Antibacterial Activity of Fe_3O_4-NPs/H_2O_2 Combination: A Promising Approach for Efficient Bacterial

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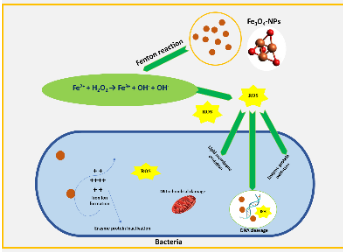

The study explored the catalytic role of ferromagnetic nanoparticles (Fe_3O_4-NPs) in generating reactive oxygen species (ROS) with H_2O_2. It aimed to assess biocidal efficacy against four bacteria strains. Fe_3O_4-NPs synthesis involved a straightforward method ensuring uniform size and morphology. Optimal ROS synthesis conditions were determined through a 4-factor Box-Behnken design, yielding pH 6, 42 ºC temperature, and specific Fe_3O_4-NPs/H_2O_2 concentrations (160 µg/mL/0.136 µg/mL). The Fe_3O_4-NPs/H_2O_2 combination induced ROS production, damaging bacterial cells via oxidative stress, showcasing potential for pathogen control and combating antibiotic resistance.

Introduction

The emergence of antibiotic-resistant bacteria poses a global health threat, necessitating innovative strategies to combat infections **[1]**. The WHO's research agenda on antimicrobial resistance (AMR) outlines critical priorities to address by 2030, aligning with CDC reports on antibiotic-resistant infections causing significant mortality **[2]**. Traditional antibiotics' declining efficacy underscores the urgency for novel antibacterial approaches. Iron oxide magnetic nanoparticles (Fe_3O_4-NPs) have emerged as promising antibacterial agents due to their unique properties and catalytic activity **[3]**. Their application in the Fenton-like reaction, generating reactive oxygen species (ROS) in the presence of H_2O_2, demonstrates potent antibacterial effects by inducing oxidative stress in bacteria **[4]**. This study investigates Fe_3O_4-NPs/H_2O_2 antibacterial activity, focusing on structural characteristics and ROS-mediated bacterial inactivation mechanisms.

Material and Methods

The study employed a rotatable central composite design (RCCD) to investigate Fe_3O_4-NPs' catalytic ROS production with H_2O_2 and their biocidal potential, analyzing factors like Fe_3O_4-NPs and H_2O_2 concentrations. A subsequent Box-Behnken design introduced additional factors (temperature, pH) to optimize ROS synthesis, evaluating Fe_3O_4-NPs and H_2O_2 concentrations, with tests conducted in microplates using chromogenic substrate TMB to identify the Fenton reaction in the presence of ROS. Absorbance measurements at specific wavelengths and stability checks were performed, employing response surface methodology for data analysis.

Results and Discussion

Figure 1 depicts the response surfaces generated through response surface methodology for these designs. In Figure 5A, the response surface for one bacterium tested in the initial experiment is displayed, aiming to investigate the occurrence of the Fenton reaction without prior optimization of variables like pH and temperature. The absence of interaction between Fe_3O_4-NPs and H_2O_2 indicates that the biocidal effect was solely due to H_2O_2, suggesting no Fenton reaction took place, leading to a lack of ROS generation with potential biocidal effects. The experimental data aligned well with a quadratic equation, where Y represents inhibition (1) or growth (0) of the microorganism tested, showcasing the predominant influence of factor A (H_2O_2) supported by statistical values like R2, Std. Dev., and C.V.

In the second experimental design, the focus shifted to determining optimal operating conditions for pH, temperature, Fe_3O_4-NPs concentration, and H_2O_2 concentration in the Fenton reaction. Figure 2 displays the UV-vis spectrum from preliminary tests confirming ROS production through reagent reactions. Characteristic peaks at 450 nm (cationic radical presence) and 370/650 nm (charge transfer complex) indicated successful reactions. Absorbance readings at 370 nm were most intense and stable, leading to a quadratic model fitting where Y represents absorbance values. Statistical parameters like R2, C.V., and Std. Dev. were significant, supporting the model's validity. Notably, quadratic parameters had no impact on the process.

The response surface in Figure 1B post fitting experimental data at 370 nm under specific pH and temperature conditions highlighted a significant interaction between Fe_3O_4-NPs and H_2O_2, favoring high nanoparticle concentrations and low H_2O_2 concentrations for optimal conditions. Additionally, Figure 1C illustrated a perturbation diagram showcasing the influence of all factors in ROS synthesis under constant pH and temperature conditions, emphasizing the importance of Fe_3O_4-NPs concentration followed by pH and temperature in influencing the process.

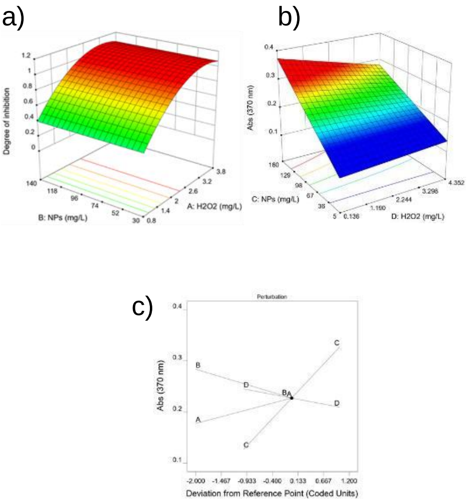

Figure 1. a) Response surface obtained from the adjustment of the experimental data of a Rotatable Central Composite Design with 2 factors in which the biocidal effect of the interaction between H_2O_2 (A) and Fe_3O_4-NPs (B) is evaluated for the case of *P. fluorescens*. b) Response surface obtained from the experimental data of a 4-factor Box-Behnken Design in which the capacity of H_2O_2 (D) and Fe_3O_4-NPs (C) to synthesize reactive oxygen species (ROS) is evaluated. The diagram shows the absorbance at the wavelength (370 nm) at which the charge transfer complexes characteristic of ROS are detected for experimental conditions of 42 ºC and pH 6. c) Perturbation diagram in which the influence of the different factors is represented by setting the values of factors A and B at T: 42 ºC and pH 6 respectively and where C and D represent the concentrations in mg/L of Fe_3O_4-NPs and H_2O_2.

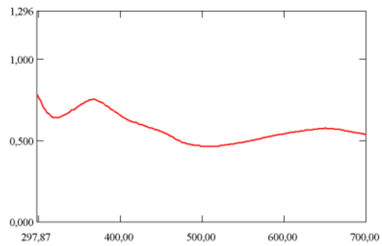

Figure 2. UV-vis spectrum where the three characteristic peaks of the reactive oxygen species obtained in the Fenton reaction produced between Fe_3O_4-NPs and H_2O_2 are identified when reacting with TMB during the preliminary tests at the following conditions: 0.00025 M Fe_3O_4, 0.1 M H_2O_2 and 0.00208 M TMB (in pH 4.5 buffer).

Conclusions

In this work, the proposed experimental design demonstrated the effectiveness of Fe_3O_4-NPs in promoting the Fenton reaction and the production of ROS with biocidal capacity. This practical application allowed for the reduction of the H_2O_2 concentration while achieving the same inhibitory effect against the four microbial strains studied. Furthermore, this work introduced the novelty of establishing the optimal operating conditions required to achieve the highest production of ROS, and consequently the highest biocidal response in combinations between Fe_3O_4-NPs and H_2O_2. These conditions were determined to be: temperature 42 ºC, pH 6, and concentrations of 0.136 µg/mL for H_2O_2 and 160 µg/mL of Fe_3O_4-NPs.

Acknowledgments

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