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Effect of Fe^{2+} Concentration on Microbial Removal of Ni and V from Spent Catalyst

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Abstract: The oil industry use huge quantities of metal catalysts in order to produce desirable products from the raw material. Once these catalysts are exhausted, they are discharged to environment representing a serious environmental concern due to their high content in metals such as Ni, V, Co, Mo, Fe among others. Different efforts have been done to minimize the negative impact of spent catalysts. Biotechnological approaches appear to be a good alternative since microorganisms have different mechanisms to survive, growth and remove metals at these severe conditions; however, different parameters need to be optimized to reach an effective treatment. As a first approach, and due to the effect of iron on microbial metal leaching and the iron content in the spent catalysts; the present study reports the effect of two different Fe^{+2} concentrations (2 g/L and 32 g/L) on the ability of seven isolates to remove Ni and V contained in a spent catalyst in 125 mL Erlenmeyer flasks containing 9k liquid medium at 30°C, 150 rpm for 7 days. Results indicated that isolates coded as MNSH1-PHGII-1 and MV-9K-4 showed the maximum Ni and V removal from spent catalyst at a Fe^{2+} concentration of 2 g/L, which corresponded to 193 and 373 mg/Kg for Ni and 560 to 1038 mg/Kg for V, respectively. Nickel removal was improved 4 - 4.5 times and V, 2.4 and 7.7 times by the isolates above mentioned, in comparison when Fe^{2+} was used at 32 g/L. According with data, isolates MNSH1-PHGII-1 and MV-9K-4 could be excellent candidates for biotechnological approaches to leach metals; however, more studies are need to optimize metal recovery.

Key words: bioleaching, iron, microorganisms, removal, spent catalysts

INTRODUCTION

Oil industries use huge quantities of metal catalysts in order to produce desirable products. These catalysts contain different metals such as Ni, V, Co, Mo, Fe, among others. Metals in the catalyst can be present in the form of metal ions, metal oxides and metal sulfides.[16] Due to their extensive use, the catalysts are deactivated and then they are considered as waste materials also known as spent catalyst. Because of their heavy metal content, spent catalyst are classified into hazardous wastes. In many countries, different to Mexico, the spent catalysts are processed for metal recovery and then disposed in a safe way. Pyrometallurgical and hydrometallurgical techniques are the most common methods to recover metals from spent catalyst.[6,17] However, both processes are expensive and therefore they have not been applied for a larger scale application. Recently, research focus has been focused on an alternative technique known as bio-hydrometallurgical process.

Bio-hydrometallurgical processes are ecofriendly and cost effective. In the past, many studies have used iron and sulphur-oxidizing microorganisms for metal leaching.[4,5,7,20] Briand et al. (1996) used *Acidithiobacillus thiooxidans* to treat a spent vanadium-phosphorous catalyst [12] Mulak et al. (2005) have studied the effect of different parameters on the rate of nickel leaching from spent nickel oxide catalyst (NiO and Al_2O_3). [24]

The leaching of metals from ores or solid wastes using bacteria depends upon the bacterial oxidation of Fe^{2+} and sulfur into Fe^{3+} and H_2SO_4 respectively. In fungi, metal leaching, depends upon the production of organic acids such as citric acid, oxalic acid etc. Most effort is focused on using Fe/S oxidizing bacteria.

Other applications of bio-hydrometallurgical processes include the recovery of metals from electronic wastes such as those reported previously [2,10] where it was demonstrated the complexity of the process. Many factors determine the process including the type of microorganisms, pH, and concentration of iron in the system, qualitative and quantitative composition of waste, toxicity of ingredients and fineness of the material. Temperature and time also play a significant role in the reaction.

As a first approach, and due to the effect of iron on metal leaching and the iron content in the spent catalyst, the present study reports the effect of two different Fe^{+2} concentrations on the ability of seven heterotrophic isolates to remove Ni and V contained in a spent catalyst in liquid culture.

