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Unlocking copper flotation efficiency: the interaction of *Citrobacter* sp. strain SKC-4 with chalcopyrite concentrate as a potential eco-friendly reagent alternative

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ABSTRACT

This research explores the viability of bioflotation as an eco-friendly alternative to conventional chemical reagents in processing copper sulfide ores. It primarily focuses on the interactions between bacterial cells and chalcopyrite concentrate. A key objective is to identify bacterial strains suitable for use as bioflotation reagents. The study specifically examines the interaction between *Citrobacter* sp. strain SKC-4, a bacterium native to sulfur-rich environments, and chalcopyrite ($CuFeS_2$) concentrates over a 30-day period. This bacterium is known for its ability to produce biosurfactants and oxidize iron and sulfur, making it an ideal candidate for various roles in bioflotation, such as biocollectors, biodepressants, and biofrothers. Experimental setups involved mixing chalcopyrite concentrates (-200+325 mesh, 25% w/v) with the bacterial strain (10% v/v) in modified LB media under aerobic conditions with constant shaking. Periodic sampling facilitated subsequent analyses. Results indicated biosurfactant production by the bacteria, as evidenced by the detection of hydroxyl (OH) groups, amine (NH) groups, and glycosidic bonds (C-O-C), along with the identification of hydrophilic C=O groups through FTIR (Fourier transform infrared) analysis, suggesting the presence of biodepressant, biocollector, and biofrother properties. Surface tension measurements consistently showed values below that of water (71 mN/m), supporting the biosurfactants' potential as flotation biofrothers and biocollectors. These findings suggest that employing *Citrobacter* sp. strain SKC-4 could substantially reduce the environmental impact of using chemical reagents in the flotation process. This study not only proposes a sustainable substitute for traditional flotation reagents but also demonstrates the potential of bioflotation to improve the efficiency and environmental sustainability of copper sulfide ore processing. The adoption of microorganisms as bioflotation reagents could transform the mineral processing industry by minimizing chemical use and environmental impact, contributing to more sustainable mining practices.

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1. Introduction

The use of bacteria as bioflotation reagents in mineral processing, particularly for copper flotation efficiency, represents a significant shift towards more sustainable and environmentally friendly practices. Traditional flotation processes rely heavily on chemical reagents, which can have detrimental environmental impacts and pose health risks. In contrast, bioflotation leverages the natural properties of microorganisms to enhance the separation of valuable minerals from impurities. One of the key advantages of bioflotation is the ability of certain bacterial strains to produce biosurfactants, which can act as biocollectors and biodepressants. These biosurfactants can reduce the surface tension of the solution, making it easier for mineral particles to attach to air bubbles and float to the surface. For copper sulfide ores, such as chalcopyrite ($CuFeS_2$), the use of bacteria has shown promise. These bacteria produce biosurfactants that can interact with the mineral surfaces, enhancing their hydrophobicity and thus improving flotation efficiency (Sanwani et al., 2015; Sanwani et al., 2015a; Jafari et al., 2016; Sanwani et al., 2016; Sanwani et al., 2021; Sanwani et al., 2021a; Sanwani et al., 2020).

The interaction between bacteria and mineral surfaces is a critical aspect of bioflotation. Studies have shown that the attachment of bacteria to mineral particles can significantly affect the hydrophobicity of the surface. For example, *Escherichia coli* (*E. coli*) has been found to decrease the hydrophobicity of chalcopyrite surfaces, leading to reduced flotation recovery rates (Li et al., 2022). Conversely, other bacterial strains such as *Acidithiobacillus*

ferrooxidans and *Leptospirillum ferrooxidans* have been used to selectively attach to pyrite, allowing for the separation of pyrite from chalcopyrite and arsenopyrite (Bleeze, 2023).

The application of bioflotation in copper flotation efficiency also involves understanding the impact of various parameters such as bacterial strain, gas used for flotation, concentration of reagents/cells, and exposure conditions. These variables can significantly influence the effectiveness of the bioflotation process. For instance, the use of different bacterial strains can result in varying degrees of mineral surface modification, affecting the overall efficiency of the flotation process (Bleeze, 2023; Kinnunen et al., 2020). By optimizing these parameters, bioflotation can offer a more sustainable and efficient alternative to traditional chemical-based flotation methods, contributing to a greener future for the mining industry.

Recent studies have significantly advanced our understanding of bioflotation using bacteria as flotation bioreagents, particularly for copper flotation efficiency. For instance, research has shown that certain bacterial strains can be highly effective in flotation tests, acting as selective and specific agents. The microbial world in copper sulfide flotation plants (CSFP) has been explored, revealing the potential of indigenous bacteria as pyrite bioreagents. These studies have identified specific bacterial phyla, such as Proteobacteria and Bacteroidetes, which are abundant in CSFP and have the capacity to adhere to pyrite, thereby enhancing the separation of valuable minerals from impurities (Arias et al., 2023). Additionally, the use of rhamnolipid biosurfactants produced by *Pseudomonas aeruginosa* has been evaluated in copper ore flotation, demonstrating their impact on metallurgical response and surface activity. These biosurfactants have been found to enhance copper recovery while slightly decreasing copper grade, highlighting their potential as sustainable alternatives to traditional chemical reagents (Biniaz et al., 2023; Asgari et al., 2024).

Therefore, this study investigated the potential of *Citrobacter* sp. strain SKC-4 as a flotation bioreagent through a fundamental analysis of its interaction with chalcopyrite concentrate, a type of copper sulfide ore. Emphasizing the characterization of bacterial-mineral interactions, the research employed techniques such as FTIR, SEM-EDS (scanning electron microscopy-energy dispersive spectroscopy), and measurements of contact angle and surface tension. By highlighting the capabilities of *Citrobacter* sp. strain SKC-4 as a potential bioflotation reagent, this research advances the development of more sustainable and environmentally friendly practices in mineral processing.

