Magnetic Field as a Tool for Enhancing β -Lactamase Activity

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β-Lactam antibiotics have been extensively employed in bacterial treatment ever since penicillin's groundbreaking discovery. Despite the proliferation of antibiotics in the pharmaceutical sector today, bacteria often evolve defense mechanisms. Chief among these is the production of β-lactamase enzymes, which degrade β-lactam antibiotics, representing a prevalent form of antibiotic resistance. Additionally, these antibiotics exhibit limited biodegradability, with only 20% breaking down naturally. Hence, finding effective methods to mitigate the presence of β-lactam antibiotics is crucial in combating antibiotic pollution.

Keywords:
ß-lactamase, magnetic field, enzyme activity, hyperactivation, secondary structure

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1 Introduction

Numerous industries use enzymatic processes as opposed to conventional chemical catalysts, because they are specific in chemical transformations, rapid and save reagents and energy (Becker et al. 2021; Choi et al. 2015). Enzymes are used to catalyze highly selective and effective chemical reactions in terms of saving energy. Despite free enzymes possessing some drawbacks, regarding poor operational stability and low resistance to process conditions, they are highly applicable in industrial processes on a large scale. To overcome such limitations, the main research is focused on protein engineering solutions. However, the most common technique widely used for enhancing enzyme activity and operational stability is immobilization onto various supports. Immobilized enzymes are more resistant for harsh and unfavorable reactions conditions due to the interactions between enzymes and their carrier structure. Such conditions as pH, high temperature and ionic strength can destabilize and alter molecular structure and performance of a certain biocatalyst. Additionally, posing as an interesting alternative is the use of physical factors, such as magnetic field, which could stimulate and affect enzyme activity with its catalytic properties. With a potential effect of magnetic field on the enzyme reaction catalysis can change and alter enzyme's kinetics. When external magnetic field is applied in a reaction catalysis, it can affect molecular structure of the enzyme, thus modifying its catalytic properties. Furthermore, exposing enzymes to magnetic field can also affect kinetic energy of unpaired electrons, that are being released in catalysis, which can impact biological processes and chemical reactions (Emamdadi et al. 2021; Wasak et al. 2019; Magazù and Calabrò 2011; Liu et al. 2010).

ß-Lactamic antibiotics are one of the most common drugs used in treating bacterial infections, since their efficiency is in their ability to inhibit important reactions, which are involved in cell wall formation. Penicillin (PEN) is an antibacterial agent, that belongs to the β-lactam antibiotics, which have strong antimicrobial activities. Hence, it is extremely used in clinical practices worldwide. The use of PEN can also cause allergic reactions and lead to death, even more it can lead to development of PEN-resistant bacterial strains. Since PEN antibiotics are widely used in the dairy industry for treating various bacterial infections, the continual abuse of PEN has been reported and documented. PEN antibiotics show limited stability in hydrolysis and can therefore cause formation of different degradation products (Li et al. 2014). As such degradation products can cause allergic reactions as well, some enzymes

can catalyze and degrade antibiotics with the advantage of excellent catalytic features, high biocompatibility and eco-friendly performance (Yang et al. 2021). Such enzymes are ß-lactamases, which are produced by bacteria that play an important role in their resistance to ß-lactam antibiotics due to their hydrolyzation of ß-lactam ring (Wang et al. 2021).

With increasing antibiotic pollution and consequently avoiding bacteria developing defense mechanisms, the urge for developing methods for successful antibiotic removal are in place (Feng et al. 2021; Wang et al. 2021; Yang et al. 2021). Therefore, any new methods for enhancing enzymatic activity, operational stability and tolerance to pH and temperature of ß-lactamase are being investigated. In our study, we investigated the PEN degradation ability of free β-lactamase. As the PEN degradation study showed promising results, the enzyme was also treated with magnetic field to achieve improved catalytic activity and stability.

2 Materials and Methods

2.1 Enzyme preparation

Enzyme β-lactamase was studied in solution and powder form. When investigating the effect of magnetic field on its activity in powder form, 1 g of β-lactamase was used. When investigating the effect of magnetic field on its activity in a solution, 1 g of β-lactamase was dissolved in 1 mL of deionized water.

2.2 Degradation of PEN with β-lactamase

The degradation process was performed in batch reactor containing PEN solution with 0.1 mg/mL concentration. When β-lactamase was added, the reaction was performed at room temperature for 24 hours. The remaining PEN concentrations were monitored via HPLC (Agilent) with an Eclipse XDB C18 column, consisting of parameters: column temperature 30 °C, flow rate 1.0 mL/min, mobile phase consisting of methanol and phosphate, injection volume was 20 µL. The degradation was detected at a wavelength of 225 nm.

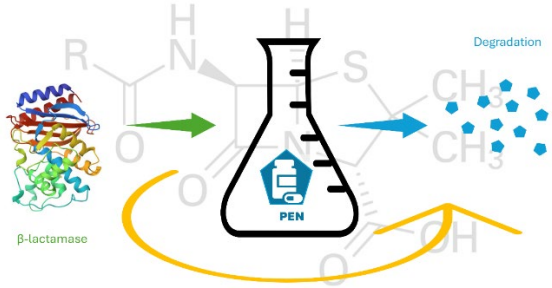

Figure 1: Schematic illustration of PEN degradation in a batch reactor using β-lactamase
Source: own.