METHODS

Spent catalyst source: Catalyst powder coded as Ni-V was provided by Mexican Petroleum Institute (IMP) located in Mexico City and it was impregnated with Ni and V. **Table 1** shows the metals content in spent catalyst used in the present study, which was determined by ICP-OES.[15] Catalyst was stored under environmental conditions until use.

Microorganism source: To evaluate the effect of Fe^{2+} concentration on metal removal from spent catalyst, seven isolates coded as MNSH2-PHGII-2, MV-PHGII-2, MV-9K-2, MV-9K-4, MV-AH-1, PRGL-MS-2 and MNSH1-9K-1 were used. Microorganisms were isolated from four samples from sites located in Guanajuato, Mexico. **Table 2** shows the origin and codification of samples. Previous studies demonstrated that all isolates showed tolerance limits greater than 200 ppm of Ni and V. [13]

Table 1. Metal content in the spent catalyst -Ni-V-

Metals	mg/Kg
Ni	427.5 ±29.5
V	2164.8 ±76.6
Fe	3994.1 ±286.8
Al	103071.6 ±5468.3
As	821.6 ±30.5
Cr	66.4 ±15.3
Mg	525.6 ±45.9
P	75.6 ±5.4
Zn	53.7 ±4.1
Mo	18.3 ±0.4

Table 2. Origin and codification of samples used as source of microorganisms.

Source of microorganisms	Codification
Valenciana mine	MV
Nopal mine wet soil 1	MNSH1
Nopal mine wet soil 2	MNSH2
Guanajuato River water	PRGL

Fresh culture: Inoculum from each microorganism was prepared as follows, isolates were grown at 24 to 48 h in 9K liquid media without metals at 150 rpm, 30°C; microbial density of each isolate was adjusted to 3×10^8 CFU/mL. An inoculum at 10% (2 mL) of each fresh culture was added to experimental sets.

Effect of Fe on Ni and V microbial removal: For each isolate, two different experimental sets were prepared a) containing 2 g/L of Fe^{2+} and b) containing 32 g/L of Fe^{2+} in 9K medium (in g/L: KH_2PO_4, 0.4; $CaCl_2 \cdot 2H_2O$, 0.2; $MgSO_4 \cdot 7H_2O$, 0.4; $(NH_4)_2SO_4$, 0.4; $FeSO_4 \cdot 7H_2O$, 33.3); pH of medium was adjusted to 2.0, using H_2SO_4 at 10%. Experimental sets were prepared in 125 mL Erlenmeyer flask containing 20 mL of 9K medium, the spent catalyst was added at 16% (w/v) pulp density and inoculated with 2 mL of fresh culture (3×10^8 CFU/mL). Flasks were incubated at 30°C, 150 rpm for 7 days. After the incubation period, microbial growth was determined by cell number counting, pH was determined according to NMX-AA-008-SCFI-2011[22] method using a digital potentiometer (PerpHect LogR meter 310). The removal of Ni and V in catalyst was determined by ICP-OES following the methodology referred below. Negative control was prepared similarly but it was not inoculated. The effect of iron concentration was based on Ni and V removal.

Digestion and analysis of metals: Samples of biotreated catalyst were subjected to metal analyses using an inductively coupled plasma optical emission spectrometer (ICP-OES, Varian Model 710-ES) after acid

digestion where 100 mg of samples were placed in 100 mL sintered silicon carbide digestion vessels, adding 6 mL of HCl and 2 mL of HNO_3. Vessels were placed in a microwave reaction system (Microwave Pro Anton Paar) with HF100 rotor at power 800 W, pressure 40 bar, temperature 210-240°C for 20 min. Digested samples were filtered using a 10 mL syringe and a 0.2 µm cellulose filter; the filtrate was collected in a 100 mL volumetric flask and set with deionized water. Thirty mL were withdrawn into plastic tubes at 4°C until analysis. Metal analysis was performed by ICP-OES at the following wavelengths (nm): Ni (231.604) and V (292.401). Metal concentrations were calculated based on different calibration curves: 0.1 to 10 ppm for Ni and V, using a commercial standard (High-Purity).[13]

