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Extraction of nickel from spent reforming catalyst through bioleaching with mesophilic and moderately thermophilic cultures

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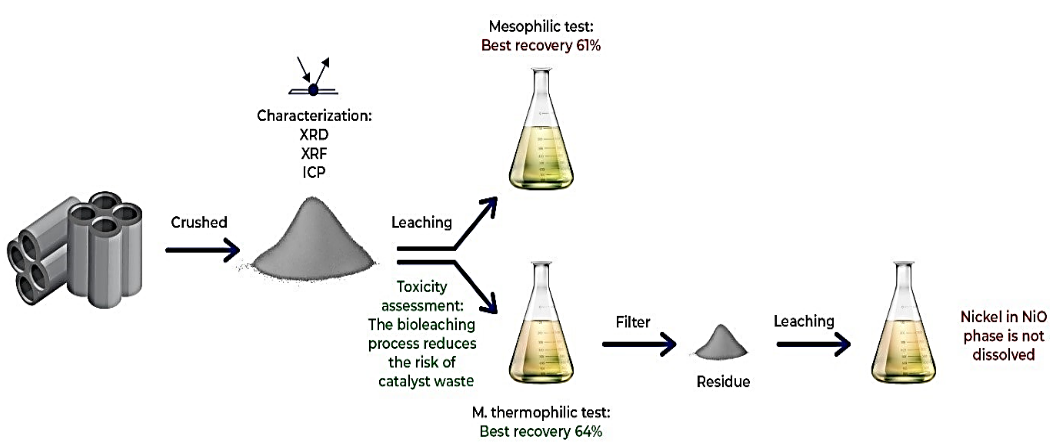
ABSTRACT

Considering the strategic relevance of nickel in a variety of industries, it is crucial to discover a method for extracting it from secondary sources. Spent reforming catalysts are hazardous to the environment, and they require proper waste management techniques. In this research, the extraction of nickel from spent catalysts was studied and it was discovered that this process can dissolve a significant amount of nickel. Due to the high calcite content in the sample, its dissolution led to an elevation in pH, which negatively impacted bioleaching. It was observed that when the sample was added to the bioleaching solution after bacterial growth, the pH of the solution elevated less, and recovery improved. Moderately thermophilic bacteria had a better capability to reduce the pH and showed 66% nickel recovery which was slightly higher than the 61% recovery observed with mesophilic bacteria. Leaching tests and XRD analysis revealed that the portion of the nickel that is in the NiO phase is resistant to conventional leaching or bioleaching treatment, but reducing the sample with H2 as the pretreatment process can enhance the recovery of nickel. Reductive pretreatment of the sample can improve recovery; however, it is not advised since it may have adverse environmental implications. The toxicity assessment test revealed that the bioleaching process with both cultures was able to reduce the risk of catalyst waste.

GRAPHICAL ABSTRACT



Keywords: *Bioleaching, Mesophile, Thermophile, Reforming catalyst, Nickel.*

1. Introduction

Reforming catalysts are essential in the petroleum refining process due to their ability to lower pressure during operations (Alper, 1994). The main disadvantage of these catalysts is their short lifetime (Tayar et al., 2020). As the industry grows, their demand is estimated to increase by 1.1% through 2040 (Alabdullah et al., 2020); in addition, they occupy 4% of all petroleum wastes (Garole et al., 2020), and they pose notableenvironmental risks. These catalysts contain heavy metals that can leach into soil and water, threatening ecosystems (Asghari et al., 2013; Kortum, 2010). Petrochemicals typically recycle, regenerate, or bury spent catalysts; however, disposal entails high costs and toxic

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emissions. Consequently, refineries are increasingly pursuing economical and sustainable management approaches (Marafi and Stanislaus, 2008). Due to the presence of metals, such as nickel, a pretreatment stage is required before disposing of the spent catalyst, making the disposal more expensive (Ferella et al., 2016). Recycling spent catalysts is an economical way to reduce their threat and obtain benefits (Lee et al., 2010).

Nickel has a wide range of applications in key industries, including steel production (Guo et al., 2022; Lv et al., 2021), batteries (Al-Thyabat et al., 2013; Saneie et al., 2022), and turbine blades (Shao et al., 2021). Nickel's primary sources are magmatic sulfides or laterites (Da Costa et al., 2013). The reduction of primary resources with increasing demand, the challenging exploration of new ore bodies, and environmental issues show the necessity for recycling (Sahu et al., 2005; Su et al., 2022). Hydrometallurgy and pyrometallurgy methods have been used to extract precious and critical metals from spent catalysts (Kolbadinejad and Ghaemi, 2023; Marafi and Stanislaus, 2008; Wang et al., 2019). In the hydrometallurgical process, metals are dissolved by different lixiviants, such as H_2SO_4, HCl, and aqua regia (Trinh et al., 2020). In some cases, oxidizing/reducing agents, such as H_2O_2, O_2, and Cl_2 have increased dissolution efficiency (Ding et al., 2019).

