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# THE EFFECT OF BENTONITE ON AFB1, AFB2, AFG2 AND T-2 MYCOTOXINS DECOMPOSITION IN SUNFLOWER OIL UNDER THE IRRADIATION OF ULTRAVIOLET LIGHT

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**ABSTRACT.** Bentonite was used as a photocatalyst in the degradation of mycotoxins in sunflower oil, under UV-illumination. The objective of this study was to investigate and evaluate the efficiency of bentonite adding to sunflower oil to decompose the solubilised B1, B2, G2 aflatoxins and T-2 toxin by UV light irradiating, as solution to increase vegetable oil quality. Identification of decontaminating process particularities consists in the formulation of mathematical model which describes the influence of process parameters on the decomposition of mycotoxins. In the present work, decontamination process was studied at laboratory scale using a plug-flow photoreactor, serial connected with a buffer vessel, at constant operating temperature and irradiating time. The kinetics of photocatalytic process was assumed to follow a pseudo-first-order rate law. The apparent photodegradation rate constant depend strongly on the present mycotoxin, the bentonite catalyst contribute to obtain much lower mycotoxin concentrations. The model predicted maximum values of apparent photodegradation constants were compared with the experimental data, the model was verified.

***Keywords:*** *Mycotoxins, Aflatoxin B1, Aflatoxin B2, Aflatoxin G2, T-2 toxin, photochemical degradation, ultraviolet light, sunflower oil, kinetics, reaction mechanism, HPLC.*

## INTRODUCTION

There is a growing negative reaction of the consumer public to added synthetic chemical compounds or existence of toxic chemicals in foods, such as for mycotoxins [1]. To approach the challenges and problems of the food industry, there are alternative possibilities of current food processing, these options usually are more sophisticated, often several ways of processing are investigated, and then the most appropriate is chosen [2]. As a method for decontaminating, the food irradiation with ultraviolet light (UV) can play an important role; there are large interests for this method. U.S. Food and Drug Administration (USFDA) and U.S. Department of Agriculture concluded that UV irradiation is safe [3, 4].

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In 2000, the FDA approved the use of UV light treatment as an alternative to thermal pasteurization of fresh fruit juices. In addition, the USFDA issued 21CFR179.41 Code [5], which approved the use of UV light in the production, processing and handling of foods. Health Canada has conducted an assessment of UV treatment of apple juice and cider, and concluded that there is no reason for concern [6]. In Europe, UV irradiation is already used to disinfect water and air in the food industry. In addition, in 2004, the National Advisory Committee for Microbiological Criteria of Food (NACMCF) of USDA has reviewed the concept of "pasteurization" of foods. This term now includes any process of treatment, or a combination thereof, which is applied to foods, to reduce contamination levels [7, 8]. Many scientific publications are available on the UV treatment; we can see that there is a need for new methods of food treatment. However, in the literature insufficient data are available to integrate basic knowledge, for example, there is insufficient data about the interaction of UV light with foods, there are available performance evaluation systems of UV technologies, there are few recommendations for UV photoreactor practical design, there are few guidelines for selecting commercial UV sources, descriptions and application prospects for successful food handling are poor. Meesuk and Vorasith [10] showed that activated betonies can absorb efficiently the sunflower oil peroxides, starting from this premise it follows logically that can be adsorbed on the surface of betonies simultaneously micotoxins and peroxides, the free radicals formed by the photochemical decomposition of peroxides can react much easier with mycotoxins.

**Process mechanism**

The case of photochemical degradation of mycotoxins in presence of bentonite (B) is based on the following assumptions: mycotoxins (MT) are fully dissolved in the liquid phase (in the sunflower oil), the reaction mass is irradiated after being sufficiently mixed to form the adsorption equilibrium with bentonite on the active surface (Langmuir adsorption isotherm), where mycotoxin with bentonite forms an adsorption complex (MTB); there are no motive forces of the mycotoxins in the excited state which are dissolved or adsorbed on bentonite, photochemical reactions are not occurring if bentonite is not added to the sunflower oil. In fact, as it will be demonstrated later, photochemical reactions take place even without the presence of bentonite. Adsorption process can be described as follows:

$$MT + B \rightarrow MTB \qquad \text{described by} \qquad r = k_1[MT][B] \qquad (1)$$

