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# Response Surface Methodology Analysis of the Effect of the Addition of Silicone Oil on the Transfer of Carbon Dioxide during Bioleaching of Mining Tailings by Native Microorganisms

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**Abstract:** The bioleaching of manganese present in mining waste after metal extraction can be catalyzed by *Leptospirillum (L.) ferriphilum* by allowing atmospheric carbon dioxide to be used in this autotrophic process and generating the subsequent recovery of silver. Bioleaching of metals is widely performed in agitated tanks; therefore, it is important to assess the mass transfer capacity of gaseous substrates, such as carbon dioxide, during the microbial processes. The main objective of this research was to evaluate the effects of the presence and concentration of a transfer vector (silicone oil) added into a stirred-tank bioreactor during bioleaching of mining tailings catalyzed by *L. ferriphilum*, determined by the combined gas/oil mass transfer coefficient of carbon dioxide ($k_{LaCO2}$) into the aqueous phase. The experiments were carried out following a Box–Behnken experimental design, evaluating the concentrations of mining waste (30%, 40%, and 50%), $Fe^{2+}$, serving as electron donor (2, 8, and 14 g/L), and silicon oil (0%, 5%, and 10%). A significant increase in $k_{LaCO2}$ was observed after the addition of the transfer vector by comparing the lowest $k_{LaCO2}$ value of 1.68 $h^{-1}$ (obtained at 50% pulp, 8 g/L $Fe^{2+}$, and 0% silicone oil) and the highest $k_{LaCO2}$ of 21.81 $h^{-1}$ (obtained at 30% pulp, 2 g/L $Fe^{2+}$, 5% silicone oil). The results showed statistically significant differences in the transfer of carbon dioxide during the bioleaching process with a transfer vector.

**Keywords:** $k_{La}$ coefficient; silicone oil; bioleaching microorganisms; tailings treatments; metal biosolubilization; *Leptospirillum ferriphilum*

## 1. Introduction

Over the years, mining activity in Mexico has generated and disposed of waste (mine tailings) in open areas, allowing valuable components to be recovered from this "garbage". However, the mismanagement of this waste represents a serious pollution problem, producing up to 65 billion tons per year [1]. Therefore, biohydrometallurgy has gained interest in the scientific community because it is a mineral treatment technique for the recovery of the base, precious metals, and metalloids of interest through the action of a biological system [2–5]. In addition to the processing of mineral concentrates, biohydrometallurgy can be used to process waste from mining activities, since, as an environmentally friendly process, it allows the reduction of toxic agents [6,7]. Bioleaching differs from conventional processes by using biogenic components, which differ concerning the biological species used during processing [8–10]. Bacteria are the most widely used in biohydrometallurgical processes [11,12], the main reason being the economic feasibility of the process due to the metabolic capacity of microorganisms when using environmental compounds for the survival of the species. Various systems can be used [13–16]; however, at an industrial level, the system with the greatest demand is the stirred reactor because it allows for control of the necessary parameters to improve the recovery or elimination of the desired compounds [17–20]. One of the most used genera of microorganisms in bioleaching processes is the *Leptospirillum* genus [21–24] because it has metabolic advantages since it uses environmental components to cover its carbon ($CO_2$) and nitrogen ($N_2$) source requirements. In addition, the generation of biofilms allows the bioconversion of mineral surfaces. Manganese and its derivative compounds are of great importance as they are widely used in the steel industry, non-ferrous metallurgy, chemicals, electronics, batteries, agriculture, and other sectors [25–27]. However, in the silver recovery industry, manganese generates a negative effect, due to its refractory properties, by reducing the efficiency of conventional processes, such as cyanidation [17].

The scaling of industrial processes begins with studies performed at the laboratory level by generating the required information to assess the viability of the process, which could be based on the recovery of precious metals, such as silver. Therefore, one of the objectives of this study was to determine the optimal conditions to obtain a greater transfer of a gaseous substrate; although bioleaching processes are influenced by factors such as weather, geology, nutrients, respiration, pH, moisture, and temperature, the modification of certain factors might affect the metabolism of microorganisms and, consequently, the efficiency of the bioleaching process.