Since bacterial processes are biodegradable, employing these substances can significantly aid in the transition to greener and more ecologically friendly operations (Asimi Neisiani et al., 2023). Bioleaching is a hydrometallurgical technique for extracting metals from ores and wastes that has several advantages, including low operational costs and little environmental risk. Utilizing bioleaching to recycle industrial wastes can be economically and environmentally advantageous, as it requires less energy and chemicals, demonstrates greater efficiency, and enables cleaner output (Khodadadmahmoudi et al., 2022; Kumar and Yaashikaa, 2020; Rizki et al., 2019). The three major microorganism groups commonly used in bioleaching processes are autotrophic bacteria, heterotrophic bacteria, and fungi. It is more economical to use autotrophic bacteria because they do not require an organic carbon source to grow. On the other hand, heterotrophic bacteria and fungi can be used at higher pHs (Asghari et al., 2013). *Acidithiobacillus* bacteria receive energy for their metabolism from the aerobic oxidation of reduced sulfur compounds (sulfides, elemental sulfur, and thiosulfates) or ferrous ions as an energy source.

Two different types of bacteria (mesophilic and thermophilic) are among the most important bacteria in the oxidative bioleaching process (Abdollahi et al., 2021; Saneie et al., 2021). It has been shown that thermophilic bacteria have faster oxidation rates and therefore higher extraction rates and efficiency (Abdollahi et al., 2024). Therefore, current industrial processes, such as tank leaching reduced have reduced cost in industrial processes, including tank leaching, and there is a growing interest in moderately thermophilic bacteria (Rohwerder et al., 2003). A culture medium, such as 9K can enhance the growth and activity of bacteria (Chen et al., 2023), while the addition of energy sources improves bioleaching extraction efficiency (Abdollahi et al., 2021). Numerous studies have been performed on the nickel-alumina catalyst's bioleaching process (Kim et al., 2010; Pathak et al., 2018; Tayar et al., 2020). *A. thiooxidans* has been shown to be efficient compared to *Desulfovibrio* by extracting 91% of nickel (Bosio et al., 2008). Tayar et al.'s research was conducted on a commercial nickel-based reforming catalyst using two acidophilic *At. ferrooxidans* and *At. thiooxidans* under various bioleaching mechanisms (one-step, two-step, and spent medium-step). Using *At. thiooxidans* in the spent medium-step, the maximum nickel extraction yield (94.4%) was achieved. The nickel extraction was reduced at pulp densities higher than 5% due to acid shortage (Tayar et al., 2020). Pathak et al.'s research indicated that spent hydroprocessing catalysts contain several metals (Al, Ni, and Mo) with varying mobility and bioavailability, posing a substantial environmental concern. Using *Acidithiobacillus ferrooxidans* (*At. ferrooxidans)* for bioleaching proved highly successful in removing these metals from the spent catalyst (Pathak et al., 2018). Kim et al. studied the effect of adaptation, initial pH, and Fe (II) concentration on process efficiency. Adapted bacteria had greater Oxidation-Reduction Potential (ORP), Fe (III)/Fe (II), and iron oxidation rates than non-adapted bacteria. In addition, the Ni and V leaching efficiencies of adapted bacteria were roughly 95%, while those of unadapted bacteria were 85%. The optimum pH and Fe(II) concentration were 2.0 and 2 g/L, but further increases in these parameters dropped the efficiency due to the formation of jarosite on the surface of the reactant particles (Kim et al., 2010).

Nickel recovery from aluminum-rich catalysts is often challenging (Pathak et al., 2019). Also, Mishra et al. were able to extract 80-90% of nickel with biological H_2SO_4 from calcinated aluminum- containing catalysts (Mishra et al., 2008). Using *A. brierleyi* Gerayeli et al. discovered that, while the bacteria expanded significantly in the presence of the wasted catalyst under ideal circumstances, only 64% of the nickel was removed from the aluminum-containing catalyst (Gerayeli et al., 2013). However, it has been shown that sulfide nickel in catalyst samples can be recovered efficiently (Bharadwaj and Ting, 2013; Srichandan et al., 2014).

In this research, an attempt was made to evaluate the effectiveness of acidophilic bioleaching to extract nickel from a spent reforming catalyst. Bioleaching was performed at two temperatures, mesophilic (34 ℃) and moderately thermophilic (45 ℃), under varied conditions (one-stage, two-stage, and two-stage fractional). It uniquely investigates the influence of pretreatment processes, including reducing the sample with H_2, to address challenges in nickel recovery, specifically from the NiO phase. Moreover, the research emphasizes environmental sustainability by employing a standard toxicology assessment test. This approach provides novel insights into optimizing bioleaching techniques to enhance nickel recovery from spent catalysts. Furthermore, the standard toxicology assessment test was carried out to determine the impact of the process on the environment.

2. Materials and methods

2.1. Sample preparation and characterization

Refinery spent catalysts were obtained from petroleum industries (Fig. 1). Bulk samples were crushed with a laboratory cone crusher (Notashsanat Co., Iran). They were then dry-milled to a particle size of -150 µm. The particle size distribution was determined through screening. Fig. S1 in Appendix depicts the particle size distribution of the sample. As demonstrated, the d_{25}, d_{50}, d_{80}, and d_{90} of the sample are 32.9, 72.0, 118.9, and 134.5 µm, respectively.

Figure 1. Spent petroleum catalyst sample used in this study

From the feed, a representative sample was prepared, powdered, and sent for ICP (Model: NexION 2000, PerkinElmer), XRD (Bruker D8-Advance), and XRF (Model: Philips PW1730) analyses, and elemental nickel was separately analyzed by atomic absorption spectrometry (AAS, Model: Trace AI1200).

