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Study of the Bioleaching and Dissolution Kinetics of Zinc from a Zinc Secondary Source Using Indigenous Acidothiobacillus Ferrooxidans

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Abstract

The increasing global demand for zinc has led to rapid depletion of its primary sources. Bioleaching, a sustainable and eco-friendly technology, offers a promising solution for metal recovery from secondary source. This study investigates the bioleaching of zinc from a secondary source, being a zinc-rich dumpsite, using indigenous *acidithiobacillus ferrooxidans*. The effects of pH, temperature, inoculum percentage and agitation speed on zinc dissolution were assessed. The results showed that optimal bioleaching conditions were pH 2.5, temperature 30°C, 20% inoculum and agitation speed 150 rpm with a maximum bioleaching of 75.5%. The dissolution kinetics using the shrinking-core model followed a mixed control; the reaction kinetics followed a first-order model, with an activation energy requirement (Ea) of 468.261 J/mol. The indigenous *A. ferrooxidans* strain demonstrated high performance and metal tolerance. This study highlights the potential of bioleaching for zinc recovery from secondary waste, contributing to economy sustainability and reduced environmental pollution.

1. Introduction

Zinc's versatility and widespread adoption make it a leading non-ferrous metal in terms of usage such as in the galvanization of steel. It is also used in sheets, anodes, castings, chemical production, micronutrients, paints, and dry cells, among others. The rising worldwide demand for zinc has resulted in the rapid exhaustion of its key sources [1]. Consequently, the efficient extraction of zinc from secondary sources would yield numerous benefits, including conservation of virgin and fossil resources utilized for energy in primary mining operations, enhanced resource efficiency, diminished landfilling and loss of zinc or other metals to landfills, waste remediation, reducing the environmental and health footprint, and boosting the economic efficiency of current infrastructure [2].

Traditional chemical approaches, including direct acid and alkaline leaching techniques for metal recovery from secondary sources, have significant drawbacks, including excessive acid use and the production of hazardous waste [3]. The increased environmental concern is a constraint to the implementation of these approaches.

Consequently, there is a pressing need to create innovative, eco-friendly, and economically viable techniques for recovering zinc from zinc-rich secondary sources.

In comparison to traditional approaches, the bioleaching process has advantageous characteristics for zinc extraction from zinc-bearing secondary materials. Studies have demonstrated the efficacy of this technique as a low-cost, environmentally responsible means of treating a range of minerals and waste products [3]. The process involves the synergistic interaction between microorganisms and metal sources, facilitating the production of organic or inorganic acids, oxidation-reduction reactions, and the release of chelating agents. [3].

There have been reports of the use of bioleaching for the extraction of metals from secondary sources. Sasiain *et al.* [4], studied the bioleaching of Zinc from Blast Furnace Cast House Dust using *acidothiobacillus ferroxidans* and recorded Maximum zinc leaching efficiencies of 63%. Rouchalova *et al.* [5], studied the bioleaching of Iron, Copper, Lead, and Zinc from Sludge Mining Sediment using *acidithiobacillus ferrooxidans* and recorded above 90% recovery of zinc during optimization. Chen and Wang [6] studied the effects of solid content and substrate concentration on the bioleaching of heavy metals from sewage sludge using *Aspergillus niger* and recorded 56% recovery of zinc after 2 days reaction time. Funari *et al.* [7], studied the bioleaching of fly ash and bottom ash from municipal solid waste. The bioleaching resulted in 100% Cu, 80% Zn and 20% Pb removal in two weeks. Sajjad *et al.* [8] studied the bioleaching of copper and zinc bearing ore using consortia of indigenous iron-oxidizing bacteria. Under optimized conditions, 77.68 ± 3.55% of copper and 70.58 ± 3.77% of zinc were dissolved.

This study examined the impact of variables like pH, temperature, inoculum %, and agitation speed on zinc bioleaching from zinc-rich secondary sources using indigenous *acidithiobacillus ferrooxidans,* aiming to optimize conditions for maximal zinc recovery. The results of this investigation are anticipated to contribute to the design of a novel, environmentally responsible, and highly efficient bioleaching technology for recovering zinc.

2. Materials and Methods

To maintain the integrity of the results, only analytical-grade reagents were used, and deionized water was employed as the throughout the investigation**.**

2.1 Sample Collection

The secondary source of zinc used in this study was municipal solid waste collected from the Dogarawa dumpsite in Zaria L.G.A, Kaduna State, Nigeria (longitude 7^0 42′ 57.44″N and latitude 11″ 4′ 54.91″E). Composite samples were obtained from five locations within the dumpsite (four diagonal points and the center) at weekly intervals over four weeks.

