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Modeling Co toxicity effects on *Acidithiobacillus ferrooxidans* for environmentally sustainable recycling of Lithium-Ion batteries

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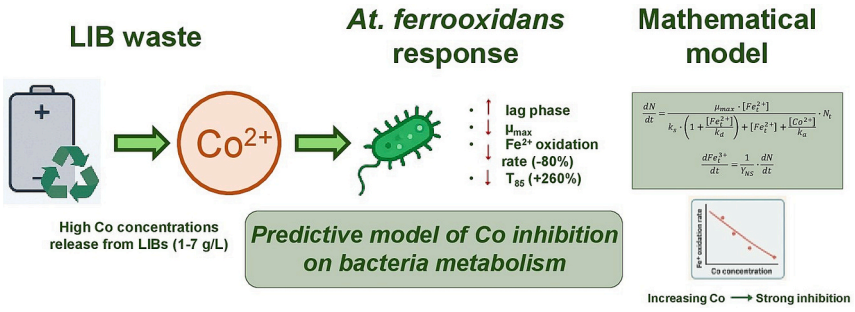
HIGHLIGHTS

- Co showed higher toxicity than Cu, Cd, Ni, Zn, As, except Cr.
- A mathematical model was built to explore Co toxicity in detail.
- Co above 5 g/L cuts Fe oxidation rate by more than 80%

GRAPHICAL ABSTRACT



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ABSTRACT

The rapid expansion of lithium-ion battery (LIB) production, driven by the rise of electric vehicles and renewable energy storage, has led to growing concerns about end-of-life management and critical material recovery. In this context, biotechnological processes represent an environmentally sustainable alternative to conventional recycling methods such as pyrometallurgy and hydrometallurgy, offering reduced impacts on both ecosystems and human health. However, the performance of bioleaching systems depends heavily on microbial tolerance to toxic metals released from LIBs.

This study focuses on assessing the toxicological effects of Co, a key strategic metal in LIBs, on *Acidithiobacillus ferrooxidans*, a model organism for bioleaching applications. Experimental findings reveal that Co exhibits greater toxicity than Cu, Cd, Ni, Zn, and As, but is less toxic than Cr. Co concentrations exceeding 5 g/L result in a 260% increase in Fe^{2+} oxidation time and an 80% reduction in the Fe oxidation rate. Additionally, elevated Co levels significantly prolong the exponential growth phase, indicating metabolic stress.

A predictive mathematical model was developed and validated to describe bacterial growth and Fe^{2+} oxidation under varying Co concentrations, achieving a determination coefficient (R^2) above 0.95. This model serves as a practical tool for optimizing process parameters in the bio-recycling of LIBs, enabling more efficient and scalable engineering applications.

These findings contribute to the advancement of greener technologies for critical raw material recovery and support the integration of bio-based methods into circular economy strategies for battery waste management.

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1. Introduction

The growing awareness of environmental and climate-related challenges has led to a global commitment toward a more sustainable future, emphasizing the urgent transition from a fossil fuel-based economy to renewable energy systems (European Commission, 2021). Given the intermittency of renewable sources, energy storage technologies have become essential components of this transition, playing a crucial role in both the energy and transport sectors, together responsible for approximately 70% of global greenhouse gas emissions (Agency, 2022).

Among various energy storage devices, LIBs have gained prominence due to their high energy density (120 W*hrs/kg), long lifespan (500–10000 cycles), wide operating temperature range (−20 to 60 °C), and absence of toxic heavy metals (such as Cd or Pb) (Al-Thyabat et al., 2013). These characteristics make LIBs particularly suitable for applications in portable electronics and electric vehicles (Fergus, 2010). Over the past two decades, LIB production has grown exponentially, alongside increasing research and development efforts (Zeng et al., 2014). However, this surge in production is also leading to a significant increase in spent LIBs, with projections estimating the generation of over 460,000 tons of waste batteries in 2025, and nearly 2 million tons by 2040 (Chen et al., 2019; Zhao et al., 2019).

LIBs contain critical raw materials such as Li and Co, whose limited availability and geographically concentrated reserves raise concerns about supply security and long-term sustainability (Chen et al., 2019; Pazik et al., 2016; Salazar and McNutt, 2021). In recognition of this, both Li and Co have been included in the European Union's list of Critical Raw Materials (European Commission, 2023). The recycling of LIBs has therefore emerged as a key strategy to reduce environmental impact and mitigate resource scarcity by recovering valuable secondary raw materials (Ferronato and Torretta, 2019; Mossali et al., 2020; Nnorom and Osibanjo, 2009).

