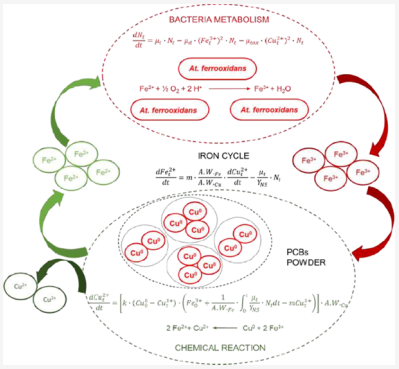
I&EC research
Industrial & Engineering Chemistry Research
pubs.acs.org/IECR
Article

Bioleaching of End-of-Life Printed Circuit Boards: Mathematical Modeling and Kinetic Analysis

Alessandro Becci,* Alessia Amato, José M. Rodríguez-Maroto, and Francesca Beolchini

Cite This: *Ind. Eng. Chem. Res.* 2021, 60, 4261−4268
Read Online

ABSTRACT: This work reports a kinetic study of the bioleaching process for Cu extraction from waste printed circuit boards (WPCBs), using iron as an oxidant agent. The kinetic study considered both the Fe^{2+} oxidation by bacteria metabolism and the chemical reaction between Fe^{3+} and Cu within WPCBs. The model was composed of a system of three differential equations characterized by three dependent variables and seven parameters. The first equation describes the bacteria abundance trend, the second one represents the variation of Fe^{2+} concentration, and the third one focuses on the Cu extraction by the biogenerated Fe^{3+}. The developed model was consistent with experimental data with an R^2 higher than 0.97. The results showed that the bacteria metabolism was 1.8−2.5 times faster than the chemical reaction. The determined mathematical model, suitable for the implementation at an industrial scale, could be an important tool to predict the bioleaching mechanism in a metal recovery process.



1. INTRODUCTION

The modern society is becoming more and more dependent on electronic devices, which are characterized by a short replacement cycle.[1] This tendency can be translated in a growing flow of new technologies to the market with a consequent increase of an electronic waste amount (e-waste).[2,3] An amount of 45 million tons of e-waste was generated in 2016 with an estimated annual growth rate of 3−4%.[4−8] There are four main sources of e-waste: small/large home appliances, hospital medical equipment, office machines, and industrial equipment/machines.[9] Within the entire e-waste quantity, the WPCB percentage is relevant (3−6%).[10−13] The WPCBs are composed for more than 40% (w/w) of metals.[10,14] Cu has an average concentration of 20% (w/w) of whole metals, and it is 10−40 times higher than that in copper-rich minerals.[4,15−17] The annual Cu production is around 15 million tons, and about 30% is used for the electric and electronic devices.[18] The Cu resulting from recycling is 40% of the total Cu production.[19] Usually, to produce 1 ton of Cu from ores, around 1400−1700 kg of standard coal is used, and 20 tons of wastewater and 3 tons of hazardous materials are generated.[15,20] According to the Bureau of International Recycling,[21] the Cu extracted from e-waste is able to save up to 85% of energy and reduce around 65% of CO_2 emissions, compared to the primary production.[19] Therefore, the Cu recovery from WPCB recycling has a triple benefit: (1) the primary resource conservation, (2) the solid waste reduction, and (3) the prevention of the environmental pollution resulting from the Cu release.[3,22,23] The most common techniques used for the e-waste recycling and metal recovery include three approaches: chemical, metallurgical, and physical. The chemical processes include pyrolysis, gasification, combustion, or the use of a chemical agent (i.e., HCl, H_2SO_4, HNO_3, ammonium, or ethylenediaminetetraacetic acid).[9,14,24] However, the acid and the gases produced during the chemical processes generate a secondary pollution. For this reason, the biotechnologies have become more and more attractive in the last decades as an eco-friendly and sustainable alternative.[9,25] The Cu extraction from WPCBs by biotechnologies is studied by several works, mainly using *Acidithiobacillus ferrooxidans* (*At. ferrooxidans*).[26−35] The mechanism for the Cu leaching from WPCBs follows eq 1:

$$Cu^0 + 2\,Fe^{3+} \rightarrow Cu^{2+} + 2\,Fe^{2+} \quad (1)$$

The main advantage of the bioleaching processes is the Fe^{2+} oxidation by bacteria metabolism (eq 2) with the possibility of the Fe^{3+} regeneration for further extractions:

$$Fe^{2+} + {}^1\!/_2 O_2 + 2H^+ \rightarrow Fe^{3+} + H_2O \quad (2)$$

The bioleaching is carried out with a WPCB concentration between 0.8 and 5% (w/v) and under these conditions: 30 °C, pH of 1.6−2.2, Fe^{2+} concentration of 4−10 g/L, and 120−200

Received: November 12, 2020
Revised: February 23, 2021
Accepted: February 24, 2021
Published: March 12, 2021

https://dx.doi.org/10.1021/acs.iecr.0c05566
Ind. Eng. Chem. Res. 2021, 60, 4261−4268

rpm. The main disadvantage of the biotechnologies is the low WPCB concentration that could be treated due to the metal toxicity on bacteria metabolism. This work aims at developing a mathematical model to combine the Fe^{2+} oxidation by bacteria metabolism and the chemical reaction between Fe^{3+} and Cu. The main innovation of this research is the integration of the bacteria metabolism with the chemical leaching, compared to the current literature that, generally, studies these two processes separately.[33,36−43] The developed model is essential to describe in detail the main mechanisms of the bioleaching process. The resulting information can be relevant to predict the possible toxicity of Cu and Fe^{3+} on bacteria metabolism and understand how the bacteria oxidation influences the kinetic of the Cu extraction. Considering both these aspects, this model could be an important tool in the perspective of bioleaching scale-up.

2. MATERIALS AND METHODS

2.1. Preparation of WPCBs. The WPCB samples, mainly from a personal computer, were prepared as follows: The material was shredded using stainless steel blades and pliers after manually removing the main parts of the electronic components (e.g., capacitors, batteries, and resistors). Thereafter, the refuse was crushed to obtain a granulometry of less than 0.5 mm, with an average Cu content of 25% (w/w), which was used for the bioleaching experiments (Table 1).

