GREEN SYNTHESIS OF IRON NANOPARTICLES USING CRUDE SURFACTIN FROM CASSAVA WASTEWATER FERMENTATION AS A REDUCING AND STABILIZING AGENT

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ABSTRACT

This study explores the eco-friendly synthesis of iron nanoparticles using crude surfactin derived from the fermentation of cassava wastewater. By optimizing parameters such as pH and iron precursor concentration, we produced spherical nanoparticles approximately 5 nm in size, stabilized by biomolecules present in the fermented broth. This sustainable method shows potential for biomedical and environmental applications, highlighting the need for future investigations into these nanoparticles' stability, magnetism, and additional biological activities.

Keywords: Surfactin 1. Biosynthesis 2. *Bacillus subtilis* 3. Iron oxide 4. Metallic nanoparticle 5.

1 INTRODUCTION

Iron nanoparticles possess remarkable physicochemical properties such as large surface area-to-volume ratios, distinctive optical and electronic characteristics, magnetism, and biological activity, such as antimicrobial, anticancer, and good biocompatibility. These properties are highly valuable in biomedical and environmental applications [1]. Traditional synthesis methods, which involve chemical reduction, often require hazardous chemicals and produce nanoparticles with inconsistent shapes and sizes, posing environmental concerns [2]. In contrast, biosynthesis has emerged as a sustainable alternative, utilizing bio-sources like surfactin, which act as both reducing and stabilizing agents [3,4].

The synthesis of iron nanoparticles using bio-sources offers an eco-friendly solution. Still, challenges remain, such as the high cost of surfactin due to expensive culture media and purification processes. This study addresses these challenges by using fermented cassava wastewater (CW) broth, rich in surfactin, as a dual-function agent for synthesizing iron nanoparticles. The research focuses on optimizing parameters like pH and iron precursor concentration to enhance the efficiency and cost-effectiveness of the process. This approach aims to advance sustainable production methods, facilitating broader applications of iron nanoparticles in various domains.

2 MATERIAL & METHODS

The CW culture medium was inoculated with a standardized culture of *Bacillus subtilis* ATCC 6633 at a concentration of 7% v/v and incubated at 150 rpm and 30°C for 72 hours [5]. Post-fermentation, the broth was centrifuged to remove cell biomass, and the resulting supernatant, called crude surfactin, was collected for nanoparticle synthesis. The crude surfactin was characterized for its surfactin content using high-performance liquid chromatography (HPLC) coupled with a mass spectrometer (LCMS-2020, Shimadzu Corp., Japan) and a photodiode array (PDA) detector, with a Kromasil® C18 column (100A, 300 mm x 4.6 mm i.d.),. Its protein content was determined using the Bradford method. For the nanoparticle synthesis, the crude surfactin was mixed 1:1 with a solution of $FeCl_2$. Three different concentrations of $FeCl_2$ were tested at three varying pH levels (as outlined in Table 1). The synthesis process was carried out over 24 hours under agitation at 150 rpm. After 24 hours, the samples were analyzed using transmission electron microscopy (TEM) and dynamic light scattering (DLS) to assess the synthesized nanoparticles.

Table 1 Experimental design of the FeNPs synthesis

Sample	$FeCl_2$ (mM)	pH
1	2.5	4
2	2.5	7
3	2.5	9
4	5	4
5	5	7
6	5	9
7	10	4
8	10	7
9	10	9

