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2ND INTERNATIONAL SCIENCE,
ENGINEERING AND TECHNOLOGY
COLLOQUIUM



THE POWER OF SCIENTIFIC INNOVATION

INTELLIGENT SOLUTIONS FOR A
SUSTAINABLE WORLD

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PREFACE

Welcome to the 2nd International Science, Engineering and Technology Colloquium 2025 (ISETC 2025)!

It is with great pleasure that we present this year's colloquium, a distinguished gathering of scholars, researchers, and professionals dedicated to the advancement of applied sciences. Held virtually on 9th July 2025, ISETC 2025 serves as a platform to highlight cutting-edge research, foster meaningful networking, encourage collaborations, and explore innovative solutions to global challenges.

This year's theme, "The Power of Scientific Innovation: Intelligent Solutions for a Sustainable World" highlights our shared goal to solve global challenges through applied science. It celebrates innovation as a key to building a more sustainable and resilient future. We're proud to feature keynote speakers and presenters who bring fresh insights, bold ideas, and breakthroughs shaping tomorrow's world.

We would like to extend our heartfelt appreciation to the Faculty of Applied Sciences, Universiti Teknologi MARA, Perak Branch, Tapah Campus, for their outstanding support and assistance throughout the planning and execution of the event. Their dedication has been instrumental to the success of this colloquium. We would like to sincerely thank all participants and researchers for their valuable contributions. We also appreciate the reviewers for their careful evaluations and helpful feedback. Your hard work and support mean a lot to us.

As editors, we are proud to present this collection of proceedings. It is an honour to bring together the interesting and meaningful work of our speakers and authors. We hope the ideas shared will lead to new discoveries, encourage more research, and help solve global challenges through science and innovation.

Warm regards,

Dr. Emi Norzehan Mohamad Mahbob

Dr. Saiyidah Nafisah Hashim

Ms. Lili Widarti Zainuddin

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ISETC 2025 Proceedings Editors

REMOVAL OF IRON IMPURITIES FROM KAOLIN USING *Bacillus cereus* SIDEROPHORES

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Abstract: The presence of iron impurities can affect the quality of kaolin, influencing its whiteness, morphology, and its refractory properties. Conventional methods such as physical and chemical processes for iron removal from kaolin have a negative impact on the environment. Therefore, this study emphasized an environmentally friendly approach using siderophores produced by *Bacillus cereus* to effectively remove iron from kaolin. The research focused on the extraction and purification of siderophores from *B. cereus* UKMCC1150 under optimal conditions. The effectiveness of siderophores in eliminating iron impurities from kaolin is then determined. The research methodology included the extraction of siderophores and cell-free supernatant, partial purification of siderophores, and bioleaching of kaolin using cell-free supernatant and partially purified siderophores. Subsequently, the whiteness index of kaolin samples is analyzed, and the percentage of iron removal is determined based on EDX analysis. The siderophore assay results demonstrated that *B. cereus* can produce a high quantity of siderophores, approximately $92.42 \pm 0.02\%$ siderophore units. The presence of catecholate-type siderophores is identified through the formation of a peak at 420 nm in the FeCl_3 assay. Furthermore, kaolin treated with partially purified siderophores shown an increased whiteness index by $2.11 \pm 0.01\%$, highlighting the potential of siderophores in enhancing the quality of kaolin. EDX analysis indicated the removal efficiency of Fe(III), where kaolin treated with partially purified siderophores has a higher removal rate compared to kaolin treated with cell-free supernatant ($79.17 \pm 0.02\%$ and $20.83 \pm 0.01\%$). SEM analysis revealed significant changes in the surface morphology of kaolin after bioleaching. Overall, this study demonstrated the ability of *B. cereus* to produce siderophores with characteristics that have the potential to improve whiteness and effectively remove iron impurities from kaolin.