Table 1. Mean Metal Concentration in WPCBs

metals	concentration % (w/w)
Cu	24.7
Zn	3.7
Al	0.4
Ni	0.03
Pb	1.3
Sn	2.1

2.2. The Bioleaching Process. The microorganism used for the bioleaching process was *At. ferrooxidans* (DSMZ 14882 T), obtained from the Deutche Sammlung von Mikroorganismenn und Zellkulturen GmbH, Germany. The bacteria culture grew in the DSMZ 882 medium; it was prepared as follows: 0.132 g of $(NH_4)_2SO_4$, 0.053 g of $MgCl_2{\cdot}6H_2O$, 0.027 g of KH_2PO_4, 0.147 g of $CaCl_2{\cdot}2H_2O$, and 49.8 g of $FeSO_4{\cdot}7H_2O$ are dissolved in 1 L of MilliQ water, and the pH is adjusted to 1.6 with a 1 M H_2SO_4 solution. The bioleaching approaches reproduced the condition reported by Becci et al.[35] Therefore, the process was conducted in three phases; The first was the bacteria growth phase, where bacteria grew without WPCBs for 48 h. At the end of this phase, a known amount of WPCBs (50 g/L) were added, and the second phase (first leaching step) started. After 48 h, 80% of the leaching solution was replaced with a new fresh medium and the third phase (second leaching step) started. The last step was conducted for 168 h. This biotechnology was carried out in three phases to avoid toxicity issues; indeed, WPCBs are at the highest concentration ever tested in the literature. The bioleaching experiment was carried out at the temperature of 30 °C and at 120 rpm in an orbital incubator (S510). The pH was monitored and adjusted to 1.6 with a 1 M H_2SO_4 solution, each day. This pH value was chosen to both prevent the jarosite precipitation on WPCBs (that reduces the leaching efficiency) and to make sure of the bacteria growth.[44−46]

2.3. Analytical Determination. For the bacteria count, a subsample (0.5 mL) was withdrawn periodically (0, 10, 24, 34, and 48 h) for both the bacteria growth phase and the first leaching step (0, 24, 72, 120, and 168 h for the second leaching step) and was fixed in formalin solution (2% final concentration). The total prokaryote abundance was determined by a direct count on an epifluorescence microscope following the procedure described by Danovaro et al.[47] As described in the method, the bacteria were stained by acridine orange solution then filtered through nucleopore 0.22 μm pore size filters. The total prokaryote count per milliliter of solution was calculated using eq 3 to determine the bacteria free in the leaching solution:

$$\text{total prokaryote count/mL} = (N{\cdot}\text{C. O. }{\cdot}d)/\text{mL} \tag{3}$$

where N is the mean bacteria number per optical section, C.O. is the maximum optical section number (12,868), d (100 times) is the sample dilution, and mL is the withdrew solution (0.5 mL).

The Fe^{2+} and Fe^{3+} concentrations were determined spectrophotometrically (Jasco model 7850) by the colorimetric thiocyanate method.[48] On the other hand, the Cu concentration was measured by an atomic absorption spectrophotometer (Techcomp, AA6000).

3. THEORETICAL BASIS

3.1. Theory and Background. The experimental results were used to fit the model and to determine the best parameter to describe the bacteria number and Fe^{2+} and Cu temporal changes. The parameters were estimated by the nonlinear least square procedure by a modification of the Levenberg−Marquardt algorithm (minpack.lm).[49,50] The bacteria growth rate (eq 5) and Fe^{2+} oxidation (eq 6) were assumed to follow the Monod model (eq 4) in the first phase (bacteria growth phase).[36−41] The cell number was limited by the product (Fe^{3+}) inhibition. The inhibition was assumed to be second order as these assumptions led to the best agreement between the observed and the predicted results:[36]

$$\mu_t = \frac{\mu_{\max}{\cdot}\text{Fe}_t^{2+}}{k_S + Fe_t^{2+}} \tag{4}$$

$$\frac{dN_t}{dt} = \mu_t{\cdot}N_t - \mu_d{\cdot}(\text{Fe}_t^{3+})^2{\cdot}N_t \tag{5}$$

$$\frac{d\text{Fe}_t^{2+}}{dt} = -\frac{\mu_t}{Y_{NS}}{\cdot}N_t \tag{6}$$

where N is the bacteria number (N°/mL), Fe^{2+} and Fe^{3+} are the iron concentrations (g/L), μ_{max} (1/h) is the maximum specific growth rate, k_S (g/L) is the substrate saturation constant, μ_d ($L^2/g^2{\cdot}h$) is the inhibition parameter due to the Fe^{3+} effects, and Y_{NS} ($N°/gFe^{2+}$) is the bacteria yield on substrate (Fe^{2+}).

Whereas, in the second (first leaching step) and the third (second leaching step) phases, the Cu leaching rate was considered and eq 7[42] was used to describe the chemical reaction between Fe^{3+} and Cu:

$$\frac{d\text{Cu}_t^{2+}}{dt} = [k{\cdot}(\text{Cu}_0^0 - \text{Cu}_t^{2+}){\cdot}(\text{Fe}^{3+} - m\text{Cu}_t^{2+})]{\cdot}\text{A. W.}_{\text{Cu}} \tag{7}$$

https://dx.doi.org/10.1021/acs.iecr.0c05566
Ind. Eng. Chem. Res. 2021, 60, 4261−4268

where k is the rate constant given by the Arrhenius equation, Cu_0^0 (mol/L, 0.197) is the initial Cu concentration in WPCBs (calculated as the maximum Cu concentration in solution as a consequence of a complete extraction from WPCBs), m is the molar ratio between the consumed Fe^{3+} and the dissolved Cu^{2+}, and $A.W._{Cu}$ is the Cu atomic weight. In a study by Becci et al.,[42] Fe^{3+} was the initial Fe^{3+} concentration (constant), because that study focused on the chemical reaction between Fe^{3+} and Cu. In our case, Fe^{3+} concentration changes during the time due to Fe^{2+} oxidation by bacteria; as a consequence, Fe^{3+} cannot be constant and it depends on bacteria metabolism as follows (eq 8):

$$Fe^{3+} = Fe_0^{3+} + \frac{1}{A.\,W._{Fe}} \cdot \int_0^t \frac{dFe^{3+}}{dt} dt = Fe_0^{3+} + \frac{1}{A.\,W._{Fe}} \cdot \int_0^t \frac{\mu_t}{Y_{NS}} \cdot N_t dt \quad (8)$$

where Fe_0^{3+} (mol/L) is the Fe^{3+} concentration at the beginning of the step and the second term is Fe^{3+} generated by bacteria metabolism, and $A.W._{Fe}$ is the Fe atomic weight. On the other hand, the differential equations, describing the bacteria abundance (eq 9) and Fe^{2+} concentration (eq 10), have an additional term compared to eqs 5 and 6, due to Cu^{2+} toxicity and Fe^{2+} formation through the chemical reaction (eq 1), respectively:

$$\frac{dN_t}{dt} = \mu_t \cdot N_t - \mu_d \cdot (Fe_t^{3+})^2 \cdot N_t - \mu_{tox} \cdot (Cu_t^{2+})^2 \cdot N_t \quad (9)$$

$$\frac{dFe_t^{2+}}{dt} = -\frac{\mu_t}{Y_{NS}} \cdot N_t + m \cdot \frac{A.\,W._{Fe}}{A.\,W._{Cu}} \cdot \frac{dCu_t^{2+}}{dt} \quad (10)$$

where μ_{tox} ($L^2/g^2 \cdot h$) is a constant parameter that represents the Cu^{2+} toxic effect on bacteria growth.

