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Research Article

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Control of Planktonic Cyanobacteria in Preventive Treatment with Hydrogen Peroxide to Maintain Recreational Activities in Freshwater Lakes

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ABSTRACT

The strategy adopted for controlling cyanobacterial biomass relies on the use of oxidative stress, following a prophylactic approach aimed at limiting the proliferation of these organisms as soon as the first signs of development appear. The preventive principle involves early intervention, that can only be carried out on small lakes of less than 25 ha. Within this framework, hydrogen peroxide is applied at a concentration of 5 mg/L when the threshold of 10 µg/L of phycocyanin is reached for two consecutive weeks. This choice helps limit the potential release of cyanotoxins caused by the oxidant, since low biomass levels correspond to smaller quantities of releasable toxins. To ensure optimal treatment efficiency, weekly analytical monitoring is carried out to rapidly detect the onset of cyanobacterial growth dynamics and enable prompt intervention. The application procedure consists of introducing hydrogen peroxide throughout the entire water body by injecting it into the water column. All activities on the lake are prohibited for a period of 24 to 48 hours. Activities may resume once the H_2O_2 concentration in the water is below 0.5 mg/L and total *Microcystins* are not detected above 0.3 mg/L using the ELISA test. For 5 years, all applications on this lake have shown a reduction of over 90% in cyanobacteria following the application of 5 mg/L of H_2O_2 on populations of *Aphanizomenon flos aquae*, *Microcystis aeruginosa*, or *Woronichinia naegeliana*, without interruption of recreational activities outside the treatment periods. This study shows the economic and sanitary interests in small shallow lakes of using H_2O_2 at low concentrations of 5 mg/l on low cyanobacteria biomasses to limit their development and to avoid the bloom phase and microcystin concentrations beyond health thresholds.

Keywords: Cyanobacteria, Cyanotoxins, Freshwater Lakes, Phycocyanin, Management, Recreative Activities, Hydrogen Peroxide.

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Abbreviations

AFNOR: Association Française de Normalisation

ANSES: Agence Nationale de Sécurité Sanitaire de l'alimentation, de l'Environnement et du travail

ARS: Agence Régionale de Santé

CYANOCOST: Action ES1105 – European Cooperation in Science and Technology

CPES: Project Interreg "Channel Payments for Ecosystem Services"

MIB: 2-methylisoborneol

Introduction

Cyanobacteria in freshwater require health monitoring, as some species produce various cyanotoxins that pose a significant risk to human health, animals and plants [1-6]. It is also noted that these toxins persist during the transfer of fresh water to marine environments, and present a risk, particularly to shellfish farming sites [7,8]. In humans, the symptoms observed are due to ingestion, skin contact or inhalation of contaminated aerosols [9]. Recreational activities such as

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swimming and water sports therefore pose risks in the event of cyanotoxins [10]. Blooms, which are becoming more frequent, lead to economic costs to produce drinking water from surface water and the cessation of recreational activities. Their prolonged presence disrupts the functioning of ecosystems [11].

Curative strategies for controlling cyanobacteria are frequently criticized due to their potential impact on the aquatic ecosystem, especially as they often take precedence over preventive measures [12]. Despite efforts to reduce nutrient inflows from catchment areas, these preventive actions have shown limited effectiveness in curbing cyanobacterial growth [13]. Local authorities, motivated by societal and economic interests, continue to develop recreational activities around their water bodies and seek curative methods that minimize disruption to the aquatic environment [14].

The control of cyanobacterial development by H_2O_2 has been studied to replace copper because of its accumulation in sediments and its toxicity in the ecosystem [15-17]. Cyanobacteria cells are affected at concentrations of 1 mg/L H_2O_2 in mesocosm studies [18]. In the natural environment, soluble organic matter interferes with the oxidant and involves higher doses of H_2O_2 [19]. Consequently, one of the objectives of this study is to apply the lowest possible concentrations of H_2O_2 to low biomasses measured weekly. Criticism of the use of this oxidant relates to the impacts on the living environment (zooplankton, fish, plants, bacteria, viruses, fungi), the replacement of species that may be potentially toxigenic and the solubilization of cyanotoxins. H_2O_2 acts immediately upon contact with the living world without sterilizing it and has been demonstrated at the level of the bacterial community [20]. Most of the biodiversity is found in the sediment and the low doses of the oxidant limit its impact by its rapid decomposition in the water column when applied to the surface [21]. The degradation of microcystins occurs within a few hours during their solubilization by the lysis of cyanobacterial cells [22].