The desorption process can be described as follows:

$$MTB \rightarrow MT + B \qquad \text{described by} \qquad r = k_{-1}[MTB] \qquad (2)$$

There is also an ineffective absorption of light, in a first step it occurs with absorption of light quanta:

$MT + h\nu \rightarrow MT^*$ described by $I_{abs}^{MT}$ (3)

After this, relaxation can occur through the phenomenon of excited state fluorescence of MT:

$MT^* \rightarrow MT + \Delta$ described by $r = k_D[D^*]$ (4)

Formed complex may also to absorb a quantum of light:

$MTB + h\nu \rightarrow MTB^*$ described by $I_{abs}^{MTB}$ (5)

After this, can occur the phenomenon of relaxation excited state fluorescence of MTB:

$MTB^* \rightarrow MTB + \Delta$ described by $r = k_D[MTB^*]$ (6)

The effectiveness photochemical processes are:
Photochemical excitation of bentonite:

$B + h\nu \rightarrow B^*$ described by $I_{abs}^{B^*}$ (7)

After this, may occur the relaxation of excited state of bentonite:

$B^* \rightarrow B + \Delta$ described by $r = k_B[B^*]$ (8)

Photochemical reaction of MT with bentonite located in the excited state:

$B^* + MT \rightarrow$ Products described by $r = k_R[B^*][MT]$ (9)

It may also take place an excitement of the adsorption complex:

$MTB + h\nu \rightarrow MTB^*$ described by $I_{abs}^{MTB}$ (10)

Relaxation of excited complex adsorption state with decomposition:

$MTB^* \rightarrow$ Products described by $r = k_R[MTB^*]$ (11)

Real system is more complex, better accuracy should be taken into account the presence of peroxides and hydroperoxides who are inherently present in the sunflower oil [9]. For simplicity, subsequent experimental measurements were made from the sunflower oil with constant peroxide index.

**Mathematical model**

We consider that $I_{abs}^{MT}$, $I_{abs}^{B}$ and $I_{abs}^{MTB}$ represents the absorbed monochromatic light by the liquid phase components (moles of photons, $s^{-1} \cdot dm^{-3}$), [*MT*], [*B*] and [*MTB*] are the current concentrations of the MT, B and MTB components at *t* reaction time ($mol/dm^3$). Speed of photodegradation process can be described by the equation:

$$r = -\frac{d[MT]}{dt} = \frac{d[Products]}{dt} = \frac{k_R K_1 c_0^B [MT]}{k_B + k_B K_1 [MT] + k_R K_1 C_0^B [MT]} \cdot I_{abs}^B \quad (12)$$

Concentration of the adsorption complex which is formed:

$$[MTB] = \frac{K_1 c_0^B [MT]}{1 + K_1 [MT]} \quad (13)$$

where $K_1 = k_1/k_{-1}$, $c_0^B = [B] + [MTB]$, $c_0^{MT} = [MT] + [MTB]$, and the reaction speed at the beginning of irradiation is described by equation ($\varphi_0$ is the reaction constant):

$$r(0) = \varphi_0^{\lambda} \frac{S}{V} I_0 \left(1 - 10^{-A_{total}(0)}\right) \quad (14)$$

Monochromatic light is absorbed by the MTB complex ($\varepsilon_{TD}$), by the bentonite ($\varepsilon_B$) and by the present mycotoxins MT ($\varepsilon_{MT}$) in the reaction mass. Considering that $L$ is the optical path through the system, the total absorbance is:

$$A_{total}(0) = (\varepsilon_{MTB}[MTB]_0 + \varepsilon_{MT}[MT]_0 + \varepsilon_B[B]_0)L \quad (15)$$

This relation expresses the total absorbed light, where $I_0$ is incident light intensity (moles of photons·s$^{-1}$·dm$^{-2}$). If $S$ is the illumination area (dm$^2$), $V$ is the active volume of the reactor (dm$^3$), then:

$$I_{abs}^{total}(0) = \frac{S}{V} I_0 \quad (16)$$

Usually, we can accept the assumption that light is absorbed in the reaction mass ($A_{total}(0)>2$) early entry into the reactor [11]. This simplification is valid for the use of bentonite, but could not be used for thin film reactor in the absence of bentonite.