In this model, only the Cu toxicity on bacteria metabolism was considered, since the other metals, which could be leached (Table 1), showed a content lower than the toxic concentration reported in literature.[38,43]

The seven parameters (μ_{max}, k_S, μ_d, μ_{tox}, Y_{NS}, k, and m) are estimated through fitting eqs 7, 9, and 10 to the experimental temporal profiles of Cu, bacteria abundance, and Fe^{2+}, respectively.

3.2. Sensitivity Analysis. The sensitivity analysis on the estimated parameter from the mathematical model aims to identify the correlation between the parameters to fit the experimental data.[51] The confidence limit at 95% through the parameters is estimated from the determined variance–covariance matrix (minpack.lm). The correlation matrix is calculated from the variance–covariance matrix.[52] One thousand combinations through all the estimated parameters are elaborated by a random generation starting from the normal distribution with the best value and the standard error of each parameters (rnorm). The negative values are excluded from the simulations. The areas of the confidence limit at 95% for all variables are displayed. R software (version 3.6.2) is applied for all the calculations and estimates in the current study.

4. RESULTS AND DISCUSSION

In the bacteria growth phase, the bacteria abundance and the Fe^{2+} concentration showed the common trend for the pure batch culture (Figure 1).[36,43,53,54] In detail, the microbial cells evidenced an exponential growth in the first 25 h of incubation,

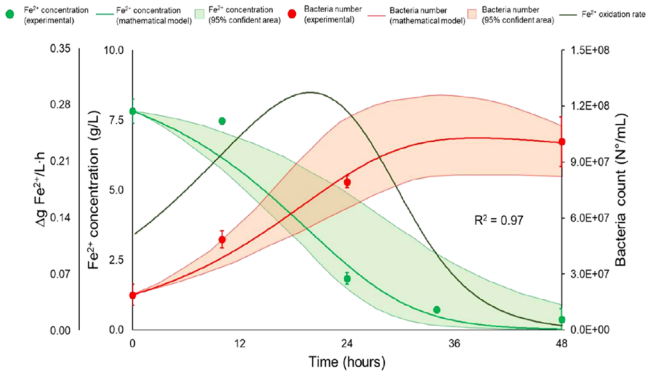


Figure 1. Bacteria count and the Fe^{2+} concentration trend during the bacteria growth phase.

followed by a stationary phase due to the decreased Fe^{2+} concentration. Table 2 reports the eqs 4 to 6 estimated parameters with confidence limits at 95%. The three parameters that describe the bacteria growth rate (μ_{max}), the substrate saturation constant (k_S), and the bacteria yield on the substrate (Y_{NS}) were in the range reported in the literature, between 0.4–1.6 × 10^{-1} 1/h, 0.3–14 g/L, and 0.25–5.9 × 10^{10} N°/g Fe^{2+}, respectively.[40,55−63] The maximum Fe^{2+} oxidation rate (0.30 g Fe^{2+} oxidized/L·h) was achieved after 20 h from the beginning, and it was reached at the end of the exponential growth phase (Figure 1).

A statistical analysis was performed to identify the correlation between the estimated parameters (Table 3).

From the results, remarkable dependence between μ_{max}–k_S and μ_d–Y_{NS} was evident with correlation coefficients of 0.99 and 0.93, respectively. The confidence helixes of the parameters were also showed to have more details on the correlation dependence and on their standard errors (Figure 2). These results confirmed the linear dependence between Y_{NS} and μ_d.

Concerning the fourth parameter, the inhibition parameter (μ_d), the estimated value was 5.40 × 10^{-5} $L^2/g^2 \cdot h$, and it was lower than reported in the literature (6.32 × 10^{-4} $L^2/g^2 \cdot h$),[36] demonstrating that the inhibition on bacteria metabolism due to the product (Fe^{3+}) was negligible.

In the second phase (first leaching step), a known amount of WPCBs (50 g/L) was added and the chemical reaction (eq 1) between Fe^{3+} and Cu^0 started. At the beginning of this phase, the Fe^{2+} and Cu^{2+} concentrations showed a rapid increase due to the chemical reaction. On the other hand, the bacteria cell showed a growth rate lower than the previous phase (Figure 3a). Table 2 reports eqs 7, 9, and 10 parameters. The estimated values for the maximum bacteria growth rate (μ_{max}, 0.20 × 10^{-1} 1/h) and the bacteria yield on the substrate (Y_{NS}, 0.53× 10^{10} N°/g Fe^{2+}) were six and two times lower than the first phase, respectively. Such decrease was mainly due to the microorganism adaptation to the new environment. The Cu^{2+} toxic effect (μ_{tox}, 7.69 × 10^{-4} $L^2/g^2 \cdot h$) on bacteria growth and metabolism was one order of magnitude higher than the Fe^{3+} product inhibition (μ_d) estimated in the bacteria growth phase. Moreover, such Fe^{3+} product inhibition resulted to be irrelevant in this first leaching step, as demonstrated by the value of 0 of the estimated parameter μ_d (Table 2).

https://dx.doi.org/10.1021/acs.iecr.0c05566
Ind. Eng. Chem. Res. 2021, 60, 4261−4268

Table 2. Estimated Parameters for the Three Phases with Confidence Limits of 95%

	bacteria growth phase		first leaching step		second leaching step	
param.	mean	St.error	mean	St.error	mean	St.error
μ_{max} (1/h)	1.26×10^{-1}	0.10×10^{-1}	0.20×10^{-1}	0.15×10^{-1}	0.42×10^{-1}	0.02×10^{-1}
k_S (g/L)	5.20	0.87	5.20	0.87	5.20	0.87
μ_d ($L^2/g^2 \cdot h$)	5.40×10^{-5}	1.01×10^{-5}	-	-	-	-
μ_{tox} ($L^2/g^2 \cdot h$)	-	-	7.69×10^{-4}	1.12×10^{-4}	1.47×10^{-4}	0.48×10^{-4}
Y_{NS} ($N°/gFe^{2+}$)	1.20×10^{10}	0.26×10^{10}	0.53×10^{10}	0.43×10^{10}	0.95×10^{10}	0.10×10^{10}
k	-	-	0.11	0.04	0.38	0.22
m	-	-	4.39	0.92	2.00	-

Table 3. Correlation Matrix for the Four Estimated Parameters in the Bacteria Growth Phase

	μ_{max}	k_S	μ_d	Y_{NS}
μ_{max}	1	0.99	0.12	0.17
k_S	0.99	1	0.10	0.15
μ_d	0.12	0.10	1	0.93
Y_{NS}	0.17	0.15	0.93	1