2. Materials and methods

2.1. Materials

2.1.1. Chalcopyrite samples

The chalcopyrite concentrate used in this study was sourced from the flotation process at PT. Freeport Indonesia in Papua. X-ray powder diffraction (XRD) analysis identified chalcopyrite as the predominant mineral, with secondary inclusions of pyrite and silica (as illustrated in Fig. 1a). The size fraction selected for investigation was -200+325 mesh, as shown in Fig. 1b, with grain size separation performed using a Retsch AS 200 type sieve. X-ray fluorescence (XRF) analysis demonstrated the elemental composition of the chalcopyrite concentrate to consist of approximately 30% copper (Cu), 35.5% iron (Fe), and 15% sulfur (S), among other elements detailed in Table 1.

2.1.2. Bacterial strain and growth medium

This study utilized a novel bacterial strain, *Citrobacter* sp. strain SKC-4, known for its ability to oxidize iron and sulfur. This strain was originally isolated from Domas Crater, Tangkuban Perahu, Bandung, where it had adapted to high-sulfur environments. The bacterium is capable of synthesizing biosurfactants and is classified as Gram-negative, characterized by red-stained cell walls and an external membrane that provides environmental defense (see Fig. 2b). For the experiments, the bacterium was cultured in a modified Luria Broth (LB) medium, which consisted of 10 g/L peptone, 5 g/L yeast extract, 10 g/L NaCl, 0.25 g/L $FeSO_4 \cdot 7H_2O$, and 0.5 g/L $Na_2S_2O_3 \cdot 5H_2O$. The medium was sterilized using an autoclave (pressure of 1.02 atm, temperature of 121°C, for 20 minutes) to ensure the elimination of any contaminants. After cooling, the medium was inoculated with 5% v/v of the pure bacterial culture and incubated for 18 days under aerobic conditions on a rotary shaker at 150 rpm and room temperature (refer to Fig. 2a). The bacterial growth phase was monitored by measuring the turbidity of the culture using a Genesys 10S UV-vis spectrophotometer at a wavelength of 600 nm. A 1 mL sample was placed in a cuvette for absorbance readings, and the data were subsequently plotted (illustrated in Fig. 2a). The colony counting of Citrobacter sp. strain SKC-4 was determined using the pour plate method on LB agar media (depicted in Fig. 2c).

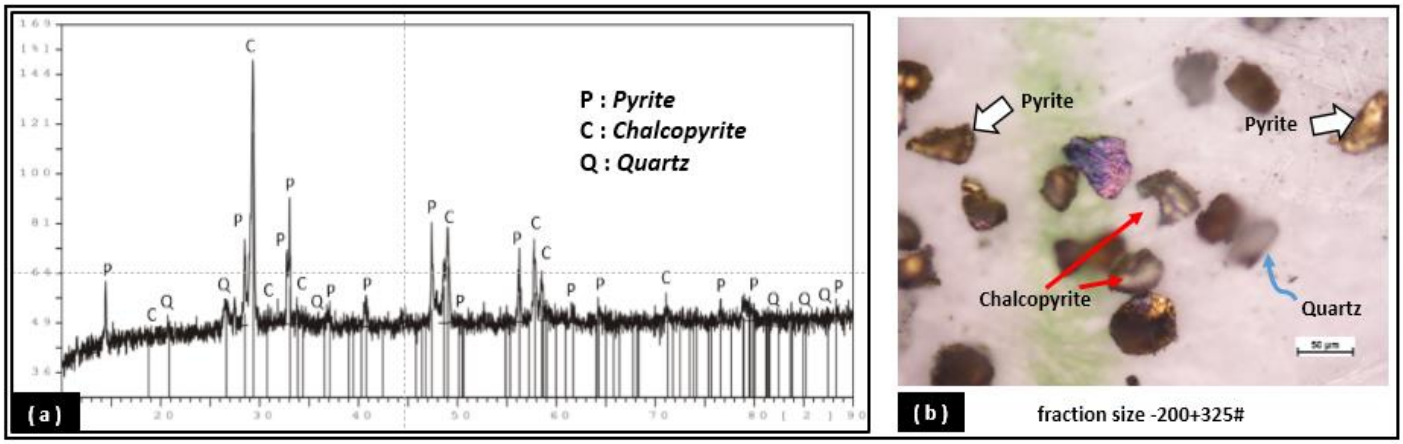


Fig. 1. XRD pattern of chalcopyrite (a); microscopic image of chalcopyrite concentrate with grain size of -200+325 mesh (b)

Table 1. Elemental composition of chalcopyrite used in this study, determined using X-ray fluorescence (XRF)

Element (wt, %)	Al	Si	S	K	Ca	V	Cr	Mn	Fe	Ni	Cu	Zn	Rb	Mo	La	Pb
Chalcopyrite ($CuFeS_2$)	1.00	3.50	15.00	1.20	0.72	0.02	0.07	0.03	35.30	0.10	30.00	0.81	0.26	11.00	0.20	0.66