2.2. Microorganisms and culture media

Mixed cultures of mesophilic (*Acidithiobacillus ferrooxidans*, *Acidithiobacillus thiooxidans*, and *Leptospirillum ferrooxidans*) and moderately thermophilic bacteria (*Acidithiobacillus caldus*, *Leptospirillum ferriphilum*, *Sulfobacillus thermosulfidooxidans*, and *Ferroplasma spp.*) were obtained from the Sarcheshmeh Copper Mine microbial culture collection in Kerman, Iran (Khodadadmahmoudi et al., 2022; Yadollahi et al., 2021). In order to enhance bacterial activity, the adaptation of bacteria to the samples with different pulp densities was assessed. Their optimal growth temperature ranged around 34°C for mesophiles and 45°C for moderate thermophiles. The culture media consisted of a 9K liquid salt solution with 3 g/L $(NH_4)_2SO_4$, 0.5 g/L K_2HPO_4, 0.5 g/L $MgSO_47H_2O$, 0.1 g/L KCl, and 0.01 g/L Ca $(NO_3)_2$. Microorganisms were incubated in rotary shakers with 140 rpm rotation speed. In addition, $FeSO_47H_2O$ (44.7 g/L) and sulfur (10 g/L) as energy sources were added.

2.3. Bioleaching experiments and analytical methods

Oxidative bioleaching tests were performed in two temperature ranges: 34°C by the mixed mesophilic culture and 45°C by the mixed moderately thermophilic culture. The spent catalyst was treated by three methods: one-stage, two-step, and two-step fractional. Five tests were conducted at mesophilic temperatures and another five at moderately thermophilic temperatures. All tests were carried out in a 250 ml shake flask, and the solid percentage was 5% in all tests.

A control experiment was carried out in 100 ml of distilled water to isolate the effect of bacteria on the leaching process. All bioleaching tests were performed in a solution consisting of 90 ml of 9K medium and 10 ml of inoculum. A bioleaching experiment without additives (Fe^{2+} and S) was studied to evaluate the effect of energy sources on bacterial growth and activity. The pH at the beginning of all the experiments was adjusted to 1.8 with sulfuric acid. This study builds on an experimental design previously used in bioleaching studies, and it has shown potential to enhance the understanding of the bacterial leaching process (Abdollahi et al., 2021; Khodadadmahmoudi et al., 2022).

In one-step tests (normal bioleaching), the spent catalyst was added to the solution immediately after the microorganisms were cultured. In the two-step, first, the bacteria reached the suitable counts and ORP (10 days), and then the spent catalyst was added to the culture medium. In the two-step fractional test, 3 g (3% solid percentage) of the spent catalyst was added to the medium immediately and after ten days, 2 g more (5% solid percentage) of the spent catalyst was added to the solution. The specifications of each test and its order are given in Table1.

Table 1. Conditions of mesophilic and moderately thermophilic bioleaching experiments.

No.	Tests	Microorganism	Pulp density (%)
1	Control	-	5
2	Bioleaching without additives	Mixed mesophile	5
3	Bioleaching	Mixed mesophile	5
4	Two-step	Mixed mesophile	0 → 5
5	Two-step fractional	Mixed mesophile	3 →5
6	Control	-	5
7	Bioleaching without additives	Mixed moderate thermophile	5
8	Bioleaching	Mixed moderate thermophile	5
9	Two-step	Mixed moderate thermophile	0 →5
10	Two-step fractional	Mixed moderate thermophile	3 →5

Bacteria cell counts were measured using a Neubauer counting chamber (0.1 1/400 mm^2) and a Zeiss optical microscope with 1000× magnification. The pH and oxidation-reduction potential (ORP, Pt vs. Ag/AgCl) values were measured at intervals of 2 to 3 days using a Mettler Toledo pH/ORP meter. The concentration of Fe^{2+} in the solution titration was determined via titration with a 1 mM potassium dichromate. The concentration of dissolved total iron (Fe_t) was determined using atomic absorption spectrometry. The amount of iron oxidation was estimated using the following formula:

$$\eta(\%) = -\frac{X_1 - X_0}{X_0} \quad (1)$$

Where X_0 is the initial concentration of Fe^{2+} and X_1 is the concentration of Fe^{2+} during the process. For each test, three replicates were performed to minimize laboratory error. Mean readings with standard deviation (SD) were reported as the final result, with a relative standard deviation (RSD) of <4% for a 1-10 μg/L multi-element solution.

2.4. Catalyst pretreatment and leaching test

Spent catalyst leaching experiments were carried out in a 500 ml glass beaker with a hot plate and a 100 ml solution volume at 350 rpm agitation speed at 85°C with 5% pulp density. Leaching tests were performed under different conditions to assess the effectiveness of the bioleaching experiments, with detailed parameters provided in Table 2. Different acids and one base were used to prepare the leaching solution in deionized water. If further crushing was needed, the sample was dry-milled. For a leaching test with a pretreated sample, the sample was submitted to a reducing environment with gas (95% argon, 5% H_2) discharge at a rate of 100ml/min at 600°C for 120 min. The temperature increasing rate was 4°C/min and for cooling the gas discharge was stopped at 200°C. Afterward, the pretreated sample was leached in the same condition as test 2. Similar to bioleaching tests, three replicates were performed for each test to minimize laboratory error. Mean readings with standard deviation error were reported as the final result.

The effect of different parameters, such as particle size and pre-treatment with sulfuric acid was investigated, because this acid is the same as the acid produced by bacteria. The leaching experiments were not primarily intended to evaluate nickel extraction efficiency but rather to understand why certain portions of nickel in the sample were resistant to bioleaching.