Current recycling technologies include pyrometallurgical and hydrometallurgical processes, both of which are widely studied and applied at industrial scale (Lv et al., 2018). However, these methods often involve high energy consumption or the generation of hazardous waste (Cerrillo-Gonzalez et al., 2020; Lv et al., 2018; Mossali et al., 2020). In response, biohydrometallurgy, an emerging field that utilizes microorganisms to produce leaching agents, offers a potentially more sustainable alternative (Roy et al., 2021c, 2021b, 2021a). This biological approach can significantly reduce the environmental footprint associated with conventional acid-based leaching processes while achieving high recovery rates of metals such as Li and Co. As such, biohydrometallurgy represents a promising avenue in the development of a circular economy for LIBs, aligning technological innovation with environmental sustainability. One of the most studied microorganisms for LIBs leaching is *At. ferrooxidans* (Mishra et al., 2008; Naseri et al., 2019a; Roy et al., 2021c, 2021b, 2021a; Sethurajan and Gaydardzhiev, 2021; Zhang et al., 2018). The main limitation of biotechnological processes is the low solid concentration (between 1 and 10%), which results from the toxicity of metals on bacterial metabolism (Becci et al., 2021; Liu et al., 2020; Wu et al., 2020; Zhang et al., 2023). While acidophilic bacteria exhibit high tolerance toward certain metals (such as Ni, Cu, and Zn (Roy et al., 2021a)), the toxicity of Co, one of the metals with the highest concentrations in LIBs (~ 19% w/w), has been poorly investigated (Wu et al., 2020). According to the literature, the average metal concentrations in LIBs are approximately 5% for Li, 14% for Mn, 8% for Ni, 6% for Al, 1% for Fe, and 5% for Cu (Bahaloo-Horeh et al., 2018; Boxall et al., 2018; Heydarian et al., 2018; Horeh et al., 2016; Kim et al., 2024; Panda et al., 2024; Roy et al., 2021c; Wang et al., 2022; Xin et al., 2016).

Co, which is present in LIBs in the form of an oxide, is extracted in biotechnological processes with acidophilic bacteria (e.g. *At. ferrooxidans*) through the following redox reactions (Eqs. 1–2) (Heydarian et al., 2018; Liu et al., 2020; Roy et al., 2021b; Zhang et al., 2023):

$$2FeSO_4 + 2LiCoO_2 + 4H_2SO_4 \rightarrow Fe_2(SO_4)_3 + 2CoSO_4 + Li_2SO_4 + 4H_2O \quad (1)$$

$$4LiCOO_2 + 6H_2SO_4 \rightarrow 2Li_2SO_4 + 4CoSO_4 + 6H_2O + O_2 \quad (2)$$

Co is an essential micronutrient for acidophilic bacteria at μM concentrations, as it plays a key role in the biosynthesis of vitamin B_{12} (Nies and Grass, 2009). However, at elevated concentrations, the redox-active nature of Co promotes the generation of reactive oxygen species (ROS), leading to oxidative stress and cellular damage (Fantino et al., 2010; Wu et al., 2020). The presence of Co has been shown to impair the cellular radical scavenging system, resulting in the accumulation of ROS, primarily superoxide anions (O_2^-) (Liu et al., 2014; Wu et al., 2020). Superoxide dismutase (SOD), a key antioxidant enzyme responsible for the dismutation of O_2^-, is inhibited by Co exposure, further exacerbating oxidative stress within the cells (Kurhaluk and Tkachenko, 2016; Wu et al., 2020).

Given the high Co concentrations typically found in LIBs and its documented toxicity toward acidophilic bacterial communities, this study aims to elucidate the effects of Co on both *At. ferrooxidans* growth dynamics and metabolic activity.