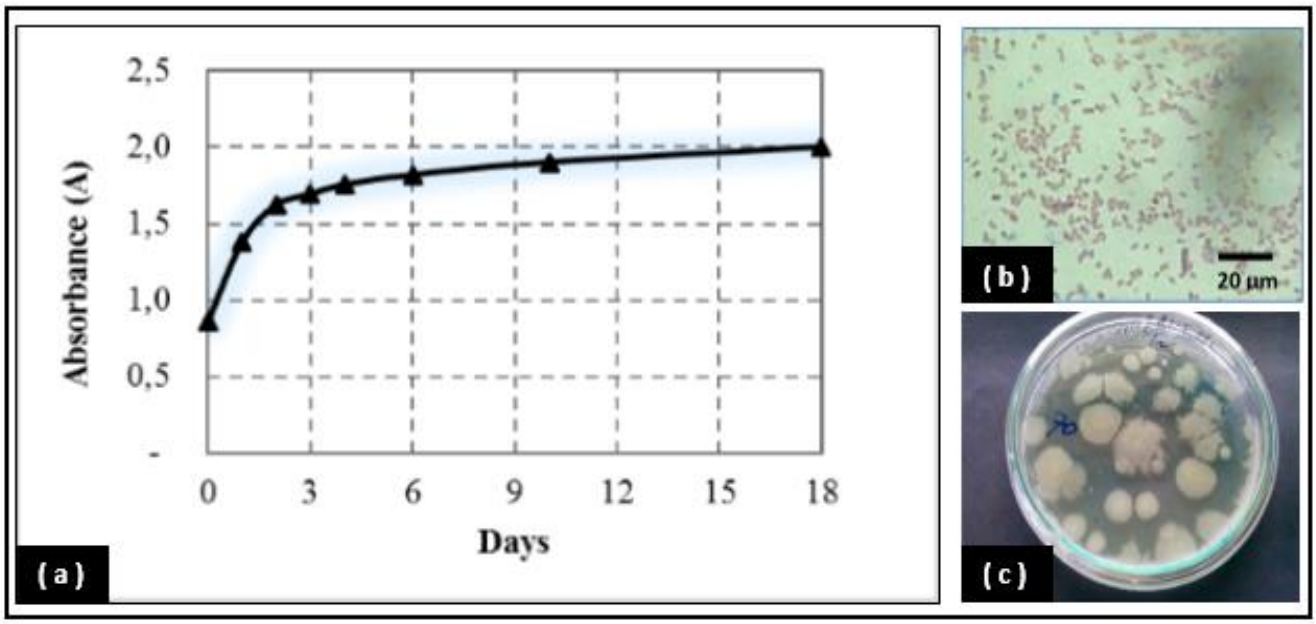


Fig. 2. Growth phase of *Citrobacter* sp. strain SKC-4 (a); Photomicrograph of *Citrobacter* sp. strain SKC-4 bacterial cells (b); Colony growth of *Citrobacter* sp. strain SKC-4 on LB Agar plates after 48 hours of incubation (c).

2.2. Methods

2.2.1. Experimental batch systems for bacteria-mineral interaction.

The interaction between bacteria and minerals was initiated once bacterial growth had reached 80% of the exponential phase. After harvesting, the spent growth medium containing bacterial cells and their metabolites was transferred into a 1-liter Erlenmeyer flask. This flask was filled with a 500-mL modified LB medium and chalcopyrite concentrate (-200+325 mesh) to facilitate batch experiments designed to investigate the bacteria-mineral interaction, as depicted in Fig. 3c. These experiments were conducted using a high solid concentration (25% w/v chalcopyrite concentrate) and 10% v/v of the previously mentioned spent growth medium. The culture underwent an adaptation process and was incubated for 30 days on a rotary shaker at 150 rpm, at room temperature, and under aerobic conditions. Sampling occurred periodically by collecting 20 mL of the suspension on days 0, 1, 2, 3, 4, 5, 6, 7, 14, 21, and 30 within a sterile environment. The samples were then subjected to a range of analytical tests. Additionally, the pH of the suspension was measured periodically using a Lutron type pH-208 digital pH meter.

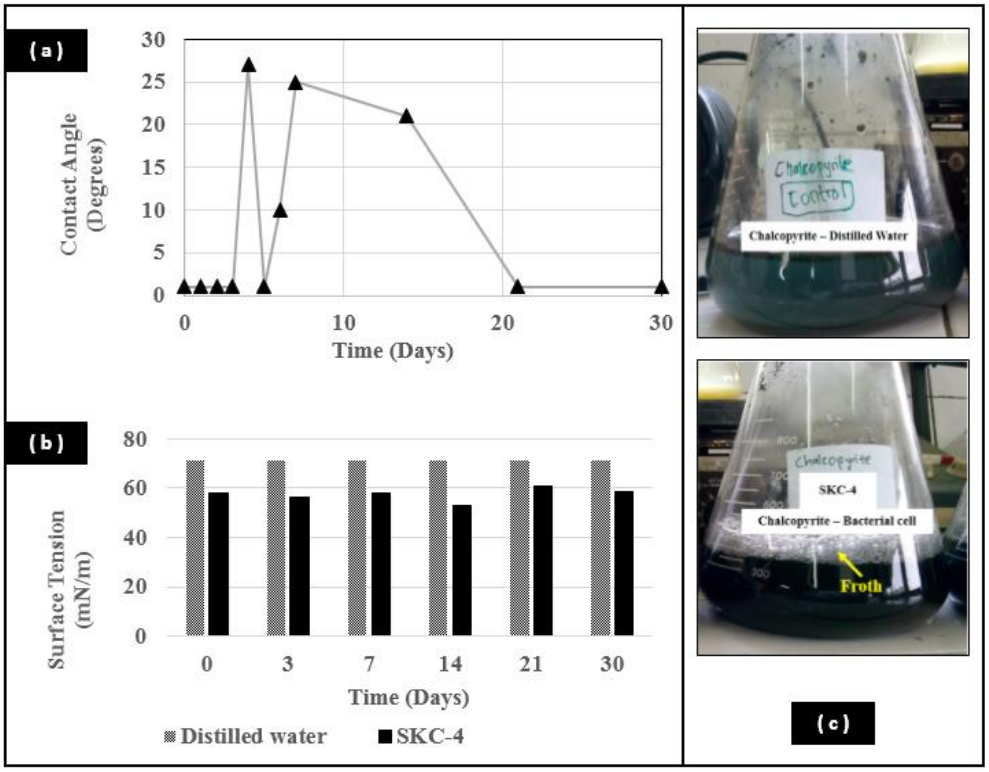


Fig. 3. Measurements of contact angle (°) in chalcopyrite concentrate after interaction with strain SKC-4 (a); Surface tension values (mN/m) during interaction (b); Appearance of froth in the solution of chalcopyrite concentrate interacting with strain SKC-4 in modified LB media (c).