2.2 Sample Preparation

The municipal waste, rich in zinc, underwent calcination at 700 °C for one hour using a muffle furnace (Thermo Fisher Scientific Muffle Furnace, USA) equipped with a temperature control system [9].

2.3 Sample Characterization

2.3.1 Elemental Composition

The elemental components of the municipal solid waste were analyzed and quantified using X-Ray Fluorescence (XRF) analysis (Powder Ultima Rigaku, Japan).

2.3.2 Mineralogical Composition

The mineralogical properties of the waste were ascertained via X-Ray Diffraction (XRD) analysis (X-MET800, England).

2.4 Bioleaching Studies

A culture of *acidothiobacillus ferrooxidans*, originally sourced from the same waste dumpsite, was utilized. The composition of the nutrient growth medium (9K) for maintaining the bacterial culture included 3 g $(NH_4)_2SO_4$, 0.5 g K_2HPO_4, 0.5 g $MgSO_4.7H_2O$, 0.1 g KCl, 0.014 g $Ca(NO_3)_2.4H_2O$, and 44.2 g $FeSO_4.7H_2O$ all of analytical British Drug Houses (BDH) grade per liter of deionized water [3].

2.4.1 Culturing of *Acidothiobacillus Ferrooxidans* from Waste

A 50g sample of waste material was collected, suspended in deionized water, and then subjected to centrifugation at 5000 rpm for 10 minutes. Subsequently, 10mL of the resulting liquid waste was inoculated into 90mL of 9K growth medium, followed by incubation at 35°C with constant agitation at 150 rpm for a period of 10 days [10].

The pH was regularly monitored, and culture purity was assessed through Gram staining and microscopy. Sub-culturing was performed by transferring 2 mL of the enriched culture into fresh 9K medium, followed by purity verification [10].

2.4.2 Bioleaching Procedure

Bioleaching was conducted in 250 mL Erlenmeyer flasks, each containing 100 mL of 9K liquid medium, subsequently, 10 g of the calcined ash sample was added and inoculated with a 10% (v/v) *A. ferrooxidans* solution containing a cell count of 1.0×10^{6}.

To ensure proper mixing and facilitate oxygen and carbon dioxide exchange, the flasks were placed in an incubating shaker at 150 rpm and maintained at 25 ± 2°C. The pH of the solution was initially set to 2 by adding a 5M sulphuric acid solution [3]. Throughout the 10-day bioleaching period, 10 mL aliquots were withdrawn daily for zinc concentration analysis using Atomic Absorption Spectroscopy (AAS) [3].

A series of experiments were conducted under diverse operating conditions to assess the impact of various factors, such as temperature (25°C, 30°C, 35°C, and 40°C), agitation speed (100, 150, 200, and 250 rpm), pH levels (1.5, 2.0, 2.5, and 3.0), and inoculum percentages (10%, 15%, 20%, and 25%), as illustrated in Fig. 1.

The effectiveness of zinc bioleaching was calculated using the equation:

$$\alpha = \frac{C.V}{Z.m} \times 100\% \quad (1)$$

where:

- **α** = Zinc leaching efficiency (%)
- **V** = Leachate volume (mL)
- **C** = Zinc concentration in leachate (g/mL)
- **m** = Mass of the sample (g)
- **Z** = Zinc content in the sample [3].