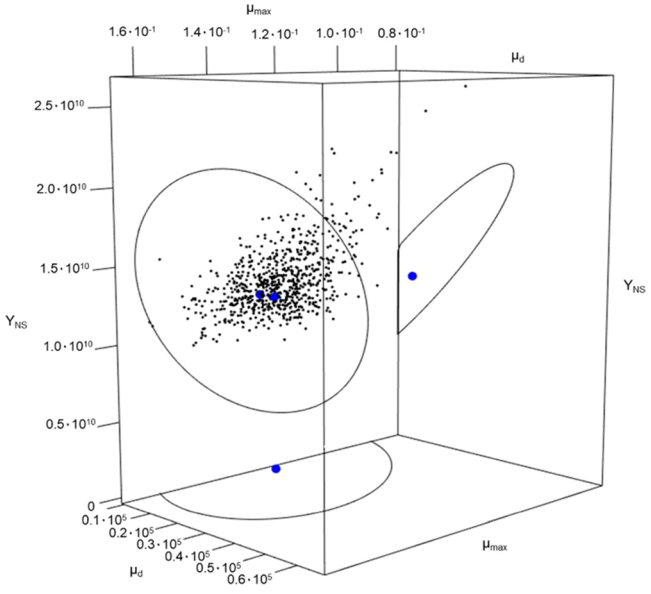


Figure 2. Confidence helixes at 95% and the random generated values to describe the bacteria growth phase.

From the experimental and mathematical modeling data, an estimation of iron generation/consumption rates through the bacteria metabolism and the chemical reaction was carried out (Figure 3b) to understand in detail the controlling steps in Cu bioleaching. In the first 14 h, the Fe^{2+} generation by the chemical reaction was faster than the Fe^{2+} consumption by bacteria metabolism. This observation can be explained by the oxidation state of Fe in the leaching solution (Fe^{3+}), which was not suitable as an energy source for bacteria metabolism. When the Fe^{3+} concentration needed for the chemical reaction decreased, a change in the controlling step was observed. From this point, the chemical reaction was controlled from the kinetic rate of the Fe^{3+} generated by bacteria metabolism. Indeed, an average Fe^{2+} oxidation (between 0.24 and 0.20 g Fe^{2+} oxidized/L·h) (bio) resulted to be 1.8 times faster than the Fe^{2+} generation (between 0.20 and 0.10 g Fe^{2+} generated/L·h) (chem). The maximum oxidation rate was achieved around 20 h after the beginning of the first leaching step, in accordance with that observed in the bacteria growth phase.

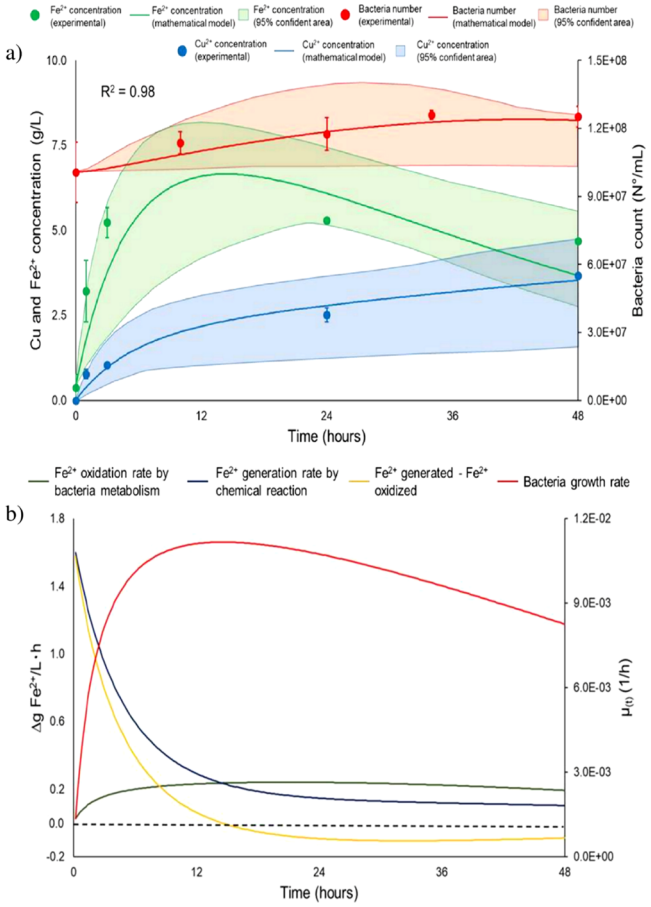


Figure 3. (a) Bacteria count and Fe^{2+} and Cu^{2+} concentration trends in the first leaching step; (b) the Fe^{2+} oxidation and generation rate by bacteria metabolism and the chemical reaction, and the time change of μ.

Also, in this case, a statistical analysis was performed to understand the dependence between the estimated parameters by the correlation matrix (Table 4).

Table 4. Correlation Matrix for the Five Estimated Parameters in the First Leaching Step

	μ_{max}	μ_{tox}	Y_{NS}	k	m
μ_{max}	1	0.95	0.98	−0.04	−0.02
μ_{tox}	0.95	1	0.92	−0.17	0.22
Y_{NS}	0.98	0.92	1	−0.06	−0.09
k	−0.04	−0.17	−0.06	1	−0.37
m	−0.02	0.22	−0.09	−0.37	1

https://dx.doi.org/10.1021/acs.iecr.0c05566
Ind. Eng. Chem. Res. 2021, 60, 4261−4268

A remarkable correlation among μ_{max}, μ_{tox}, and Y_{NS} is evident, while k and m are not significantly correlated with the other parameters. The estimated parameters k and m, which describe the Cu leaching trend through bioleaching, were quite different from the parameter reported by Becci et al.,[42] 3.08 and 3.22, respectively, which concern Cu chemical leaching. With regards the stoichiometric ratio (m) between Fe^{3+} and Cu^{2+}, the value of 3.22 was in the 95% confidence limit (Figure 4). The estimated rate constant (k) reported a value of one