2.2.2. Quantification of bacterial colonies (CFU, Colony-Forming Units)

The pour plate counting method was utilized on agar plates composed of modified LB media enhanced with 2% agar to quantify bacterial colonies. Initially, 1 mL of the bacterial inoculum was added to a test tube containing sterile 0.85% NaCl physiological solution, followed by vortexing for approximately one minute to achieve a homogeneous mixture. Next, 1 mL of this suspension was taken using a micropipette and subjected to serial dilution in test tubes containing 9 mL of the same physiological solution. From each serial dilution, 1 mL was transferred and gently poured onto pre-cooled agar plates (temperature maintained between 40-50°C) with a diameter of 9 cm (Fig. 2c). The bacterial suspension was then uniformly dispersed within the agar medium. These plates were subsequently incubated at room temperature for 48 hours to facilitate colony growth. To assess the impact of chalcopyrite minerals on bacterial growth, identical procedures were implemented, with the addition of minerals to the media. Bacterial colony quantification was conducted in triplicate, and the data presented are the average of these three measurements.

2.2.3. Assessment of enzymatic activity using Fluorescein Diacetate (FDA) assay

The enzymatic activity was evaluated using a modified version of the FDA assay method described by Green et al. (2006). This assay was performed at regular intervals over a 30-day period to monitor the interaction between bacteria and minerals. In brief, a 1 mL sample of the suspension was transferred into a 125 mL Erlenmeyer flask. To this, 50 mL of Phosphate Buffer Saline (PBS) and 0.50 mL of FDA substrate were added. The mixture was then incubated at 37°C for 60 minutes. Subsequently, 50 mL of acetone was added to halt the FDA hydrolysis process, and the mixture was thoroughly mixed before being filtered through filter paper. The resulting filtrate (10 mL) was centrifuged at 3000 rpm for 15 minutes. The clear filtrate was then placed into a cuvette, and the absorbance was measured using a Genesys 10S UV-Vis spectrophotometer at a wavelength of 490 nm.

2.2.4. Assessment of contact angles

Contact angles were determined using the drop-test method. This procedure involved applying 30 µL of a suspension, derived from the bacteria-mineral interaction, onto samples of mineral powder collected during the interaction process. To separate the solid and liquid phases resulting from this interaction, centrifugation was performed using an Eppendorf 5415 C at 12,000 rpm for 2 minutes. This step was crucial to ensure co-precipitation of bacterial cells with the mineral solids. The resulting contact angles were subsequently measured using AutoCAD software. Analyzing contact angles on powder substrates is instrumental in understanding bacterial adhesion to mineral surfaces and assessing changes in the hydrophobicity of the mineral. An increase in the contact angle suggests a rise in the hydrophobicity of the mineral surface.

2.2.5. Surface tension analysis of the suspension

The surface tension of the suspension, following interaction with minerals, was measured at defined intervals: incubation days 0, 3, 7, 14, 21, and 30. For each measurement, approximately 30 mL of the suspension was sampled. Prior to analysis, the homogenization was performed to ensure the suspension homogenization. The measurements were carried out using a K10ST Krüss Digital Ring Tensiometer, utilizing the Du Nouy ring method. This analysis was pivotal for detecting the presence of biosurfactants produced as a result of bacterial interaction, which potentially act as frothing agents and contribute to the stabilization of the solution's surface tension. Surface tension measurements were conducted at ambient room temperature. To ensure accuracy and reliability, the procedure was repeated three times for each sample, and the results were averaged.

2.2.6. Fourier Transform Infrared (FTIR) spectroscopic analysis

This analysis was conducted to identify both inorganic and organic chemical bondings in samples containing bacterial cells and chalcopyrite, as well as in the initial chalcopyrite sample. Initially, approximately 2 mg of dry solid powder sample was thoroughly blended with 200 mg of potassium bromide (KBr) to achieve a homogeneous mixture. This mixture was then compressed into pellets using a manual hand press. The pellets were analyzed using an FTIR spectrometer (FTIR Prestige 21, Shimadzu, Japan), which recorded the spectral data over a wavenumber range of 4000 to 400 cm^{-1}. FTIR sampling was conducted on days 0, 3, 7, 14, and 30 during the interaction of the bacteria with chalcopyrite minerals. The spectral results obtained were subsequently compared with the spectrum of the initial chalcopyrite to assess the chemical modifications resulting from the bacterial interaction.

2.2.7. Scanning electron microscopy (SEM) and energy-dispersive spectroscopy (EDS) analysis

To characterize the samples morphology, elemental composition, and structure, SEM-EDS analysis was conducted. Sample preparation involved preserving bacteria on the mineral surfaces using 2.5% glutaraldehyde, maintained at 4^0C for 24 hours. Post-fixation, the samples underwent rinsing with phosphate buffer solution, with vortexing and subsequent centrifugation at 12,000 rpm for 2 minutes, repeated twice to separate solids. A graduated dehydration process followed using acetone in increasing concentrations of 25%, 50%, 75%, and 100%, with each stage lasting 15 minutes. After drying, the samples were analyzed using a JEOL JSM-6510 (LA) SEM operated at an accelerating voltage between 10 kV and 15 kV (Chaerun et al., 2004).