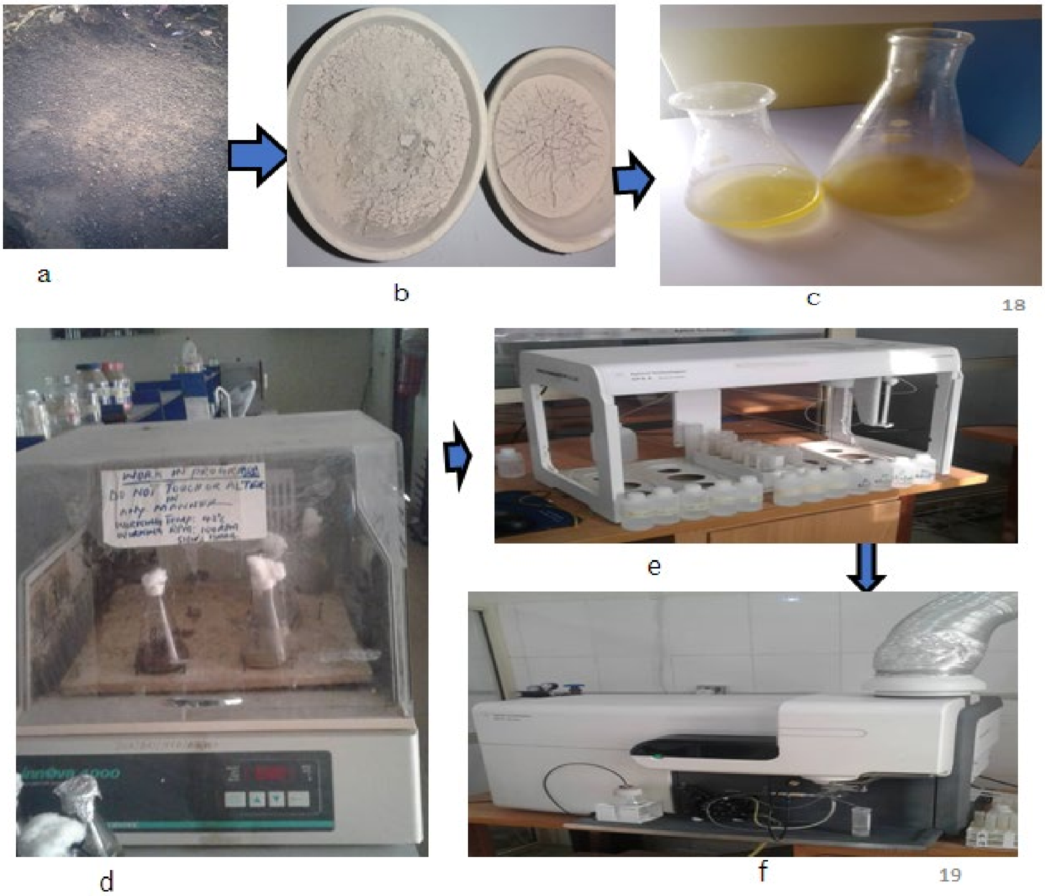

Fig. 1 *Description of bioleaching process (a)Raw zinc municipal waste; (b) calcined sample in a crucible; (c) culture media (d) Incubator shaker; (e) filtered sample on a rake; (f) AAS machine used for the analysis of zinc content in the liquor*

3. Results and Discussion

Study of bioleaching and dissolution kinetics of zinc from zinc-rich secondary source using indigenous *acidothiobacillus ferrooxidans* was conducted and the results presented. The tables and figures below summarize the key findings of the research. The discussion that follows provides interpretation of the results and highlights some importance implications of the acquired results.

3.1 Physiochemical Analysis

The final result of physiochemical tests of the secondary source of zinc for this research is reported in Table 1. The slightly alkaline pH of 7.6 indicated its feasibility for zinc bioleaching utilizing *acidithiobacillus ferrooxidans* [11]. The result in this study is consistent with the pH range of 6.5-7.5 reported by Kumar *et al.* [11]. Also, the loss of mass on ignition observed is extremely low compared to the range of value of 20-40% reported by Kumar *et al.* [11] which imply the lack of organic contaminants that might impair the bioleaching process [12]. However, the value in the present research is closer to the range of 5-15% reported by Pathak *et al.* [20].

Table 1 *Physiochemical analysis of the sample*

Parameter	Value
pH	7.6
Loss of mass on ignition (%)	2.5±0.005

3.2 Chemical Characterization

The result of the mineralogical characterization using XRD is provided in Fig.2. The result reveals that the sample includes quartz, graphite, anhydrite, whereas the result of elemental analysis of the sample using XRF is shown in Table 2. The result showed that the sample contains 20.239% Zn, 0.078% Si, 4.235% Fe, 0.082% Al, 0.426% Cu, 0.025 % S and 17.470% V which verified the sample as a possible secondary source for zinc bioleaching due to the high proportions of Zinc.