In an aerobic bioreactor, the measurement of the mass transfer capacity of gaseous substrates is of the highest importance since it relates to the productivity of the system. Thus, many empirical equations can be used to obtain the mass transfer coefficient $k_{La}$, and several studies have reported its determination. Hence, numerous studies have analyzed the transfer of gaseous substrates through the variation of operating design and control parameters [28–31]. Additionally, some research has studied the main characteristics that focus on mass transfer in systems where carbon dioxide dissolution is used for the growth of microorganisms [32–35]. Due to the autotrophic properties of the microorganisms used in the bioleaching process (such as *L. ferriphilum*), the supply of carbon dioxide in a gaseous state can be a very important factor in the efficiency of the process; therefore, monitoring its transfer and the effects caused by the variables of operation for the optimal growth of the biological species can affect its solubility. Therefore, a continuous supply of carbon dioxide is important, and the carbon dioxide transfer rate (CDOTR) should be identified and, if possible, calculated to control the process and sizing of a bioreactor. On the other hand, it should be mentioned that several studies have been carried out on biphasic partition bioreactors (TPPB), where silicone oil is added as a non-aqueous phase (NAP) to a biological process to improve the transfer of gaseous substrates (low solubility in water) to microorganisms [36]. This research evaluated a system consisting of mineral residues (% pulp), ferrous sulfate (% $w/v$), as well as silicone oil and indigenous microorganisms, which generate an important variation in the rheological behavior and interactions between components. The main objective of this research was to study the effect of the addition of silicone oil on the combined gas/oil mass transfer coefficient of carbon dioxide ($k_{LaCO2}$) into the aqueous phase during the manganese bioleaching process in a stirred-tank reactor and on the solubility of gaseous substrates under conditions unconducive to carbon dioxide transfer but favorable to bacterial metabolism. Interactions between variables were analyzed using the response surface method.

## 2. Materials and Methods

Tailings obtained from the "La Encantada" mine, located in Ocampo, Coahuila, Mexico, were used for this research. These tailings are byproducts obtained from a cyanidation process that is currently being performed to recover silver.

### 2.1. Materials

The following reagents were purchased from Sigma-Aldrich (St. Louis, MO, USA): $(NH_4)SO_4$, $MgSO_4{\cdot}7H_2O$, KCl, $K_2HPO_4$, $Ca(NO_3)_2$, $FeSO_4{\cdot}7H_2O$, and $H_2SO_4$.

### 2.2. Processing Parameters

To evaluate the effect of $CO_2$ transport and the process parameters on manganese bioleaching and subsequent silver recovery, a stirred reactor was used (BioFlo III batch/continuous fermenter, New Brunswick Scientific, Edison, NJ, USA). The processing parameters were the concentration of mining waste (30%, 40% and 50% $w/v$), the electron donor concentration ($FeSO_4{\cdot}7H_2O$: 2%, 4%, 8% and 14% $w/v$), and the concentration of silicone oil (0%, 5% and 10% $v/v$). The measurement of dissolved carbon dioxide was performed at the beginning of the bioleaching process.

### 2.3. Inoculum

A consortium isolated from a humid area of the mine was used (in greatest proportion was isolated *Leptospirillum ferriphilum*; ITDBC6, GenBank: MZ190834.1). It was grown in 9K medium [37] containing: 3 g/L $(NH_4)SO_4$, 0.1 g/L KCl, 0.5 g/L $K_2HPO_4$, 0.5 g/L $MgSO_4{\cdot}7H_2O$, 0.01 g/L $Ca(NO_3)_2$, and 44.22 g/L $FeSO_4{\cdot}7H_2O$. The pH was adjusted to 3 with 2 N $H_2SO_4$, incubated at 30 °C, and stirred at 150 rpm in 500 mL Erlenmeyer flasks with baffles.

### 2.4. Dissolved Carbon Dioxide

Quantification of carbon dioxide dissolved ($DCO_2$) was made using a Carbon Dioxide Gas-Sensing Electrode (WD-35802-10, pHoenix Electrode Company, Houston, TX, USA) with a controller ($DCO_2$ 7685, microprocessor, B&C Electronics, Denver, CO, USA). Two flasks containing distilled water with 0% and 100% $DCO_2$ were used: (a) Nitrogen ($N_2$) was bubbled for 20 min in one of the flasks until the electrode reading indicated the minimum concentration (0–0.05 mg/L), and (b) simultaneously, the air was bubbled into the other flask to reach 100% $DCO_2$ (≈1450 mg/L).

### 2.5. Statistical Design

Table 1 shows Box–Behnken response surface experimental design was used to evaluate three parameters: energy source ($FeSO_4{\cdot}7H_2O$) (g/L), silicone oil (%, $v/v$), and mining waste (%, $w/v$); these parameters presented a considerable expense in the scaling up of the bioleaching process. Silicone oil was used to evaluate its effect on $k_{LaCO2}$.