Applying H_2O_2 to control low biomass cyanobacteria offers several advantages: it helps manage cyanobacterial growth, protects users from various symptoms linked to water consumption and aerosol exposure, and preserves both biodiversity and the ecological balance of the aquatic ecosystem, especially in the face of recurring high cyanobacterial biomass that can result from inaction [23-26].

Reservoirs dealing with eutrophication for more than a century have undergone extensive experimentation, including management of watershed nutrient loading, hydrological manipulation, nutrient removal, hydro physical and physical control, chemical treatments, and oxidative stress-based approaches [27,28]. This study validates the work of Matthijs in a natural setting, demonstrating that H_2O_2 application is effective for controlling cyanobacterial biomass and promoting other algal families, particularly when used at low concentrations targeting low cyanobacterial biomass [29].

Materiel and Methods

Lake Tanchet in Les Sables d'Olonne, France, is a 6.5 ha urban lake, averaging 1.7 m deep, affected by eutrophication and high turbidity. Used year-round for boating and occasional fishing, it follows cyanobacteria health guidelines. The lake has no macrophytes, is fully silted, and is separated from the sea by a dike, with its outlet draining directly into the marine environment through a threshold and a small pre-reservoir area upstream.

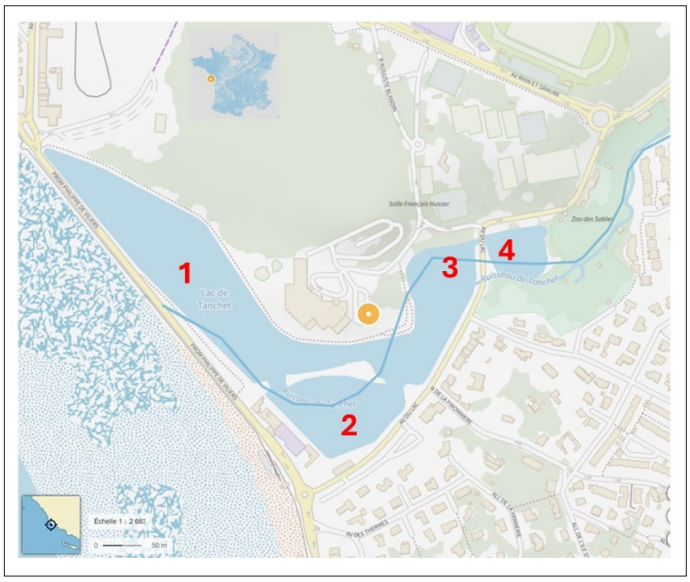

Photo 1: Sampling points at Lake Tanchet - Geoportail Map 2026

Batch test on raw water from the lake at differentconcentrations of H_2O_2 to define the consumption kinetics up to 0.5 mg/L.

Physico-chemical monitoring of phycocyanin, chlorophyll, conductivity, pH, O_2, Secchi disk by profiles in the water column weekly from May to October. Multiparameter probe are YSI or Aquaread SDEC and for phycocyanin and chlorophyll with probe Trios or Trilux.

Measurements of the phycocyanin and chlorophyll pigments are carried out using probes at four points in the lake selected according to the frequency of accumulation phenomena linked to the wind or in sheltered areas. The different objectives are to know the distribution of cyanobacteria with phycocyanin and to quickly detect the species present [30,31]. A sample is taken in the centre of the lake using a one-meter tube, on the first meter of the water column, corresponding to a volume of one liter sampled [31,32]. The collected sample is then divided into three fractions, with and without preservatives (alkaline lugol), to allow the identification, counting of algal species and analysis of microcystins [32].

Phytoplankton were analyzed using the Nageotte cell technique on samples taken from the top meter of water at the centre of each cumulative transect [31,32]. The samples are concentrated on a polycarbonate filter with a porosity of 1µm. Identification and counting by direct microscopy in number of cells then converted into biovolume according to the AFNOR standard XP T 90-330 in 2025 [32].