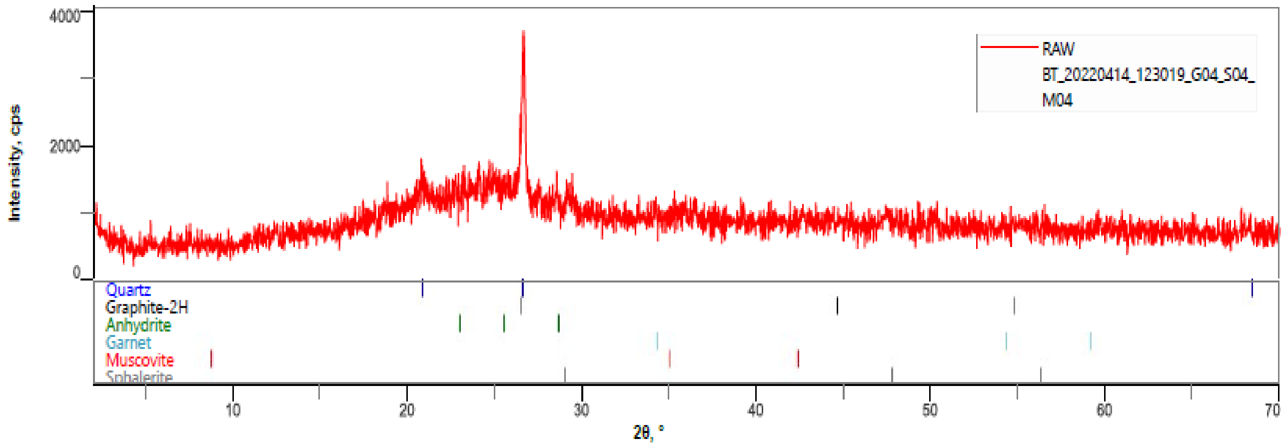

Fig. 2 *Mineralogical phase analysis results of the sample*

Table 2 *Elemental analysis by X-ray fluorescence (XRF) of the sample*

Oxide composition	ZnO	SiO_2	Fe_2O_3	TiO_2	Al_2O_3	CuO	SO_3	V_2O_5
	25.19	37.374	6.055	0.710	7.943	0.097	7.610	0.044
Element	Zn	Si	Fe	Ti	Al	Cu	S	V
Composition	20.239	0.078	4.235	4.203	0.082	0.426	0.025	17.470

3.3 Bioleaching Studies

3.3.1 Effect of Temperature

From the result in Fig. 3, the highest zinc bioleached was observed at temperature of 30^0C. At temperature of 40^0C, the rate of zinc bioleaching decreased tremendously due to decrease in microbial activities, hence inability of the bacterial to withstand the temperature. At 25°C, the zinc dissolution rate was observed to be low, likely resulting from a slower gas transfer rate that made the leaching operation more challenging [13]. These results agree with those conducted by previous studies [13,14].

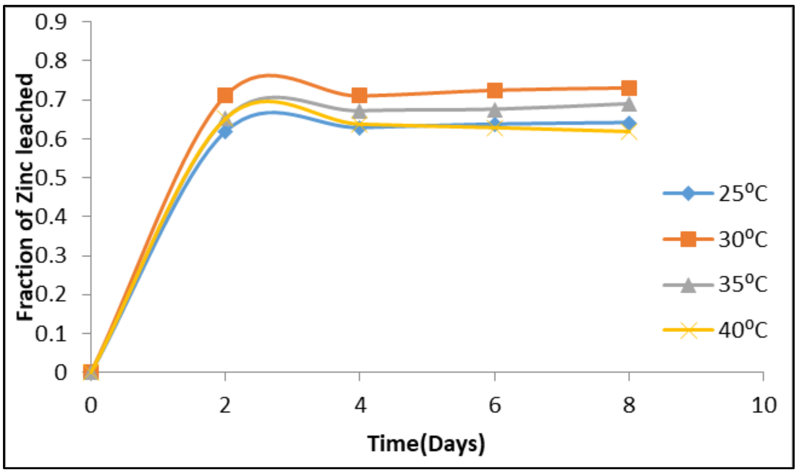

Fig. 3 *Effect of temperature on the bioleaching of zinc using acidothiobacillus ferrooxidans*

Generally, the rate of bioleaching increase rapidly on the second days which implies quick adaptation of the *acidothiobacillus ferrooxidans* to the sample environment and the subsequent leveling off indicates that the easily accessible zinc metals have been solubilized. This result is in agreement with those reported by Pathak *et al.* [20] where the bioleaching efficiency of municipal solid waste using *acidothiobacillus ferrooxidans* increase rapidly in the second and third days and then level off [20].

3.3.2 Effect of pH

Fig. 4 shows the experimental result conducted to determine the effect of pH. The experimental condition here consists of 250 mL Erlenmeyer flasks with 100 mL medium, 10 g of the sample, 20% (v/v) inoculum, temperature of 30^0C, flask shake speed 150 rpm and pH range of 1.5-3.0. At pH 1.5, the rate of dissolution was lower compared to that of pH 2.0, supporting the claim by Nancucheo and Johnson [15], that lower pH inhibits bacterial. This result is supported by the experiment using *acidithiobacillus ferrooxidans* conducted by Bhaskar et al. [13] with the optimum pH being 2.0.

