WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 6.805
Volume 5, Issue 5, 213-224. Research Article ISSN 2277– 7105

THE EFFECT OF STATIC MAGNETIC FIELD ON THE PRODUCTION OF AMYLASE ENZYME UNDER SOLID STATE FERMENTATIONS FROM FIVE DIFFERENT FUNGAL GENERA

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Article Received on
05 March 2016,

Revised on 26 March 2016,
Accepted on 17 April 2016

DOI: 10.20959/wjpr20165-6142

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ABSTRACT

The effect of static magnetic field on the production of amylases under solid state fermentations of (*Alternaria* sp., *Aspergillus niger*, *Fusarium* sp., *Mucor* sp., and *Penicillium* sp.) was investigated. The above species were exposed to the Northern pole, Southern pole and both poles together. The substrate used for fungi growth was bread only and it was cut into small pieces and sterilized by autoclaving. Static magnetic field of 100 gauss was subjected to the above fungal species for seven days of fermentation at 28 °C. After seven days, the medium was mixed with 100 ml of phosphate buffer saline, and filtered through Whatman filter paper No 1. The crude extract was tested for the amylases' specific activities and the results were statistically analysed. The results showed that Northern pole significantly decreased specific activity of amylase enzyme of *Alternaria* sp., *Fusarium* sp., and *Penicillium* sp. which were (2.50, 2.12, and 3.27) U/mg respectively. While Southern pole significantly increased that of *Fusarium* sp. (3.84 U/mg) and *Mucor* sp. (3.36 U/mg), while it significantly decreased that of *Alternaria* sp. (2.77 U/mg) and *Penicillium* sp. (3.88 U/mg). The effect of both poles was significantly decreased that of *Alternaria* sp. (2.62 U/mg) and *Penicillium* sp. (2.26 U/mg), whereas significantly increased that of *Fusarium* sp. (3.84 U/mg) and *Mucor* sp. (3.83 U/mg). This study clearly demonstrated that there are significant effects of the electrostatic magnetic field in increasing or decreasing the enzymes activities of the fungal species.

KEYWORD: Magnetic field, Fungi, solid state fermentations and Amylase.

INTRODUCTION

Some studies revealed that the influence of the magnetic field of cellulolytic microorganisms regarding metabolism. The influence of the magnetic field of the microorganisms was recognized since (1937), when Kimball found that the wine yeast cells were not affected after exposure for several periods of time to the magnetic field.[1] *Staphylococcus aureus* growth rate was increased when exposed to a magnetic field for (3-6) HR, while there was no effect after 7 hours of exposure. The growth rate was unchanged when *Serratia marcescens* was exposed to the magnetic field for 6 hours, but it increased after exposition to a long time.[2] The morphology of *Pseudomonas aeruginosa* was changed by the influences of different levels of static magnetic field. The growth of *Escherichia coli*, was enhanced by a static magnetic field and this enhancement of the growth is proportional to the increase of the magnetic field frequency.[3] Many researchers have found that the magnetic field affects the growth of bacteria, which includes an increase in mass and cell division. Exposure of *Escherichia coli* to an AC field (0−2 mT, 16 and 50 Hz), shows shortened at generation time.[4] Powerful magnetic fields (5.2 − 6.1 T) caused delayed cell death in stationary cultures of *Bacillus subtilis*.[5] Whether or not an AC magnetic field exerts an inhibitory or else a stimulatory mode of action based on a complex manner on the frequency and the field strength. Many studies have shown that the influence of static magnetic fields on biological systems and time-varying magnetic or electromagnetic fields of transport of membrane cat-ions and other function, the influences of exposures to different magnetic fields are not identical, some reports display an inhibitory effect by the fields, others show activation, while others showed no significant effects on the transport of cat-ion. In recent years, many studies indicated the effect of magnetic field on Ca^{2+} influx across the cell membrane or intracellular Ca^{2+} movements. Significantly exposed to the time-varying magnetic field suppressed the increase in [Ca^{2+}] and partially inhibited the K^+ influx through Ca^{2+}-dependent K^+ channels, proposing the inhibition of Ca^{2+} influx and/or Ca^{2+} release by the exposure. However, the inhibition of K^+ influx is due to a direct magnetic field exposure on K^+ channels rather than suppression of the increase in [Ca^{2+}].[6] Liburdy found that the magnetic field affects Ca^{2+} uptake across the cell membrane, but the release of Ca^{2+} from its stores is not affected. This is because eddy current induced by the magnetic field or electric field could not enter the outer cell membrane. The cell membrane is bilayers that act as an electric insulator.[7] Magnetic fields influence the charge of the cell membrane, which open up the membrane channels. These channels are similar to windows and doors of a house. By opening cell

channels, nutrients are capable to enter the cell, and waste is more easily removed from the cell. This would assist to equilibrate and restore optimum cell function.