2.5. Standard toxicity assessment test

If the concentration of hazardous metals surpasses a certain level, the waste is considered hazardous and harmful. The Toxicity Characteristic Leaching Procedure (TCLP) is proposed for risk assessment of solid wastes from an environmental perspective (Hira et al., 2018). Initial catalyst powder and ultimate residue were subjected to the normal TCLP examination. The original catalyst powder and the final residue were added to the buffer solution (5.7 mL glacial acetic acid and 64.3 mL of 1 N NaOH per 1 L of solution with a pH of 4.93) in a solid-to-liquid ratio of 1:20 with 30 rpm shaking for 18 hours. After filtration, the final sample solutions were examined with ICP-MS to determine the elemental content.

Table 2. Different acidic and basic leaching tests and their conditions.

Test Number	1	2	3	4	5	6
Particle size (μm)	-150	-150	-38	-150	-150	-150
Leaching agent	H_2SO_4	H_2SO_4	H_2SO_4	HNO_3	HCl	NaOH
Molar concentration	3	3	3	3	3	1
Time (min)	120	180	180	180	180	120

2.6. Kendall's tau-b (τB)

For studying the correlation between operational parameters Kendall's tau-b correlation can be used (Mohammadzadeh et al., 2023; Saneie et al., 2021). Kendall's tau-b calculates the correlation coefficient between two parameters by taking into account their interdependence and intercorrelations. The value of τ indicates the strength of the correlation, with 1 indicating a perfect positive correlation, -1 indicating a perfect negative correlation, and 0 indicating no correlation (Kendall, 1938). Kendall's Tau-b, which is included in IBM SPSS 26, was used in this study to assess the correlation between the bioleaching parameters.

3. Results and discussion

3.1. Characterization of sample

The results of XRF and ICP-MS analyses are shown in Table 3. As it is evident, the sample mainly consisted of Al_2O_3 (65.20%) and CaO (12.57%). The high CaO content in the sample can be problematic, since if it dissolves, the pH will rise. As for Ni bioleaching, the sample pH remained low; therefore, pH in the solutions was an essential parameter in this research. The sample contained a considerable amount of nickel (9.2%). Other influential elements in the bioleaching process were cobalt (119 ppm), arsenic (2.2 ppm), antimony (1.28 ppm), and silver (0.42 ppm).

Table 3. XRF and ICP analyses of the spent catalyst sample.

XRF analysis (%)				ICP analysis (ppm)										
Ni	Al_2O_3	MgO	CaO	Ag	As	Co	Fe	Mg	Mn	Mo	P	S	Sb	Zn
9.2	65.20	0.63	12.57	0.42	2.2	119	1026	1080	32	5.8	164	1160	1.28	430

Fig. S2 in Appendix shows the XRD results of the sample. According to the XRD analysis, the dominant phases of this sample are Corundum (Al_2O_3), Grossite ($CaAl_4O_7$), Bunsenite (NiO), Quartz (SiO_2), pure Nickel, and Gibbsite (Al $(OH)_3$).

3.2. Bioleaching tests by the mesophilic culture

The bioleaching results with the mesophilic culture are shown in Fig. 2. As the sample had a high amount of CaO, the pH in the control test increased quickly in the first days to more than 6. The ORP stayed the same in the whole process for the control test, which confirms the absence of any reducing or oxidizing agents in the sample.

The role of bacteria in bioleaching tests is to convert ferrous ions to ferric and produce sulfuric acid using elemental sulfur, thiosulfate, or metal sulfides (Donati and Sand, 2007). Increasing the ratio of ferric to ferrous ions is the primary factor that increases the ORP throughout the process. Because of this, it was expected that the ORP would rise as bacterial activity increased. On the other hand, sulfur oxidation leads to the formation of sulfuric acid, which results in lower pH as bacterial activity increases. The reduction role of ferrous ions in nickel oxide leaching increased the dissolution rate of nickel (Pichugina et al., 2002). Consequently, it can be stated that bacterial activity would increase nickel dissolution.

It is evident that in all bioleaching tests, the ORP increases, and pH decreases as the bacterial count rises. The pH in fractional tests did not increase significantly during the process. The sample contained CaO and other acid-consuming compounds, which resulted in a pH increase in control and normal bioleaching tests. However, in fractional bioleaching tests, when the sample was added to the solution, it had high cell counts, and η, resulting in no rise in pH. The recovery in the control test reached 20% in 25 days and stayed the same until the end of the process. All bioleaching tests' recovery was the same at the end of the process. Fractional tests performed better, but the optimal recovery difference compared with other tests was negligible. This result shows that the dissolution of nickel in the process was partly related to ORP and pH. Approximately 40% of the nickel remained undissolved, even under conditions of low pH and high ORP. The fractional bioleaching was able to recover 61% of the nickel from the sample.

Moosakazemi et al. (2019) showed that two-stage dissolution (acidic leaching with HCl on the residue of alkaline leaching with NaOH) on a spent catalyst of nickel, nickel oxide, and alumina are the main constituents, can lead to 95% dissolution of nickel. However, even with high levels of effective factors (such as the temperature of 90 °C, HCl concentration of 4 M, and liquid-to-solid ratio of 10:1), exploratory one-step leaching studies with HCl indicated limited Ni extraction. Hence, Ni as the active phase is not only distributed on the surface of the catalytic support but also within it (Moosakazemi et al., 2020). Similar studies on the bioleaching of spent catalysts show that metal oxides, such as NiO interfere with the dissolution process (Tayar et al., 2020).