2. Materials and methods

2.1. Toxicity test

At. ferrooxidans (DSMZ 14882 t) was provided as pure culture by Deutche Sammlung von Mikroorganismenn und Zellkulturen GmbH (DSMZ) of the Leibniz Institute. Bacteria were cultured in DSMZ 882 medium, containing 0.132 g/L $(NH_4)_2SO_4$, 0.053 g/L $MgCl_2{\cdot}6H_2O$, 0.027 g/L KH_2PO_4 and 0.147 g/L $CaCl_2{\cdot}2H_2O$, as well as electron donor $FeSO_4{\cdot}7H_2O$ at a concentration of 20 g/L and H_2SO_4 for a pH value of 1.5. The toxicity of Co towards *At. ferrooxidans* was tested by inoculating the bacteria (10% v/v) in a modified growth medium containing different concentrations of soluble Co^{2+} (1, 3, 5, 7 g/L). Co was dissolved in the medium as $CoSO_4{\cdot}7H_2O$. The maximum concentration of 7 g/L was chosen by simulating a complete leaching of LIBs at a concentration of 4% (w/v), based on the average Co concentration reported in the literature. Previous studies have shown that the Co leaching yields started to decrease at this LIB concentration in biotechnological processes (Heydarian et al., 2018; Kim et al., 2024; Naseri et al., 2019a,b; Panda et al., 2024; Roy et al., 2021b; Wang et al., 2022). Bacteria were inoculated in the different modified mediums at their exponential growth phase. The cultures were then kept shaken (120 rpm) for 96 hrs (4 days) at room temperature, and pH was re-established to a pH value of 1.5 once a day to avoid the Fe^{3+} precipitation (Amato et al., 2020; Becci et al., 2020). All the experiments were carried out in double. Despite this, the reproducibility between replicates was high (SD < 10%), supporting the reliability of the results.

2.2. Analytical methods

The quantitative analysis of the Fe concentration and its speciation was carried out through a spectrophotometric determination. Samples were withdrawn periodically (15, 19, 24, 39, 43, 48, 63, 72 and 96 h) for the Fe^{3+} determination, it was useful to study the bacteria activity and metabolism during the time. The Fe^{3+} present in the samples was coloured with potassium thiocyanate (KSCN) (ratio sample:KSCN of 1:1 (v/v)) and spectrophotometrically detected at 480 nm (Jasco model 7850) (Becci et al., 2021; Welcher and Hahn, 1964).

The estimation of the total bacterial number (N°/mL) was carried out through the direct count technique (Becci et al., 2021; Danovaro et al., 2002; Zimmermann, 1977). Samples were taken after 15, 24, 48, 72 and 96 h to evaluate bacterial growth over time. The analysed samples were firstly fixed in formalin, then diluted to obtain a final count of

approximately 10–20 bacteria per optical field. Two replicates at the final dilution were prepared for each initial sample. Each replicate was firstly incubated with Acridine Orange (final concentration of 0,025% w/v) and then pressured-filtered on a sterile polycarbonate membrane (0.22 μm). The membranes were then positioned on sterile glass microscope slides.

Slides were analysed using an epifluorescence microscope at λ = 450–490 nm and 1000X magnification. As described in the protocol, the bacterial number of each replicate was obtained as an average of the bacterial counts obtained from 20 different and random optical fields. The N°/mL for each replicate was then calculated through the following equation (Eq. (3):

$$\frac{N^{\circ}}{mL} = \frac{N \bullet CO \bullet d}{mL} \tag{3}$$

where N is the mean bacteria number per optical section, CO is the maximum optical section number (12,868), d is the sample dilution, and mL is the withdraw solution (0.5 mL).

2.3. Mathematical model to predict Co toxicity on at. Ferrooxidans

The experimental results were used to fit the model and to determine the best parameters to describe the bacteria number and Fe^{3+} temporal profile. The parameters were estimated by the nonlinear least square procedure by a modification of the Levenberg − Marquardt algorithm (Marquardt, 1963; Moré, 1978). In the model used, the bacterial growth rate (Eq. (4) and the Fe^{3+} production rate (Eq. (5) were assumed to follow the model described by Harvey and Crundwell (1997) (Eq. (6) (Harvey and Crundwell, 1997). This model is a modification of the Monod equation (Monod, 1949), that relates the bacterial growth to the use of limiting nutrient Fe^{2+}, the inhibition of the product (Fe^{3+}) factor and the toxicity factor of the heavy metal.

$$\frac{dN_t}{dt} = \mu \bullet N_t \tag{4}$$

$$\frac{dFe_t^{3+}}{dt} = \frac{1}{Y_{NS}} \bullet \frac{dN_t}{dt} \tag{5}$$

$$\mu = \frac{\mu_{max} \bullet \left[Fe_t^{2+}\right]}{k_s \bullet \left(1 + \frac{\left[Fe_t^{3+}\right]}{k_d}\right) + \left[Fe_t^{2+}\right] + \frac{[Co]}{k_a}} \tag{6}$$

Where N is the bacterial number (N°/mL), Fe^{2+} and Fe^{3+} are the concentrations of the Fe species (g/L), Co is the concentration of Co (g/L), μ_{max} (1/h) is the maximum specific growth rate, k_s (g/L) is the substrate saturation constant, k_d (g/L) is the inhibition parameter due to the Fe^{3+} effects, k_a (g/L) is the toxicity index due to Co and Y_{NS} (N°/gFe^{2+}) is the bacteria yield on substrate (Fe^{2+}).