Keywords: *Bacillus cereus*, siderophore, iron, kaolin, bioleaching

INTRODUCTION

Kaolin, primarily composed of the mineral kaolinite ($\text{Al}_2\text{Si}_2\text{O}_5(\text{OH})_4$), is widely utilized in various industries due to its inherent properties, including natural whiteness. However, raw kaolin often contains impurities, such as iron oxides. The presence of these iron impurities significantly negatively impacts the quality of unprocessed kaolin, diminishing its whiteness index, altering its morphology, and affecting its refractory properties. This discoloration limits kaolin's applicability in numerous industrial and pharmaceutical sectors (Jing et al., 2021, Thirumalai et al., 2020). Traditional purification methods, such as physical techniques like grinding and magnetic separation, can remove coarser impurities but are ineffective at removing fine colour-imparting particles like iron and titanium oxides. Conventional leaching methods also demand high energy and cost. Chemical methods, including oxidation-reduction and acid dissolution, while more effective for iron removal, involve potentially hazardous reagents and can reduce beneficial organic content (Cuadros, 2017).

A more promising and environmentally friendly alternative is biotechnology, specifically utilizing microorganisms for biological leaching to remove iron ions. This bioprocess can involve microbial reduction of Fe(III) to Fe(II). More efficiently, the iron impurity, particularly Fe(III), can be removed by siderophores, specialized low molecular weight compounds (500-1500 Da) synthesized by microorganisms, particularly under iron-deficient conditions. Siderophores act as iron chelating agents with a high affinity for ferric oxide (Fe(III)) (Ferreira et al. 2019). The *Bacillus* genus is

well-suited for this purpose as its species are capable of producing siderophores in high quantities (Maleki et al., 2018). While siderophore production varies among strains and is influenced by factors, *Bacillus cereus* was selected based on existing research, which demonstrated its ability in reducing Fe(III) oxide impurities in kaolin on a larger scale compared to other *Bacillus* species (Yong et al., 2023).

Therefore, this study aimed to explore the use of siderophores produced by *Bacillus cereus* UKMCC1150 for effective and environmentally friendly removal method of iron from kaolin. The specific objectives were to extract and partially purify siderophores from *B. cereus* cultured under optimum conditions, examine the effectiveness of these siderophores in removing iron impurities from kaolin, and evaluate the impacts of bioleaching process with siderophores on the whiteness index, iron content, and morphology of kaolin.

MATERIALS AND METHODS

Preparation of Cell-free Supernatant

An overnight bacterial culture was prepared by inoculating a bacterial colony from *B. cereus* UKMCC1150 agar plate into LB broth and incubated at 37°C for 16 hours. The overnight bacterial culture was then subcultured into fresh LB broth at 1:100 ratio and incubated at 30°C at 250 rpm for 48 hours. After incubation, the bacterial culture was centrifuged, and the cell-free supernatant was collected and stored at 4°C.

Partial Purification of Siderophore

Siderophores were extracted from cell-free supernatant using the phenol-chloroform method (Meyer, 1978). Briefly, 0.5 g/L FeCl₃ was first added to stabilize the siderophore. Then, the supernatant was mixed with an equal volume of 1:1 phenol-chloroform solution. This mixture was then centrifuged, and the collected organic layer was further extracted with 2 volumes of ethyl acetate and half a volume of deionized water. The aqueous phase was then collected and dried at 50°C. The dried powder was dissolved in acidic water (pH 4) and added to chloroform and 3% 8-hydroxyquinoline. The aqueous layer was collected and freeze-dried. Freeze dried siderophores were then dissolved in deionized water and stored at 4°C.

Determination of Siderophore Production

Siderophore production was determined using the CAS liquid assay (Lee et al., 2022). Briefly, 0.5 mL of sample was mixed with 0.5 mL of CAS mixture solution and vortexed. The mixture was left for 3 hours in the dark at room temperature. Absorbance at 630 nm was measured. The calculation of siderophore production (% siderophore units) was performed using the formula: $[(A_r - A_s) / A_r] \times 100$ % unit, where A_s is the absorbance of the sample mixture and A_r is the reference absorbance. The assay was performed in triplicates.

