Photodynamic Effect. Experience of Application of Photosensibility Series for Monitoring Microbiological Water Pollution

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Abstract— *The kinetics of the destruction of standard museum strains of microorganisms as a result of photodynamic action of red light and a number of non-toxic photosensitizers in the process of water conditioning has been studied experimentally. Prokaryotic cells of Escherichia coli ATCC 35218, eukaryotic cells of Candida albicans ATCC 24433 were used as the objects of the study. Eosin H, sodium fluorescein, methylene blue and riboflavin (vitamin B2) in concentrations of 10 mg/l served as photosensitizers. A photodynamic effect was established with respect to microorganism cells, leading to their death in the presence of photosensitizers and red light. It has been shown that riboflavin and fluorescein are the most effective for eukaryotes (on the example of Candida albicans ATCC 24433), which help to reduce the number of colonies of cells in 2 hours of observations by more than 3.0 and 11.0 times, respectively. It was found that the death of prokaryotic cells in the case of Escherichia coli ATCC 35218 is most effective in causing methylene blue, riboflavin (vitamin B_2). For 2 hours of observations in their presence due to photodynamic action, microflora decreases in 36.0 and 90.0 times, respectively. The photodynamic effect of eosin against the microorganisms under study was the smallest, which is explained by the peculiarities of its chemical structure, including phenolic groups, which are known to exhibit an antioxidant effect. It is shown that fluorescein and methylene blue are most promising for effective lethal action against pathogenic microflora in pool water. Riboflavin is most effective for purification of drinking water used for cooking and drinking in public, including pre-school and school meals, which will allow not only to exclude the possibility of mass poisonings, but also to provide a daily intake of vitamin B_2 with a glass of water.*

Keywords— photodynamic effect, methylene blue, riboflavin, fluorescein, red light, death of museum strains of Escherichia coli ATCC 35218, Candida albicans ATCC 24433.

I. INTRODUCTION

Water used for food purposes requires special water treatment procedures that ensure the death of microorganisms. One of the new promising ways to combat microbiological water pollution along with chlorination and ozonation is considered a photodynamic method involving the use of light and photosensitizers. The method is based on photo induced by the sensitizer the formation of active forms of oxygen, which due to the activation of free radical processes cause the death of microorganisms.

The beginning of studies of the photodynamic effect is connected with the works of O. Raab and G. von Thappeyner [1, 2], who in 1897 discovered that infusoria and other protozoans, stained with acridine derivatives, stop their growth and die under illumination. This phenomenon was called the photodynamic effect (action) (FD), which denoted the influence of light on the dynamics of cell growth, their mobility and death. It was soon shown that. For photodynamic damage of cells, in addition to the dye and light, oxygen is needed. The photodynamic effect is found in all living organisms. During the 20th century, primary mechanisms of photodynamic cell death were studied [3, 4]. It is shown that multiple lesions are induced in procaryotes as a result of photodynamic action: loss of ability to form colonies, damage to DNA, proteins, cell membranes. For the manifestation of the photodynamic effect, the presence of a photosensitizer is necessary, which increases the sensitivity of tissues and cells to light. The critical effect of the photosensitizer is the formation of active forms of oxygen in

the body, the action of which as a result of photooxidation of most biologically significant structures: amino acids (methionine, histidine, tryptophan, etc.), nucleosides, lipids, polysaccharides leads to damage and cell death.