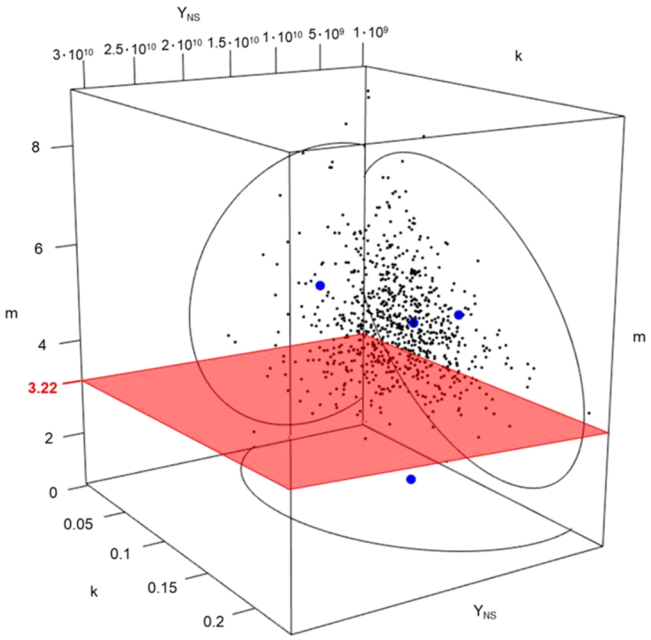


Figure 4. Confidence helixes at 95% and the estimated value to describe the first leaching step. The red plane represents the value of m reported by Becci et al. (2019).[42]

order of magnitude lower than reported by Becci et al.;[42] this record was mainly due to the lower agitation rate in the bioleaching experiment than the chemical leaching test, and this determined a low frequency of collisions in the correct orientation between Fe^{3+} and Cu^0.

In the third and last phases (second leaching step), 80% of the leaching solution resulting from the previous step was removed and replaced with a new fresh medium. For this reason, the microorganism abundance and Cu^{2+} concentration decreased and Fe^{2+} concentration increased than the first leaching step at the end. The bacteria count and Fe^{2+} showed the same observed trend in the bacteria growth phase, with a slower kinetic (Figure 5a). Indeed, the microorganism exponential growth rate lasted 84 h, and the Fe^{2+} reached the concentration close to zero after 100 h from the beginning. The slowest kinetic trend for bacteria abundance and Fe^{2+} oxidation was confirmed by the mathematical model. The estimated parameters μ_{max} and Y_{NS} reported values lower than the bacteria growth phase (Table 2). The achieved value of 0.42×10^{-1} 1/h for the maximum specific growth rate was comparable with that reported (0.61×10^{-1} 1/h) by Cabrera et al.[38] that studied the influence of Cu^{2+} at the 10 g/L concentration on bacteria metabolism. This result confirmed the reliability of this model and demonstrated that Cu is the main factor to affect the bacteria growth rate.

Furthermore, in this step, the bacteria abundance temporal profile showed the death phase after 120 h from the beginning.

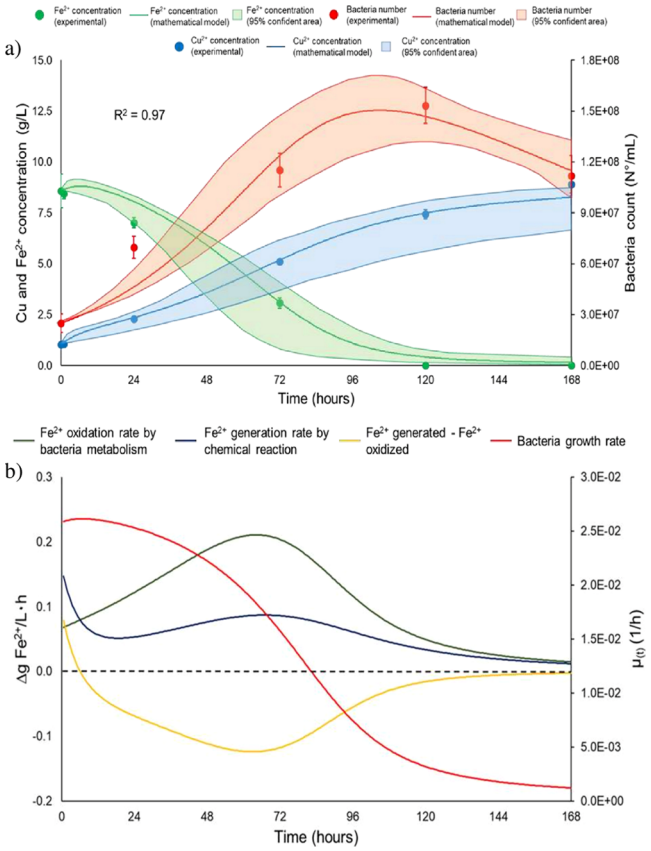


Figure 5. (a) Bacteria count and Fe^{2+} and Cu^{2+} concentration trends in the second leaching step; (b) the Fe^{2+} oxidation and generation rate by bacteria metabolism and the chemical reaction, and the time change of μ.

The decrease of the cell number was mainly due to the lower Fe^{2+} concentration as an energy source for microorganism.[54] The estimated parameter for the Cu toxic effect (μ_{tox}) reported a record of five times lower than the previous phase. These results demonstrated that the leaching in more steps allowed the highest bacteria adaptation to the new substrates and an increasing leaching efficiency.

Concerning the assessment of iron production/consumption rates, the Fe^{2+} oxidation by bacteria showed in the first 96 h a kinetic rate 2.5 times faster than the chemical reaction (Figure 5b). The two kinetics (bacteria metabolism and the chemical reaction) were comparable after 120 h from the beginning when the bacteria cells started to decrease, and Cu leaching was mostly completed. This result was another attestation of the highest microorganism adaptation.

The correlation matrix (Table 5) shows a lower correlation between the parameters except for the μ_{tox}–Y_{NS} correlation (0.89). Another important result from the correlation matrix was the univocal results for the stoichiometric ratio (m = 2)

Table 5. Correlation Matrix for the Four Estimated Parameters in the Second Leaching Step

	μ_{max}	μ_{tox}	Y_{NS}	k
μ_{max}	1	0.11	0.15	−0.33
μ_{tox}	0.11	1	0.89	−0.43
Y_{NS}	0.15	0.89	1	−0.25
k	−0.33	−0.43	−0.25	1

https://dx.doi.org/10.1021/acs.iecr.0c05566
Ind. Eng. Chem. Res. 2021, 60, 4261−4268

between Fe^{3+} and Cu^{2+}. This result demonstrated that the concentration of the other metals in WPCBs decreased due to the first leaching step, so the stoichiometric ratio between Cu^{2+} and Fe^{3+} was equal to that reported in eq 1.[42] On the other hand, the rate constant k reported a higher value than the first step leaching, but all the possible value achieved in the previous step fell into the 95% confidence limit of the determined k in the second leaching step (Figure 6).