The equation which describes the initial absorption of light by bentonite is:

$$I_{abs}^T(0) = \frac{S}{V} I_0 \frac{\varepsilon_T [B]_0}{\varepsilon_{MT}[MT]_0 + \varepsilon_B [B]_0 + \varepsilon_{MTB}[MTB]_0} \left(1 - 10^{-A_{total}(0)}\right) \quad (17)$$

Theoretically, the relation between $r(0)$ and $[MT]_0$ is predictable [12], where $r(0) = 0$ if $[MT]_0 = 0$:

$$r(0) = \frac{k_R K_1 c_0^T [MT]_0}{k_B + k_B K_1 [MT]_0 + k_R K_1 c_0^B [MT]_0} \cdot \frac{S}{V} \cdot \cdot I_0 \cdot \frac{\varepsilon_T [B]_0}{\varepsilon_{MT}[MT]_0 + \varepsilon_B [B]_0 + \varepsilon_{MTB}[MTB]_0} \cdot \left(1 - 10^{-A_{total}(0)}\right) \quad (18)$$

Simplified: $$r(0) = \varphi_0 \frac{S}{V} I_0 \left(1 - 10^{-A_{total}(0)}\right) \tag{19}$$

but $r(0) = 0$ if $[D]_0 \to \infty$ [13]. Function has a maximum ($r_{max}$, $[D]_0^{opt}$), which can be calculated from the following relation, where:

$$b = k_B K_1 + k_R K_1 c_0^B, \quad c = \varepsilon_{MT} - \varepsilon_{MTB}, \quad d = \varepsilon_B [B]_0 + \varepsilon_{MTB} c_0^{MT}$$

$$\frac{dr(0)}{d[MT]_0} = 0 \quad \text{hence} \quad [MT]_0^{opt} = \left(\frac{k_B d}{bc}\right)^{1/2} \tag{20}$$

Relations are valid for monochromatic light with a specific $\lambda$, and for polychromatic light may be used an integration [14], where we can define the initial integration constant ($\Phi_0$) and the total initial rate of degradation of mycotoxins ($R_0$), resulting in the following equation:

$$\sum_{\lambda} rr_0^{\lambda} = RR_0 = \sum_{\lambda} \varphi_0^{\lambda} \frac{S}{V} I_0 \left(1 - 10^{-A_{total}(0)}\right) \tag{21}$$

The used light source was with mercury vapour, emission spectral bands are characteristic of mercury. Reaction rate equation at the beginning of irradiation ($rr_0$) and location of maximum ($rr_0^{max}$, $[MT]_0^{opt}$) depend on the wavelength and intensity of light emitted by the source. Knowing that MT had significant absorbance at the characteristic wavelength of the UV source, it became possible to carry out practically decontamination. The integral reaction speed $RR_0$ in response to polychromatic light source was obtained by summing $rr_0^{\lambda}$ ($RR_0 = \sum rr_0^{\lambda}$). Function maximum ($RR_0^{max}$) at a concentration of dissolved MT, for known dissolved MT concentration can be predicted the $[MT]_0^{opt}$ if all experimental constants are known ($s$, $V$, $I_0^{\lambda}$), physical constants ($\varepsilon_{MT}$, $\varepsilon_B$, $\varepsilon_{MTB}$) and chemical constants ($K_1$, $k_{d,r}$). Kinetic model reveals that the rate of degradation of mycotoxins depends very much on the active surface affinity of bentonite. The ratio between the bentonite and mycotoxins must be adjusted according to need of process, but basically it cannot be used high doses of bentonite, there appear major difficulties of pumping. The scientific literature contains very few data on the optical properties of mycotoxins, suspensions of bentonite and bentonite-sunflower oil organogels. In limited area of concentrations of investigated mycotoxins can be performed the degradation kinetics linearization, especially if it seeks to minimize changes of essential fatty acids, which logically require a relatively short irradiation times. We can write the equation:

$$-\frac{d[MT]}{dt} = k_{apMT}[MT] \tag{22}$$

For each mycotoxin in part, $[MT]$ represent the actual concentration of mycotoxin, $k_{apMT}$ is the pseudo-first order transformation constant, *t* is the time of irradiation.