Analyses of total microcystins by 1 or 10 mg/L or 0.01 to 1 mg/L ELISA strip test (Eurofins Abraxis).

H_2O_2 analyses in the field by Quantofix TM method using Macherey-Nagel TM strips, reading from 0.5 to 25 mg/l, detection limit at 0.5 mg/l, with a QUANTOFIX RELAX reader. Another more sensitive method was used: Lovibond Water Testing method, Lovibond R reagents in pellets for a concentration of 0.03 to 3 mg/l of H_2O_2. Measurement with Lovibond's MD 610 photometer at 530 nm.

Hydrogen peroxide is 35% and stored in 20 L canisters.

Progress

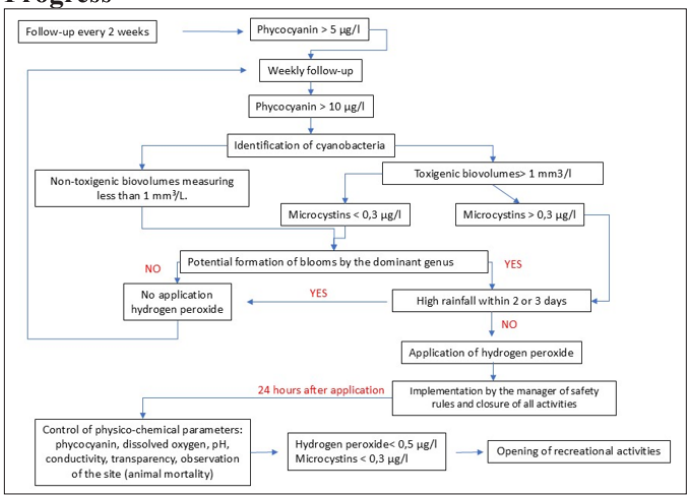

Figure 1: Methodology for monitoring and managing the health of bathing waters and nautical activities in relation to planktonic cyanobacteria, as implemented at Tanchet Lake in 2024.

Installation of a safety enclosure around the lake and cessation of all activities on the water. Diffusion of peroxide hydrogen by the boat's engine over the entire lake at 4 p.m.

The next day (+ 15 hours): Measurements of the different parameters with the multi-parameter probe on the defined transects, peroxide hydrogen analysis on cumulative samples and microcystin analyses using ELISA strips.

After 7 days: continuation of weekly monitoring of physico-chemical measurements and phytoplankton analyses.

The imponderables for the application are that hydrogen peroxide must be consumed within 48 in batch tests, that there is a growth dynamic expressed by the measurement of phycocyanin above 10 mg/l for 2 weeks.

H_2O_2 is applied to the total surface of the lake.

Results

Application and effects of hydrogen peroxide treatments (Figure. 2)

First Application: June 7, 2024

The initial hydrogen peroxide treatment was carried out at a concentration of 5 mg/L, specifically targeting the dominant cyanobacterial species, *Aphanizomenon flos aquae*. Within 48 hours, this application led to a significant decrease in cyanobacterial cell counts, achieving a 99% reduction per milliliter. Phycocyanin levels also dropped by 72.7%, as indicated in Table 1. These changes effectively suppressed cyanobacteria growth, resulting in phycocyanin concentrations remaining below 10 µg/L for eight weeks. During this period, there was a rapid proliferation of phytoplankton, predominantly Chlorophyceae species such as genera *Pediastrum*, *Scenedesmus*, and *Coelastrum*. Chlorophyll concentrations declined sharply from 88.7 mg/L to 11.4 mg/L over ten days.