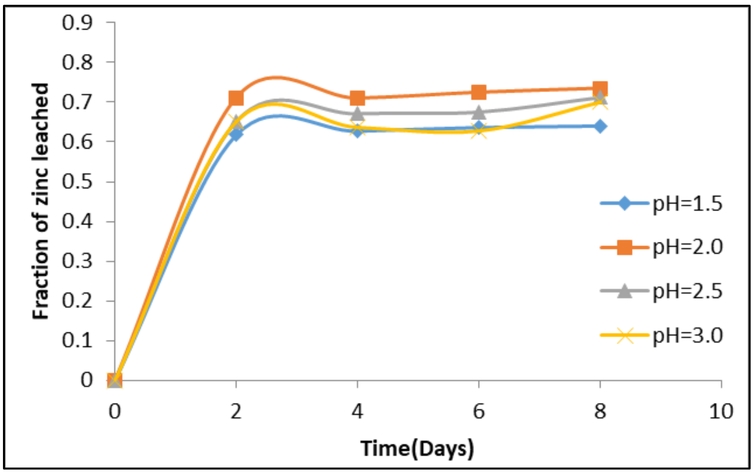

Fig. 4 *Effect of pH on the bioleaching of zinc using acidothiobacillus ferrooxidans*

Generally, the rate of bioleaching increase rapidly on the second day which implying rapid adaptation of the bacteria to the experimental environment and then leveling off subsequently, which indicates that the quickly accessible zinc metals have been leached. This is in consistence with those reported by Pathak et al. [20], where the bioleaching efficiency of municipal solid waste using *acidothiobacillus ferrooxidans* increase rapidly in the second and third day and then level off [20].

3.3.3 Effect of Inoculum Percentage

In the present studies, the inoculum percentage 10%, 15%, 20% and 25% were studied and the results is as shown in Fig. 5. From the result, 10% inoculum showed the lowest zinc dissolution while the highest bioleaching was observed at 20% inoculum percentage. At 25% inoculum, the rate of zinc dissolution slightly decreased, this may be as a result of increased competition for nutrients; oxygen limitation can consequently lead to excessive acid production and hence bring about pH imbalance leading to reduction in bioleaching efficiency. Generally, increase in inoculum percentage leads to increase in microbial activities such as increase in acid production, better adaptation and hence increase in zinc dissolution.

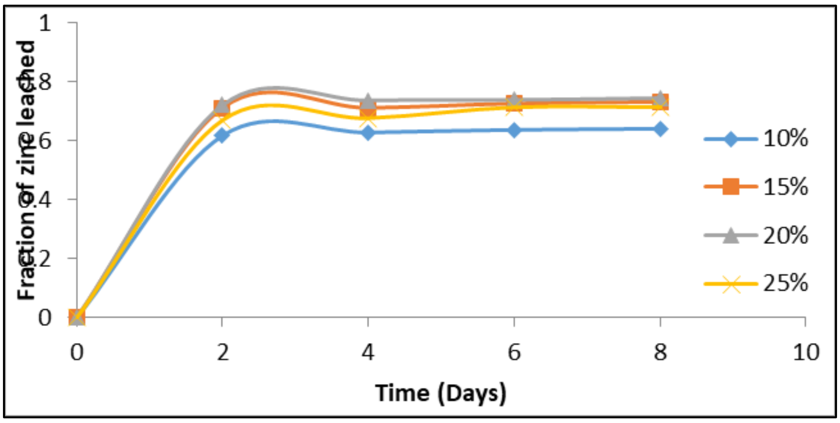

Fig. 5 *Effect of inoculum percentage on the bioleaching of zinc using acidothiobacillus ferrooxidans*

3.3.4 Effect of Shake Flask Speed

Shake flask speed in the laboratory bioleaching research correlates to agitation speed in the bioreactor of industry, which is of significance to process engineers. In the current research the influence of shake flask speed at many variations like 100 rpm, 150 rpm and 200 rpm and 250 rpm was explored. Fig. 6 illustrates the rate of zinc bioleached at varied flask speed. At 150 rpm, the rate of zinc bioleached is rather high, as the agitation at this speed maintains the bacterium in touch with the zinc-rich municipal solid waste sample; while preventing it from settling down at the bottom. At a speed of 250 rpm, the dissolution process was hindered by turbulence, leading to cell rupture and detachment from the sample, which consequently reduced the rate of dissolution.