**Table 1.** Experimental design of the Box-Behnken response surface (Ferrous Sulfate, Silicon Oil, Mining Waste).

| Run | Ferrous Sulfate (g/L) | Silicon Oil (%, *v*/*v*) | Mining Waste (%, *w*/*v*) |
|---|---|---|---|
| 1 | 14 | 5 | 50 |
| 2 | 8 | 0 | 50 |
| 3 | 8 | 5 | 40 |
| 4 | 14 | 10 | 40 |
| 5 | 8 | 5 | 40 |
| 6 | 8 | 10 | 50 |
| 7 | 8 | 5 | 40 |
| 8 | 8 | 5 | 40 |
| 9 | 2 | 10 | 40 |
| 10 | 8 | 10 | 30 |
| 11 | 2 | 5 | 50 |
| 12 | 8 | 0 | 30 |
| 13 | 14 | 5 | 30 |
| 14 | 2 | 5 | 30 |
| 15 | 2 | 0 | 40 |

Bioleaching Experiment

The experiments were performed in a Bioflo III batch/continuous bioreactor. The tested variables were the concentration of $FeSO_4 \cdot 7H_2O$ (*w*/*v*), silicone oil concentration (*v*/*v*), and % mining waste concentration (*w*/*v*). A total volume of 80% of the bioreactor capacity (4.5 L) was used. The experiment began by adding mining waste to the bioreactor, followed by 9K medium (different concentrations of $FeSO_4 \cdot 7H_2O$) and then silicone oil. The reactor flanges were closed, and it was verified that the $CO_2$ and $N_2$ inlets were not clogged by solute. After the reactor was homogenized, 2 N $H_2SO_4$ was added to adjust the medium to the pH (3.0 ± 0.2) required by the bacterial strain. Once the temperature of 30 °C was reached, the dissolved carbon dioxide electrode was inserted in the vessel and the bioreactor was purged with pure nitrogen ($N_2$). When $CO_2$ concentration reached 0 mg/L, aeration with pure carbon dioxide at 0.5 volume of gas per volume of liquid per minute (VVM) began, and changes in the concentration of solubilized carbon dioxide were measured every 10 s for 0.194 h.

### 2.6. Evaluation of $k_{LaCO2}$: Gassing-Out Method

The evaluation of $k_{LaCO2}$ in bioreactors is crucial to establish aeration efficiency and to assess the effects of operation variables on the supply of gaseous substrates such as carbon dioxide. Some research has focused on the transfer flux of gaseous substrates, such as oxygen, in bioreactors [31,38], which could apply to assess $CO_2$ transfer.

Equation (1) establishes the mass balance for dissolved carbon dioxide in the liquid phase with ideal mixing:

$$\frac{dC}{dt} = CDOTR - CDOUR \tag{1}$$

where $\frac{dC}{dt}$ is the accumulation of carbon dioxide over time, *CDOTR* represents the flow of carbon dioxide transfer from the gas to the liquid, and *CDOUR* is the carbon dioxide consumption rate by microorganisms; the last term can be expressed by Equation (2):

$$(q_{CO_2}) \cdot (C_x) \tag{2}$$

where $q_{CO_2}$ is the specific carbon dioxide consumption rate of the microorganisms ($CO_2$ demand) and $C_x$ is the biomass concentration.

$k_{LaCO2}$ was estimated through a biological method, which determines the concentration of dissolved carbon dioxide in the medium by carbon dioxide absorption or desorption. After a step-change in the inlet gas concentration, the dynamic change in the concentration of dissolved carbon dioxide was analyzed. Only the dynamics of the liquid phase were considered in this research, assuming a completely mixed system. The concentration of carbon dioxide is described by Equation (3).

$$\frac{dC}{dt} = -k_{LaCO2}(C^* - C) \tag{3}$$

where $C^*$ is the concentration in equilibrium with the gas phase (mg/L) and $C$ is the concentration at different time intervals (mg/L).