There are two types of photodynamic processes. In the photodynamic effect of type I, the photoexcited molecules of the sensitizers of S pass into the excited singlet state of 1S * and then into the long-lived triplet state of 3T * and react with the substrate RH and the molecules of the medium, in particular, with water. Intermediate free-radical intermediates are formed, which then interact with oxygen and give a complex mixture of highly active products of a radical nature that continue reactions of free radical oxidation and damage biostructures. One of the damaging factors is singlet oxygen 1O2, which can destroy cells in the immediate vicinity of the photosensitizer molecules. Oxidizing ability of singlet oxygen is 2 orders of magnitude higher than that of normal oxygen. It can damage all the major components of cells. In nucleic acids, it attacks mainly a pair of thymine and uracil, and also causes cross-linking of DNA-DNA or DNA-protein, single-strand breaks of DNA. These effects are exacerbated by the fact that enzymes that repair DNA are particularly sensitive to singlet oxygen. However, in interphase cells, DNA is not a primary target for PD effects, since photosensitizers usually localize in the cytoplasm and do not penetrate the nucleus [3, 4]. In proteins, disulfide bonds, cysteine, histidine, tyrosine, tryptophan and phenylalanine are most easily photocontained, especially if they are located on the surface of globules and are accessible to the photosensitizer. They usually play a key role in enzymatic activity, and therefore proteins are very sensitive to photodynamic effects. Proteins lose activity as a result of photoinduced disruption of the structure of the active site, internal cross-links or intermolecular cross-links with other proteins, lipids, RNA and DNA. In type I photodynamic reactions, the radical pairs formed during electron transfer are relatively stable in an aqueous medium, where the reverse electron transport is difficult. In nonpolar lipid media, the lifetime and solubility of IO2 are higher. Consequently, type I photodynamic reactions are easier in the cytosol, and type II in the lipid phase of biomembranes. Thus, the photodynamic reactions with the participation of hydrophilic photosensitizers predominantly proceed according to the first type, and the hydrophobic photosensitizers according to the second type. Type II reactions dominate the damaging effects of most photosensitizers, including porphyrins, chlorins, phthalocyanines, and so on.

The development of oxidative stress, the disruption of the functions of cells and, as a result, their death are due to the intense generation of reactive oxygen species: superoxide radical anion (O2-), hydroxyl, hydroperoxyl radicals (OH •, HO2 •), hydrogen peroxide (H2O2), singlet oxygen (IO2). The photodynamic effect manifests itself both with UV irradiation, but especially light acts in the red wavelength range (620-780) nm. Since 1903, the study of the potential therapeutic value of the photodynamic effect began, the skin cancer was first cured with eosin staining and bright sunlight [5], since 1970 photodynamic therapy has been widely used to treat tumors [6, 7], in treatment of periodontal diseases [8-15].

Methylene green, acridine orange and proflavine, methylene blue and toluidine blue, indocyanine green, Bengal pink, eosin, curcumin, chlorine, porphyrins, phthalocyanines, chalcogen-containing benzophenoxazinium dyes, conjugates of nanoparticles with methylene blue, porphyrin or chlorine are used as photosensitizers. Cationic photosensitizers are most effective, since a positive charge enhances the interaction of the dye with the negatively charged surface of the microorganism.

In recent years, the prospects for using the photodynamic effect for purifying drinking water, controlling microbial contamination of water in aquariums and basins have been extensively studied [16-21].

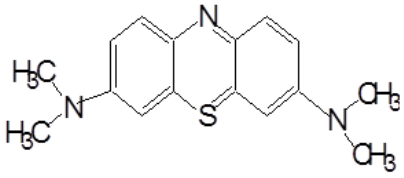
This paper presents the experience of inactivation of a number of microorganisms in water due to the photodynamic effect using red light and a number of non-toxic photosensitizers in the process of water conditioning for food purposes.

The dynamics of growth and death of standard museum strains of microorganisms of different types: prokaryotic cells of Escherichia coli ATCC 35218, and also cells of eukaryotes Candida albicans ATCC 24433 were studied.

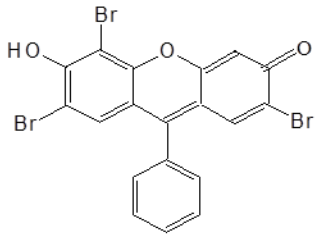
The bacterium Escherichia coli (E. coli) is found in the intestines of humans and warm-blooded animals. Most strains of E. coli are harmless, but some strains, for example, O157: H7, O121, O104: H4 and O104: H21, synthesize potentially deadly toxins that can contribute to human infection by nutritional methods with low food hygiene. The ability of virulent strains to survive for some time in the environment makes them an important indicator for investigating the presence of traces of fecal contamination in water.

Representatives of the genus Candida (primarily Candida albicans) are classified as conditionally pathogenic varieties of fungal infection. Microorganisms of the genus Candida are part of the normal microflora of the mouth, esophagus, vagina and large intestine of most healthy people. The disease is caused not only by the presence of fungi of the genus Candida, but by their multiplication in large numbers or by the entry of more pathogenic strains of the fungus. Most often, candidiasis occurs in people with a decrease in general and local immunity.

Methylene blue (I), eosin (II), sodium fluorescein (III) and riboflavin (vitamin B2) (IV) were used as sensitizers. The compound formulas are shown in Scheme 1.