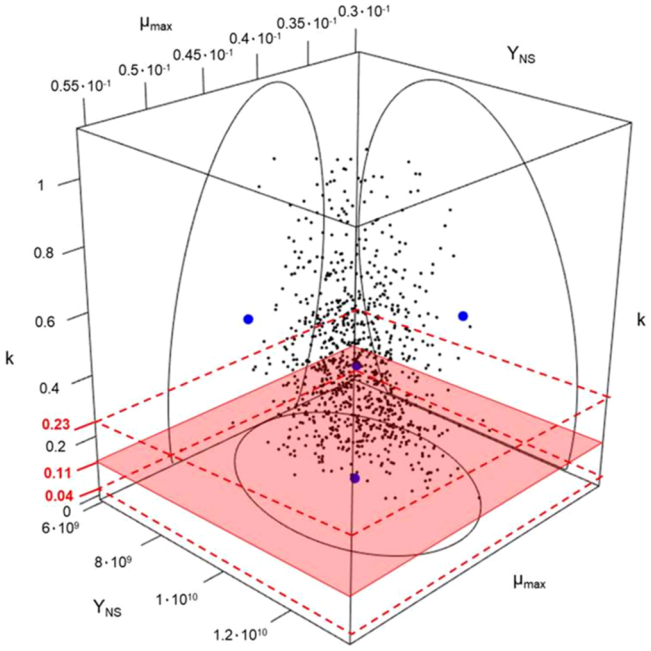


Figure 6. Confidence helixes at 95% and the estimated value to describe the second leaching step. The red plane represents the best, the maximum, and the minimum value of k estimated for the first leaching step.

5. CONCLUSIONS

In this study, the kinetics and the mechanism of the Cu bioleaching from WPCBs by *At. ferrooxidans* was studied in detail. The following conclusions can be deduced from the present work:

1. The developed mathematical model was in a very good agreement with the experimental data, as confirmed by a R^2 value higher than 0.97 in all the three considered phases.
2. In the first phase of the first leaching, the fastest step was the chemical reaction between the Fe^{3+} produced in the bacteria growth phase and Cu within WPCBs. After 14 h from the beginning, bacteria metabolism resulted to be faster than Cu chemical extraction. The achieved results showed that the microorganism activity was 1.8−2.5 times faster than the chemical reaction.
3. Leaching carried out in a multistage operation allowed to increase the microorganism adaptation to the substrates; the parameters showed as the maximum specific growth rate (μ_{max}) and the bacteria yield on substrates (Y_{NS}) increased their values in the second leaching step than the first leaching step, and the Cu toxic effect (μ_{tox}) decreased in the second one.
4. This work demonstrated that the WPCB (50 g/L) and the maximum Cu^{2+} (8.91 g/L) concentrations did not have very significant toxic effects on bacteria metabolism. The Fe^{2+} oxidation rate by microorganism decreased from a value of 0.30 g/L·h, in the bacteria growth phase, to 0.21 g/L·h in the last one.

The mathematical model for Cu recoverable from WPCBs was essential information for the development of innovative bioleaching processes. Understanding in detail as the microbial metabolism influenced the chemical leaching could be useful for the implementation of the bioleaching approaches in the industrial scale.

AUTHOR INFORMATION

Corresponding Author

Alessandro Becci − *Department of Life and Environmental Sciences, Università Politecnica delle Marche, Ancona 60131, Italy;* orcid.org/0000-0001-5886-1557; Email: a.becci@pm.univpm.it

Authors

Alessia Amato − *Department of Life and Environmental Sciences, Università Politecnica delle Marche, Ancona 60131, Italy*

José M. Rodríguez-Maroto − *Departamento de Ingeniería Química, Facultad de Ciencias, Universidad de Málaga, Málaga 29071, Spain*

Francesca Beolchini − *Department of Life and Environmental Sciences, Università Politecnica delle Marche, Ancona 60131, Italy;* orcid.org/0000-0002-6211-6354

Complete contact information is available at: https://pubs.acs.org/10.1021/acs.iecr.0c05566

Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

Part of the work has been realized thanks to staff exchange within the H2020-MSCA-RISE-2017 project e.THROUGH.

REFERENCES

(1) Sodha, A. B.; Tipre, D. R.; Dave, S. R. Optimisation of biohydrometallurgical batch reactor process for copper extraction and recovery from non-pulverized waste printed circuit boards. *Hydrometallurgy* **2020**, *191*, 105170.

(2) Li, Z.; Diaz, L. A.; Yang, Z.; Jin, H.; Lister, T. E.; Vahidi, E.; Zhao, F. Comparative life cycle analysis for value recovery of precious metals and rare earth elements from electronic waste. *Resour., Conserv. Recycl.* **2019**, *149*, 20−30.

(3) Moosakazemi, F.; Ghassa, S.; Mohammadi, M. R. T. Environmentally friendly hydrometallurgical recovery of tin and lead from waste printed circuit boards: Thermodynamic and kinetics studies. *J. Cleaner Prod.* **2019**, *228*, 185−196.

(4) Zhang, S.; Li, Y.; Wang, R.; Xu, Z.; Wang, B.; Chen, S.; Chen, M. Superfine copper powders recycled from concentrated metal scraps of waste printed circuit boards by slurry electrolysis. *J. Cleaner Prod.* **2017**, *152*, 1−6.

(5) Schneider, E. L.; Hamerski, F.; Veit, H. M.; Krummenauer, A.; Cenci, M. P.; de Andrade Chaves, R.; Hartmann, W. L.; de Mattos Dias, M.; Robinson, M. D. M.; de Vargas, A. S. Evaluation of mass loss in different stages of printed circuit boards recycling employed in temperature controllers. *Mater. Res.* **2019**, *22*, 1−7.