If *CDOUR* = 0, the integration yields Equation (4):

$$ln\left(\frac{C^* - C}{C^* - C_0}\right) = -k_{LaCO2}\,(t - t_0) \tag{4}$$

The biological absorption methodology comprises the elimination of carbon dioxide in the liquid phase through nitrogen bubbling until the carbon dioxide concentration is equal to zero. Then, the liquid makes contact with pure carbon dioxide, and the variation in carbon dioxide concentration over time is measured. The biological desorption methodology comprises supplying pure carbon dioxide until the concentration of dissolved carbon dioxide saturation is achieved. Then, nitrogen is introduced into the tank, thus decreasing the concentration of dissolved carbon dioxide, and is recorded as a function of time. Under these conditions ($C = C_0$ at $t_0 = 0$ and $C_0 = 0$), Equation (5) can be expressed as follows:

$$-\,ln\left(\frac{C}{C_0}\right) = k_{LaCO2}(t) \tag{5}$$

On the other hand, when carbon dioxide has been removed from the system (by bubbling with nitrogen) and pure carbon dioxide is fed to the system again ($t_0 = 0$; $C_0 = 0$), Equation (6) can be expressed as follows:

$$ln\left(1 - \frac{C}{C^*}\right) = -k_{LaCO2}(t) \tag{6}$$

where $C^*$ is the concentration of carbon dioxide saturation over time and it is a function of the solid fraction (mineral waste, pulp concentration). Equations (5) and (6) describe the dissolved carbon dioxide when restarting pure carbon dioxide or when carbon dioxide is removed from the system; in both cases, $k_{LaCO2}$ can be determined from the slope of the graph of ln f ($C_L$) vs. time.

In this study, two variables important for bacterial proliferation were set constant, the temperature at 30 °C and agitation at 300 RPM, to make the biological process more cost-efficient. In addition, the electrode response was not taken into consideration for the calculations.

The variation in the energy source was derived from the pre-activation of the *L. ferriphilum* strain, thus generating a selective attack on the mineral surface. The amount of mining waste varied, with the goal being to treat the largest amount of residue to make the process more efficient, since most studies on bioleaching processes use a maximum of 10% pulp. While variation in the volume of silicone oil affects the rheological properties of the system during the bioleaching process, it also alters the transfer of carbon dioxide. It was assumed that the mass-transfer dynamics account for the average of both gas–liquid interfaces: $CO_2$-water and $CO_2$-silicone oil. Finally, a combined gas/oil mass transfer coefficient was measured in the aqueous phase.

To evaluate $k_{LaCO2}$, nitrogen was supplied in the bioreactor until the concentration of 0 mg/L was achieved. Afterward, the $CO_2$ feed valve was released, and the volume of pure carbon dioxide (0.5) per volume of liquid per min (VVM) was maintained for each experimental run. Measurements of carbon dioxide concentration were taken every 10 s until a constant value was reached. The pH was set at the start of the process (3.0 ± 0.2) with 2 N $H_2SO_4$ to ensure consortium survival. In addition, it was assumed that the $CO_2$ probe measures the average solubility of both liquid phases: water and silicone oil.

### 2.7. Statistical Analysis

Statistical analysis was performed with Minitab version 18. A Box–Behnken design was used. All data are presented as mean ± SEM; $p < 0.05$ was considered to indicate statistical significance.

### 2.8. Tailings Characterization

For the analysis in the inductively coupled plasma optical emission spectrometry (ICP-OES, Perkin Elmer 5300DV, Waltham, MA, USA), 100 g of sample were dried at 50 °C for 24 h. After that, the sample was pulverized in an agate mill for 3 min, then 2 mL of $HNO_3$ were added and the sample was heated at 70 °C for 30 min. Then, 6 mL of HCl were added and heated again at 70 °C for 2 h. Afterward, 17 mL were taken and centrifuged at 3000 rpm. Finally, the supernatant was analyzed in the ICP-OES. The target metals were Mn and Ag.

## 3. Results and Discussion

### *3.1. Combined Gas/Oil Mass Transfer Coefficient of Carbon Dioxide ($K_{LaCO2}$)*

The results of the Box–Behnken design showed an increase in the solubility of carbon dioxide and the transfer rate during the analysis of $k_{LaCO2}$ with the highest obtained value at 2 g/L ferrous sulfate, 5% silicone oil, and 30% mining waste (21.8 $h^{-1}$), compared to the lowest $k_{LaCO2}$ measurement where the run was 8 g/L ferrous sulfate, 0% silicone oil, and 50% mining waste (1.69 $h^{-1}$), as seen in Figure 1. Reaching the saturation concentration in a shorter time allows the maintenance of good solubility in the gaseous substrate.