3. Results and discussion

3.1. Analysis of suspension pH, bacterial proliferation, and enzymatic activity

The interaction with chalcopyrite concentrate resulted in noticeable pH shifts in the suspension, influenced by bacterial growth and metabolic activities (refer to Fig. 4a). Initially, the pH of the mixture comprising bacteria and chalcopyrite remained stable at 4.6 until day 14. Subsequently, a gradual increase in pH was observed, reaching a peak of 7.7, whereas the control system containing only distilled water and chalcopyrite maintained a constant pH of approximately 3. Prior to exposure to the mineral, the bacterium, having achieved 80% of its exponential growth phase, was quantified at a significant concentration of 6.5 x 10^{10} cfu/mL. Upon initial exposure to chalcopyrite concentrate, the bacteria exhibited a decrease in survival, with counts dropping to 10,800 cfu/mL. However, by the second day, the bacterial count rebounded to 60,500 cfu/mL, indicating that a subset of the bacterial population was able to survive and proliferate after prolonged interaction with the chalcopyrite concentrate.

The FDA assay was employed to evaluate the enzymatic activity of the bacterial strain SKC-4 during its interaction with chalcopyrite minerals. Initially, the FDA values were negative, suggesting a suppressed enzymatic response due to high bacterial mortality caused by concentrated chalcopyrite. However, from the second day, FDA values turned positive and remained elevated until day fourteen, indicating a resurgence in enzymatic activity. This pattern reversed, and FDA values declined to negative levels by the study's end, as shown in Fig. 4c. Although a significant number of bacteria perished upon initial exposure, a subset adapted, surviving in the high sulfur environment and synthesizing biosurfactants. This

adaptation facilitated the oxidation of iron and sulfur by the bacteria, enhancing their survival and contributing positively to the FDA values. The strain's ability to thrive in sulfur-rich conditions and produce biosurfactants supported this bacterial growth. The presence of SiO2 in the samples also aided bacterial proliferation. The subsequent decline in FDA values after day fourteen correlated with a reduction in viable bacteria. After twenty-one days, the interaction dynamics with chalcopyrite were primarily influenced by the biosurfactants. Overall, the FDA assay provided critical insights into microbial activity, highlighting its role in catalyzing the oxidation of metal sulfide ores, which is essential for understanding the interactions in this system (Tao and Dongwei, 2014).

This study suggests that even when bacterial growth declines, the production of the FDA enzyme can be attributed to mechanisms related to bacterial stress responses and metabolic regulation. Under conditions such as nutrient depletion or environmental stress, bacteria may shift their metabolic focus from growth to survival, involving the upregulation of enzymes linked to secondary metabolism. These enzymes, crucial for adaptation and survival, might be enhanced through regulatory pathways that maintain cellular functions under stress (Gonzalez and Aranda, 2023). For instance, FDA hydrolase may be upregulated as part of a stress response to prioritize essential metabolic processes. Factors such as pH and temperature might also influence enzyme activity, potentially increasing production to maintain metabolic balance during adverse conditions.

Furthermore, measuring Fluorescein Diacetate (FDA) enzymatic activity during bacterial interactions with minerals such as chalcopyrite provides critical insights into microbial ecology and biogeochemical processes. FDA hydrolysis is a well-established method to quantify total microbial activity, reflecting the collective enzymatic activity of viable cells and offering a direct indicator of their metabolic state and viability under mineral-induced stress conditions (Adam and Duncan, 2001; Patle et al., 2018). This method is particularly useful for assessing how bacterial communities respond and adapt to mineral presence, crucial for understanding processes such as bioleaching or bioremediation (Bararunyeretse et al., 2017; Sırt Çıplak and Akoğlu, 2020). Moreover, FDA enzymatic activity can indicate metabolic shifts in bacteria due to changes in nutrient availability or environmental stress, as it measures a broad range of hydrolytic enzymes, providing a comprehensive overview of microbial activity. This non-specific enzyme measurement is advantageous in complex environments where multiple enzymatic processes occur simultaneously (Szabó et al., 2022). The FDA assay is valued for its rapidity and sensitivity, enabling timely assessments of microbial activity in dynamic systems where conditions can quickly alter microbial behavior. By capturing transient changes in microbial activity, this method facilitates a deeper understanding of the functional state of bacterial communities and their interaction mechanisms with minerals (Szabó et al., 2022).