Methylene blue (I)



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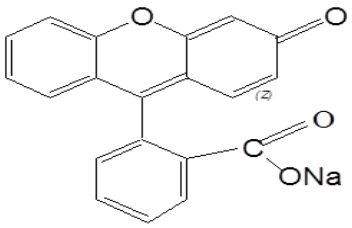
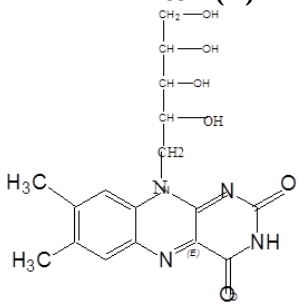


Fluorescein sodium (III)



Riboflavin (vitamin B_2) (IV)

SCHEME 1. FORMULAS OF PHOTOSENSITIZERS

The compounds studied are used in medicine, as a rule, as antiseptic, antimicrobial agents. Fluorescein is widely used as a diagnostic tool for the detection of lesions of the cornea of the eye, as a preparation for fluorescent angiography [22].

II. EXPERIMENTAL PART

In sterile tubes, a working concentration of microorganisms (1.5 kd / ml Candida albicans ATCC 24433 and 75.0 kts / ml Escherichia coli ATCC 35218) was prepared. One of the photosensitizers, methylene blue (I), eosin (II), fluorescein (III) and riboflavin (IV), and a culture of microorganisms were added to the flasks with sterile saline solution at comparable concentrations of 10 mg / l. The contents of the reaction vessels were mixed, which provided a high concentration of oxygen in the liquid.

The flasks were placed under a lamp emitting red light with a wavelength (620-780 nm), with a power of 250 watts. From prototypes (irradiation with light in the presence of photosensitizer) and control (red light irradiation) at 2, 5, 10, 20, 30, 40, 60 and 120 minutes, 0.1 ml samples were taken by a sterile pipette, introduced into sterile Petri plates with meat-peptone agar and thoroughly rubbed over the entire surface with a sterile spatula. The crops were incubated in a thermostat for 24 hours. CFU counts were made on the surface of the agar. In each of the control points, the reliability of the difference in the mean indicators was determined by the Student's criterion [23].

Results and its discussion When studying the effect of photosensitizers on Candida albicans ATCC 24433 and Escherichia coli ATCC 35218, it was shown that, without irradiation, the number of microorganisms of Candida albicans decreased by 10-12% (Figure 1a, curve 1) for 2 hours of observation, for Escherichia coli by 20% (Figure 1b, curve 1). When samples are exposed in a red light flux without a photosensitizer, the number of colonies decreases by 3.0 and 1.5 times, respectively, for the mentioned microorganisms (Fig. 1a, curve 2, Fig. 1b, curve 2).