The aim of the present study was to investigate the effect of static magnetic field on amylases production under solid state fermentation.

MATERIALS AND METHODS

Fungal species: The fungi species, *Penicillium* sp., *Alternaria* sp., *Fusarium* sp., *Mucor* sp., and *Aspergillus niger* were obtained from the Department of Biology/ College of Science/ University of Baghdad. The fungi species were identified after growing on Potato Dextrose Agar (PDA) medium by observing the growth characteristics (color, texture, appearance and diameter of colonies) and microscopic (microstructure).[8][9] All the cultures were maintained on PDA slants. Then, stored in refrigerator and sub-cultured regularly at an interval of three months.

Static magnetic field: A special magnetic bar of thickness (2.9 cm) with single field strength of 100 Gauss which was measured by a Gauss meter. The magnetic bars (North Pole, South Pole, or both poles) were put on the side of the cultured flasks using adhesive tape.

Spores' suspension preparation

Spores' suspension was prepared according to [10] with slight modifications as follows:

- Plates containing PDA medium inoculated with fungal isolates were incubated at 28 °C for 3-4 days.
- Spores were harvested by adding 5 ml of DW on the plate.
- The spore's suspension was transferred by a micropipette to a flask containing sterilized bread and incubated at 28 °C for 5 days.
- One hundred ml of DW was added to the flask and mixed vigorously by hand.
- The suspension of spores was filtered through sterile cotton wool.
- The suspension was centrifuged at (3000 rpm for 5 min). Then, the supernatant was discarded and the spores then washed twice by DW and further re-centrifuged.
- Then, 1 ml of DW was added to the deposit and mixed vigorously.
- The following equation was used to estimate the number of spores /ml in original suspension: Spores /ml = (Average no. of spores / 5) × (25) × (10^4) × (Dilution Factor).

Effect of magnetic field poles on enzyme activity under solid state fermentation

To test the effect of the magnetic field on the fungi cultures under solid state fermentation the following steps were carried out

- Bread loaves were cut into pieces (approximately 1 cm^3) using a bread loaf knife.
- Twelve culture flasks were loaded with 30 g of bread pieces and autoclaved at 121 °C for 15 min.
- Each flask was inoculated with 10^8 spores/ml of fungus.
- The twelve flasks were divided into four groups as follows

Three flasks used as control, three flasks were put under the effect of northern pole, three flasks were put under the effect of southern pole and three flasks were put under the effect of both poles.

- The flasks were incubated at 28 °C for 7 days with shaking every day.

Crude enzyme extraction

After 7 days of fungal fermentation, extraction of crude enzyme was done as follows

- To each culture flask, 100 ml of phosphate buffer (pH 7) was added and shake vigorously to suspend the bread pieces.
- The whole content of the flask was filtered through sterile cotton wool and centrifuged at (3000 rpm for 5 min).
- The supernatant was assembled and filtered by filter paper Whatman No.1 under vacuum.
- The filtrate was stored at 4 °C.

Assay of amylase activity

Amylase activity was assayed as described by [11]

- Thirteen test tubes were prepared. One tube was used as a blank and the others were used to assay amylase activity.
- To each tube, (1 ml) of starch solution was added.
- To each tube, (1 ml) of enzyme extract was added, except the blank to which (1 ml) of DW was added, the tubes were mixed by swirling and incubated at 37 °C for exactly 3 min.
- To each tube, (1 ml) of color reagent (DNS) was added and boiled in water bath for 15 min.

- The tubes were cooled on ice for 3 min.
- To each tube, (9 ml) of DW were added with mixing.
- The absorbance for all the test tubes was measured at 540 nm using spectrophotometer.