Bioleaching of Kaolin

A total of 100 mL of cell-free supernatant was added to flasks containing 10 g of autoclaved kaolin. For bioleaching with partially purified siderophore, 4 mL of siderophore solution and 96 mL of deionized water was added to flasks containing 10 g of autoclaved kaolin. The flasks were incubated for 5 days at 30°C and 250 rpm. After 5 days of incubation, the kaolin samples were filtered and dried at 80°C for 24 hours.

Analysis of Treated Kaolin

Kaolin samples were analyzed using a Chroma Meter (Minolta, CR-400) before and after the leaching process. Color properties were described using L*, a*, and b* parameters, where L* represents lightness, a* is the red-green axis, and b* is the yellow-blue axis. The whiteness index was calculated using the Stensby equation based on the CIELAB parameters (Yong et al. 2023). Each sample was analyzed three times.

Purified and raw kaolin samples were analyzed using a Scanning Electron Microscope (SEM) to observe particle morphology and an Energy-dispersive X-ray (EDX) system to determine chemical composition, specifically the

percentage of Fe(III). The Fe(III) removal rate was calculated using the formula: $((X_i - X_f) / X_i) \times 100 \%$, where X_i and X_f are the initial and final weight percentages of Fe obtained from EDX analysis.

RESULTS AND DISCUSSION

Bacillus cereus UKMCC1150 was shown to effectively produced siderophores, with a high percentage of siderophore units detected: $92.42 \pm 0.02\%$ before purification and $84.05 \pm 0.01\%$ after partial purification. This production was higher than the positive control ($81.82 \pm 0.01\%$), indicating *B. cereus*'s strong capability for siderophore synthesis in LB broth. Analysis using the FeCl_3 assay confirmed the presence of catecholate-type siderophores in *B. cereus*, identified by a peak in the 400-420 nm wavelength range (Figure 1). Siderophores are known to be low molecular weight compounds (500-1500 Da) that act as potent iron chelating agents with a high affinity for Fe(III). Microorganisms, like *B. cereus*, synthesize siderophores, especially under iron-deficient conditions, as a strategy to acquire iron from their environment (Ferreira et al., 2019).

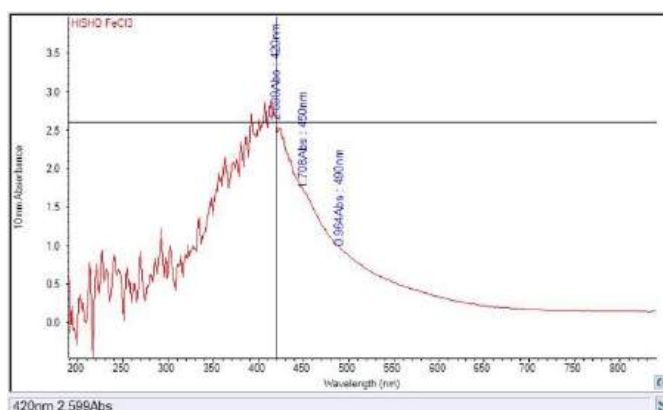


Figure 1. The presence of catecholate-type siderophore in *B. cereus*

When kaolin samples were treated with the partially purified siderophores from *B. cereus*, there was a noticeable impact on the kaolin's whiteness index. Kaolin treated with partially purified siderophores showed an increase in the whiteness index by $2.11 \pm 0.01\%$, rising from $52.78 \pm 0.01\%$ (untreated) to $54.89 \pm 0.05\%$. This highlights the potential of these siderophores to enhance the quality of kaolin in terms of its colour properties. While this increase was observed, it was less significant compared to some previous studies (Yong et al., 2023; Zegeye et al., 2013).