RESULTS

As can be observed in **Fig. 1 and 2,** the removal of Ni and V from spent catalyst at 16 % (w/v) pulp density in 9K medium, in general, was higher at 2 g/L Fe^{2+} than that observed for 32 g/L Fe^{2+}. At 2 g/L of Fe^{2+}, four isolates showed a higher Ni removal where isolates coded as MNSH1-PHGII-1 and MV-9K-4 showed the maximum Ni removal which corresponds to 193 and 373 mg/Kg, respectively **(Fig. 1)**

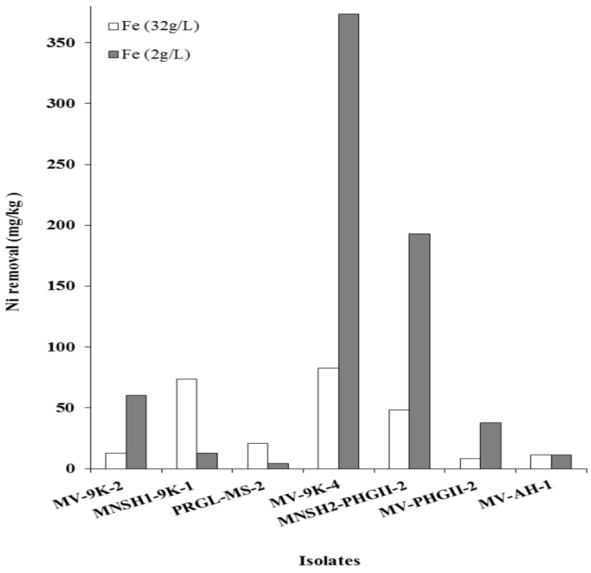

Fig.1: Effect of Fe^{+2} on Ni removal from spent catalyst Ni-V at 16 % (w/v) pulp density after 7 days, 30°C, 150 rpm in 9K medium.

In comparison, Ni removal was improved 4 - 4.5 times by the isolates above mentioned than when iron was added at 32 g/L. Different, isolates coded as MNSH-1-9K-1 and PRGL-MS-2 showed a removal of 73.6 and 20.56 mg/Kg at 32 g/L. Literature reports the effect of different concentrations of ferrous sulfate on bioleaching (5-20 g/L) where it was observed that 10 g/L of ferrous sulfate concentration were enough to bioleach metals from sewage sludge in the following order: Zn: 69%> Cu: 52%> Cr: 46%> Ni: 45. Authors also showed that using 10 g/L ferrous sulfate, indigenous iron-oxidizing microorganisms can down pH to cause significant metal solubilization.[1] Boult et al. (1994) reported high dissolution of Mn, Cu, Zn, Al at

Fe^{2+} concentrations up to 260 mg/L in the highly acid Afton Goch river which is polluted by mine drainages.[21] Present study showed that at 2 g/L of Fe^{2+}, Ni removal from spent catalyst was improved using heterotrophic bacteria possibly due to increased dissolution of Ni contained in spent catalyst; however, more studies are needed.

According with literature, main parameters which affect the bioleaching kinetics and metabolic activity of microorganisms are pH, concentration of Fe^{2+} in the system, qualitative and quantitative composition of waste, the toxicity of the ingredients (for microorganisms) and the degree of grinding material, temperature, time [11], medium composition has also a remarkable effect on cell wall composition and consequently the cell affinity to specific metals [18], also inoculum size could affect the metal removal by microbial populations. However, some microorganisms such as *Bacillus subtilis* are not affected, for example, by iron salts since the organism appears to need excessive amounts of iron for abundant growth. [8]