(6) Baldé, C. P.; Forti, V.; Gray, V.; Kuehr, R.; Stegmann, P. *The global E-waste monitor 2017: Quantities, flows and resources*; United Nations University, International Telecommunication Union, and International Solid Waste Association: 2017.
(7) Li, X.; Ren, Q.; You, X.; Yang, Y.; Shan, M.; Wang, M. Material flow analysis of discarded refrigerators from households in urban and rural areas of China. *Resour., Conserv. Recycl.* **2019**, *149*, 577−585.
(8) Evangelopoulos, P.; Kantarelis, E.; Yang, W. Waste electric and electronic equipment: Current legislations, waste management, and recycling of energy, materials, and feedstocks. In *Sustainable resource recovery and zero waste approaches*; Elsevier: 2019; 239−266, DOI: 10.1016/b978-0-444-64200-4.00017-7.
(9) Kaya, M. *Electronic waste and printed circuit board recycling technologies*; Springer: 2019, DOI: 10.1007/978-3-030-26593-9.
(10) Vermeşan, H.; Tiuc, A.-E.; Purcar, M. Advanced recovery techniques for waste materials from IT and telecommunication equipment printed circuit boards. *Sustainability* **2020**, *12*, 74.
(11) Moyo, T.; Chirume, B. H.; Petersen, J. Assessing alternative pre-treatment methods to promote metal recovery in the leaching of printed circuit boards. *Resour., Conserv. Recycl.* **2020**, *152*, 104545.
(12) Awasthi, A. K.; Zeng, X. Recycling printed circuit boards. In *Waste Electrical and Electronic Equipment (WEEE) Handbook*; Woodhead Publishing: 2019; 311−325, DOI: 10.1016/b978-0-08-102158-3.00011-2.
(13) Lin, C. S. K. *Chemistry and chemical technologies in waste valorization*; Springer: 2018; 189−228, DOI: 10.1007/978-3-319-90653-9.
(14) Pietrelli, L.; Ferro, S.; Vocciante, M. Eco-friendly and cost-effective strategies for metals recovery from printed circuit boards. *Renewable and Sustainable Energy Rev.* **2019**, *112*, 317−323.
(15) Qiu, R.; Lin, M.; Ruan, J.; Fu, Y.; Hu, J.; Deng, M.; Tang, Y.; Qiu, R. Recovering full metallic resources from waste printed circuit boards: A refined review. *J. Cleaner Prod.* **2020**, *244*, 118690.
(16) Hsu, E.; Barmak, K.; West, A. C.; Park, A.-H. A. Advancements in the treatment and processing of electronic waste with sustainability: A review of metal extraction and recovery technologies. *Green Chem.* **2019**, *21*, 919−936.
(17) Xiu, F.-R.; Zhang, F.-S. Recovery of copper and lead from waste printed circuit boards by supercritical water oxidation combined with electrokinetic process. *J. Hazard. Mater.* **2009**, *165*, 1002−1007.
(18) Manjengwa, E.R. Evaluating the economics of and business models for metal recycling from waste printed circuit boards in a South African context. Diss. Stellenbosch, Stellenbosch University, 2019.
(19) Bonnin, M.; Azzaro-pantel, C.; Domenech, S.; Villeneuve, J. Multicriteria optimization of copper scrap management strategy. *Resour., Conserv. Recycl.* **2015**, *99*, 48−62.
(20) Zeng, X.; Duan, H.; Wang, F.; Li, J. Examining environmental management of e-waste: China's experience and lessons. *Renewable Sustainable Energy Rev.* **2017**, *72*, 1076−1082.
(21) *Bureau of International Recycling*. Retrieved from: BIR - Non-Ferrous Metals: https://www.bir.org/the-industry/non-ferrous-metals (accessed November 10, 2020).
(22) Akcil, A.; Erust, C.; Ozdemiroglu, S.; Fonti, V.; Beolchini, F. A review of approaches and techniques used in aquatic contaminated sediments: metal removal and stabilization by chemical and biotechnological processes. *J. Cleaner Prod.* **2015**, *86*, 24−36.
(23) D'Adamo, I.; Ferella, F.; Gastaldi, M.; Maggiore, F.; Rosa, P.; Terzi, S. Towards sustainable recycling processes: Wasted printed circuit boards as a source of economic opportunities. *Resour., Conserv. Recycl.* **2019**, *149*, 455−467.
(24) Li, H.; Oraby, E.; Eksteen, J. Extraction of copper and the co-leaching behaviour of other metals from waste printed circuit boards using alkaline glycine solutions. *Resour., Conserv. Recycl.* **2020**, *154*, 104624.
(25) Fonti, V.; Amato, A.; Beolchini, F. *Urban Biomining: New challenges for a successful exploitation of WEEE by means of a biotechnological approach*; AND THE ENVIRONMENT, 2015, 329−358.
(26) Ilyas, S.; Lee, J.-c.; Chi, R.-a. Bioleaching of metals from electronic scrap and its potential for commercial exploitation. *Hydrometallurgy* **2013**, *131-132*, 138−143.
(27) Joshi, V.; Shah, N.; Wakte, P.; Dhakephalkar, P.; Dhakephalkar, A.; Khobragade, R.; Naphade, B.; Shaikh, S.; Deshmukh, A.; Adhapure, N. Comparative bioleaching of metals from pulverized and non-pulverized PCBs of cell phone charger: advantages of non-pulverized PCBs. *Environ. Sci. Pollut. Res.* **2017**, *24*, 28277−28286.
(28) Liang, G.; Tang, J.; Liu, W.; Zhou, Q. Optimizing mixed culture of two acidophiles to improve copper recovery from printed circuit boards (PCBs). *J. Hazard. Mater.* **2013**, *250-251*, 238−245.
(29) Hubau, A.; Minier, M.; Chagnes, A.; Joulian, C.; Perez, C.; Guezennec, A.-G. Continuous production of a biogenic ferric iron lixiviant for the bioleaching of printed circuit boards (PCBs). *Hydrometallurgy* **2018**, *180*, 180−191.
(30) Zhu, N.; Xiang, Y.; Zhang, T.; Wu, P.; Dang, Z.; Li, P.; Wu, J. Bioleaching of metal concentrates of waste printed circuit boards by mixed culture of acidophilic bacteria. *J. Hazard. Mater.* **2011**, *192*, 614−619.
(31) Choi, M.-S.; Cho, K.-S.; Kim, D.-S.; Kim, D.-J. Microbial recovery of copper from printed circuit boards of waste computer by *Acidithiobacillus ferrooxidans*. *J. Environ. Sci. Health, Part A: Toxic/Hazard. Subst. Environ. Eng.* **2004**, *39*, 2973−2982.
(32) Annamalai, M.; Gurumurthy, K. Enhanced bioleaching of copper from circuit boards of computer waste by *Acidithiobacillus ferrooxidans*. *Environ. Chem. Lett.* **2019**, *17*, 1873−1879.
(33) Yang, Y.; Chen, S.; Li, S.; Chen, M.; Chen, H.; Liu, B. Bioleaching waste printed circuit boards by *Acidithiobacillus ferrooxidans* and its kinetics aspect. *J. Biotechnol.* **2014**, *173*, 24−30.
(34) Wu, W.; Liu, X.; Zhang, X.; Zhu, M.; Tan, W. Bioleaching of copper from waste printed circuit boards by bacteria - free cultural supernatant of iron − sulfur - oxidizing bacteria. *Bioresour. Bioprocess.* **2018**, *5*, 1−13.
(35) Becci, A.; Amato, A.; Fonti, V.; Karaj, D.; Beolchini, F. An innovative biotechnology for metal recovery from printed circuit boards. *Resour., Conserv. Recycl.* **2020**, *153*, 104549.
(36) Kumar, S. R.; Gandhi, K. S. Modelling of Fe^{2+} oxidation by *Thiobacillus ferrooxidans*. *Appl. Microbiol. Biotechnol.* **1990**, *33*, 524−528.
(37) van Scherpenzeel, D. A.; Boon, M.; Ras, C.; Hansford, G. S.; Heijnen, J. J. Kinetics of ferrous iron oxidation by *Leptospirillum* bacteria in continuous cultures. *Biotechnol. Prog.* **1998**, *14*, 425−433.
(38) Cabrera, G.; Gómez, J. M.; Cantero, D. Kinetic study of ferrous sulphate oxidation of *Acidithiobacillus ferrooxidans* in the presence of heavy metal ions. *Enzyme Microb. Technol.* **2005**, *36*, 301−306.
(39) Nyavor, K.; Egiebor, N. O.; Fedorak, P. M. The effect of ferric ion on the rate of ferrous oxidation by *Thiobacillus ferrooxidans*. *Appl. Microbiol. Biotechnol.* **1996**, *45*, 688−691.
(40) Boon, M.; Ras, C.; Heijnen, J. J. The ferrous iron oxidation kinetics of *Thiobacillus ferrooxidans* in batch cultures. *Appl. Microbiol. Biotechnol.* **1999**, *51*, 813−819.
(41) Shahcheraghi, S. H.; Shahcheraghi, S. H.; Tavakoli, M. R.; Javanshir, S.; Hosseini, S. M. Kinetic study of the bioleaching process of Miduk copper sulfide minerals by modeling. *Int. Miner. Process.* **2012**.
(42) Becci, A.; Amato, A.; Rodríguez Maroto, J. M.; Beolchini, F. Prediction model for Cu chemical leaching from printed circuit boards. *Ind. Eng. Chem. Res.* **2019**, *58*, 20585−20591.
(43) Cabrera, G.; Gómez, J. M.; Cantero, D. Influence of heavy metals on growth and ferrous sulphate oxidation by *Acidithiobacillus ferrooxidans* in pure and mixed cultures. *Process Biochem.* **2005**, *40*, 2683−2687.
(44) Boon, M.; Meeder, T. A.; Thöne, C.; Ras, C.; Heijnen, J. J. The ferrous iron oxidation kinetics of *Thiobacillus ferrooxidans* in continuous cultures. *Appl. Microbiol. Biotechnol.* **1999**, *51*, 820−826.
(45) Crundwell, F. K. The kinetics of the chemiosmotic proton circuit of the iron-oxidizing bacterium *Thiobacillus ferrooxidans*. *Bioelectrochem. Bioenerg.* **1997**, *43*, 115−122.