3 RESULTS & DISCUSSION

The fermentation of cassava wastewater by *Bacillus subtilis* produced 451,4 ± 17,6 mg L^{-1} of surfactin. Protein characterization of crude surfactin showed a concentration of 174 mg L^{-1} of total protein. The translucent yellow solution of $FeCl_2$ with crude surfactin turned reddish brown at the end of 24 h of incubation. Figure 1 shows the absorbance spectra between 300 and 700 nm for each tested condition in Table 1. Studies have reported that the absorption curves of FeNPs showed intense peaks in the 320–540 nm range, determined by the type of electronic transitions of iron ions [6]. Three main peaks can be identified in the spectra (marked in pink as areas 1, 2, and 3). The first peak may be related to proteins and the crude surfactin in the broth, which commonly absorb light in this region. The second absorbance peak was determined between 400 and 450, which may indicate the formation of Fe_3O_4 nanoparticles [7]. The third peak, exhibiting maximum absorbance at approximately 510 nm, corresponds to the surface plasmon resonance band of hematite nanoparticles (Fe_2O_3) [6]. It can be suggested that the synthesized nanoparticles are a mixture of iron oxides; however, a more detailed investigation is required to confirm this composition.

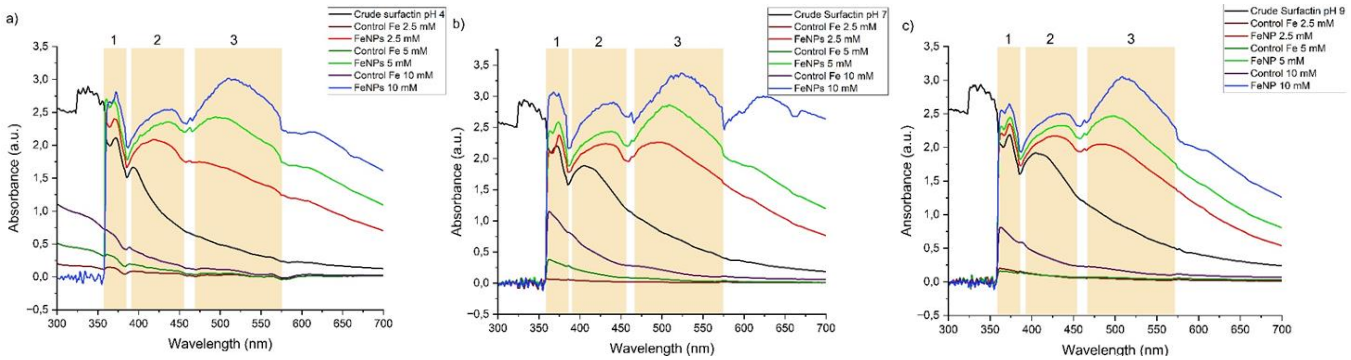

Figure 1 UV-visible spectra (300 – 700 nm range) of crude surfactin, iron precursor and FeNPs in pH 4 (a), 7 (b), and 9 (c)

The tested pH levels produced similar UV-Vis spectrum responses (Figure 1). Among them, pH 7 resulted in the highest absorbance and was therefore selected for further investigation of particle size and stability using TEM and DLS. DLS analysis (Table 2) reveals particles with a hydrodynamic size between 366.6 and 469.2, smaller than the particle sizes found in samples of just surfactin or the iron precursor at pH 7. This suggests that the precursor and surfactin interacted to form smaller particles.

Table 2 Synthesis conditions and their respective hydrodynamic size and PDI

Sample	$FeCl_2$ (mM)	pH	Hydrodynamic Size (nm)	PDI
2	2.5	7	469.2	0.577
5	5	7	366.6	0.315
8	10	7	375.7	0.371
Crude surfactin	0	7	1702.0	1.000
Iron precursor	10	7	841.2	0.745

The TEM images, as shown in Figure 3, provided an insight into the morphology of the nanoparticles. These images revealed that the nanoparticles exhibited a spherical shape and ranged in size from 5.8 nm to 60.5 nm. Additionally, the formation of nanoparticle aggregates, or clusters, was observed. It was found that the actual size of the nanoparticles is slightly smaller than the size measured by DLS. This is because DLS measures the hydrodynamic diameter, which includes surface-bound molecules and the adsorbed water layer and can interpret clusters as a single particle [3].