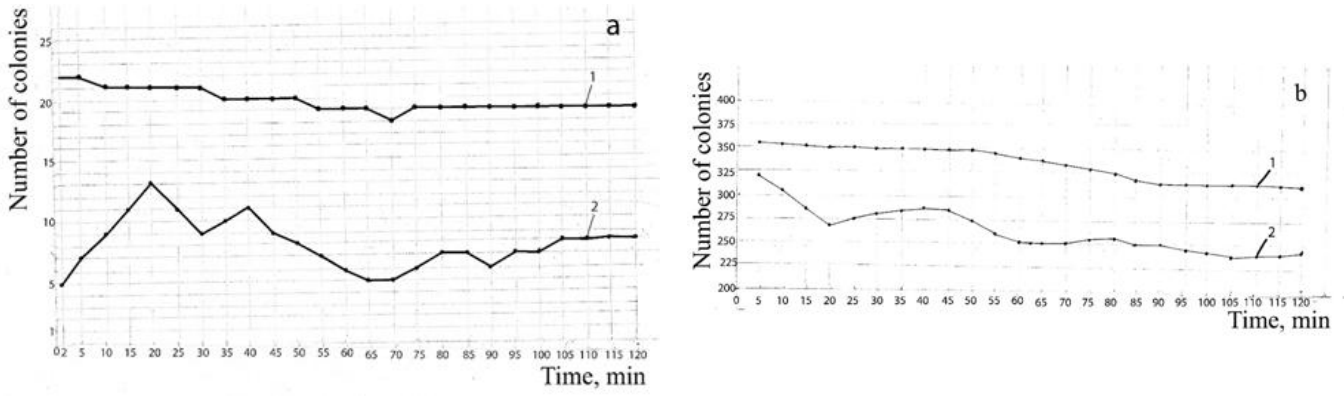


FIG. 1. A) DYNAMICS OF CHANGES IN THE NUMBER OF MICROORGANISMS CANDIDA ALBICANS ATCC 24433

Under the influence of red light. The initial concentration is 1.5 thousand / ml, T = 20 ° C, the stirring speed is 100 rpm; b) Dynamics of changes in the number of microorganisms of Escherichia coli ATCC 35218 under the action of red light. The initial concentration is 75 thousand / ml, T = 20 ° C, the stirring speed is 100 rpm.

Reducing the number of colonies of microorganisms under the influence of red color is explained by the photodynamic effect caused by the presence in the cells of endogenous photosensitizers, which include hemoglobin, porphyrins, which form part of a number of enzymes and vitamins.

In the presence of exogenous photosensitizers, there is a more significant decrease in the number of microorganisms. Comparison of a number of photosensitizers made it possible to establish differences in their action. Photo 1 shows the number of colonies of Candida albicans ATCC 24433 at 2 hours exposure in red light in the presence of fluoscecein (III), eosin (II) and methylene blue (I).

Photo 1 shows the photodynamic effect of fluorescein in comparison with culture control without irradiation and control after 2 min and 2 hours of irradiation.

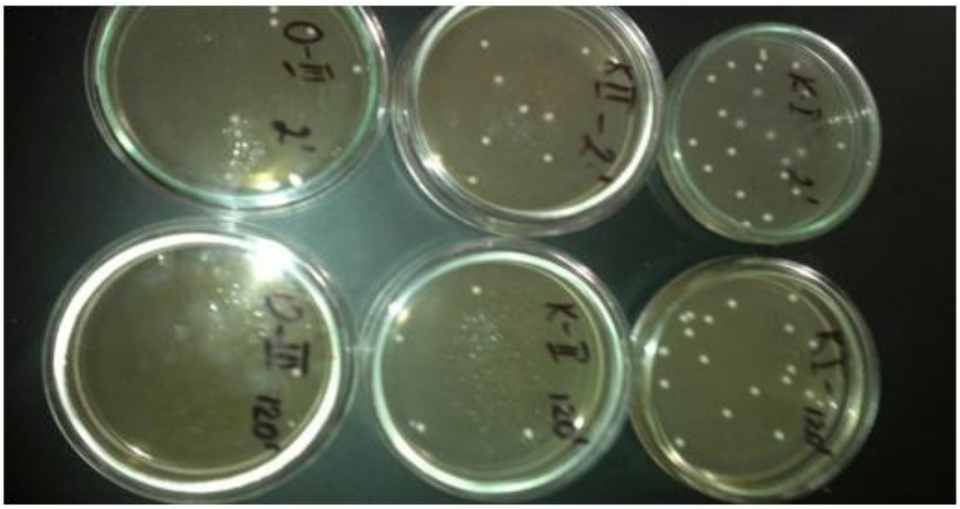


PHOTO 1. Influence of fluorescein (III) (left) upon irradiation with red light on the growth dynamics of colonies Candida albicans ATCC 24433. The concentration of the photosensitizer is 0.10 mg / l; Exposure time 2 hours. Red light (λ = 620-780 nm), lamp power 250 watts. T = 20 ° C, stirring speed 100 rpm.

A study of the growth kinetics of eukaryotic cells of Candida albicans ATCC 24433 in the presence of the photosensitizers under study showed that PD exhibited all the dyes (Fig. 2a, b).

From Fig. 2a, it is seen that eosin (II) was the least effective. Obviously, this is due to the peculiarities of the chemical structure of the compound. In the structure of eosin there are phenolic groups, which are known to act as antioxidants, capable of directly interacting with free radicals [24, 25], which leads to a decrease in the intensity of free radical processes. Reducing the number of reactive oxygen species leads to a decrease in PD. The effect of methylene blue (I) and riboflavin

(IV) is comparable, fluorescein (III) is the most effective, leading to almost complete death of microorganisms in 2 hours (Fig. 2a). The remaining dyes indicated above were inferior to fluorescein on average by 30% (Fig. 2).