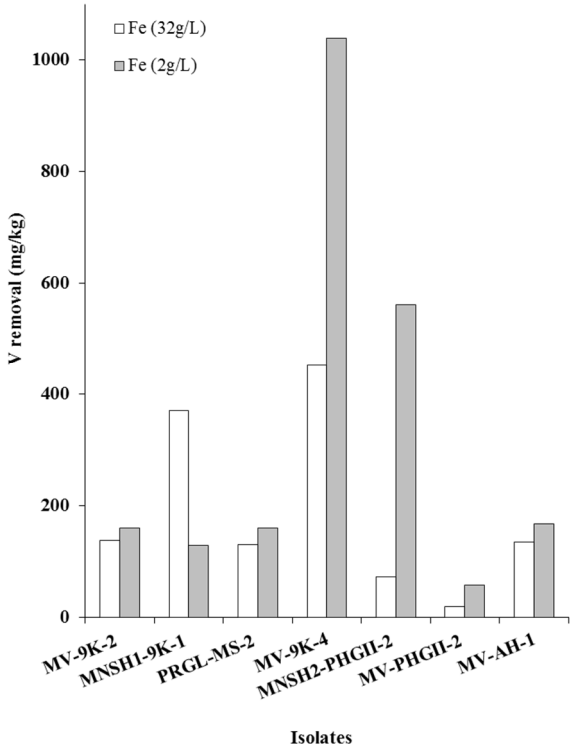

Fig.2: Effect of Fe^{+2} on V removal from spent catalyst Ni-V at 16 % (w/v) pulp density after 7 days, 30°C, 150 rpm in 9K medium.

Regarding to pH, as can be observed in Fig. 3 more important pH changes were observed when Fe^{2+} was added at 2 g/L, specifically, cultures coded MV-9K-4 and MNSH2-PHGII-2 decreased the pH from 3.7-3.74 to 3.25 and 3.45 respectively, probably this was the reason why both cultures had a higher metal removal. The rest cultures increased lightly the pH reaching values up to 4.08, in this case could be due to metal bioaccumulation, during this process the metals are precipitated, resulting in alkalization of the outer surface of the cell thereby increasing the pH of the medium.[3] Hocheng et al. (2012) explored an innovative metal biomachining (processes that use microorganisms as a tool to remove metal from a work piece) using *Acidithiobacillus ferrooxidans* to remove metals of samples cut into squared of 2 cm x 2 cm of 0.2 mm sheet of cooper, nickel and aluminium, where the first step is the oxidation of Fe^{2+} to Fe^{3+}. At the beginning, the initial concentration of Fe^{2+} decreased from 0.072 M to 0.0024 M increasing Fe^{3+} concentration and the

pH decreased from 2.5 to 1.0; then the produced Fe^{3+} oxidized the metals.[9] Previous situation could be similar than that observed in our study at 2 g/L, different to that observed at 32 g/L where no important pH changes were observed.