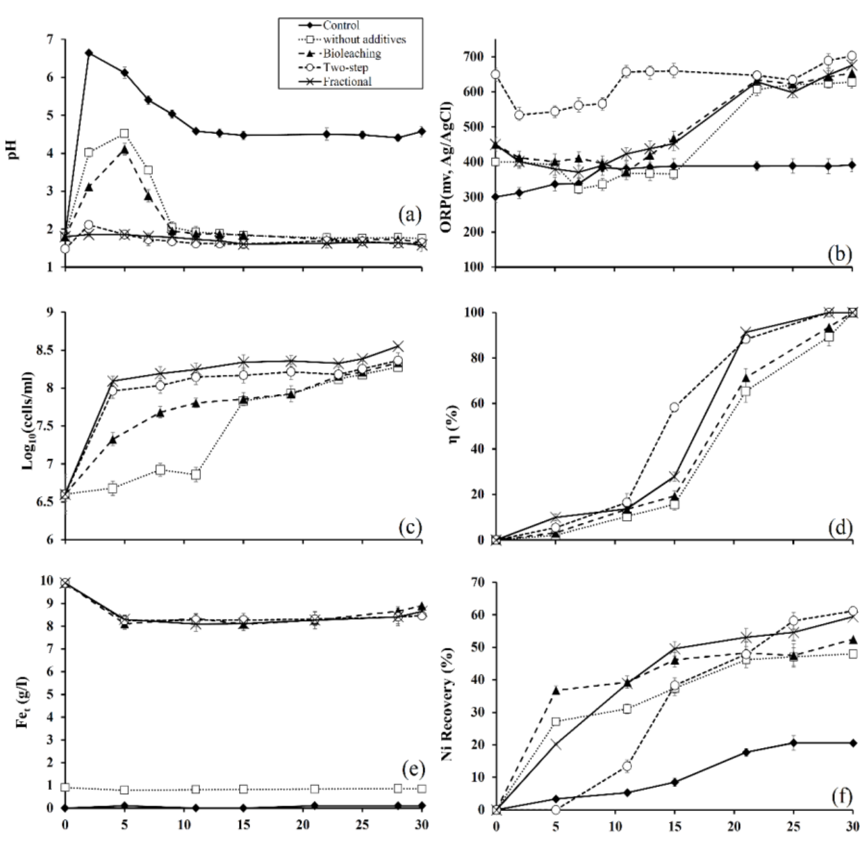

Fig. 2. Spent catalyst bioleaching results at 34°C; (a) pH, (b) ORP (mV), (c) bacterial counts (cells/mL), (d) iron oxidation (%), (e) total iron concentration (g/mL), and (f) nickel recovery (%).

3.3. Bioleaching tests by the moderately thermophilic culture

The bioleaching results with a moderately thermophilic culture are shown in Fig. 3. Similarly to the mesophilic culture, the pH was raised on the first day in the control test due to acid-consuming compounds. As the sample included a high concentration of CaO, the pH of the control test rose sharply within the first few days. In the control test, the ORP remained constant during the whole procedure, indicating the absence of any reducing or oxidizing agents. Thermophilic bioleaching tests were able to lower the pH more rapidly compared to mesophiles. Also, the Fe^{3+}/Fe^{2+} ratio reached the optimal value more rapidly. It has been shown that moderately thermophilic bacteria have a higher ability to increase ORP and decrease pH compared to mesophilic bacteria (Abdollahi et al., 2021; Saneie et al., 2021). The pH level of the solution changed less when the sample was added to the culture after a period of time. In this way, a lower pH can inhibit the deposition of jarosite and enhance recovery. Nickel dissolution is highly pH-sensitive (Agrawal et al., 2012), with thermophilic bioleaching experiments exhibiting lower pH levels. Nevertheless, the recovery in the moderately thermophilic condition was only slightly higher than in the mesophilic treatment. The fractional bioleaching was able to recover 61% of the nickel in the sample. The two-step fractional bioleaching was able to recover 66% of the nickel in the sample. As previously stated, it can be challenging for nickel to dissolve in acidic circumstances before aluminum. Consequently, utilizing thermophilic bacteria to increase the acidity of a solution is ineffective. In addition, because these bacteria function at a higher temperature, it is not cost-effective to utilize them to extract nickel from these catalysts. Based on past studies, nickel in spent catalysts can be recovered by conventional bioleaching methods (Meshram et al., 2019). However, it seems that a portion of the nickel in the catalyst used in this research cannot be extracted by conventional bioleaching methods.