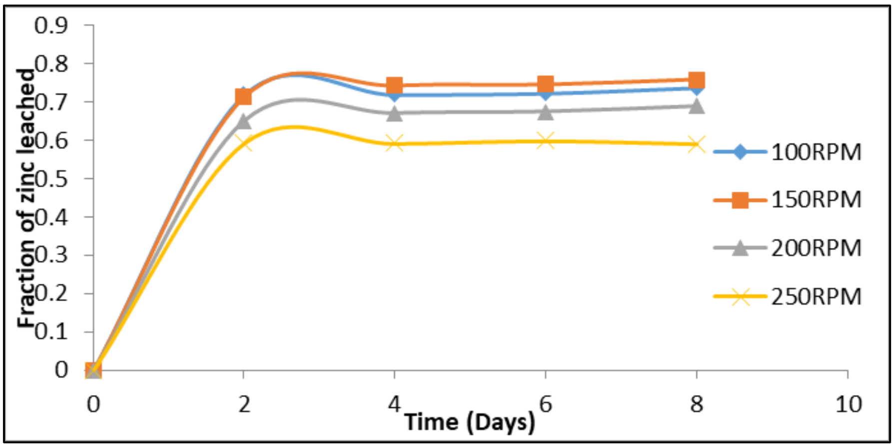

Fig. 6 *Effect of agitation on the bioleaching of zinc using acidothiobacillus ferrooxidans*

3.4 Dissolution Kinetics Studies

The dissolution kinetics of zinc from the zinc-rich secondary source was determined by subjecting the leaching results to three shrinking core model (SCM) summarized by the equations below.

$$1-(1-\alpha)^{1/3}=Kct \quad (2)$$

$$1-\frac{2}{3}\alpha-(1-\alpha)^{\frac{2}{3}}=Kpt \quad (3)$$

$$1-(1-\alpha)^{\frac{2}{3}}+\frac{y}{6}[(1-\alpha)1/3+1-2\,(1-\alpha)^{2/3}=Kmt \quad (4)$$

In this context, α represents the percentage of waste that underwent reaction, t denotes the duration in days, and y is a constant value of 1 [16-18]. When the leaching results were subjected to chemical reaction control (Eq. 2), diffusion controlled process (Eq. 3), and mixed controlled process (Eq. 4) respectively, Equation 4 gave the best fit with a correlation coefficient (R^2) of 0.986. Hence, the result of the fitting test for the zinc bioleaching kinetic is as represented in Fig. 7.

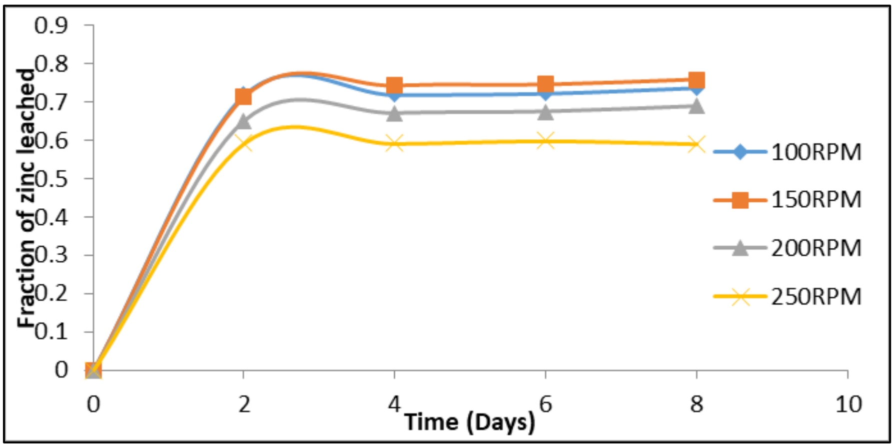

Fig. 7 *Effect of agitation on the bioleaching of zinc using acidothiobacillus ferrooxidans*

The experimental rate constant, K was estimated from the slopes of the straight lines of fig. 8, and the results subjected to Arrhenius equations,