For a PFR (tubular) reactor, the equation that describes the operation can be described as follows [15]:

$$-\frac{d[MT]}{dV}=-\frac{r_{MT}}{\nu_0}, \tag{23}$$

where $V=\frac{\pi}{4}\left(d_0^2-d_i^2\right)l$, and $-r_{AMT}=-\frac{d[MT]}{dt}$. (24)

In previous relations *V*, $\nu_0$, $d_0$, $d_i$ and *l* are the volume, volume flow, inner diameter, outer diameter and the length of photochemical reactor. From the last two equations we get:

$$-\frac{d[MT]}{dl}=\frac{\pi/4\left(d_0^2-d_i^2\right)}{\nu_0}\left(-\frac{d[MT]}{dt}\right) \tag{25}$$

The obtained model is:

$$-\frac{d[MT]}{dl}=\frac{\pi/4\left(d_0^2-d_i^2\right)}{\nu_0}\left(\frac{a[B]_0/[MT]_0}{1+b([B]_0/[MT]_0)+c([B]_0/[MT]_0)^2}\right)[MT] \tag{26}$$

## EXPERIMENTAL SECTION

In the reactor system [16], in the continuous mixing reactor which are previously cleaned, dried, was added 500 grams of Top Floris extra pure sunflower oil, which is a special product of SC Expur SA, and it was checked for lack of mycotoxins in the laboratory of Sapientia University Miercurea Ciuc. After dosing the sunflower oil, was started the carbon dioxide gas adding, so that in both reactors to reach a sufficient amount remove of atmospheric oxygen, gas dosing was continued until the end of experiments. Through a hole in the top of the batch reactor was added with a Hamilton microsyringe the amount of mycotoxin standard solution to achieve the initial concentration of mycotoxins. After setting the working parameters, after the start of pumping (50-1000 ml/minute), the UV source was ignited, which measured power output are 29,8 mW/cm$^2$ at a distance of 5 cm, and then at regular intervals were extracted samples in special vessels, which being filled, sealed and stored at -24 °C in the dark until analytical determinations were carried out. Samples were processed as follows: using centrifuge

Hettich Micro 20 (13000 rot./min, 5 minutes), bentonite was separated, we split (if necessary) the higher density oil phase, which was pipetted into an extraction bottle, it was determined the mass, after this was performed methanol extraction of mycotoxins in several steps, resulting mixtures were separated by centrifugation (Hettich Universal 32, 3400rot./min, 5 minutes), alcoholic liquid phases were pipetted and unified. Later, separated bentonite was extracted in several steps, for the extraction of adsorbed mycotoxin, and then unified. Since resulted alcoholic phases were sometimes cloudy, was needed a new set of centrifugations to perfect the separation. Alcoholic extracts thus obtained were subjected to evaporation, their mass was determined, and portions of alcoholic extracts was injected into an apparatus Varian Pro Star HPLC type, using fluorescent and UV detector, using Supelcosil LC 18 column, with flow rate of 0,9 ml/min, without derivatization, because previous derivatization tests did not provide the expected results. For accurate determinations using liquid chromatography (HPLC), it was necessary an adequate preparing of the apparatus, every time was given enough time for conditioning and stabilizing all components of the HPLC equipment. HPLC equipment was controlled by Varian Star Chromatography Workstation Version 6.00 software. The used eluent was a mixture of water, methanol and acetonitrile, mixture in the ratio of 130:70:40. Fluorescence detector was set for excitation at 365 nm, and the emission at 435 nm. Based on HPLC chromatograms provided by the HPLC, we can evaluate the concentration of MT in the samples, which is proportional to the area under the signal curve offered by that component.

## RESULTS AND DISCUSSION

By using the bentonite as an additive to the reaction mass, after the experiments was carried out, extraction of MT from bentonite was necessary, with the unification of the extracts, followed by partly evaporation of methyl alcohol. The values of apparently degradation constants of MT depending on the mass ratio between bentonite (B) and MT (g B/μg MT) are indicated in figure 1.