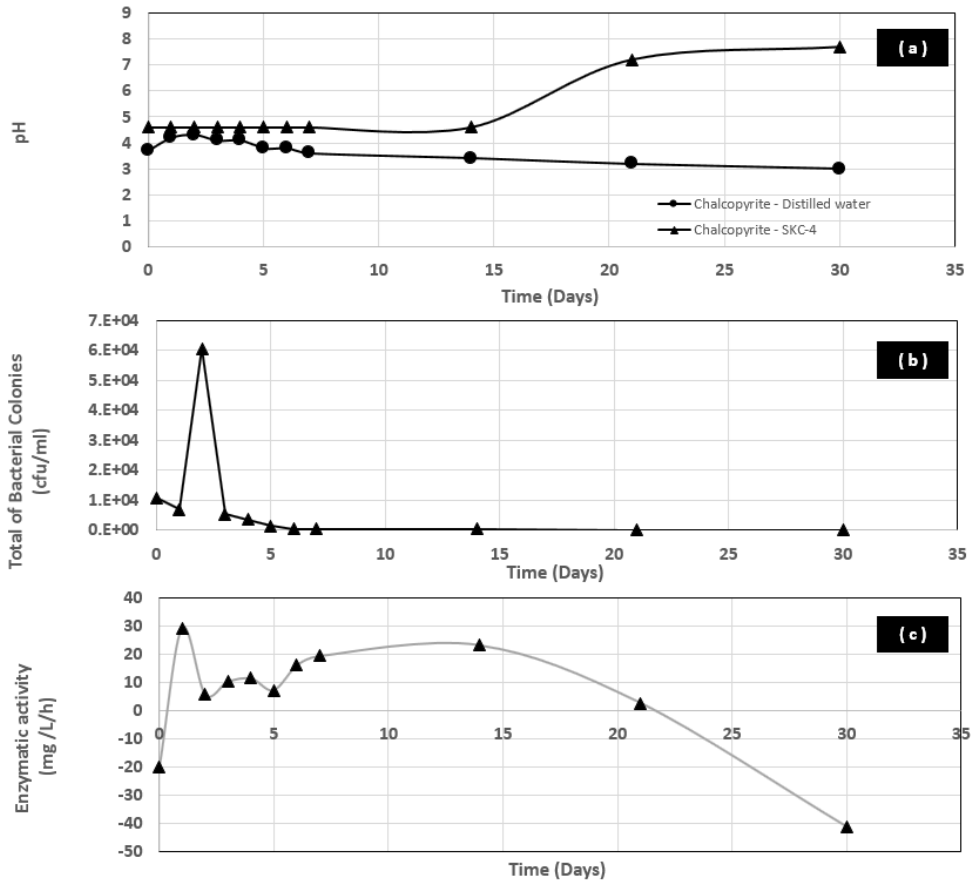


Fig. 4. pH levels of the SKC-4 bacterial interaction solution with chalcopyrite concentrate (a); Colony count of strain SKC-4 post-interaction with chalcopyrite concentrate (b); Fluorescein diacetate (FDA) enzymatic activity during interaction with chalcopyrite concentrate (c).

3.2. Contact angle and surface tension analysis

Contact angle measurements involving *Citrobacter* sp. strain SKC-4 and chalcopyrite concentrate from the initial interaction up to day three demonstrated a shift towards increased hydrophilicity, evidenced by a contact angle of 1°. By the fourteenth day, the mineral surface exhibited increased hydrophobicity with a contact angle of 27°, consistent with observations on days seven and fourteen. This shift is associated with the enzymatic activities of the

live bacteria, as depicted in Fig. 4c. Surface tension assessments are indicative of biosurfactant production, observed through froth formation in the bacterial-mineral interaction solution, as seen in Fig. 3c. These biosurfactants, potentially serving as frothing agents, significantly reduced the surface tension below that of distilled water (71 mN/m), reaching a minimum of 53 mN/m by day fourteen, with notable froth stability. Biosurfactants, primarily generated by bacterial activity, play a crucial role in facilitating interactions between bacteria and chalcopyrite, as noted by Ismail et al. (2021). Their capacity to decrease surface tension and enhance the bioavailability of hydrophobic substrates profoundly impacts bioflotation processes, according to Sharma et al. (2023). Additionally, biosurfactants are integral to biofilm formation, which promotes bacterial adhesion to mineral surfaces and optimizes bacterial interactions with chalcopyrite, as described by Safar et al. (2020).

During their interaction with chalcopyrite minerals, the bacterial strain SKC-4 synthesizes chemical compounds known as biosurfactants, which is evidenced by the observable froth formation depicted in Fig. 3c. These biosurfactants function by decreasing the surface tension of water through the disruption of hydrogen bonds at the water's surface. This reduction is facilitated by the amphiphilic nature of biosurfactants, which align their hydrophilic (polar) groups towards the water surface and hydrophobic (non-polar) groups away from it. Biosurfactants are categorized based on molecular weight into two types: low molecular weight biosurfactants, known for their efficiency in reducing surface tension, and high molecular weight biosurfactants, which are noted for their enhanced binding or adhesion properties to mineral surfaces. These compounds include various substances that can lower surface tension, such as foaming agents and alcohols, contributing significantly to their functional properties (Jahan et al., 2020).

3.3. Fourier transform infrared (FTIR) spectroscopic analysis

FTIR spectroscopy was utilized to analyze chalcopyrite concentrates before and after interaction with *Citrobacter* sp. strain SKC-4, revealing consistent peaks across various functional groups including OH (hydroxyl), NH (amines), CH, CO_2, C=O (carbonyl), carboxylic acids, CC, and polysaccharides. While the wavenumbers of these peaks remained stable, variations in their intensities were observed (Fig. 5). The analysis identified a C-H bond, indicative of fatty acids within bacterial cells, which contributed to the increased hydrophobicity of the mineral surface initially. By day 30, the more pronounced presence of OH and carboxylic acid groups at 3417 cm^{-1} and 1643 cm^{-1} respectively, suggested a shift towards increased hydrophilicity of the minerals. Additionally, spectral features between 2350-2363 cm^{-1} were consistent with CO_2 reactions, monosubstituted aromatic compounds, and Si-H bonds, indicating interactions between silica, bacterial cells, and biosurfactants. Throughout the interaction period, the presence of hydroxyl groups at 3417 cm^{-1} was consistently observed in all samples. The glycosidic bonding (C-O-C), typically seen around 1039 cm^{-1}, confirmed biosurfactant production. By the 30th day, a decrease in peak intensity suggested a reduction in viable bacteria. Prominent sharp peaks between 1023-1086 cm^{-1} characterized the interaction with chalcopyrite, and a distinctive peak at 795 cm^{-1} indicated a reaction involving iron oxide, primarily from the Fe element in FeS_2. These results support the bacterium's ability to oxidize iron and sulfur, emphasizing its potential utility in producing biosurfactants that could replace traditional collectors in bioflotation, enhancing the hydrophobicity of chalcopyrite minerals (Chaerun et al., 2020; Dhar et al., 2021).