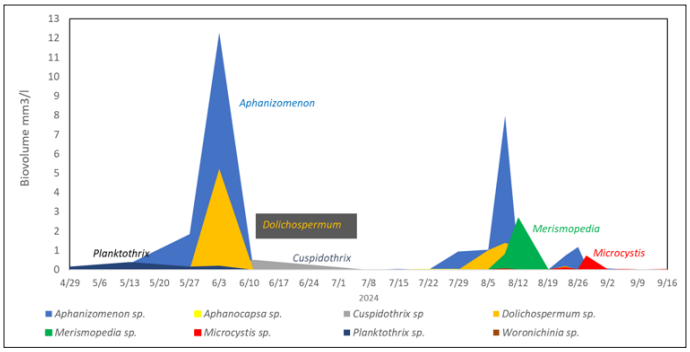

Figure 2: Expression of cyanobacteria cells in biovolume at point 1

Table 1: Impact of hydrogen peroxide on cyanobacteria and phycocyanin at Tanchet lake in 2024

Date	07/06/24	09/08/24	23/08/24	06/09/24
H_2O_2 Quantity	5mg/L	2.5mg/L	2.5mg/L	5mg/L
% reduction on cell mm3/L biovolume	99,96	99,08	72.34	65,28
% reduction on phycocyaninfrom mg/L	70,2	71,76	38,95	37,32
standard deviation on phycocyaninfrom the 4 lake sampling areas	14.39	4.49	26.91	33.29
Toxigenic biovolume of cyanobacteria mm3/L	18,22	12,01	0,92	0,08
Number cells of cyanobacteria by mL	193 667	328 220	12 320	1020

Second Application: August 9, 2024

A subsequent treatment was performed at a lower concentration of 2.5 mg/L. At the time of application, the lake harbored a mixed cyanobacterial population, with *Aphanizomenon flos aquae* numbering 100,000 cells/ml and *Merismopedia minutissima* at 210,000 cells/ml. The intervention resulted in a 91% reduction in cell count per milliliter and a 72% decrease in phycocyanin. Ten days following the treatment, chlorophyll levels further decreased to 7.4 mg/l. After this application, *Microcystis* emerged as the predominant genus (14,000 cells/ml), while *Aphanizomenon flos aquae* measured 16,200 cells/ml. Due to the continued presence of *Microcystis*, a third hydrogen peroxide application was necessary.

Third Application: August 23, 2024

The third H_2O_2 treatment was applied at 2.5 mg/L. This

intervention resulted in a 72.3% reduction in cyanobacterial cell numbers and a 39% decrease in phycocyanin levels within 48 hours. Importantly, no microcystin were detected following the application.

Fourth Application: September 6, 2024

Persistent *Microcystis* presence and a low, heterogeneously distributed cyanobacteria population (fewer than 1,000 cells/ml) prompted a fourth application of hydrogen peroxide at a concentration of 5 mg/l. This treatment achieved an 82% reduction in cell counts and a 37% decrease in phycocyanin, with no microcystin detected after 48 hours.

Summary of Effects

The average phycocyanin values measured across the four designated zones of the lake typically reflect a reduction of around 70%. Over the six years of hydrogen peroxide applications, similar dynamics were observed. Cyanobacteria distribution in the lake was heterogeneous, with standard deviation values for measurements ranging from 4.49 to 33.29. *Aphanizomenon* remained the dominant genus from May to September, while *Merismopedia* and *Microcystis aeruginosa* reached peak abundance in August (Figure. 3). Water column profiles confirmed the predominance of *Aphanizomenon* and *Dolichospermum* near the lake surface, whereas *Merismopedia* and *Microcystis* were distributed throughout the water column.

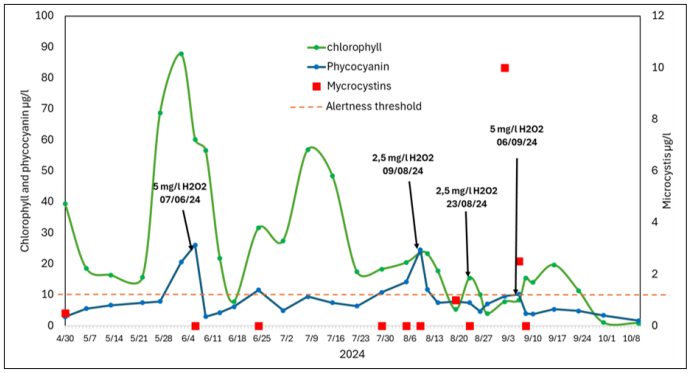

Figure 3: Evolution of pigments and microcystins in the middle of the lake Tanchet

H_2O_2 applications led to a 10% reduction in lake oxygenation (Figure 4). This decrease did not adversely affect fish populations and was also observed as part of the natural algae dynamics, even in the absence of treatments.