Amylase specific activity (U/mg)

One unit of amylase enzyme can be defined as the amount needed for liberation of (1.0 mg) of maltose from starch in 3 min at pH 6.9 at 20°C.

- The absorbance of all sample tests obtained above was put in the slope of the standard curve (Figure 2-3) to give the liberated maltose from starch in mg.
- The total activity of amylase in unit/ml can be obtained using the following equation

$$\textbf{Amylase activity (U/ml)} = \frac{\textbf{Maltose released (mg)} * \textbf{df}}{(1)}$$

Where

Df = Dilution Factor

1 = Enzyme volume (ml)

- Ultimately, the enzyme specific activity was estimated by dividing the enzyme total activity on the protein, total activity obtained from bovine serum albumin standard curve (Figure 2-1) according to the following equation.

$$\textbf{Amylase specific activity (U/mg)} = \frac{\textbf{Amylase total activity (U/ml)}}{\textbf{Protein concentration (mg/ml)}}$$

Statistical Analysis: All the experiments were performed in triplicates. The statistical analysis was done using GenStat program. Least significant difference (LSD) was determined for each fungus to both enzymes (Amylase and Protease). The mean of specific activity for each treatment was calculated and subtracted from the control of this treatment manually. If the result of this subtraction is more than LSD then the difference is significant. Alternatively the difference will not be significant if the subtraction results are less than LSD.

RESULTS

Effect of Magnetic Field on Amylase Specific Activity

Effect of Magnetic Field on the Amylase Specific Activity of *Alternaria* sp.

The effect of magnetic field on specific activity of amylase of *Alternaria* sp. was investigated. It was measured after 7 days of fermentation on solid medium (bread cubes) at

28 °C. The Control of all experiments was the solid medium without exposure to the effect of the magnetic field. The crude extract of the Control flasks was assayed and the specific activity was (3.66 U/mg). As shown in the Figure (3-6), the Southern and Northern Poles decreased the amylase specific activity (2.77 U/mg) and (2.50 U/mg) respectively. The two poles together (South + North) decreased the specific activity as well (2.62 U/mg). Results in Table (3-1) revealed that the Least Significant Differences (LSD) was (0.375), significant decrease in specific activity of the amylase enzyme obtained in all treatments, Southern, Northern, and Both Poles when compared to the Control.

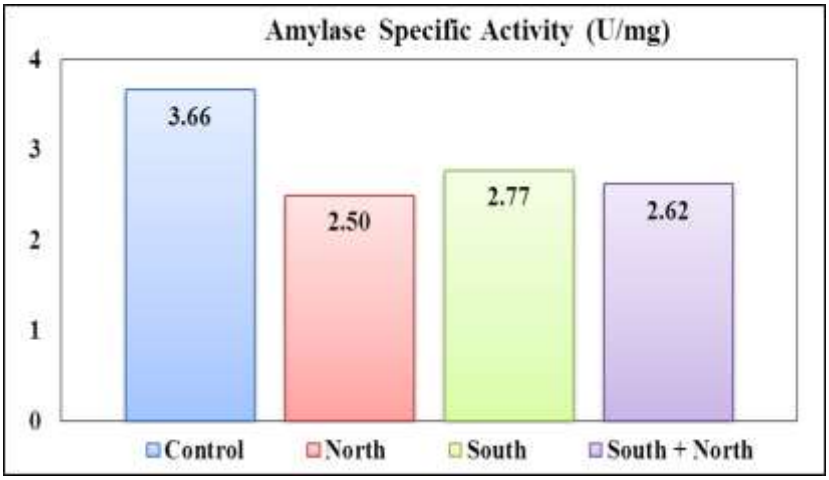

Figure (3-6): Effect of magnetic field on amylase specific activity of *Alternaria* sp.

Table (3-1): Effect of magnetic field on the amylase specific activity of *Alternaria* sp.