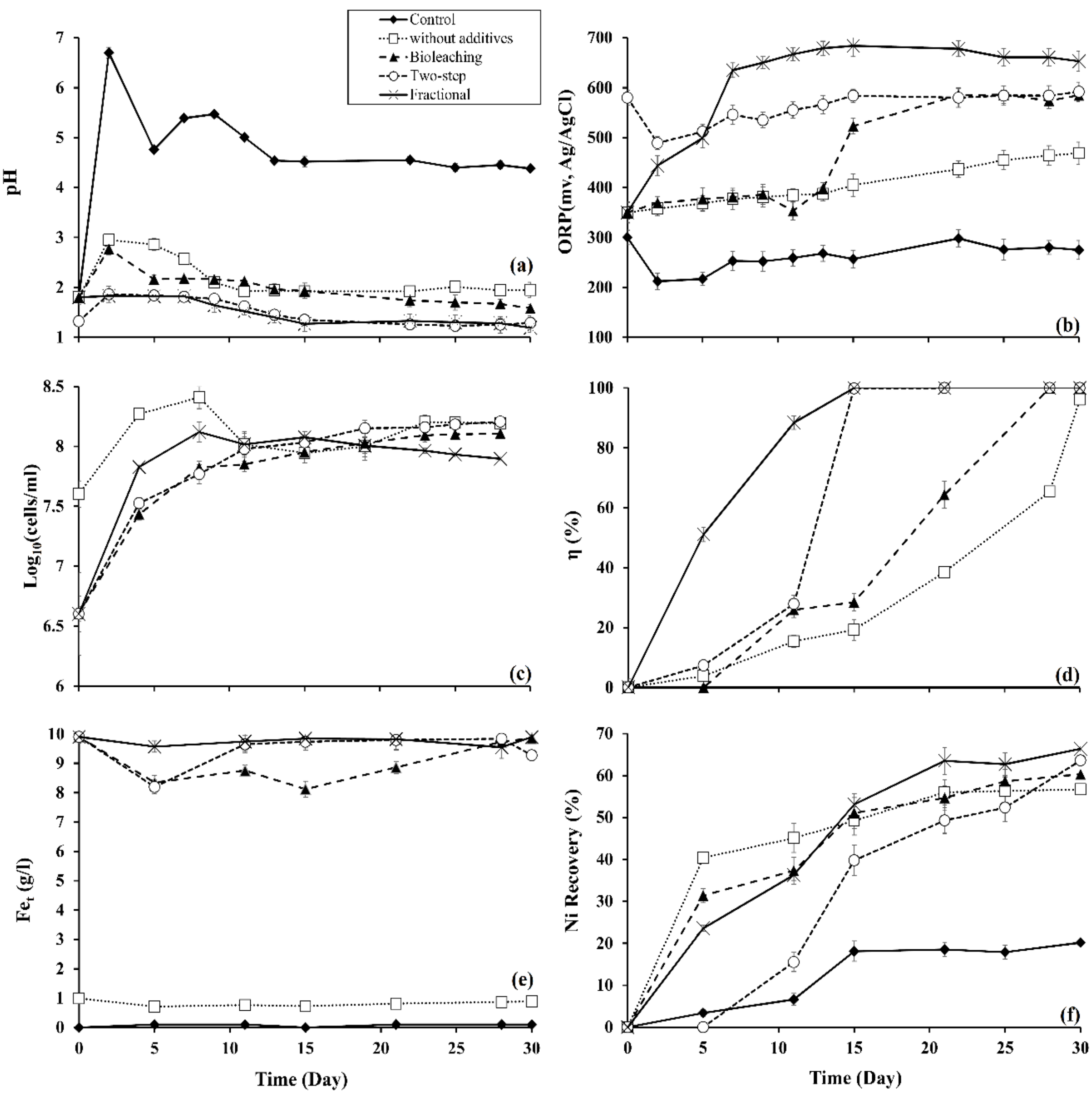

Fig. 3. Spent catalyst bioleaching results at 45°C; (a) pH, (b) ORP (mV), (c) bacterial counts (cells/mL), (d) iron oxidation (%), (e) total iron concentration (g/mL), and (f) nickel recovery (%).

3.4. Investigating the cause of the low recovery of nickel in bioleaching treatment

As mentioned before, part of the nickel content in the catalyst sample might not be recovered because it's in the active matrix and has turned into NiO (Nazemi et al., 2012) . Fig. S3 in Appendix shows the X-ray diffractograms for the residue of the fractional thermophilic bioleaching test, which had the best recovery among all bioleaching tests. The nickel remaining in the bioleaching residue is in the form of NiO. By comparing the XRD result of the feed and residue sample, it can be said that all the nickel content in the form of Ni was recovered and only the part of nickel that remained in the sample was in the oxidic phase (NiO). Therefore, six leaching tests with high levels of effective parameters were conducted to evaluate the potential of acid or base leaching for nickel recovery. Fig. 4 demonstrates the result of leaching experiments.

Acid leaching with H_2SO_4 (the same acid that is produced by bacteria) could only recover 70% of the nickel in the sample with high acid molarity and temperature after 180 minutes. It is noteworthy that 69.5% of nickel was recovered after two hours, which shows that part of the nickel cannot be recovered with this method. Compared with the best bioleaching result, 64.9% recovery with thermophile bacteria, it seems that both methods have similar efficiency. Also, Sharma et al compared bioleaching with chemical leaching of nickel from catalysts and especially at lower pH, both methods had similar recovery (Sharma et al., 2015). Further particle size reduction only deteriorated the results and even changing the acid to HNO_3 or HCl was not completely effective. HCl was the most efficient leaching agent, the better recovery of nickel with HCl is due to the pitting action of Cl^- to the passive film on the nickel's surface, which can result in the generation of $Ni(H_2O)_5Cl^+$ and $Ni(H_2O)Cl_2$ (Tran et al., 2022). Nickel recovery with sodium hydroxide was negligible, showing that basic conditions are not suitable for the dissolution of nickel.

Various pretreatment methods have been shown to improve the recovery of metals from catalysts (Bharadwaj and Ting, 2013; Karim and Ting, 2023). To further investigate this issue, a new leaching test with a pretreated sample with H_2 as a reducing agent was performed under the same condition as the leaching experiment No.2 (best H_2SO_4 recovery.) H_2SO_4 was chosen for further investigation because it closely resembles the acid naturally produced by bacteria during bioleaching. It was shown that reduction pretreatment can enhance the recovery of nickel (Tayar et al., 2020). 92% of the nickel was recovered in the reduced sample, showing that for complete recovery of nickel, it should be reduced with reducing agents before leaching or bioleaching treatments. Fig. S4 in Appendix shows the X-ray diffractograms for the residue of the pretreated spent catalyst sample leaching. No nickel in any phase was detected, as most of it was dissolved.