The dynamics of eukaryotes (Figure. 5) are reached by $H_2O_2$with very different responses. Before the first application of H_2O_2, the water body is strongly colonized by eukaryotes, going from 80,000 cells/ml to 2,000 in 3 weeks, validated by chlorophyll which evolves from 40 to 18 µg/L without application of oxidant but with a disturbance of 40 mm of rain (Figure. 6). The second application on 9 August, i.e. 9 weeks after the first, occurred after 90 mm of rain, the medium eutrophied (60 µg/L of chlorophyll and 72,000 cells/ml of eukaryotes) but the Eukaryotic/Prokaryotic trend was reversed at the end of July with the dynamics of other cyanobacteria species and the presence of *Microcystis*. H_2O_2 regulates phytoplankton dynamics but in parallel with other microorganisms that have not been identified.

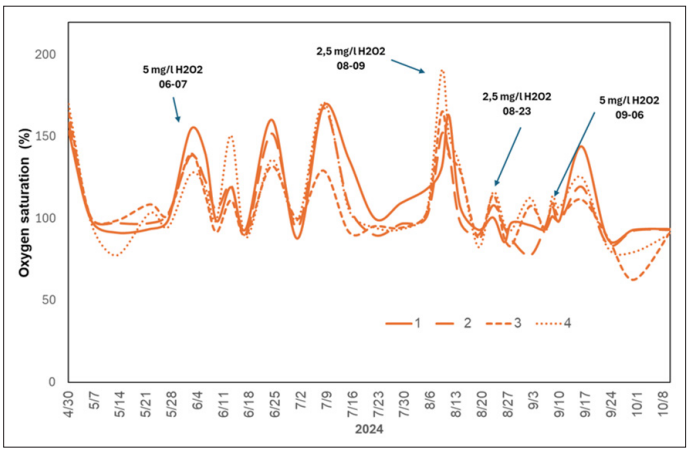

Figure 4: Oxygen saturation monitoring (-0,5 m) in four zones of Tanchet lake

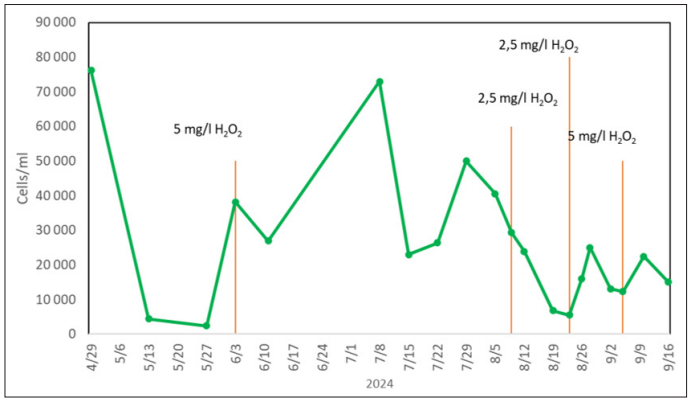

Figure 5: Dynamics of eukaryotes in number of cells/ml

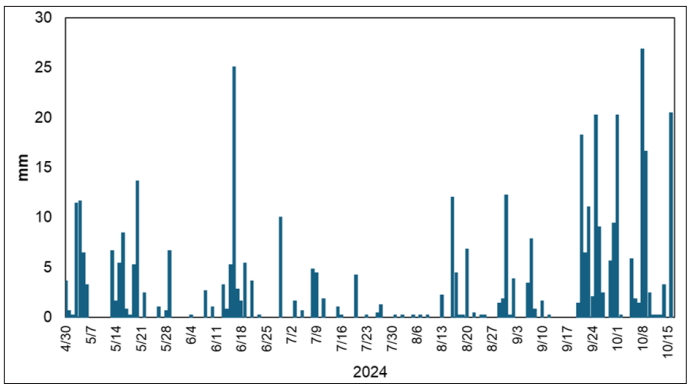

Figure 6: Daily cumulative rainfall on Tanchet lake

Discussion

To mitigate the oxidative effect of H_2O_2 on the ecosystem, two applications at 2.5 mg/l were tested in 2024 instead of 5 mg/l. The results on the environment were different because of the biomass and the species (Table 1). It is therefore difficult to conclude the specific impact of H_2O_2 without taking into account that of the microbial loop [33,34]. Batch tests show a reduction of cyanobacteria to 48% with a concentration of 2.5 mg/L instead of 98% at 5 mg/L of H_2O_2, whereas in the natural environment the results differ due to a heterogeneous distribution of cyanobacteria on the lake (Table 1). This variation in results may be due to a lower light intensity in September than in August [35].