We adjusted the experimental data to the proposed model, based on data from $k_{ap}$ corresponding to bentonite additive use (B concentration expressed in g/l reported to the MT concentration expressed in μg/kg), the effects of the bentonite presence are differentiated: in case of AFB1 are obtained increase with 315% of the reaction speed, in case of AFB2 are obtained an increase with about 26%, in case of AFG2 are obtained an increase with about 44% and in case of T-2 are obtained an increase with about 65%. Parameters of individual models with 95% confidence level and using the sum of the squares error method (SSE) as a function of error were obtained by nonlinear regression using Polymath 5.0 software package.

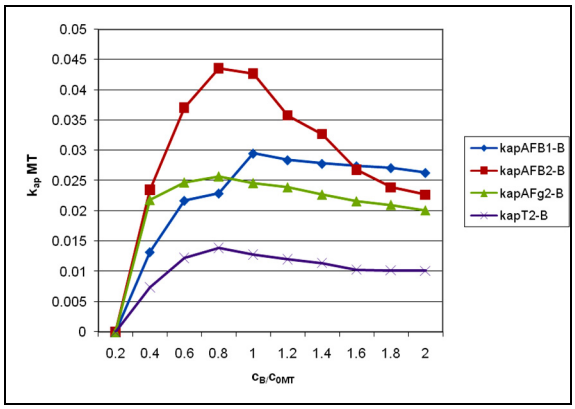


**Figure 1.** Values of apparently degradation constants of MT depending on the mass ratio between bentonite (B) and MT (g B/μg MT)

$k_{apAFB1}$

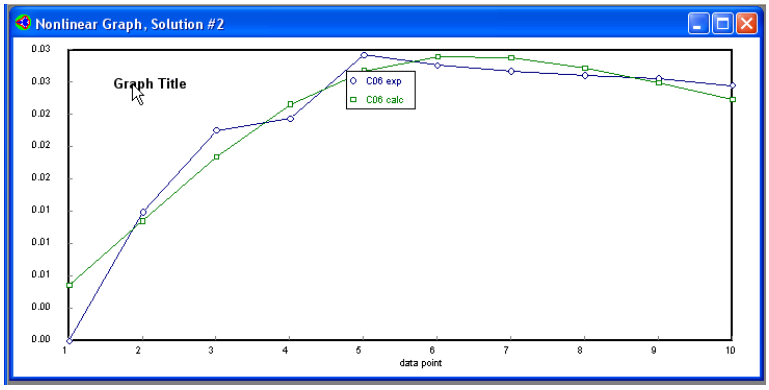


**Figure 2.** AFB1-B experimental data adjustment

$k_{apAFB2}$

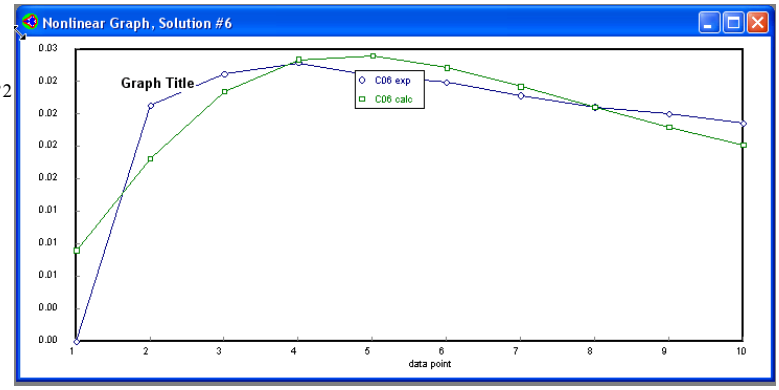


**Figure 3.** AFB2-B experimental data adjustment

We adjusted the experimental data from the proposed model; the results are shown in Figures 25, 26, 27 and 28, in order of AFB1, AFB2, AFG2 and T-2. The obtained values for the parameters *a*, *b* and *c* is presented in table 3.