Based on the result, it can be concluded that a portion of the spent catalyst sample cannot be recovered with conventional leaching or bioleaching methods. Although the pretreatment method may be effective for enhancing the recovery, this method emits CO_2 and is performed at high temperatures, which can be an environmental problem. Also, pyrometallurgy has high energy consumption and greenhouse gas emissions (Liu et al., 2019). The use of fungi that utilize the redoxolysis mechanism can be beneficial, as they use reduction reactions and microbial oxidations, to enhance fungal leaching (Dusengemungu et al., 2021). Heterotrophic cultures have been proven to be more efficient in the bioleaching of spent catalysts in some cases (Srichandan et al., 2013). However, Santhiya and Ting were able to extract 62.8% of nickel from the spent catalyst, but it has not been studied what nickel compounds remained in the sample (Santhiya and Ting, 2005). Further research into optimizing extraction methods could provide a more sustainable and effective method for recovering valuable metals from spent catalysts.

3.5. Toxicity assessment

It is known that spent catalysts may include a variety of elements hazardous to the environment (Pathak et al., 2018). The leachability of harmful elements and compounds can serve as a reliable environmental indicator for solid waste. After executing the TCLP and elemental analysis, it was determined that the pretreatment with H_2 reduction for the spent catalyst powder had diminished its potential danger (Table 4). As demonstrated, the concentration of several elements, including Co, Cr, Mn, Pb, and Zn, in the original sample exceeds the allowed limit. This indicates that catalyst waste poses a significant threat to the environment. Bioleaching with thermophilic or mesophilic microorganisms has significantly reduced the sample's threat to the environment. The only remaining element in the sample that exceeds the criteria is nickel, which was recovered at a rate of about 60% during the procedure. Because nickel was not completely dissolved during the bioleaching process, the waste is still categorized as hazardous. However, the fact that all other metals are below the threshold cannot be ignored. Also, the leachate may require treatment as a result of the hazardous metals' presence, including neutralizing acidity or detoxifying harmful elements before discharge. Following this procedure and employing alkaline leaching, a complete nickel extraction flowsheet may be defined. Thus, the established route is both more profitable and less hazardous to the environment.

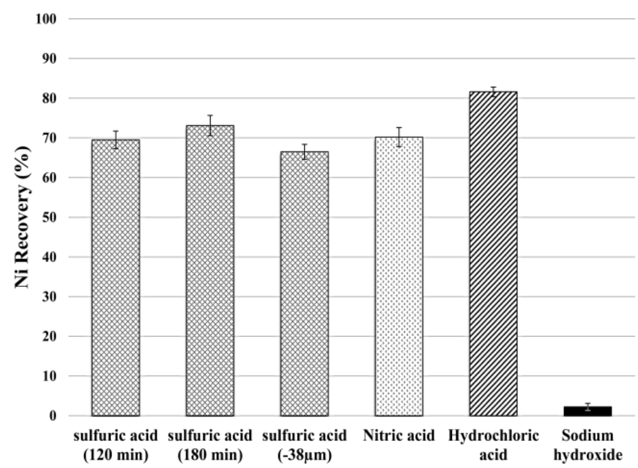

Fig. 4. Results of nickel recovery with different leaching agents in acidic and basic conditions at an RPM of 350, a temperature of 85°C, and a pulp density of 5%.

3.6. Correlation coefficient of bioleaching parameters

The final results of pH, ORP, bacterial count, bio-oxidation index (B_i), and nickel recovery was examined and the results are shown in Table 5. The bio-oxidation index can be calculated using the following equation:

$$B_i = \frac{\beta}{t_i} \quad (2)$$

Where β is the optimum bio-oxidation extent of the initial ferrous iron in the selected experiment and t_i is the time (days) that β reaches its peak value. Higher B_i indicates a more rapid bio-oxidation rate in the selected test. Based on the results, it can be seen that in the mesophilic culture, nickel recovery has no full correlation with other parameters. Also, the observed correlation between nickel recovery and B_i, pH, and ORP in the mesophilic culture can be due to random chance as correlations are significant at less than 0.05 level. However, in total it can be said that by increasing B_i and ORP and decreasing pH, nickel recovery would be enhanced. Unlike the mesophilic culture, thermophilic bacteria nickel recovery shows a complete correlation with B_i, pH, and ORP, and there is a low chance that this observation is due to chance. Moreover, in both cultures, pH and ORP have a completely negative association. Hence in total, it can be said that, by decreasing pH, ORP, B_i, and nickel recovery would be reduced and bacterial count has no effect on the bioleaching process.

Table 4. Toxicity test assessment data.