The five parameters (Y_{NS}, μ_{max}, k_s, k_p and k_a) were estimated by fitting the equations (Eq. 4–6) to the experimental data obtained in the bacterial growth and activity analysis. The derived equations were solved using the Levenberg–Marquardt algorithm (Kenneth Levenberg, 1944). In the Levenberg-Marquardt algorithm the parameters of the model are chosen so that the residual deviation between observed and estimated data is minimized (Kenneth Levenberg, 1944).

2.4. Sensitivity analysis

A sensitivity analysis was performed on the parameters estimated by the mathematical model to investigate their correlations and assess how they influence the model's fit to the experimental data. The 95% confidence intervals for the parameters were derived from the variance–covariance matrix, computed using the minpack.lm package. The corresponding correlation matrix was obtained directly from this variance–covariance matrix. To explore parameter uncertainty, 1,000 random parameter sets were generated using a normal distribution centred on the best-fit values, with standard deviations equal to the estimated standard errors (via the rnorm function). Negative parameter values were excluded from the simulations. The 95% confidence regions for all variables were then visualized. A global sensitivity analysis of the parameters was also performed using the Wasserstein–Bures index to identify which parameters had the greatest impact on the final outcome (using the gsaot package). All calculations and parameter estimations were carried out using R software (version 2025.05.1 + 513).

3. Results and discussion

The study was conducted through a series of experiments involving varying concentrations of Co, alongside a control culture. From the experimental data, two key parameters were derived to aid in the interpretation of results:

- T_{85}: the time required to oxidize 85% of the Fe^{2+} substrate (h), and
- BP (Bacterial Production): the amount of Fe^{2+} oxidized per bacterium per h (mg Fe^{2+}_{oxid}/bacterium·h).

The values of these parameters for each ion concentration and culture conditions are summarized in Table 1.

It is evident that the presence of Co reduced the oxidative capacity of the studied bacterial species (*At. ferrooxidans*), and furthermore, cell growth was inhibited with increasing metal concentrations in the medium (Fig. 1). The Fe^{2+} oxidation profile in the presence of different Co concentrations are shown in Fig. 1b. Increasing metal concentrations led to a progressive extension of the oxidation time: approximately 24 h in the control (this result is consistent with findings reported in previous studies (Becci et al., 2021; Cabrera et al., 2005a; Pourhossein and Mousavi, 2018; Tavakoli et al., 2021)), with about a 60% increase in the time required to oxidize 85% of the substrate at 1 g/L Co, and up to a 260% increase at the highest Co concentration tested (Co 7 g/L) (Table 1). A concentration of 5 g/L of Co produced effects comparable to those reported in previous toxicity assays conducted with 10 g/L of Cu and Cd, and 30 g/L of Ni, all resulting in approximately 70 h for the oxidation of 85% of Fe^{2+}. Conversely, a concentration of 3 g/L of Co resulted in T_{85} (around 50 h) values comparable to those observed for Zn and Cr at the concentration of 30 and 0.4 g/L, respectively (Cabrera et al., 2005a). Similar trends were also observed for BP. These findings suggest that Co, even at significantly lower concentrations, exhibits a toxicological impact similar to that of Cu, Cd, Zn and Ni at higher concentrations, thereby indicating a relatively higher acute toxicity, except for Cr. The presence of Cr^{3+} inhibits the ferro-cytochrome *c* reductase located in the cell membrane, thereby decreasing the ability of *At. ferrooxidans* to oxidize Fe^{2+}. In contrast, Co does not inhibit the enzymes involved in Fe^{2+} transport but leads to an increased production of ROS, resulting in oxidative stress (Wu et al., 2020). This delay is mirrored by a reduction in cell growth, as shown in the examples presented in Fig. 1a, where high Co concentrations result in a pronounced lag phase. However, *At. ferrooxidans* exhibits greater resistance to Co compared to other bacteria. At concentrations around 7 g/L (~ 120 mM), the bacterium is still able to grow, although its growth rate and metabolism are reduced. In contrast, other bacteria such as *L. ferriphilum*, *E. coli*, and *G. sulfurreducens* can tolerate only lower concentrations, up to a

Table 1
T_{85} and BP for a pure culture of *At. ferrooxidans* in the presence of Co.

