numerous cyanide-degrading microorganisms, optimal cyanide biodegradation occurred under mesophilic conditions. The decline in both growth and degradation above 35 °C is likely due to thermal stress, as higher temperatures may cause enzyme and protein transport denaturation.

Higher temperatures can damage enzymes such as cyanide dihydratase, cyanide hydratase, and nitrilases, which are particularly sensitive to conformational changes leading to a reduction in catalytic efficiency due to denaturation (Luque-Almagro *et al.*, 2018). Similar inhibitory effects of cyanide degradation due to increasing temperature have been reported for *Pseudomonas pseudoalcaligenes* CECT5344 and other cyanotrophic bacteria (Ebbs, 2004; Sáez *et al.*, 2019). Despite a large reduction in biomass, interestingly, cyanide degradation was still relatively high at 40°C (61.3%). This indicates that cyanide removal was not only a function of cell numbers but also the specific activity of cyanide-degrading enzymes that may tolerate higher temperatures, as reported in other cyanide biodegradation systems (Arevalo *et al.*, 2022; Justo Arevalo *et al.*, 2022).

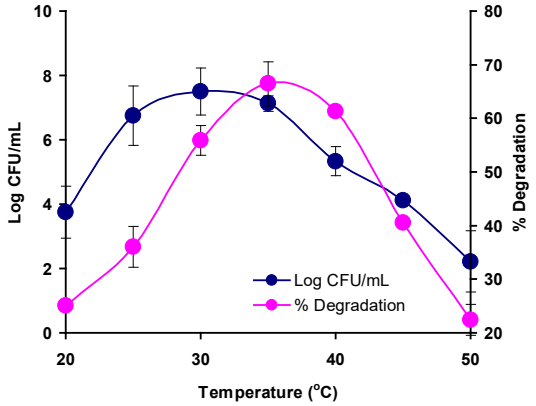


Figure 4. Effect of temperature on bacterial growth and cyanide degradation. Bacterial growth (log CFU/mL) and cyanide degradation (%) were determined at different incubation temperatures. Error bars represent mean ± standard deviation (SD) of triplicate determinations (n = 3).

Effect of cyanide concentration

Initial cyanide concentration had a strong inhibitory effect on bacterial growth and cyanide degradation, where at the lowest concentration tested (100 mg/L) the bacterium achieved 5.84 log CFU/mL and the highest degradation efficiency of 78.3%. Growth increased slightly to 6.75 log CFU/mL at 200 mg/L, which indicates that moderate cyanide concentrations may still allow microbial growth and cyanide degradation. At higher cyanide concentrations, a gradual decrease in both biomass and degradation efficiency was observed, where the growth decreased to 5.08 log CFU/mL at 500 mg/L with only 31.4% degradation. At higher cyanide concentrations, an almost complete suppression of both growth and cyanide degradation occurred, especially at cyanide concentrations from 700 mg/L onwards, with cell densities below 1.5 log CFU/mL and degradation efficiencies of about 21–23% (**Figure 5**). This phenomenon is a clear substrate inhibition, where, similar to the toxicant phenol, cyanide functions as both a nutrient source and a potent inhibitor at high concentrations.

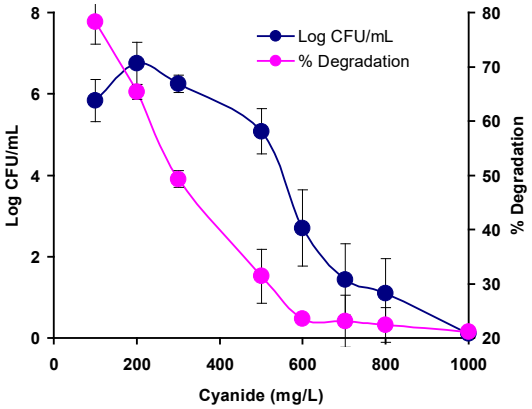


Figure 5. Effect of initial cyanide concentration on bacterial growth and cyanide degradation. Bacterial growth (log CFU/mL) and cyanide degradation (%) were evaluated at varying initial cyanide concentrations. Error bars represent mean ± standard deviation (SD) of triplicate determinations (n = 3).