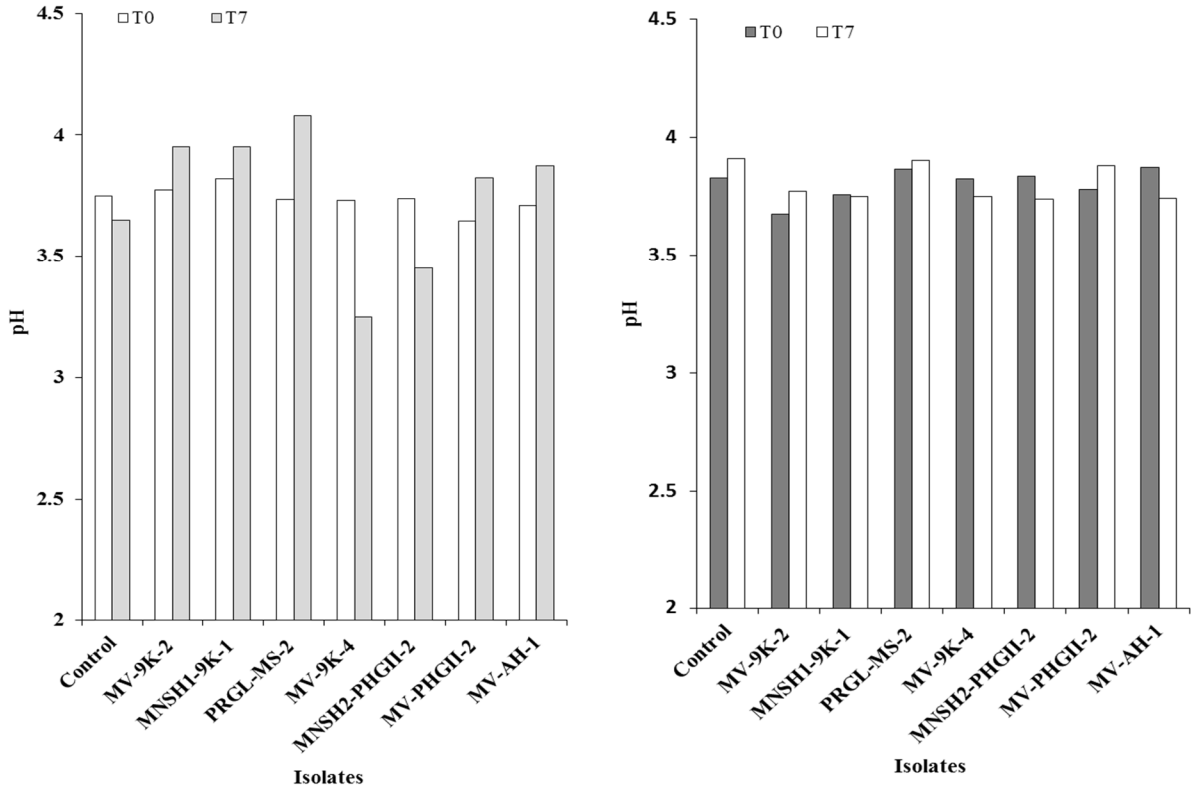

Fig. 3: pH changes during Ni and V removal by isolates on spent catalyst Ni-V at 16% (w/v) pulp density after seven days of incubation at 30 °C and 150 rpm in 9K medium containing A) 2 g/L Fe2+ and B) 32 g/L of Fe^{2+}.

Figure 4 shows the microbial behavior of isolates in presence of spent catalyst at 16% pulp density when two Fe^{2+} concentrations were used. As can be observed on Fig. 4A, isolates MV-9K-4 and MNSH2-PHGII-2 were able to grow reaching a count of 8.12×10^7 CFU/mL and 7.24×10^7 CFU/mL respectively. Pina et al (2010) studied the kinetics of ferrous iron oxidation by chemoautotrophic *Sulfobacillus thermosulfidooxidans* observing that ferrous iron oxidation rate and the microbial specific growth rate are a function of the initial ferrous iron concentration. A maximum overall oxidation rate of 0.697 g L^{-1} h^{-1} was observed in the experiments carried out at 10 g/L Fe^{2+}, and it did not change at higher concentrations (15 and 20 g/L).[19] It is necessary more investigation to optimize the parameters for Fe^{2+} oxidation, since the produced ferric ions play a key role as oxidizing agents for the dissolution and removal of metals, although in heterotrophic microorganisms is not well documented. Other isolates were able to grow at the same conditions but in a less extent, only isolate coded as PRGL-MS-2 decreased its microbial count at the end of 7 days of incubation, this isolate previously was identified as a yeast by microscopic observation. In

eukaryotic organisms a potential mechanism of iron toxicity includes its role in damage to DNA and membrane.[23] This could be the reason of lower growth of most isolates.

In the **Figure 4B,** also microbial growth was observed but it was less than that observed at 2 g/L, isolates MNSH-1-9K-1, MV-9K-4 and MNSH2-PHGII-2 showed a higher count, newly the isolate coded PRGL-MS-2 was the only that showed a decrease in its microbial count.