Co^{2+} concentration (g/L)	T_{85} (h)	BP (mgFe^{2+}_{oxid}/ bacterium·h)
0	24 ± 1	0.190 ± 0.002
1	39 ± 5	0.13 ± 0.03
3	54 ± 9	0.10 ± 0.03
5	72 ± 9	0.07 ± 0.02
7	87 ± 7	0.05 ± 0.01

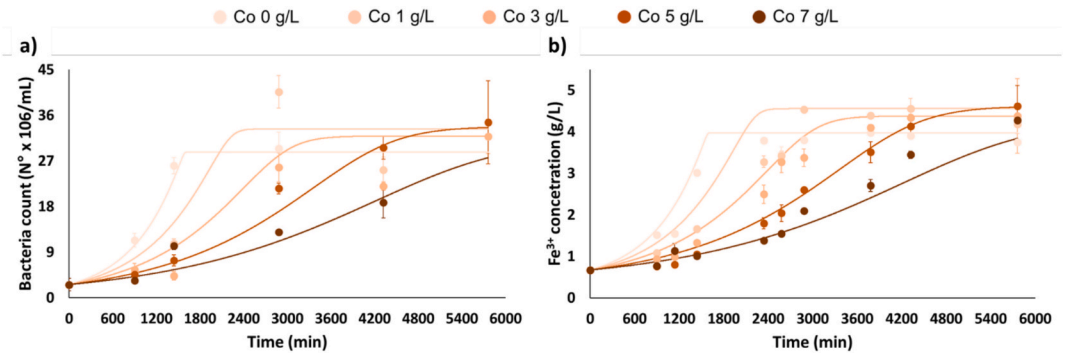

Fig. 1. Evolution of bacterial growth (a) and the Fe^{3+} concentration trend (b) in the presence of several concentrations of Co (from 0 to 7 g/L). The points and the lines are the experimental and the differential equations (Eq.s 4–6) results, respectively. The bacteria growth is carried out for 4 days at room temperature and with 4 g/L of Fe^{2+}. Reported are mean values and standard errors calculated from two independent replicates.

maximum of 5–10 mM Co (Dulay et al., 2020; Fantino et al., 2010; Nies and Grass, 2009; Wu et al., 2020).

Bacterial counts (N°/mL) and Fe^{3+} concentrations (g/L) obtained from these experiments were used to calibrate the differential equations (Eq.s 4 and 5). Model parameters were estimated by minimizing the sum of squared errors between experimental data and model predictions (Harvey and Crundwell, 1997). The parameters k_s, k_d, k_a, Y_{NS} in the differential equations were held constant across all tested conditions, as previous studies have shown that these parameters are not significantly influenced by metal-induced toxicity on bacterial growth (Harvey and Crundwell, 1997). In contrast, the maximum specific growth rate (μ_{max}) exhibited a marked decrease with increasing inhibitor concentrations in the medium (Becci et al., 2021; Cabrera et al., 2005b, 2005a; Harvey and Crundwell, 1997).

The numerical solutions, in which all parameters were held constant except for μ_{max}, closely aligned with the experimental data, yielding coefficients of determination (R^2) greater than 0.95 (Fig. 1). This high level of agreement indicates that the model reliably captures the dynamics of bacterial growth and Fe metabolism.

The parameters that describe the bacteria growth rate (μ_{max}), the substrate saturation constant (k_s), the inhibition parameter due to the Fe^{3+} effects (k_d) and the bacteria yield on the substrate (Y_{NS}) were in the range reported in the literature, between 0.06 and 0.25 1/h, 0.024 and 2.02 g/L, 0.06 and 0.315 g/L and 0.25 – 5.9 × 10^7N°/g Fe^{2+}, respectively (Boon et al., 1999; Cabrera et al., 2005b; Chavarie et al., 2009; Kumar and Gandhi, 1990; Molchanov et al., 2007; Nemati et al., 1998; Rashidi et al., 2012). On the other hand, the k_a value (3 ± 1 g/L) estimated in this study is higher than the value previously reported for assessing As toxicity (2.0 ± 0.2 g/L), suggesting that Co exerts a slightly greater inhibitory effect on the metabolic activity of *At. ferrooxidans* compared to As (Harvey and Crundwell, 1997). The global sensitivity analysis showed that the parameters exerting the greatest influence on the results for bacterial growth and Fe^{2+} oxidation are Y_{NS} (bacteria yield on substrate) and k_a (the Co-induced toxicity index). Other parameters that strongly affect the final outcomes are the μ_{max} values determined for each individual Co concentration, whereas the remaining parameters (k_s and k_d) exhibited a minor influence on the results.