Treatment	Value
Control	3.660
North	2.50*
South	2.77*
Both	2.62*
L.S.D 0.05	0.375

***The mean difference is significant at the 0.05 level.**

Effect of Magnetic Field on the Amylase Specific Activity of *Aspergillus niger*

The effect of magnetic field on the specific activity of amylase of *Aspergillus niger* was studied. After 7 days of fermentation on solid medium (bread cubes) at 28 °C the study revealed that the specific activity of the Control was (4.23 U/mg), the Southern and Northern Poles decreased the specific activity (3.74 U/mg) and (2.96 U/mg) respectively. The two poles together (South + North) decreased the specific activity as well (3.28 U/mg). Table (3-

2) showed that the LSD was (1.322) and there was no significant difference for specific activity obtained in all treatments.

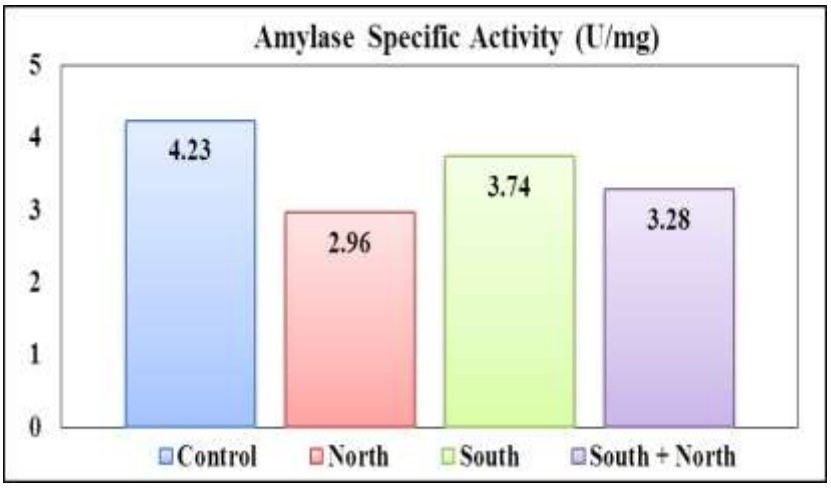

Figure (3-7): Effect of magnetic field on amylase specific activity of *Aspergillus niger*.

Table (3-2): Effect of magnetic field on amylase specific activity of *Aspergillus niger*.

Treatment	Value
Control	4.23
North	2.96
South	3.74
Both	3.28
L.S.D 0.05	1.322

***The mean difference is significant at the 0.05 level.**

Effect of Magnetic Field on the Amylase Specific Activity of *Fusarium* sp.

The effect of magnetic field on amylase specific activity of *Fusarium* sp. was studied. The results in Figure (3-8) showed that after 7 days of fermentation on solid medium (bread cubes) at 28 °C. The Southern Pole increased amylase specific activity (3.84 U/mg) and the two poles together (South + North) as well (3.84 U/mg). On the contrary the Northern Pole decreased specific activity (2.12 U/mg). Compared with the Control (2.61 U/mg) (without MF). Table (3-3) showed that the LSD was (0.4597) and the treatments of Southern and Both Poles recorded significant increases in amylase specific activity, while Northern Pole gave a significant decrease in amylase specific activity, compared with the Control.

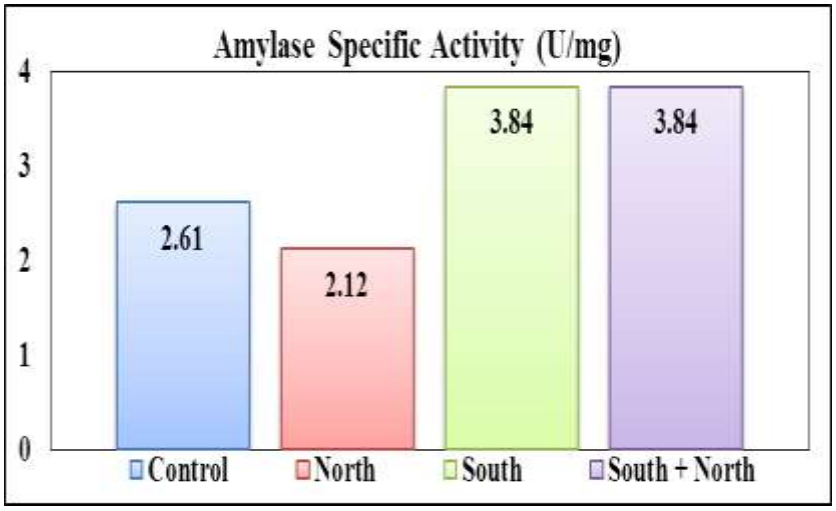

Figure (3-8): Effect of magnetic field on amylase specific activity of *Fusarium* sp.