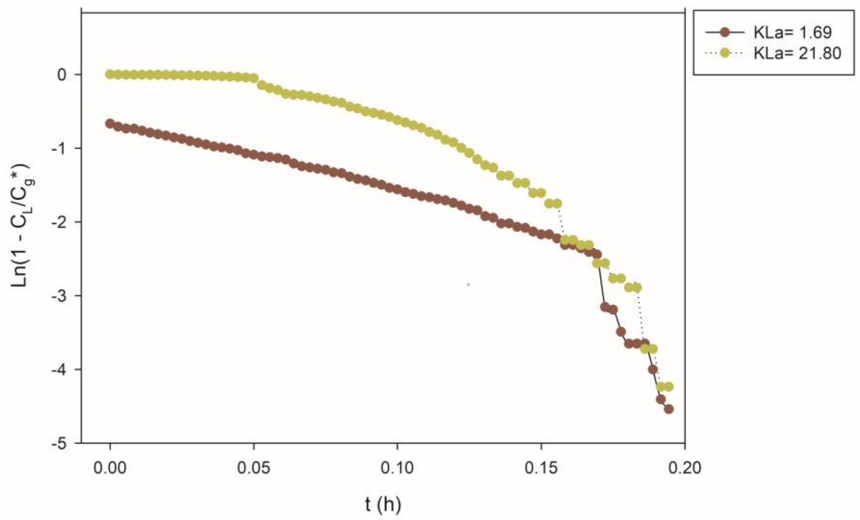


**Figure 1.** $k_{LaCO2}$ as a time function. The analyzed variables were $FeSO_4 \cdot 7H_2O$ (2, 4, 8, and 14 q/L $w/v$), silicone oil (0%, 5%, and 10% $v/v$), and mining waste (30%, 40%, and 50% $w/v$). Comparison between the highest value of $k_{LaCO2}$ (21.8 $h^{-1}$) and the lowest value of $k_{LaCO2}$ (1.69 $h^{-1}$). $C_g$* is the concentration of carbon dioxide saturation over time.

This means that each experimental design that increased the $k_{LaCO2}$ value by decreasing the time to reach the saturation concentration showed a better transfer of carbon dioxide from the gas phase to the aqueous phase. It is important to highlight that this system is multiphase, having not only the gaseous and liquid phases present in the process but also a solid and biological phase.

### *3.2. Effect of the Operating Parameters of the Bioleaching Process on the $K_{LaCO2}$*

This research demonstrates a directly proportional relationship between the use of pure carbon dioxide as a carbon substrate and its absorption and transport by silicone oil. An inversely proportional relationship between $k_{LaCO2}$ and the concentration of mining residues was also obtained; this phenomenon is shown in Figure 2.

The regression coefficients of the model are shown in Table 2, indicating the impact of the operating variables and the interaction between the variables on the transfer of carbon dioxide. Additionally, the importance of absorption and transport by silicone oil was confirmed, as the presence of this transfer vector (at >5%) maintained the solubility of $CO_2$ > 300 mg/L in systems with elevated concentrations of residues. This indicates that silicon oil has a positive effect on the homogeneity of aeration and mixing in the bioleaching system. In this way, the operating costs of bioleaching in an agitated tank could be reduced, since the mass transfer of gas to pulp is improved by diffusion in the gaseous substrate, thus improving the efficiency of the process. Furthermore, the $k_{LaCO2}$ value was stable even with an elevated concentration of mining waste and a greater addition of silicone oil, which enables the use of higher amounts of pulp in batch bioleaching operations by reducing costs.