The estimated parameters and the resulting differential equations were used to analyse the oxidation rate of Fe^{2+} driven by bacterial metabolism under varying Co concentrations. In the control test (Co 0), the maximum Fe^{2+} oxidation rate was observed approximately 25 h after the onset of bacterial growth, reaching a value of about 4.6 mg Fe^{2+}/L·h (Fig. 2). The results show a progressive decline in the Fe^{2+} oxidation rate with increasing Co concentrations, culminating in a minimum rate of approximately 0.8 mg Fe^{2+}/L·h at the highest Co level tested (Co 7 g/L), corresponding to a reduction of roughly 82%. This minimum rate was reached around 70 h after the beginning of bacterial growth (Fig. 2). These findings are consistent with previous observations

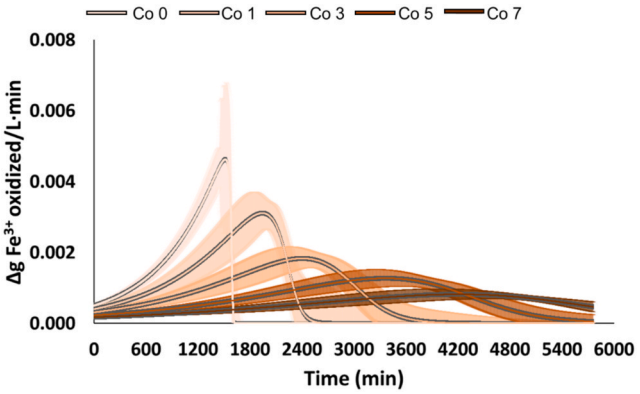

Fig. 2. Fe^{2+} oxidation rate by bacteria metabolism at different Co concentrations (0, 1, 3, 5, 7 represent Co conc. in g/L). The bacteria growth is carried out for 4 days at room temperature and with 4 g/L of Fe^{2+}. The graph shows the mean values (lines, oxidation rate calculated using the model's best-fit parameters) and the corresponding standard deviations (shaded areas, oxidation rate computed using the 1,000 parameter sets estimated by the model).

and results obtained using T_{85} and BP parameters.

To validate and strengthen the reliability of the parameters estimated by the mathematical model, a statistical analysis was conducted to evaluate the correlations among them. The estimated values for the maximum specific growth rate (μ_{max}) showed a consistent decrease with increasing Co concentration. The correlation analysis revealed a strong relationship among most of the μ_{max} values, with correlation coefficients exceeding ± 0.65. However, μ_{max} values at Co 0 exhibited no significant correlation with those at Co 5 and Co 7, as indicated by correlation coefficients close to 0.

To further support these observations, confidence ellipses between the μ_{max} of the control condition (Co 0) and those obtained at different Co concentrations (Co 1, Co 3, Co 5, and Co 7 g/L) were also analysed, providing more detailed insights into the correlation patterns and associated standard errors (Fig. 3a). A significant correlation (r = 0.79) was observed between k_s and k_d (Table 1). This relationship is clearly illustrated in Fig. 3b, where an increase in k_s corresponds to a proportional increase in k_d. In contrast, k_a did not show any relevant correlation with k_s or k_d. However, k_a exhibited a strong negative correlation with μ_{max} across different Co concentrations. This correlation became more pronounced with increasing Co levels, ranging from −0.76 to −0.91, indicating that higher Co concentrations led to a more marked decrease in μ_{max}, thereby reflecting an increase in Co-induced toxicity.

Given the observed decrease in μ_{max} with increasing Co concentrations, a correlation between these two parameters was investigated. A non-linear regression analysis was performed to fit the determined μ_{max}