Table (3-3): Effect of magnetic field on amylase specific activity of *Fusarium* sp.

Treatment	Value
Control	2.61
North	2.12*
South	3.84*
Both	3.84*
L.S.D 0.05	0.4597

***The mean difference is significant at the 0.05 level**

Effect of Magnetic Field on the Amylase Specific Activity of *Mucor* sp.

The effect of magnetic field on specific activity of amylase of *Mucor* sp. was measured. The results after 7 days of fermentation on solid medium (bread cubes) at 28 °C were indicated. In the Figure (3-9) the specific activity of the Control was (1.78 U/mg). The Southern and Both Poles increased the specific activity (3.36 U/mg) and (3.83 U/mg) respectively. The Northern pole decreased specific activity (1.70 U/mg). Results in Table (3-4) indicated that the LSD was (0.565) and the Southern and Both Poles recorded a significant increase in specific enzyme activity, while there was no significant difference obtained in Northern pole when compared with the Control.

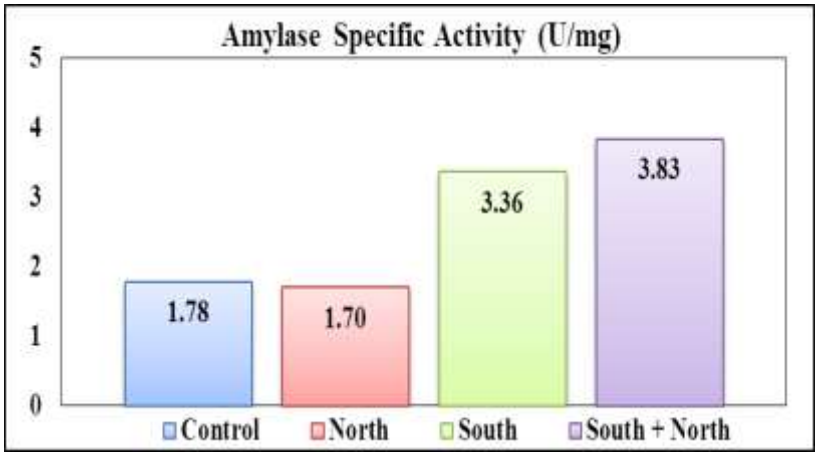

Figure (3-9): Effect of magnetic field on amylase specific activity of *Mucor* sp.

Table (3-4): Effect of magnetic field on amylase specific activity of *Mucor* sp.

Treatment	Value
Control	1.778
North	1.70
South	3.36*
Both	3.83*
L.S.D 0.05	0.565

***The mean difference is significant at the 0.05 level**

Effect of Magnetic Field on the Amylase Specific Activity of *Penicillium* sp.: The effect of magnetic field on specific activity of amylase of *Penicillium* sp. was tested. After 7 days of fermentation on solid medium (bread cubes) at 28 °C, the specific activity was measured. The Control specific activity was (4.60 U/mg). As seen in the Figure (3-10), the Southern and Northern Poles decreased the specific activity (3.88 U/mg) and (3.27 U/mg) respectively. As well the two poles together (South + North) decreased the specific activity (2.62 U/mg). Results in Table (3-5) showed that the LSD was (0.508) and there was a significant reduction in enzyme specific activity in all treatments as compared with the Control.

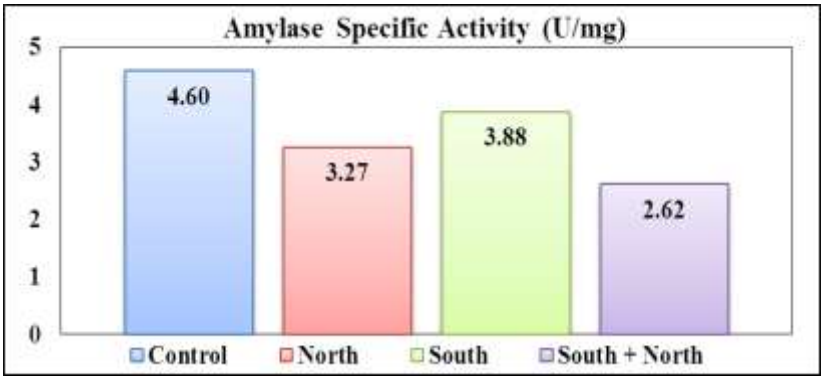

Figure (3-10): Effect of magnetic field on amylase specific activity of *Penicillium* sp.