$k_{apAFG2}$

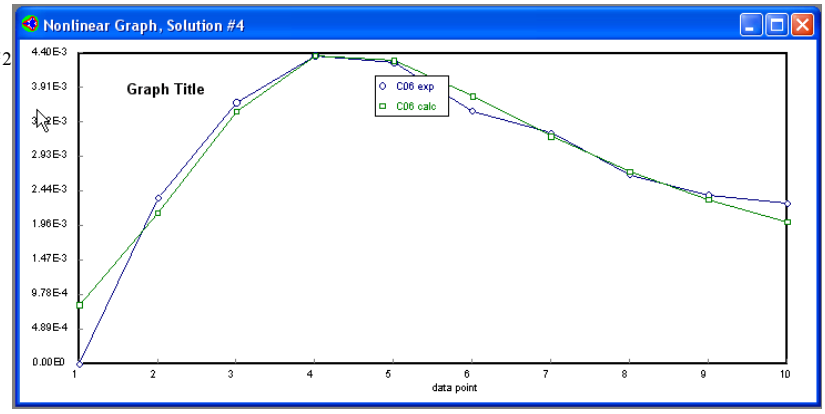


**Figure 4.** AFG2-B experimental data adjustment

$k_{apT-2}$

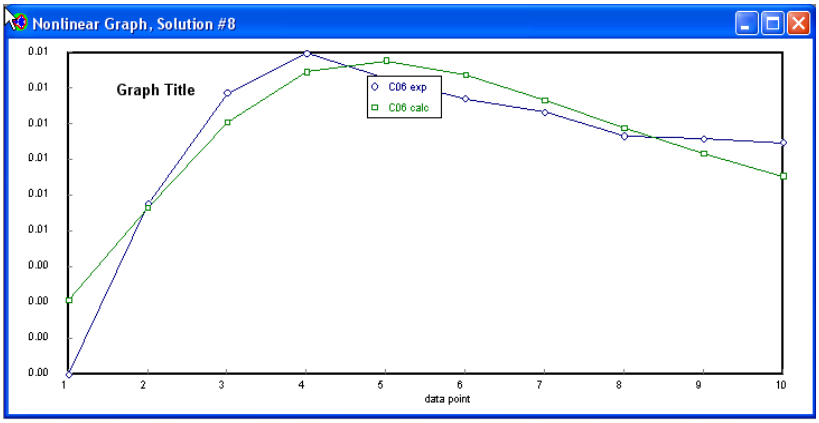


**Figure 5.** (T-2)-B experimental data adjustment

**Table 1.** Obtained values for the parameters *a*, *b* and *c* for MT photodegradation (AFB1, AFB2, AFG2, and T-2) in the presence of bentonite (B)

| Parameter | AFB1 | AFB2 | AFG2 | T-2 |
|---|---|---|---|---|
| *a* | 0,0153 | 0,0013 | 0,0360 | 0,0092 |
| *b* | 0,7131 | 0,3353 | 1,5101 | 0,7614 |
| *c* | 0,2177 | 0,2563 | 0,5732 | 0,3502 |

## CONCLUSIONS

The proposed model for prediction of AFB1 photodegradation in the presence of bentonite has a maximum for $k_{apAFB1}$ at around 1,3 for the $[B]_0 / [AFB1]_0$ ratio, which is in good agreement with experimental data obtained for AFB1, which resulted for $k_{apAFB1}$ a 1,0 value of the ratio. The proposed model for prediction of AFB2 photodegradation in the presence of bentonite has a maximum for $k_{apAFB2}$ at around 1,2 for the $[B]_0 / [AFB2]_0$ ratio, which is in good agreement with experimental data obtained for AFB2, which resulted for $k_{apAFB2}$ a 1,0 value of the ratio. The proposed model for prediction of AFG2 photodegradation in the presence of bentonite has a maximum for $k_{apAFG2}$ at around 0,8 for the $[B]_0 / [AFG2]_0$ ratio, which is in very good agreement with experimental data obtained for AFG2, which resulted for $k_{apAFG2}$ a 0,8 value of the ratio. The proposed model for prediction of T-2 photodegradation in the presence of bentonite has a maximum for $k_{apT-2}$ at around 1,0 for the $[B]_0 / [T-2]_0$ ratio, which is in good agreement with experimental data obtained for T-2, which resulted for $k_{apT-2}$ a 0,8 value of the ratio. The constructed models may be used to estimate the B/MT concentrations ratio requirements for the photochemical degradation of mycotoxins in each hand. On the other hand, it becomes obvious need to be operated the sunflower oil refining such as decontamination of mycotoxins needs amounts of present bentonite ratios to be within certain limits, depending on the nature of mycotoxins. The value of B/MT ratio needed for decontamination at the same time requires restrictions on the choosing of circulation pumps.

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