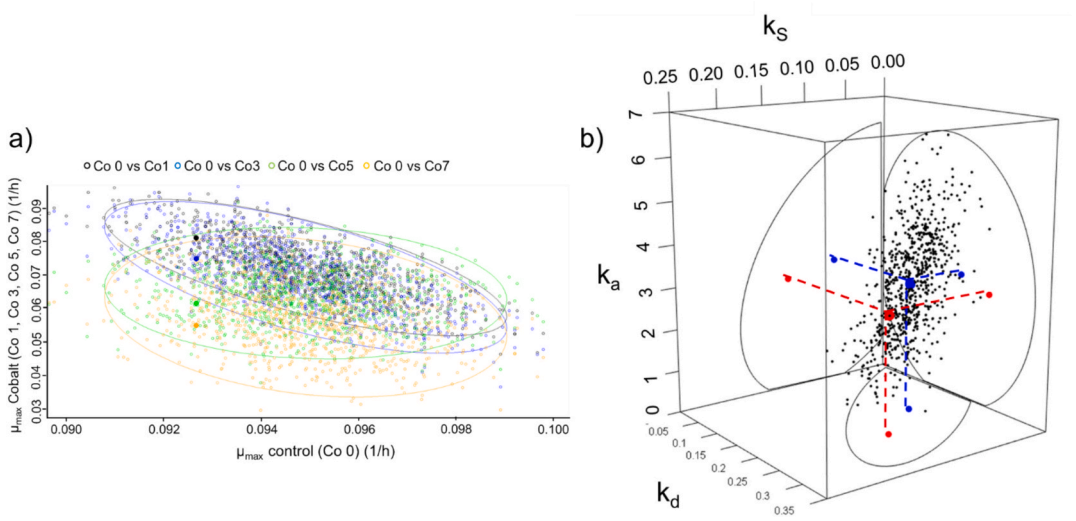

Fig. 3. Shown are the 95% confidence ellipses along with 1,000 randomly generated parameter values:(a) Distribution of values around the estimated μ_{max} at different Co concentrations; (b) Parameter space for constants k_s, k_d and k_a as determined by the mathematical model, red points represent the best-fitting parameter sets, while blue points indicate the centres of the ellipses.

at different Co concentration by the mathematical model using the characteristic equation for non-competitive inhibition (Eq. (7), describing the apparent maximum specific growth rate (Arsalan and Younus, 2018; Cabrera et al., 2005b).

$$\mu^{*}_{max} = \frac{\mu_{max} \bullet k_I}{[Co^{2+}] + k_I} \tag{7}$$

In this context, μ^{*}_{max} represents the maximum specific growth rate estimated for each experiment at different Co concentrations, k_I is the inhibition constant, and [Co^{2+}] denotes the Co concentration in the medium. An inhibition constant was calculated for each of the 1000 possible combinations of μ_{max} values obtained from the mathematical model and the sensitivity analysis at varying Co concentrations, yielding a mean k_I value of 11 ± 5 (Fig. 4a). The k_I value obtained in this study supports the previous findings based on the T_{85} and BP parameters. Specifically, this value is lower than the inhibition constants reported for Cu, Cd, Zn, and Ni in earlier work (with a value of 20.92, 47.01, 131.52 and 89.23, respectively) (Cabrera et al., 2005b), confirming that Co is more toxic to *At. ferrooxidans* than these metals. However, Co appears to be less toxic than Cr, that reports a k_I value of 0.42 (Cabrera et al., 2005b, 2005a). The determined k_I value allowed for accurate estimation of μ_{max} at each Co concentration, with a coefficient of determination (R^2) greater than 0.97 (Fig. 4b). Thanks to the determined k_I value, it will be possible to calculate μ_{max} at different Co concentrations and, by applying the previously developed and validated model equations, to study and predict the bacterial growth and metabolism. This will allow the model to be used for the design and optimization of the biotechnological process for Co recovery from LIBs.

Finally, all possible scenarios resulting from the mathematical model and sensitivity analysis were evaluated, based on 1,000 parameter combinations. The resulting output distributions were analysed to identify the probability ranges corresponding to the various outcomes by combining all possible solutions. The results show that the full range of modelled outcomes effectively describes the experimental data in detail. In addition, the likelihood of the simulations converging to the same final outcome was estimated.

Regarding the 1,000 combinations of parameters in the control experiment (Co 0), 100% of the simulations predicted Fe^{2+} oxidation efficiencies greater than 95% within the first 24–26 h. For the parameter sets corresponding to Co concentrations of 1 and 3 g/L, over 97% of the simulations predicted Fe^{2+} oxidation by bacteria exceeding 95% within 96 h. For Co concentrations of 5 g/L, 85% of the simulations predicted Fe^{2+} oxidation above 90%, while the remaining 15% fell within an efficiency range of 70–90%. Finally, for Co concentrations of 7 g/L, only 20% of the simulations indicated Fe^{2+} oxidation above 90%, whereas the remaining 80% fell within the 50–90% range, with a higher probability (~20%) of achieving efficiencies between 85% and 90% (Fig. 5).