Table (3-5): Effect of magnetic field on amylase specific activity of *Penicillium* sp.

Treatment	Value
Control	4.60
North	3.27*
South	3.88*
Both	2.62*
L.S.D 0.05	0.508

***The mean difference is significant at the 0.05 level.**

DISCUSSION

The statistical analysis of the results in Figures (3-(6, 7, 8, 9, 10)) showed that the Southern and Both Poles treatments significantly increased the amylase specific activity of *Fusarium* sp. and *Mucor* sp. and conversely significantly decreased amylase specific activity of *Alternaria* sp. and *Penicillium* sp. compared with the Control. On the other hand, the Northern Pole treatment significantly decreased the amylase specific activity of *Alternaria* sp., *Fusarium* sp., and *Penicillium* sp.

It is well known that there are many factors affecting the enzyme activity. These include temperature, pH, substrate concentration and the presence of activator or inhibitor.

In the present study it was attempted to show the effect of the magnetic field on the enzyme activity of *Alternaria* sp., *Aspergillus niger*, *Fusarium* sp., *Mucor* sp. and *Penicillium* sp.

The effect of the magnetic field of the Southern pole seems to potentiate the action of (Ca^{2+}) ion leading to increase in amylase specific activity in *Fusarium* sp. and *Mucor* sp. It seems that other fungi (*Alternaria* sp., and *Penicillium* sp.) respond to the action of the southern pole of the magnetic field differently, which could be explained by the difference of the amino acid composing these proteins' active sites which led to this decrease of their activity by the effect of the magnetic field.

The action of both poles of the magnetic fields which led to increase the specific activity of amylase of *Mucor* sp. and *Fusarium* sp. could be due to the Southern pole only as it competes with Northern pole and suppressed its effect.

The most reasonable explanation of the decrease of enzyme specific activity (amylase) after exposing the fungi to the Northern the magnetic field is attributable to the probable interface of the negative charge of magnetic field with the release of the stimulating effect of (Ca^{2+}).

The Moving Charge Interaction (MCI) model p suggests that electron motion affect enzyme activity. Theoretical models have depicted existence of low frequency (ELF) magnetic field interactions with Biosystems at ion cyclotron resonance which is, at frequencies corresponding to charge to mass ratios of ions such as Ca^{2+}, Mg^{2+} and K^{+} (Bruce *et al.*, 1992). Experimental investigations detect that low frequency electromagnetic fields affects living systems through the calcium signaling pathways and systolic calcium oscillator.[12] The theories on the effects of MFs on the activities of enzymatic refer to changes of motion of ions at the active site (Edmonds, 1993).

Blank and Soo,[13] demonstrated the EMF interaction mechanism with Na^{+}- K^{+} ATPase and suggested to be due to acceleration of the electron, regardless of the direction the electrons move regularly and the threshold force producing the effect on the enzyme . Therefore, it is very likely that the influence of the field on the acid phosphatase could include electron density at the anti-ferromagnetically coupled binuclear Fe(III)-Mn(II) center and the active site.

CONCLUSIONS

- Magnetic field poles (100 Gauss) showed different effects on amylase specific activity of *Alternaria* sp., *Aspergillus niger*, *Fusarium* sp., *Mucor* sp., and *Penicillium* sp.
- The Northern pole had a negative effect on the amylase specific activity of *Alternaria* sp., *Fusarium* sp., and *Penicillium* sp.
- The Southern pole had a positive effect on amylase specific activity of *Fusarium* sp., and *Mucor* sp. While it had a negative effect on amylase specific activity of *Alternaria* sp. and *Penicillium* sp. as compared with the Control under the same conditions.
- The two poles together (South + North) showed negative effects on both amylase specific activities of *Alternaria* sp. and *Penicillium* sp. While they showed positive effects on amylase specific activity of *Fusarium* sp. and *Mucor* sp. as compared with the Control under the same conditions.

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