The limit to 0.5 mg/L of H_2O_2 is retained in this methodology for the ease of its control by strip tests. To meet the standards for toxicity in the natural environment of H_2O_2, the Lovibond Water Testing method shows that the threshold of less than 0.1 mg/L of H_2O_2 is obtained only after 16 h. (Figure. 7), confirming access to the water body after 48 hours without any health risk for users.

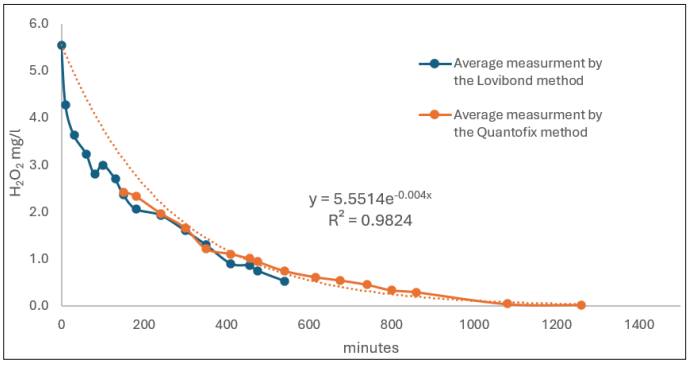

Figure 7: Kinetics of H_2O_2 consumption from lake of Tanchet by 2 analysis methods

This method also showed the persistent production of H_2O_2 in blooms by the presence of eukaryotes in the lake up to values greater than 1 mg/L in H_2O_2. Could this persistence of H_2O_2 have an impact on population dynamics either between prokaryotes and eukaryotes or between the different genera of cyanobacteria in the environment?

The application of hydrogen peroxide in a natural environment requires particular vigilance, particularly with regard to its mode of application, storage and transport [36]. The effectiveness of H_2O_2 is conditional on intervention on low algal biomasses, which presupposes regular and precise monitoring of the water body, in particular by weekly monitoring of the phycocyanin and chlorophyll pigments at different points on the site.

Uncertainties remain about the long-term consequences of the use of H_2O_2, including the potential disruption of other organisms such as viruses or fungi. However, the analysis of weekly phytoplankton monitoring carried out over ten years – including four on a first water body and six on Lake Tanchet – did not reveal any significant change in algal composition. This observation applies both to the body of water dominated by the genera *Limnothrix* and to those, such as lake Tanchet, where we find *Aphanizomenon*, *Microcystis* and *Woronichinia*.

The use of H_2O_2 affects the functioning of the microbial loop, so it seems essential to deepen the assessment of these impacts using metagenomics or meta transcriptomics approaches, to better understand the dynamics of recolonization of the different communities present in the aquatic environment, beyond the nutrient resource alone [37].

Conclusions

During the summer of 2019, Lake Tanchet was confronted with a major episode of proliferation of *Microcystis* with toxins. This situation led to the suspension of nautical activities for several weeks, due to a concentration of microcystins exceeding 50 µg/L and marked olfactory nuisances, attributed to molecules such as geosmin and MIB. Faced with this health problem, a six-year experiment was conducted, based on the curative application of H_2O_2. This protocol made it possible to control the high biomasses of cyanobacteria observed during the 2019 and 2020 episodes, while promoting the resumption of recreational activities in an environment where the presence of cyanobacteria and cyanotoxins remained a concern.

Analysis of the effects of the treatment shows that, despite repeated applications of H_2O_2, eukaryotes persist in the environment. Thus, the oxidant, used at a concentration of 5 mg/L, does not lead to the total elimination of phytoplankton and therefore does not have a sterilizing effect on the environment.

Each treatment reduced microcystin concentrations to at least the analytical detection limit of 0.1 µg/l.

No fish mortality was noted during these operations.

Acknowledgement

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