The known toxicity of cyanide towards respiratory enzymes and cellular energy metabolism is the ability of cyanide to bind easily to iron-containing proteins like cytochrome c oxidase, which inhibits electron transport and decreases ATP generation required for cellular growth and biodegradation processes (Baxter and Cummings, 2006; Asadi *et al.*, 2019). Very few works have modeled the substrate-inhibiting effect of cyanide on bacterial growth rates and degradation rates of cyanide. In one such study, substrate inhibition has been reported in cyanide-degrading *Serratia marcescens* AQ07, in which the growth kinetics were best described by inhibitory models such as Haldane and Luong (Karamba *et al.*, 2018).

The consortium showed a peak growth at about 200 mg/L cyanide, followed by a rapid decrease at concentrations above 300 mg/L. This profile is characteristic of cyanide-degrading microorganisms that have a limited tolerance threshold, and have been similarly observed in another study where the microbial fuel cell strain MC-1 exhibits Haldane kinetics (Wu *et al.*, 2018). In another similar study, Luque-Almagro et al. (Luque-Almagro *et al.*, 2018) found that while cyanotrophic bacteria are capable of using cyanide as a nitrogen source, concentrations in excess overwhelm the detoxification systems and result in substrate inhibition. The near-complete inhibition of growth at 1000 mg/L is also observed in several studies, presenting a limit for most microbial-assisted cyanide remediation. In the future, more substrate inhibition models other than Haldane, like Luong, Aiba, or Han-Levenspiel, should be tested to describe substrate inhibition kinetics.

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Effect of metal ions

Heavy metals were found to strongly inhibit bacterial growth and cyanide degradation. Among the metal ions tested, mercury and silver were found to be the most toxic metals tested. A reduction in bacterial growth of 0.54 and 0.38 log CFU/mL, respectively. For cyanide degradation, a drastic decline to only 3.0–3.3% was observed. Cadmium was also highly toxic, which limited growth to 1.15 log CFU/mL and cyanide degradation to 20.15%. However, the inhibitory effect of copper, lead, cobalt, and nickel was less profound with higher cell densities (1.78-2.96 log CFU/mL) and degradation efficiencies (16.6-28.9%). Of the metals tested, lead (Pb^{2+}) and copper (Cu^{2+}) were the least detrimental to cyanide degradation, with removal efficiencies above 25% (**Figure 6)**.

Both mercury and silver exhibit strong inhibition, which is in agreement with the well-known affinity of these metals for sulfhydryl (-SH) groups of proteins, inactivating important metabolic pathways associated with cyanide assimilation and detoxification (Nies, 1999; Bruins et al., 2000). Similarly, silver ions lead to severe growth inhibition by damaging the membrane integrity, respiratory enzymes, and DNA replication. The inhibition by metals suggests a probable direct inhibition of cyanide-degrading enzymes such as cyanide dihydratase, cyanide hydratase, nitrilase and cyanidase systems (Sáez *et al.*, 2019; Vallenas-Arévalo *et al.*, 2019; Alvarado-López *et al.*, 2022; Ajao *et al.*, 2024).

The decreased cyanide degradation upon exposure to toxic metal ions was also reported in other studies on cyanide-degrading bacteria. Karamba et al. (Karamba *et al.*, 2018) reported that heavy metals, especially mercury exposure, significantly inhibited the cyanide biodegradation rates in *Serratia marcescens* AQ07. The effect of heavy metals was demonstrated on growth and cyanide removal efficiency to show that successful biodegradation of cyanide depended on active microbial metabolism. Also, Baxter and Cummings (Baxter and Cummings, 2006) noted that metal toxicity is a significant limitation in the biological treatment of mining effluents, as cyanide is found together with dissolved metals that hinder microbial activity. The relatively low toxicity of copper, lead, cobalt, and nickel could be explained by the ability of microorganisms to tolerate or partially detoxify these metals by mechanisms such as extracellular sequestration, biosorption, efflux pumps, metallothionein production and intracellular compartmentalization (Marlida *et al.*, 2025).