Crucially, the analysis of chemical composition using EDX demonstrated the effectiveness of the siderophores in removing iron impurities. The initial weight percentage of Fe(III) in untreated kaolin was $2.4 \pm 1.6\%$ (Table 1). After treatment with partially purified siderophores, the Fe(III) content significantly decreased to $0.5 \pm 0.8\%$, resulting in a high Fe(III) removal rate of $79.17 \pm 0.02\%$. This removal rate was substantially higher than that achieved using cell-free supernatant ($20.83 \pm 0.01\%$), with Fe content reduced to $1.9 \pm 1.1\%$. This confirms that the partially purified siderophores have a higher affinity for iron compared to the cell-free supernatant and are more effective in removing Fe(III) impurities from kaolin. This aligns with the understanding that siderophores specifically chelate Fe(III).

Table 1. EDX analysis for Fe composition in kaolin

Sample	Fe (Wt%)	Fe(III) removal rate %
Untreated kaolin	2.4 ± 1.6	
Kaolin + cell-free supernatant	1.9 ± 1.1	20.83 ± 0.01
Kaolin + partially purified siderophore	0.5 ± 0.8	79.17 ± 0.02

In the SEM analysis, untreated kaolin was seen as particles with varying sizes and thicknesses, smooth edges, and a regular, fixed shape (Figure 2). Kaolin treated with cell-free supernatant from *B. cereus* exhibited some changes in morphology, including non-uniform size and sharp edges, suggesting damage. However, these changes were described as less distinct compared to the siderophore treatment. In contrast, kaolin treated with partially purified siderophores displayed significant changes to the surface morphology. The images revealed damage and showed that some particles appeared fractured or broken apart after being leached for 5 days at 30°C. These particles looked fragmented and irregular, and these effects were more evident than those seen with the cell-free supernatant. Overall, the SEM analysis confirmed visually that both the cell-free supernatant and, more notably, the partially purified siderophores interacted with the kaolin particles, altering their surface morphology and structure.

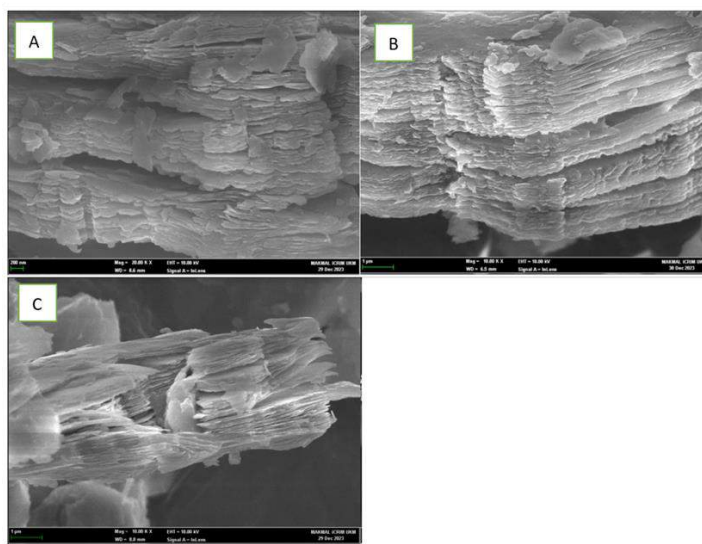


Figure 2. SEM figures of kaolin particles under 1000x magnifications. (a) Untreated kaolin, (b) kaolin + cell-free supernatant, and (c) kaolin + partially purified siderophore

CONCLUSIONS

Raw kaolin's quality is negatively impacted by iron impurities, limiting its use in industries and pharmaceuticals due to reduced whiteness and altered properties. This study investigated an environmentally friendly biological approach using siderophores produced by *Bacillus cereus* UKMCC1150 to address this. *B. cereus* demonstrated a high capacity for siderophore production, and these were identified as the catecholate-type. Treatment of kaolin with partially purified siderophores led to a slight increase in the kaolin's whiteness index. Analysis showed a significant reduction in Fe(III) content, with a high removal rate achieved using partially purified siderophores. Overall, this research highlights the potential of *Bacillus cereus* siderophores for effectively improving kaolin quality by enhancing whiteness and significantly removing iron impurities.

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Sekian, terima kasih.

"BERKHIDMAT UNTUK NEGARA"

Saya yang menjalankan amanah,

Setuju.

27.1.2023

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