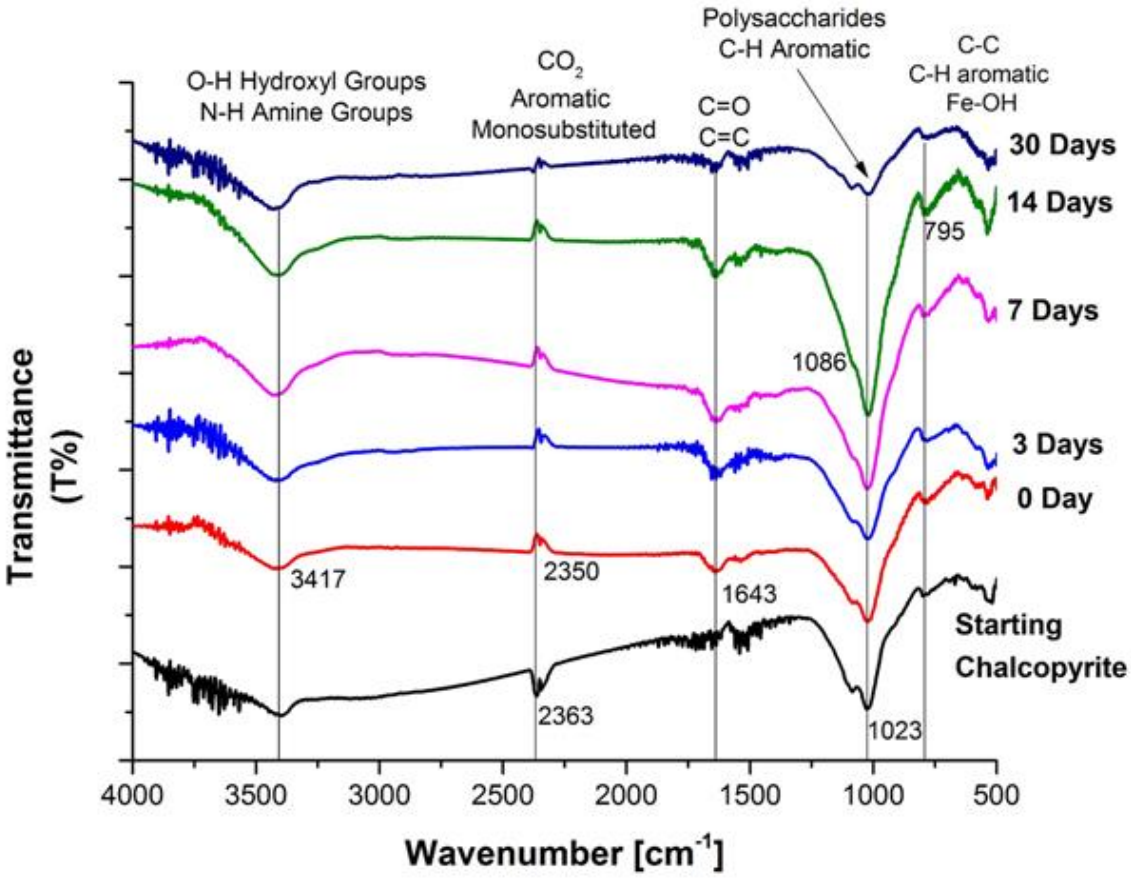
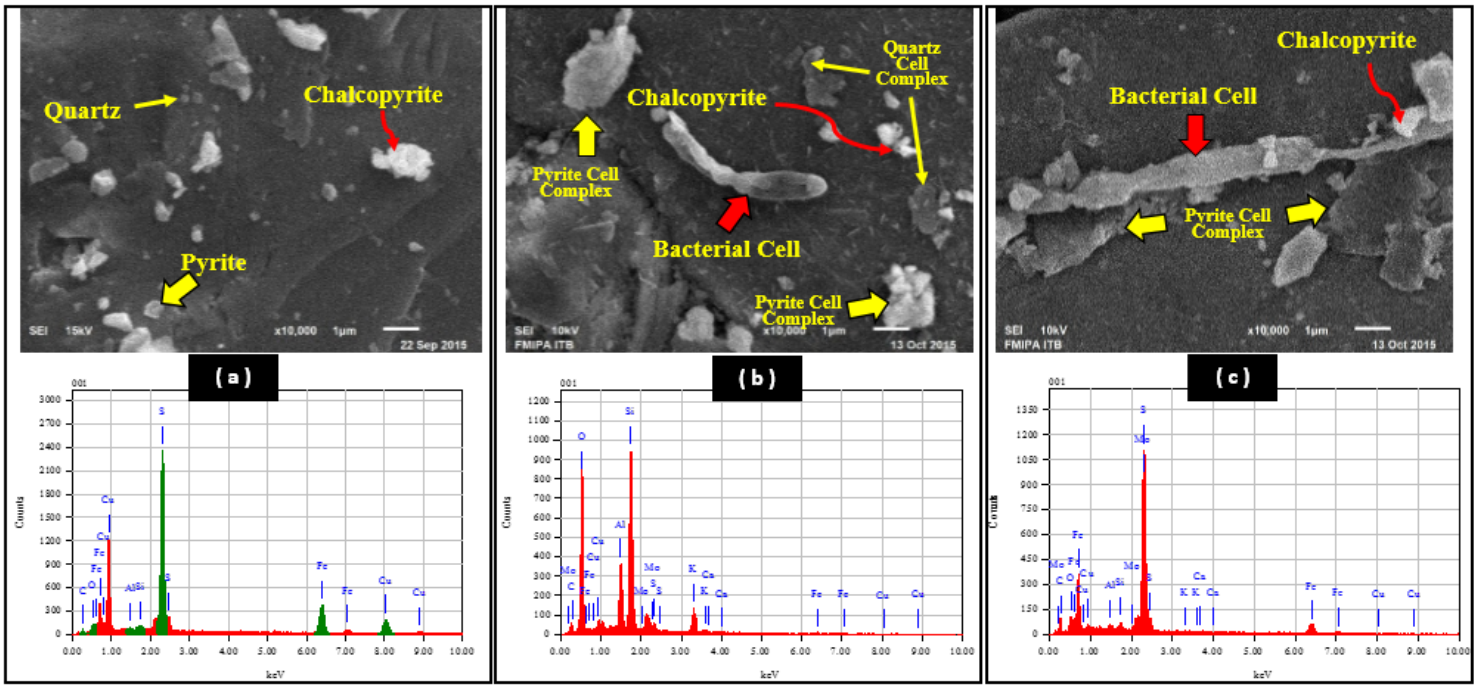