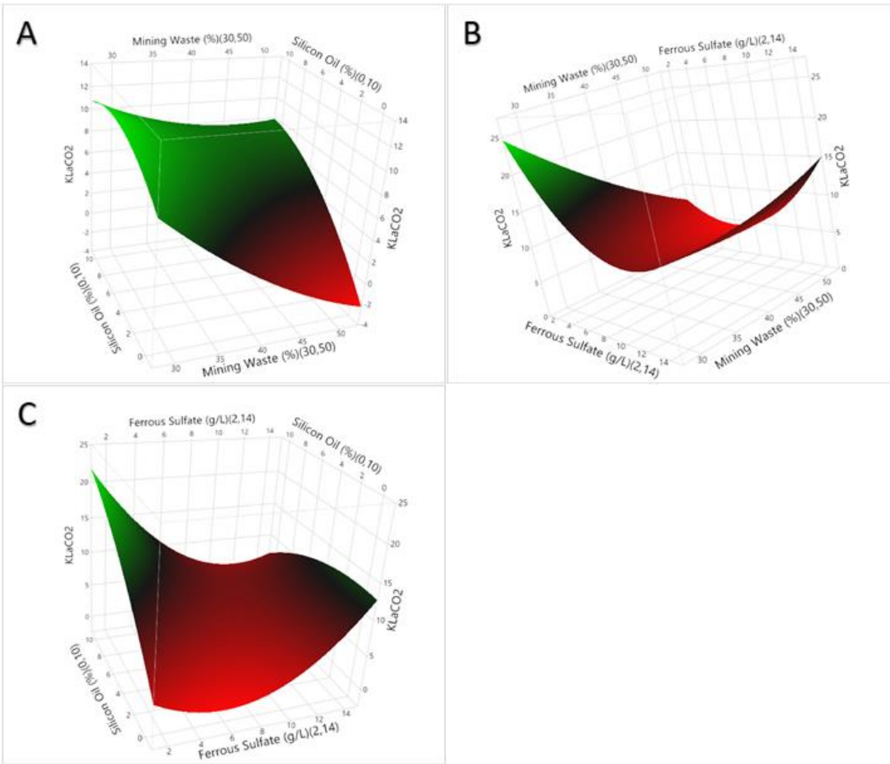


**Figure 2.** Response surfaces of (**A**) silicone oil-mining waste vs. $k_{LaCO2}$, (**B**) mining waste-ferrous sulfate vs. $k_{LaCO2}$., and (**C**) ferrous sulfate-silicone oil vs. $k_{LaCO2}$.

**Table 2.** Response surface regression coefficients: $K_{laCO2}$ vs. ferrous sulfate, silicon oil, and mining waste (coded coefficients).

| Factor | Coef | EE Coef | T Value | $p$-Value | FIV |
|---|---|---|---|---|---|
| Intercept | 7.19 | 1.67 | 4.31 | 0.008 | - |
| Ferrous Sulfate (g/L) (2, 14) | −1.78 | 1.02 | −1.75 | 0.141 | 1.00 |
| Silicon Oil (%) (0, 10) | 3.13 | 1.02 | 3.06 | 0.028 | 1.00 |
| Mining Waste (%) (30, 50) | −2.70 | 1.02 | −2.64 | 0.046 | 1.00 |
| Ferrous Sulfate (g/L) × Ferrous Sulfate (g/L) | 3.97 | 1.51 | 2.64 | 0.046 | 1.01 |
| Silicon Oil (%) × Silicon Oil (%) | −2.09 | 1.51 | −1.39 | 0.223 | 1.01 |
| Mining Waste (%) × Mining Waste (%) | 1.27 | 1.51 | 0.85 | 0.436 | 1.01 |
| Ferrous Sulfate (g/L) × Silicon Oil (%) | −3.14 | 1.45 | −2.17 | 0.082 | 1.00 |
| Ferrous Sulfate (g/L) × Mining Waste (%) | 3.57 | 1.45 | 2.47 | 0.056 | 1.00 |
| Silicon Oil (%) × Mining Waste (%) | 1.29 | 1.45 | 0.89 | 0.413 | 1.00 |

Note: The quadratic model shows a correlation coefficient of 0.8917.

Interactions between the pulp density and the concentration of $Fe^{2+}$ and silicone oil are shown in Figure 3 with the following highlights: (a) the use of silicone oil promoted the solubility of pure carbon dioxide used as a carbon source for microorganisms in the bioleaching process, even with an increase in the solid phase concentration (mining residues); (b) the decrease in $k_{LaCO2}$ was inversely proportional to the addition of silicone oil, indicating important interactions between residue concentrations of 40% and 50% (the maximum concentration used in this study), such values had a negative effect on the solubility of pure carbon dioxide during processing; and (c) as the silicone oil concentration increases, mass transfer through the interface is favored; therefore, the solubility of carbon dioxide can be maintained even at pulp concentrations >45%.