$$K=Ae^{-Ea/RT} \quad (5)$$

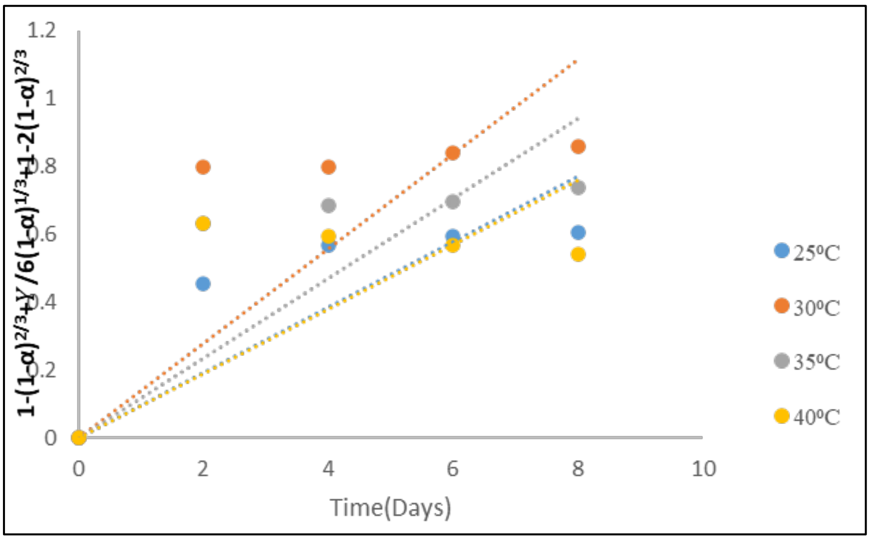

Fig. 8 *Plot of* $1-(1-\alpha)^{2/3}+Y/6(1-\alpha)^{1/3}+1-2(1-\alpha)^{2/3}=k_{m}t$ *for temperature of acidothiobacillus ferroxidans*

Where A is a constant related to the geometry needed, K is the rate constant, R is the gas constant (8.314 JmolK^{-1}), T is temperature in Kelvin (K)

$$\ln K = \frac{-Ea}{R}\left(\frac{1}{T}\right) + \ln A \quad (6)$$

When lnK is plotted versus the inverse of the temperature (1/T) as shown in Fig. 9, the value of the slope (m) is equal to $\frac{-Ea}{R}$.Where R is a constant (8.314 Jmol^{-1}K^{-1}) [18,19]. The activation energy (Ea) was estimated to be 468.261 Jmol^{-1}

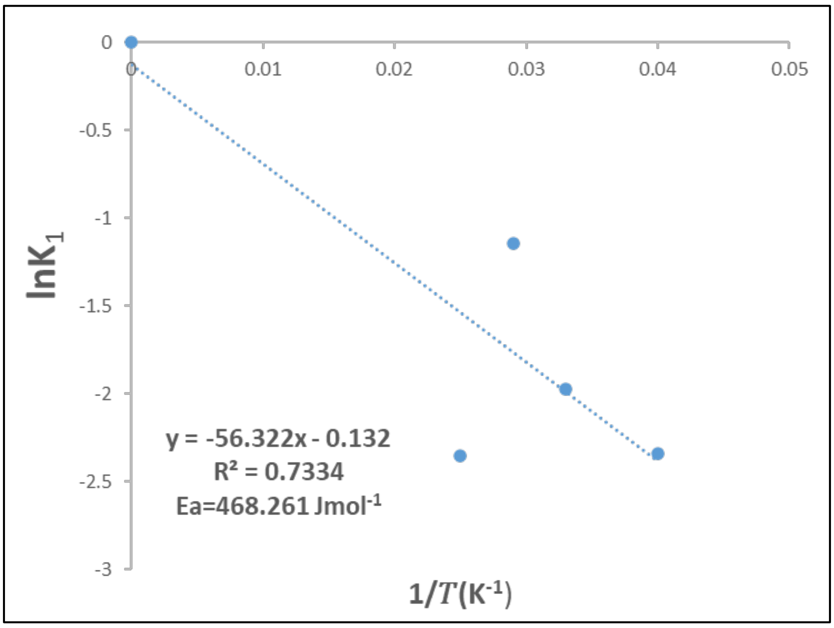

Fig. 9 *Plot of lnK against 1/T*

Analysis of the dissolution data reveals that the shrinking core model accurately describes the process, suggesting that the bioleaching kinetics are governed by a combined diffusion and chemical control mechanism. Similarly, the result of the fitting test concerning the initial pH showed that the data fitted perfectly into equation (4), as shown in Fig. 10