Fig. 5. FTIR spectra of chalcopyrite concentrate after interacting with the strain SKC-4 in modified LB media obtained after a 30-day incubation at 25°C on a rotary shaker set to 150 rpm.

3.4. Scanning Electron Microscopy (SEM) and Energy-Dispersive Spectroscopy (EDS) assessments

SEM-EDS analyses of chalcopyrite concentrates, conducted before and after interaction with bacteria (illustrated in Fig. 6a, 6b, and 6c), demonstrated that bacterial activity increased the porosity of the minerals and enhanced flocculation, facilitating more rapid sedimentation. A significant decrease in copper (Cu) content was observed in the chalcopyrite post-interaction on days 3 and 30. Specifically, Cu content decreased dramatically from 29.21% initially to 0.64% by day 3 and further to 0.51% by day 30. This reduction indicates that prolonged exposure to biosurfactants produced by the bacteria effectively leached copper from the chalcopyrite into the solution. Additionally, contact angle measurements confirmed that the biosurfactants generated by *Citrobacter* sp. strain SKC-4 increased the hydrophobicity of the chalcopyrite surface.

The chalcopyrite concentrate in this study primarily consists of the mineral chalcopyrite ($CuFeS_2$), accompanied by minor quantities of pyrite (FeS_2) and silica (SiO_2). The high copper content (29.21%) in the concentrate can be detrimental to bacterial survival, impacting their ability to adhere to chalcopyrite compared to pyrite and silica. This differential adhesion was apparent in SEM-EDS analyses conducted on day 3 (Fig. 6b), which revealed more pronounced bacterial adhesion to silica, resulting in the formation of a complex floc of silica and bacterial cells that settled rapidly. By day 30 (Fig. 6c), a similar floc formation was observed with pyrite, indicating significant bacterial adhesion. The success of the bioflotation process, aimed at selective mineral separation, heavily relies on the interaction between bacterial cells and mineral surfaces. The adsorption of bacteria on minerals is facilitated through electrostatic interactions and chemical bonds involving hydrocarbon chains and metal ions. This bacterial adhesion can create a biofilm on the mineral surfaces, modifying their surface characteristics and influencing the flotation process's effectiveness (Dunne, 2002).



Component	C	O	Al	Si	S	K	Ca	Fe	Cu	Total
Chaycopyrite	11.76	2.64	0.30	0.42	31.11	0	0	24.57	29.21	100
Chaycopyrite 3 –SKC-4	6.33	47.80	7.15	26.09	0.69	9.56	0	1.73	0.64	100
Chaycopyrite 30 –SKC-4	22.31	4.81	0.50	0.66	38.74	0.18	0.26	32.03	0.51	100

Fig. 6. SEM-EDS analysis of chalcopyrite concentrate prior to bacterial adaptation (a); SEM-EDS analysis of the interaction between the strain SKC-4 and chalcopyrite concentrate on day 3 (b); SEM-EDS analysis of the interaction between the strain SKC-4 and chalcopyrite concentrate on day 30 (c).

4. Conclusion

This study robustly demonstrates the potential of *Citrobacter* sp. strain SKC-4 as a viable bioflotation reagent for the processing of copper sulfide ores, presenting a sustainable alternative to conventional chemical reagents. The investigation highlighted the bacterium's ability to interact effectively with chalcopyrite concentrate, underlining its utility in enhancing the efficiency of copper extraction processes. Notably, the production of biosurfactants and the ability to oxidize iron and sulfur by *Citrobacter* sp. strain SKC-4 contribute to its functionality as a biocollector, biodepressant, and biofrother. These properties were substantiated through comprehensive experimental analyses, including FTIR and SEM-EDS, which confirmed the presence of functional groups essential for bioflotation processes and supported the observed reduction in surface tension in the bioflotation system.

Despite these promising findings, the study faces limitations, including the scale of the experiments which were confined to laboratory settings. The transition from laboratory-scale to industrial-scale application remains untested and presents a significant challenge due to the complexity of scaling biological processes. Furthermore, the long-term stability and consistency of the bacterial activity under varied mineral processing conditions have not been fully explored. Future research should focus on addressing these limitations by conducting pilot-scale studies to evaluate the performance of *Citrobacter* sp. strain SKC-4 in real-world mineral processing environments. Additionally, exploring the genetic and metabolic pathways responsible for the production of

biosurfactants and other useful compounds by this bacterium could lead to the enhancement of its bioflotation capabilities. Investigating the interaction of *Citrobacter* sp. strain SKC-4 with other types of sulfide minerals could broaden the applicability of this bioflotation approach, potentially revolutionizing the mineral processing industry by reducing its environmental footprint and enhancing the sustainability of resource extraction practices.

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Conflict of interest

The authors declare there is no conflict of interest in this research.

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