4. Conclusions

This study demonstrated that Co is a toxic metal for the metabolism of *At. ferrooxidans*. The estimated parameters (T_{85}, BP, k_a, and k_I) revealed that Co exhibits higher toxicity than Cu, Cd, Zn, Ni, and As, based on comparisons with values reported in previous studies on the

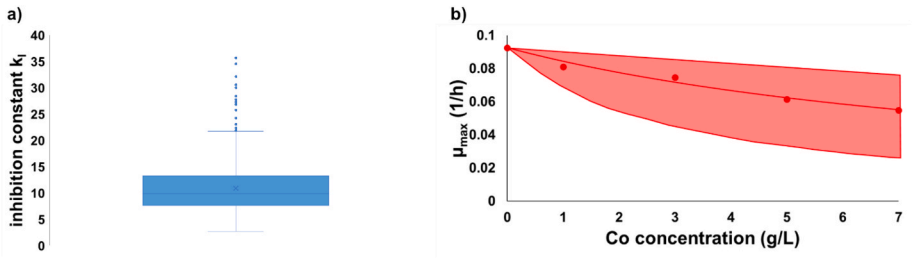

Fig. 4. (a) Boxplot of the 1000 k_I values estimated using the non-competitive inhibition equation; (b) Range of estimated μ_{max} at different Co concentrations corresponding to different k_I values. Points represent the best-fit μ_{max} values obtained from the mathematical model (Eqs. (4)–(6). The line shows the theoretical values calculated using the non-competitive inhibition equation with average parameter estimates, while the shaded area represents the range of the 1,000 theoretical values computed using the same equation.

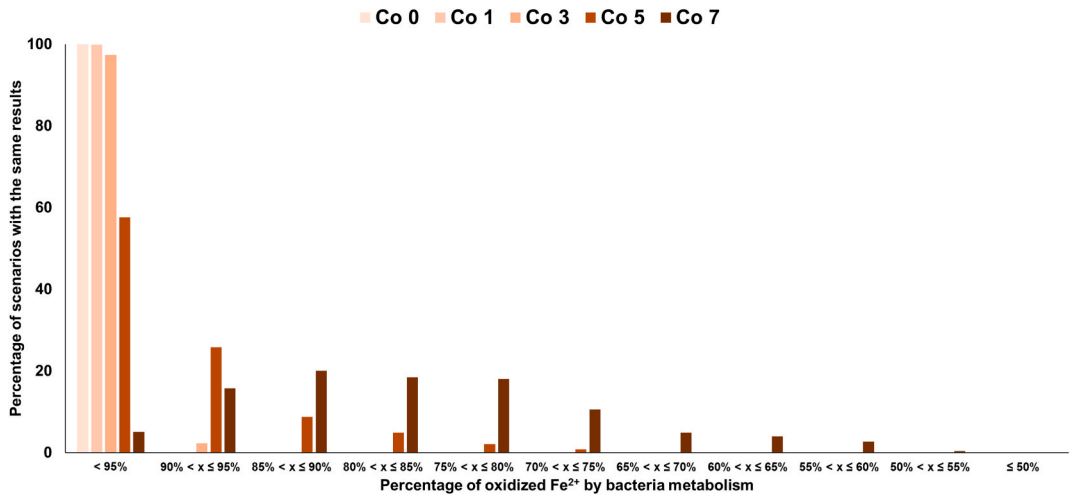

Fig. 5. Probability of achieving Fe^{2+} oxidation efficiencies under different parameter combinations at varying Co concentrations.

same bacterial species. Cr was the only metal found to be more toxic than Co. At Co concentrations above 5 g/L, bacterial metabolism and growth were significantly inhibited. The Fe^{2+} oxidation time increased by more than 260% compared to control conditions, while the Fe^{2+} oxidation rate decreased by approximately 80%. Additionally, the presence of Co led to a prolonged lag phase in bacterial growth. These effects of Co on the slowdown of bacterial growth and metabolism would result in a considerable increase in the time required to complete a biotechnological process using *At. ferrooxidans* to treat end-of-life LIBs, leading to higher economic costs and reduced environmental sustainability of such an approach, thereby making biotechnologies less attractive for industrial-scale development.

Furthermore, the mathematical model developed in this study accurately simulated the temporal dynamics of both Fe^{2+} oxidation and bacterial growth. This represents a valuable starting point and a useful model; however, it will need to be further developed to investigate the combined toxic effects of Co with other metals present in LIBs (e.g., Li, Ni, Mn, and Al). Therefore, this work should be regarded as an initial step toward studying the interactions among these metals and assessing whether they result in a greater or lesser inhibition of Fe oxidation. Furthermore, the ongoing replacement of Co with Ni in the production of new batteries, given the latter's lower toxicity, could further enhance the applicability of biotechnological approaches for end-of-life LIB treatment, promoting them as a more environmentally sustainable alternative.

CRediT authorship contribution statement

Alessia Amato: Writing – original draft, Visualization, Resources, Project administration, Methodology. **Alessandro Becci:** Writing – original draft, Visualization, Validation, Software, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Francesca Beolchini:** Writing – original draft, Supervision, Resources, Project administration, Funding acquisition, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they 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 A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.biortech.2026.134312.

Data availability

Data will be made available on request.

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