https://dx.doi.org/10.1021/acs.iecr.0c05566
Ind. Eng. Chem. Res. 2021, 60, 4261−4268

(46) Daoud, J.; Karamanev, D. Formation of jarosite during Fe^{2+} oxidation by *Acidithiobacillus ferrooxidans*. *Miner. Eng.* **2006**, *19*, 960−967.
(47) Danovaro, R.; Manini, E.; Dell'Anno, A. Higher abundance of bacteria than of viruses in deep mediterranean sediments. *Appl. Environ. Microbiol.* **2002**, *68*, 1468−1472.
(48) Welcher, F. J.; Hahn, R. B. Semi-micro qualitative analysis. *Encycl. Chem. Technol.* **1964**.
(49) Marquardt, D. W. An algorithm for least-squares estimation of nonlinear parameters. *J. Soc. Ind. Appl. Math.* **1963**, *11*, 431−441.
(50) Moré, J. J. The Levenberg-Marquardt algorithm: Implementation and theory. In *Numerical Analysis*; Springer: Heidelberg, Berilin, 1978: pp. 105−116, http://ci.nii.ac.jp/naid/110001769136.
(51) Homma, T.; Saltelli, A. Importance measures in global sensitivity analysis of nonlinear models. *Reliab. Eng. Syst. Saf.* **1996**, *52*, 1−17.
(52) Beolchini, F.; Veglió, F. Kinetic modeling of pyrrothite ore leaching by ferric iron and related statistical analysis. *Ind. Eng. Chem. Res.* **1999**, *38*, 3296−3299.
(53) Chen, B.-Y.; Liu, H.-L.; Chen, Y.-W.; Cheng, Y.-C. Dose−response assessment of metal toxicity upon indigenous *Thiobacillus thiooxidans* BC1. *Process Biochem.* **2004**, *39*, 737−748.
(54) Moreira, I. V.; Yamane, L. H.; Siman, R. R. *Influence of printed circuit boards' components on Acidithiobacillus ferrooxidans-LR growth*; 17th Int. Waste Manag. Landfill Symp., 2019.
(55) Pagella, C.; Perego, P.; Zilli, M. Biotechnological H_2S Gas Treatment with *Thiobacillus ferrooxidans*. *Chem. Eng. Technol.* **1996**, *19*, 79−88.
(56) Halfmeier, H.; Schäfer-Treffenfeldt, M.; Reuss, W. Potential of Thiobacillus ferrooxidans for waste gas purification. Part 2. Increase in continuous ferrous iron oxidation kinetics using immobilized cells. *Appl. Microbiol. Biotechnol.* **1993**, *40*, 582−587.
(57) Halfmeier, H.; Schäfer-Treffenfeldt, W.; Reuss, M. Potential of Thiobacillus ferrooxidans for waste gas purification. Part 1. Kinetics of continuous ferrous iron oxidation. *Appl. Microbiol. Biotechnol.* **1993**, *40*, 416−420.
(58) Chavarie, C.; Karamanev, D.; Godard, F.; Garnier, A.; Chimique, D. D. G.; Polytechnique, E. Comparison of the kinetics of ferrous iron oxidation by three different strains of *Thiobacillus ferrooxidans*. *Geomicrobiol. J.* **2009**, *11*, 37−41.
(59) Nagpal, S. A Structured model for *Thiobacillus ferrooxidans* growth on ferrous iron. *Biotechnol. Bioeng.* **1997**, *53*, 310−319.
(60) Rashidi, A.; Safdari, J.; Roosta-Azad, R.; Zokaei-Kadijani, S. Modeling of uranium bioleaching by *Acidithiobacillus ferrooxidans*. *Ann. Nucl. Energy* **2012**, *43*, 13−18.
(61) Nemati, M.; Harrison, S. T. L.; Hansford, G. S.; Webb, C. Biological oxidation of ferrous sulphate by *Thiobacillus ferrooxidans*: A review on the kinetic aspects. *Biochem. Eng. J.* **1998**, *1*, 171−190.
(62) Molchanov, S.; Gendel, Y.; Ioslvich, I.; Lahav, O. Improved experimental and computational methodology for determining the kinetic equation and the extant kinetic constants of Fe(II) oxidation by *Acidithiobacillus ferrooxidans*. *Appl. Environ. Microbiol.* **2007**, *73*, 1742−1752.
(63) Zilouei, H.; Shojaosadati, S. A.; Khalilzadeh, R.; Nasernejad, B. Bioleaching of copper from low- grade ore using isolated bacteria and defined mixed cultures. *Iran. J. Biotechnol.* **2003**, *1*, 162−168.

https://dx.doi.org/10.1021/acs.iecr.0c05566
Ind. Eng. Chem. Res. 2021, 60, 4261−4268