2.3 Effect of magnetic field on β-lactamase activity

The effect of treatment with constant magnetic field with 50 mHz frequency was studied on the activity of enzyme β-lactamase. The activity of β-lactamase was investigated in forms of a solution and powder, while the effect of exposed time (2 and 20 min) was studied, as well.

2.4 β-lactamase activity assay

β-Lactamase activity was assessed spectrophotometrically. The reaction that contained 570 µL of β-lactamase enzyme in 50 mM HEPES buffer was initiated with 30 µL of substrate nitrocefin. β-Lactamase activity was monitored by measuring the increase in absorbance at 482 nm for 1 min at room temperature.

3 Results

3.1 Enzymatic degradation of PEN with β-lactamase

The catalytic performance of β-lactamase for degradation of PEN was determined in water solution at room temperature. As shown in **Figure 2**, PEN degradation with β-lactamase was determined using enzyme concentration of 0.01 mg/mL. PEN concentration was 0.1 mg/mL. From the results shown in **Figure 2** we can observe that the degradation of PEN with enzyme β-lactamase was slowly improving. After 10 min 10% of PEN degraded and after 6 and 24 hours 18% and 22% degradation of PEN was observed, respectively.

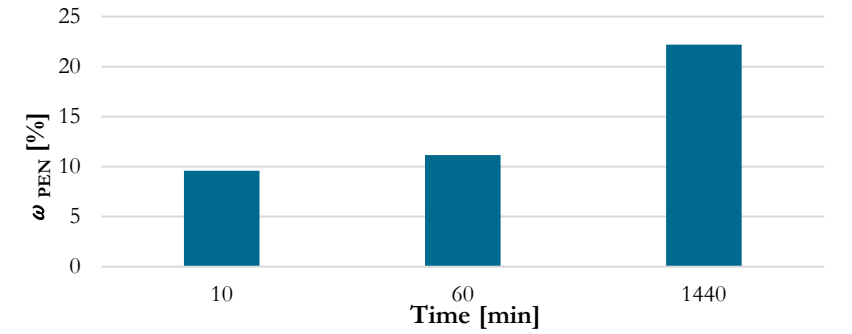

Figure 2: PEN degradation by enzyme β-lactamase after incubation for 24 hours.
Source: own.

3.2 Effect of magnetic field on β-lactamase activity

As the enzyme shows great promises in degradation studies, it will be immobilized onto nanostructured carriers and used for applications, where it can easily be separated with the use of an external magnetic field. Hence, we investigated the impact of a continuous magnetic field on the activity of β-lactamase in both solution and powdered forms. Our analysis revealed that magnetic treatment did not deactivate the enzyme; instead, it preserved its activity with only minimal loss compared to the non-exposed enzyme. The peak activity of the treated enzyme in powdered form under a constant magnetic field was attained after 20 minutes, resulting in a 96% retention rate, while for the enzyme in solution form, the highest activity was achieved after the same duration, with a retention rate of 99% (**Figure 3**).

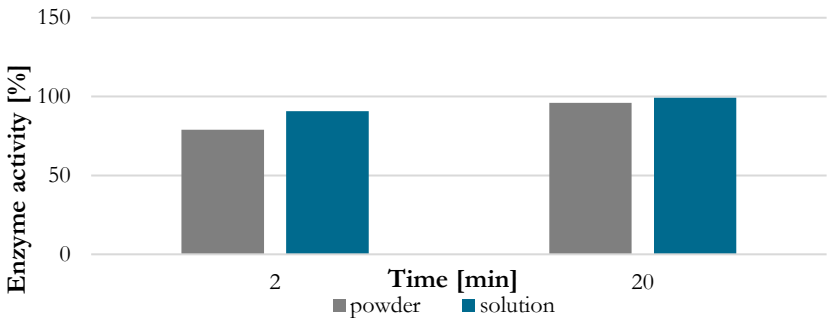

Figure 3: Enzyme activities of β-lactamase in powder form and in solution after constant magnetic field treatment.
Source: own.

Based on the findings, it can be concluded that the enzyme's activity is influenced by the frequency of exposure, potentially leading to alterations in its structure when subjected to a magnetic field. It is widely acknowledged that electromagnetic fields have the capacity to dynamically impact proteins and the ions within their composition (Wasak et al. 2019; Liu et al. 2010). The observed outcomes could stem from the distinctive structural characteristics of the enzyme and its catalytic function. The magnetic field's ability to modify physiochemical properties, such as conductivity or dielectric constant, may contribute to changes in the enzyme's structure. This factor is crucial for maintaining the stability of the enzyme's three-dimensional structure and its catalytic efficacy. The interaction between an external magnetic field and the enzyme's three-dimensional arrangement could trigger structural adjustments, thereby influencing the geometry of the enzyme's active sites.

4 Conclusions

Utilizing magnetic fields to regulate enzymatic activity appears to hold significant promise for enhancing the catalytic performance of enzymes. This approach not only boosts the enzymatic efficiency but also enhances the operational stability of the enzymes. Our research demonstrates that exposing β-lactamase to an external magnetic field can effectively modify its activity, maintaining its functionality without any adverse effects on enzyme performance.

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