Contaminants	Concentrations (ppm)			TCLP threshold limit (ppm)
	Original sample	Final mesophilic residue	Final thermophilic residue	
Ag	0.42	0.32	0.22	5
As	2.2	3×10^{-3}	10^{-3}	5
Cd	1.3	0.02	5×10^{-3}	1
Co	119	0.02	8×10^{-3}	1
Cr	31	0.187	0.532	5
Cu	95	0.03	3×10^{-3}	250
Mn	32	1.1	0.4	5
Mo	5.8	6×10^{-3}	--	30
Ni	92643	39732	36933	5
Pb	34	0.06	0.04	5
Sb	1.28	8×10^{-3}	6×10^{-3}	150
V	1	10^{-3}	2×10^{-3}	250
Zn	430	0.08	9×10^{-3}	100

Table 5. Kendall's tau-b correlation coefficient between different parameters of (a) mesophile and (b) thermophile bioleaching tests.

a	pH	ORP	Bacterial Count	Recovery	B_i
pH	1.000	-1.000	0.333	-0.667	-0.816
ORP	-1.000	1.000	-0.333	0.667	0.816
Bacterial Count	0.333	-0.333	1.000	0.000	0.000
Recovery	-0.667	0.667	0.000	1.000	0.816
B_i	-0.816	0.816	0.000	0.816	1.000

b	pH	ORP	Bacterial Count	Recovery	B_i
pH	1.000	-1.000	0.333	-1.000	-1.000
ORP	-1.000	1.000	-0.333	1.000	1.000
Bacterial Count	0.333	-0.333	1.000	-0.333	-0.333
Recovery	-1.000	1.000	-0.333	1.000	1.000
B_i	-1.000	1.000	-0.333	1.000	1.000

4. Concluding remarks

The bioleaching process for recycling the spent reforming catalysts was examined, and it was discovered that this process can dissolve a significant amount of nickel. The dissolution of calcium, which made up a considerable portion of the sample, resulted in a pH increase in the bioleaching experiments, which had a negative impact on microbial metabolism. It was observed that with the two-step bioleaching technique, the pH elevation was less considerable, and nickel recovery improved. Bioleaching with moderately thermophilic bacteria yielded a slightly higher nickel recovery than with mesophiles which was mainly due to the faster metabolism of thermophiles for better oxidation, and also the elevated temperature of the system. Using the moderately thermophilic and mesophilic bioleaching process, 64% and 61% of the nickel in the sample were dissolved, respectively. Leaching tests with various leaching agents with high temperature, rpm, and molarity showed that the portion of the nickel in the NiO phase cannot be recovered via conventional leaching or bioleaching tests. Although reductive pretreatment of the sample can enhance the recovery, this method is not recommended as it may have negative environmental effects. Further investigation is required for enhancing the recovery of nickel with fungal bioleaching. The toxicity assessment test revealed that the bioleaching process with both cultures is able to reduce the risk of catalyst waste. All hazardous elements, except nickel, were successfully dissolved, though nickel was not fully recovered. Therefore, an additional leaching procedure is necessary to maximize nickel recovery.

The datasets generated during the current study are not publicly available due to not being published yet but are available from the corresponding author on reasonable request.

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Declaration of Competing Interest

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

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Appendix

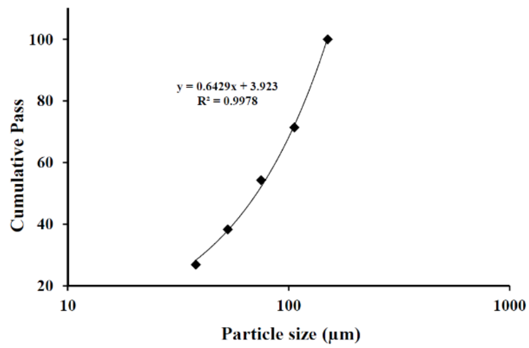

Fig. S1. The particle size distribution of the spent catalyst sample.

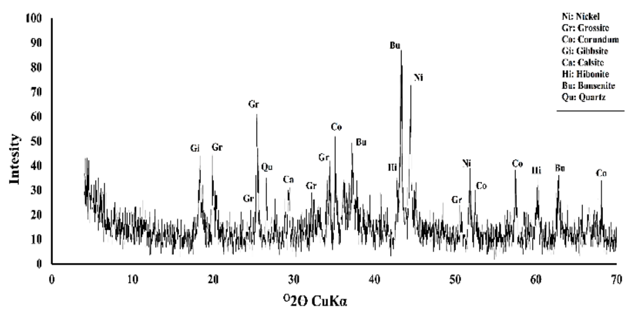

Fig. S2. X-ray diffractogram of spent catalyst sample.

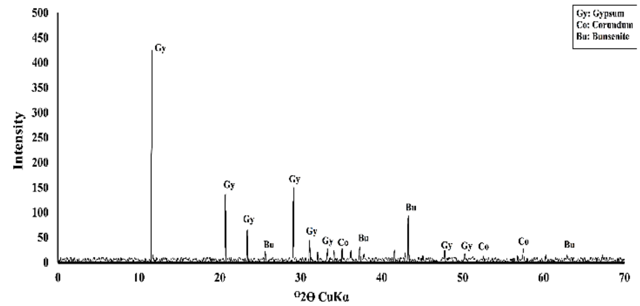

Fig. S3. X-ray diffractogram of residue of the fractional moderately thermophilic bioleaching test.

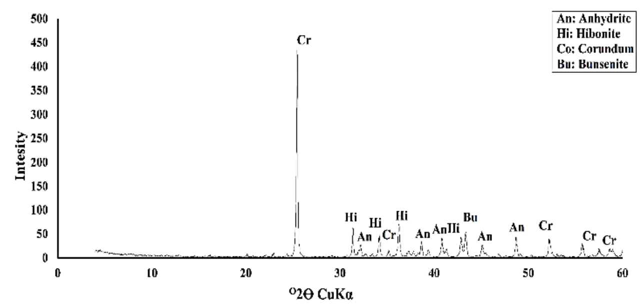

Fig. S4. X-ray diffractogram of the pretreated spent catalyst sample of leaching test.