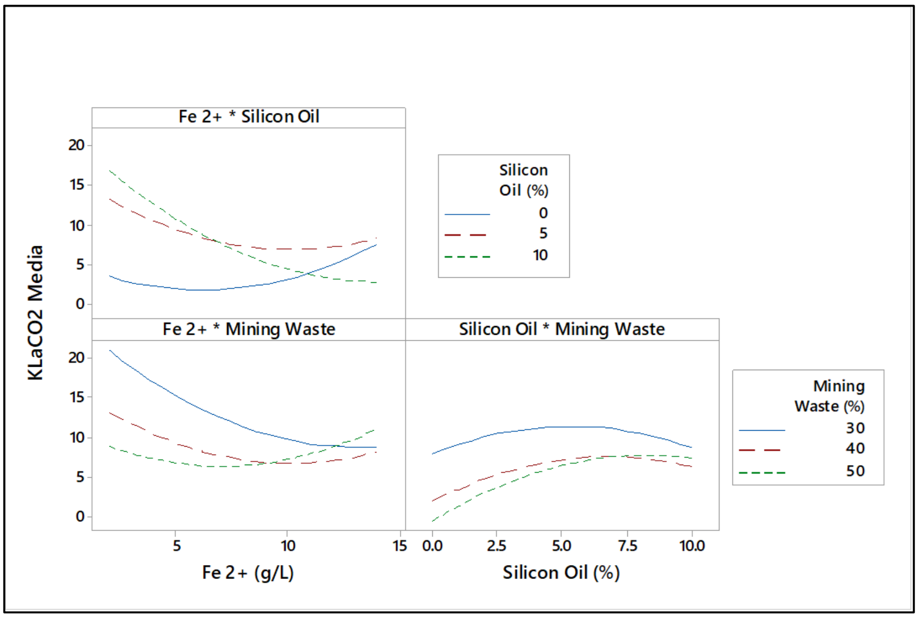


**Figure 3.** Interaction plot (adjusted media) between silicone oil–mining waste–ferrous sulfate. Crossing between parameters indicates an interaction concerning the response ($k_{LaCO2}$).

This is confirmed by plots in Figure 4 showing that the use of different concentrations of silicone oil (≥5%) at high pulp concentrations (>40%) could mitigate the decrease in $k_{LaCO2}$ by maintaining the $CO_2$ concentration above the minimum value. Access to the carbon source is indicative of the growth of the bioleaching microorganisms, and a high concentration improves the efficiency of the microbial catalysis of the mineral degradation. In this manner, operational benefits could be obtained, since it has been shown that bacteria of the *Leptospirillum* genus can perform catalytic functions under autotrophic conditions [24,39,40]; therefore, carbon dioxide requirements during processing would remain constant, even at minimum concentrations, because of the transfer vector (silicone oil), which might help the bioleaching of the material by avoiding drops in $k_{LaCO2}$ [41]. The conditions of the bioleaching process with the help of silicone oil allow the pure carbon dioxide applied in the gaseous phase to solubilize, thus transforming it into carbonic acid, which is mainly used by the microorganisms through the metabolic pathway for this genus [42].

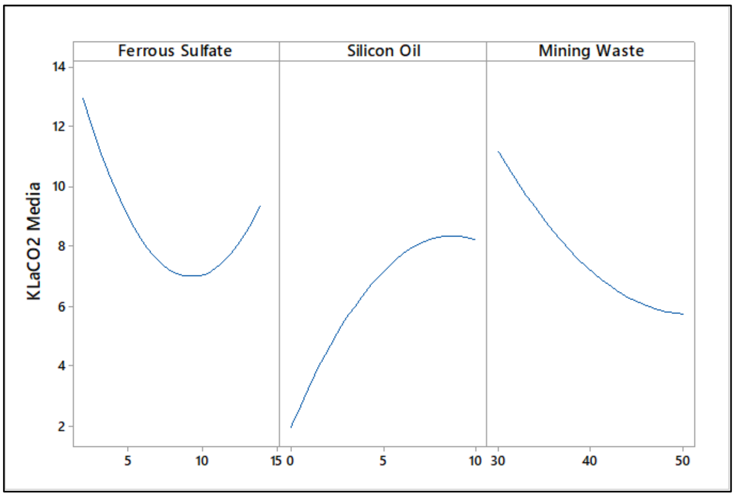


**Figure 4.** $k_{LaCO2}$ main effects plot (adjusted media) of ferrous sulfate (g/L), silicon oil (%), and mining waste (%).

The relationship between temperature and agitation speed affects the transfer of gaseous substrates. However, these parameters were not varied in this study due to the optimum operating conditions for microorganisms, previously postulated at 300 RPM and 30 °C [27]. This research shows that the use of a transfer vector under the aforementioned operating conditions helps to maintain stable $k_{LaCO2}$.