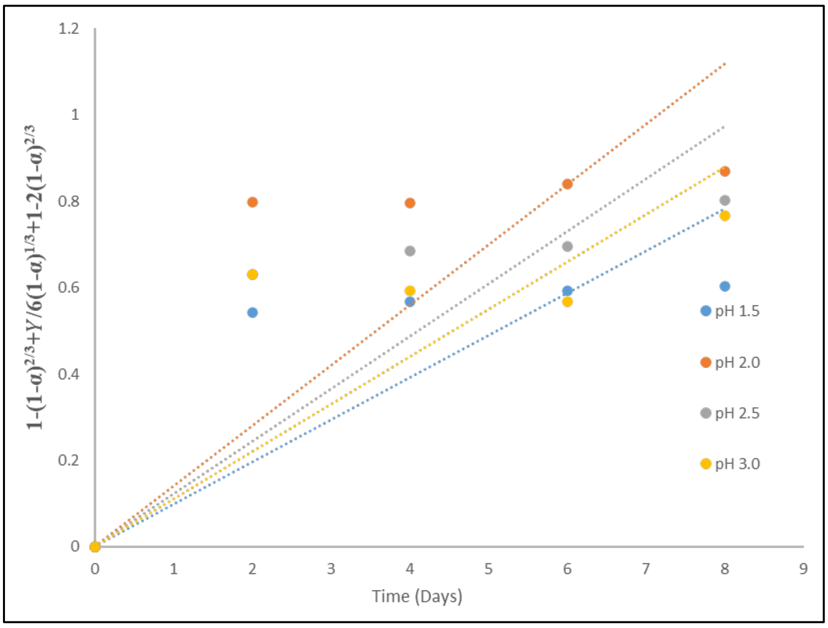

Fig. 10 *Plot of $1-(1-\alpha)^{2/3}+ Y/6(1-\alpha)^{1/3}+1-2(1-\alpha)^{2/3} = k_m t$ for pH of acidothiobacillus ferrooxidans*

The experimental rate constant K_2 for zinc dissolution by *Acidotiobacillus ferrooxidans* was calculated from figure 10 using the slope of the straight line at different pH [17]. The plot of lnK_2 against ln [H_2SO_4] is illustrated in Fig. 11, with a slope of 0.8899. This shows that the order of reaction for zinc bioleaching with respect to H+ ion concentration is 0.8899 with correlation coefficient of 0.8689. This conclusion agreed with those reported by Muktar et al. [19].

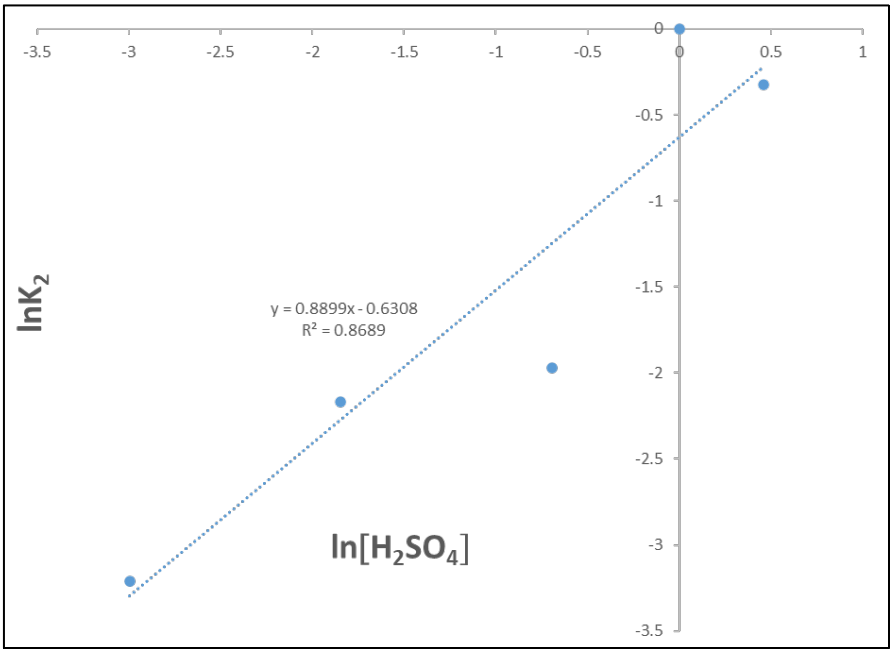

Fig. 11 *Plot of lnK₂ against ln[H₂SO₄]*

4. Conclusion

The experimental data presented above yields the following key findings: Optimal bioleaching conditions were achieved at pH 2.5, 30°C, 20% inoculum, and a flask shake speed of 150 rpm, resulting in a maximum bioleaching efficiency of 75.5%. Furthermore, the dissolution kinetics adhered to a shrinking core model, characterized by a mixed control mechanism with R^2= 0.7334, Ea of 468.261Jmol$^-$ and the order of reaction is 0.8899. Considering the favorable activation energy obtained in this research, the design is recommended for further development and scaling up for potential applications in zinc manufacturing.

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Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of the paper.

Author Contribution

***Edoka F.N.** played a key role in designing and executing the experimental setup for the bioleaching process and contributed to the development of the introduction and methodology sections of the manuscript; **Omoniyi K.I.** provided overall supervision and guidance throughout the project, and also edited and revised the manuscript to ensure its publication readiness. **Suleiman I.** and **Ugwoke A.O**. collaborated on the writing of the results and discussion sections of the manuscript, providing valuable insights and analysis. **Ella E.E.** was responsible for isolating and characterizing the indigenous acidithiobacillus ferrooxidans strain used in the study. **Asuke F.** conducted a comprehensive kinetic study of the bioleaching process, analysing the data and results, and contributing to the writing of the results and discussion sections of the manuscript.*

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