Among the three concentrations of iron precursor tested, the lowest concentration produced the smallest nanoparticles. The presence of surfactin and proteins stabilizes the nanoparticles, forming a corona that prevents their growth and aggregation [4]. With a lower concentration of the iron precursor, relatively more biomolecules are available to cover the nanoparticles, forming smaller nanoparticles.

The synthesis of iron nanoparticles using crude surfactin is a promising method due to its eco-friendly nature, eliminating the need for high temperatures, pressure, and toxic chemical reagents. Moreover, utilizing surfactin in its raw form bypasses the costs associated with purification. Biosynthesis has also been shown to produce nanoparticles with valuable biological activities, such as antibacterial properties and enhanced biocompatibility, which are crucial for biomedical applications. Future research should focus on examining other properties of these nanoparticles, including stability, magnetism, and potential biological activities, to further explore their application potential.

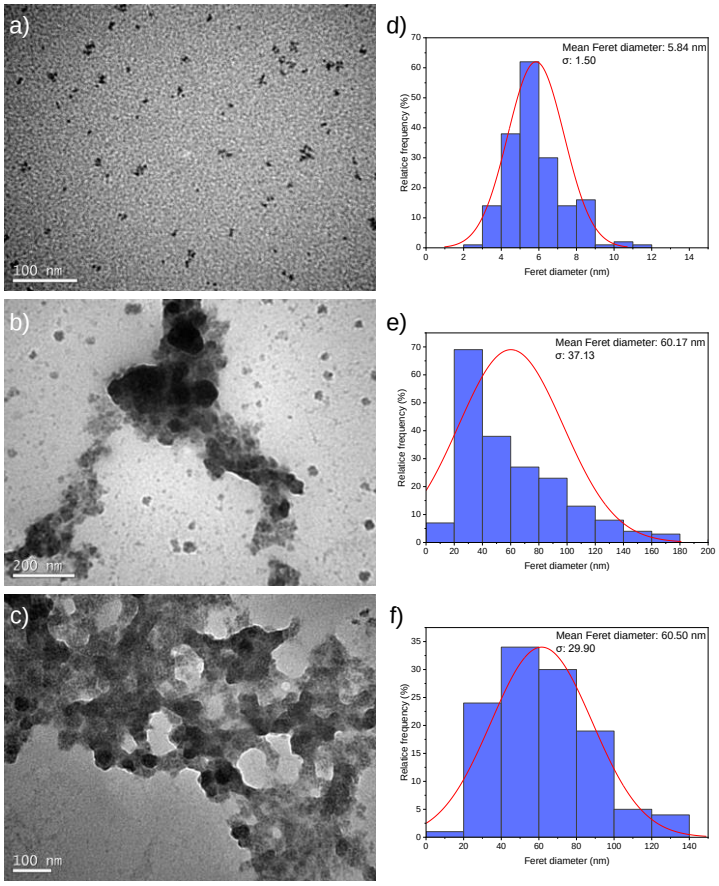

Figure 2 TEM images of the nanoparticles synthesized at 2.5 mM (a), 5 mM (b), and 10 mM (c) of $FeCl_2$. The relative frequency distribution of the Feret diameter for nanoparticles at 2.5 mM (d), 5 mM (e), and 10 mM (f) of $FeCl_2$

4 CONCLUSION

This study successfully demonstrated using fermented cassava wastewater broth, rich in surfactin and proteins, as a dual-function agent for nanoparticle synthesis. By optimizing key parameters such as pH and iron precursor concentration, we successfully produced spherical nanoparticles with sizes around 5 nm. The hydrodynamic size measured was larger than the actual size, indicating, along with TEM images, that the nanoparticles were stabilized by biomolecules present in the fermented broth, such as surfactin and proteins. Future research should delve deeper into the properties of these iron nanoparticles, focusing on their stability, magnetism, and additional biological activities. Such investigations will help to fully understand and maximize their potential applications across various domains, ultimately contributing to the advancement of sustainable nanoparticle production methods.

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