Copper is a required trace element in many enzymatic processes, but is toxic at high concentrations. Thus, copper may have supported the highest growth (2.96 log CFU/mL) of all the metal treatments. Lead showed the highest degradation efficiency (28.88%), which confirms that the microorganism has some tolerance to Pb^{2+} and can maintain limited cyanide degradation activity despite metal stress. The strong correlation between cell density and degradation efficiency suggests that cyanide removal was growth-associated. But, even under high metal stress, degradation was not completely abolished, indicating that some enzyme activity remained, but that cell proliferation was significantly inhibited. Such observations have been reported for cyanide-degrading *Pseudomonas, Bacillus* and *Serratia* species exposed to metal-containing wastewaters where partial cyanide degradation occurred despite marked reductions in biomass (Luque-Almagro *et al.*, 2018; Asadi *et al.*, 2019).

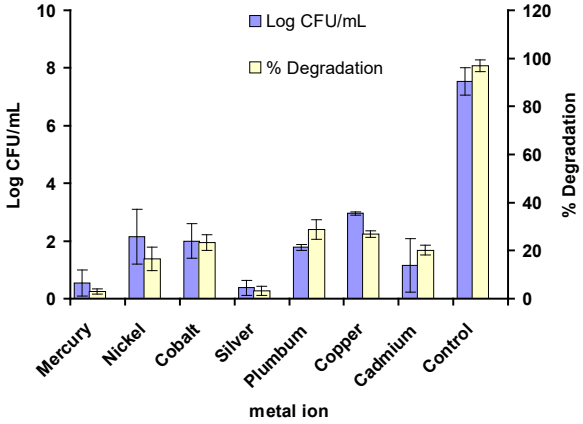


Figure 6. Effect of selected heavy metals on bacterial growth and cyanide degradation. Bacterial growth (log CFU/mL) and cyanide degradation (%) were determined in the presence of different metal ions. Error bars represent mean ± standard deviation (SD) of triplicate determinations (n = 3).

Growth and degradation profile

A clear relationship between bacterial proliferation and cyanide removal using an initial cyanide concentration of 200 mg/L over the 72 h incubation period shows that cyanide decreased progressively to 55.0 mg/L with a corresponding removal value of approximately 72.5%. Concomitantly, bacterial growth increased from 0.58 log CFU/mL at inoculation to 6.98 log CFU/mL at the end of the 72 h incubation period. A short lag period was observed during the first 12 h. This is followed by a rapid exponential phase between 12 and 36 h, in which the density increased from 1.09 to 5.90 log CFU/mL. This increase in density period coincides with the most substantial reduction in cyanide concentration, from 185.2 to 115.7 mg/L (**Figure 7**), which indicates active cyanide utilization by the bacterial consortium.

There exists a strong negative correlation between cyanide concentration and bacterial growth, which indicates that cyanide degradation was largely growth-associated. Similar growth-related cyanide degradation patterns were reported for cyanide-degrading bacteria such as *Serratia marcescens* AQ07 where biomass accumulation was accompanied by increased cyanide removal due to enhanced metabolic activity and enzyme production (Karamba *et al.*, 2018). In general, microorganisms require nitrogen in the exponential growth phase to produce proteins and reproduce. With the only source of nitrogen being cyanide, an enzymatic conversion to ammonia and formate by the action of cyanide hydratase, cyanide dihydratase, nitrilase, or cyanidase enzymes is needed (Ebbs, 2004; Luque-Almagro *et al.*, 2018). Thus, the fast cell proliferation from 12 to 48 h may indicate the increase in the synthesis of cyanide-degrading enzymes, leading to faster detoxification. The bacterial consortium showed the highest rate of cyanide removal between 24th and 48th hour, during which cyanide concentration was decreased by about 83 mg/L. This is the phase of logarithmic growth, indicating that optimal biodegradation was maximal at the time when cells were metabolically most active. The same has been observed for *Pseudomonas pseudoalcaligenes* CECT5344 and other cyanotrophic bacteria, with cyanide rates of degradation being maximum during active growth and decreasing as cultures reached the stationary phase (Luque-Almagro *et al.*, 2018). The decrease in cyanide removal rate after 48 hours may be due to the depletion of easily metabolized

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nutrients, or the accumulation of metabolic by-products, or a decrease in enzyme synthesis and the lower availability of cyanide as a substrate. In many biodegradation systems, the substrate concentrations are much lower than the concentrations that support maximum enzymatic activity. This phenomenon makes it increasingly difficult to remove residual cyanide concentrations (Asadi *et al.*, 2019). Similar behavior has been observed in cyanide-degrading *Serratia, Bacillus* and *Pseudomonas* species, where residual cyanide was detected even with significant biomass accumulation (Baxter and Cummings, 2006; Karamba *et al.*, 2018).