Additionally, and as part of a previous study reported by our research team, isolates coded as MV-9K-4 and MNSH2-PHGII-2 were identified as *Rhodotorula mucilaginosa* with a GenBank accession number KJ848328) and *Microbacterium liquefaciens* with a GenBank accession number KJ848325, respectively.[13,15]

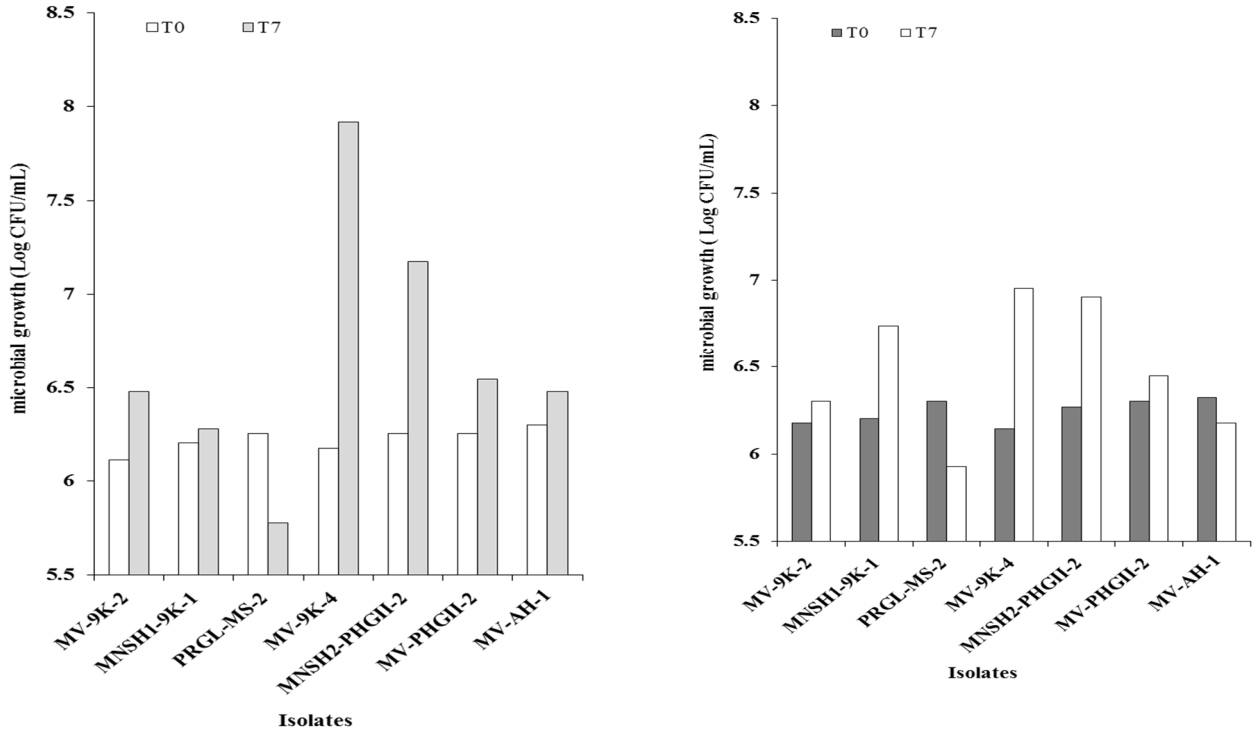

Fig.4 Microbial growth during Ni and V removal from spent catalyst Ni-V at 16% (w/v) pulp density after seven days of incubation at 30 °C and 150 rpm in 9K medium containing a) 2 g/L of Fe2+ and b) 32 g/L of Fe^{2+}.

CONCLUSIONS

Iron concentration has an important effect on metal removal; lower Fe^{2+} concentration (2g/L) improved the removal of Ni and V by isolates MNSH1-PHGII-1 and MV-9K-4 from spent catalyst where Ni removal was improved 4 and 4.5 times and V, 2.4 and 7.7 times in comparison to 32 g Fe^{2+}/L.

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