Table 3 shows the percentages of manganese removal after 24 h of the bioleaching process applied to the mining waste; the results were compared to a sample without biological treatment used as a control. Table 3 also shows the results of silver extraction, which were obtained by characterizing each sample of mining residue after being treated at the laboratory level with 1000 ppm of sodium cyanide for 72 h, determining the amount of silver that was able to be solubilized by cyanidation after the bioleaching process of mining residue.

**Table 3.** Manganese removal and silver extraction after the bioleaching process using the response surface Box–Behnken experimental design.

| Run | Ferrous Sulfate (g/L) | Silicon Oil (%, *v*/*v*) | Mining Waste (%, *w*/*v*) | Manganese Removal (%) | Silver Extraction (%) |
|---|---|---|---|---|---|
| 1 | 14 | 5 | 50 | 21.26 | 26.49 |
| 2 | 8 | 0 | 50 | 2.35 | 33.93 |
| 3 | 8 | 5 | 40 | 15.69 | 15.00 |
| 4 | 14 | 10 | 40 | 27.49 | 21.64 |
| 5 | 8 | 5 | 40 | 14.03 | 10.25 |
| 6 | 8 | 10 | 50 | 17.93 | 19.83 |
| 7 | 8 | 5 | 40 | 15.04 | 28.01 |
| 8 | 8 | 5 | 40 | 6.86 | 27.45 |
| 9 | 2 | 10 | 40 | 21.30 | 19.15 |
| 10 | 8 | 10 | 30 | 33.34 | 18.21 |
| 11 | 2 | 5 | 50 | 13.44 | 17.81 |
| 12 | 8 | 0 | 30 | 21.29 | 40.20 |
| 13 | 14 | 5 | 30 | 27.43 | 35.72 |
| 14 | 2 | 5 | 30 | 21.16 | 18.95 |
| 15 | 2 | 0 | 40 | 21.40 | 16.69 |

Table 4 shows that the concentration of ferrous sulfate and the addition of silicone oil have a significant positive effect ($p < 0.05$) on the removal of manganese; this could be related to mechanisms of action that might be generated on the surface of the mineral when solubilizing surface components through direct and indirect oxidation [26]. After the cyanidation process, analysis of the silver extraction showed no significant effect from the evaluated factors ($p > 0.05$), which could indicate that the residence time of the process was insufficient.

**Table 4.** Regression coefficients of responses of the bioleaching process (coded factors).

| Response | Intercept | Lineal | | | Quadratic | | | Interactions | | | $R^2$ |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | $X_1$ | $X_2$ | $X_3$ | $X_1{}^1$ | $X_2{}^2$ | $X_3{}^3$ | $X_1X_2$ | $X_1X_3$ | $X_2X_3$ | |
| Mn | 94.4 | 0.72 | **6.02** * | **−6.03** * | 3.22 | 1.12 | 2.68 | 5.18 | 0.39 | 0.88 | 0.86 |
| Ag | 132.0 | 4.84 | −4.93 | −1.88 | 0.09 | 3.39 | 6.90 | −2.07 | −2.02 | 1.97 | 0.67 |

Note: * Indicates significant difference ($p < 0.05$). $X_1$ = Ferrous Sulfate [g/L], $X_2$ = Silicon Oil [% *v*/*v*], $X_3$ = Mining Waste [% *w*/*v*]. Mn = Manganese Removal [%], Ag = Silver Extraction [%].

## 4. Conclusions

1. The obtained results indicate that, in the manganese bioleaching process, the operating parameters used (concentration of ferrous sulfate, silicon oil concentration, and mining waste concentration) have a significant effect on the combined gas/oil mass transfer of $CO_2$ into the aqueous phase. Also, there is an inversely proportional relationship between pulp density and $k_{LaCO2}$ and a directly proportional correlation between the absorption and transport by silicone oil and $k_{LaCO2}$.

2. The interaction between the operating variables indicates the importance of the activity of silicone oil on the solubility of carbon dioxide in the system by allowing the gaseous substrate to remain for a longer time and increasing its usage by microorganisms, even during stress conditions.
3. Improving the transfer of $CO_2$ using silicone oil during the bioleaching of Mn allowed us to maintain the survival of the microbial consortium under adverse conditions, which enables the use of pulp percentages greater than 10% ($w/v$), which is highly useful in the characterization of the bioleaching process at the bioreactor level and promising for future scaleup.
4. The evaluated operation conditions were extreme for the survival of the consortium, but it should be noted that there was a directly proportional effect upon the increase in $k_{LaCO2}$ and the removal of manganese.

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