In general, the growth profile shows the typical microbial growth pattern of lag, exponential, and stationary phases, while the corresponding cyanide depletion profile confirms efficient biodegradation of cyanide at the initial concentration of 200 mg/L. The close temporal correlation between biomass formation and cyanide disappearance indicates that cyanide degradation was mainly growth-associated and dependent on active microbial metabolism. The results show that the isolate is a good candidate for biological treatment of cyanide-contaminated wastewater under moderate cyanide concentrations.

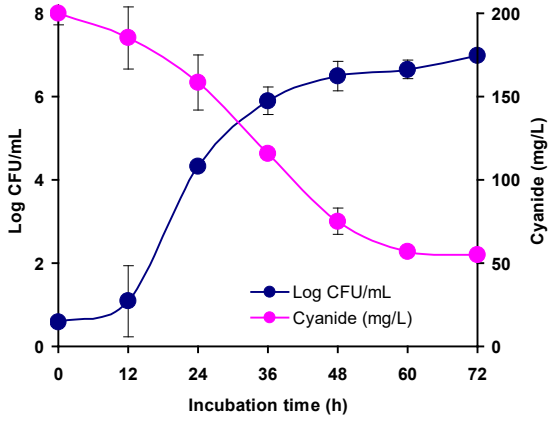


Figure 7. Growth profile and cyanide degradation by the bacterial isolate in medium containing an initial cyanide concentration of 200 mg/L. Bacterial growth (log CFU/mL) and residual cyanide concentration (mg/L) were monitored over the incubation period. Error bars represent mean ± standard deviation (SD) of triplicate determinations (n = 3).

CONCLUSION

The bacterial consortium, under laboratory conditions, has exhibited a significant potential for biodegradation of cyanide. The environmental and nutritional factors strongly influenced the growth and degradation performance, with the most favorable conditions being glucose, pH 7.2, and temperatures of 30–35°C. The consortium isolated from volcanic soil was able to degrade large quantities of cyanide and, at the same time, achieved significant biomass growth, which points to a close association of cyanide degradation with microbial metabolic activity. High levels of cyanide and heavy metals inhibited growth and degradation efficiency, but the consortium still had measurable cyanide-removal activity under inhibitory conditions. Further, the analysis of the growth profile confirmed its ability to reduce cyanide concentrations from 200 mg/L to 55 mg/L within 72 h. The observed tolerance to cyanide and performance under different environmental conditions indicate that microorganisms inhabiting volcanic soils have adaptive mechanisms that make them suitable for cyanide detoxification. These results demonstrate the potential application of microbial consortia from volcanic soils as eco-friendly and low-cost biocatalysts for the treatment of cyanide-containing industrial effluents. Future work should focus on molecular approaches for characterization of the consortium, identification of the cyanide-degrading enzymes, kinetic modeling of the biodegradation, and evaluation of the performance in pilot and field-scale treatment systems.

CONFLICT OF INTERESTS

The authors have no competing financial interests or personal relationships to disclose.

ETHICS STATEMENT

Not applicable

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AUTHORS' CONTRIBUTIONS

M.R contributed to conceptualization, methodology, analysis, and writing of the original draft. F.I.N. conducted the methodology, investigation, and data analysis. M.M. provided investigation, resources and data collection.

DATA AND MATERIALS AVAILABILITY

Data available upon request

AI STATEMENT

In the preparation of this work, the authors used generative AI technologies solely for the purpose of language editing and refinement. All authors reviewed and edited the content. The authors are responsible for the contents of the publication.

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Abbreviations:

CFU mL^{-1} colony forming units per millilitre

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