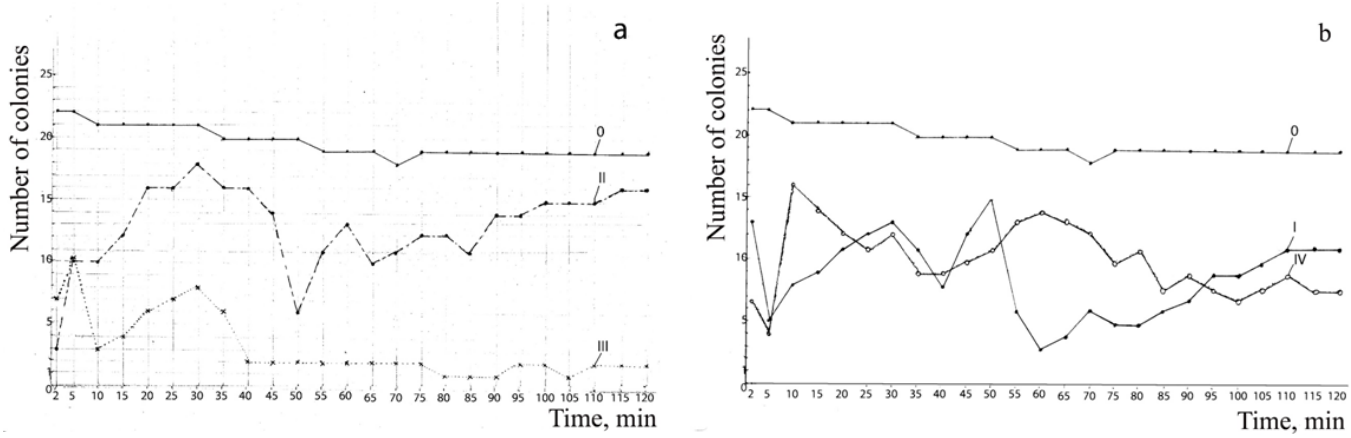


FIG. 2. a) Dependence of changes in the number of Candida albicans ATCC 24433 cells (initial concentration 1.5 kd / ml), without dye (0), in the presence of eosin (II), fluorescein (III). Irradiation with red light (λ = 620-780 nm), lamp power 250 W. The concentration of the photosensitizer is 0.10 mg / ml; T = 20 ° C, stirring speed 100 rpm. b) Dependence of changes in the number of cells of Candida albicans ATCC 24433 (initial concentration 1.5 thousand / ml), without dye (0), in the presence of methylene blue (I), riboflavin (IV). Irradiation with red light (λ = 620-780 nm), lamp power 250 watts. The concentration of the photosensitizer is 0.10 mg / l; T = 20 ° C, stirring speed 100 rpm.

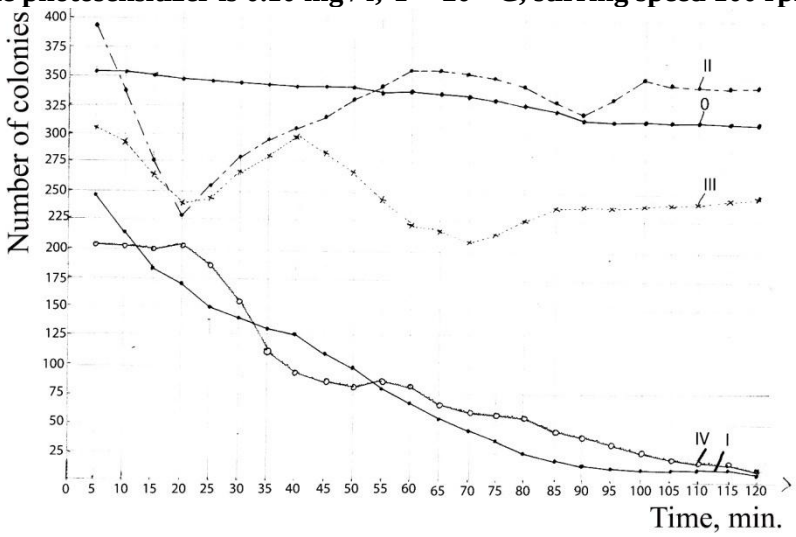


FIG. 3. Dependence of changes in the number of cells of Escherichia coli ATCC 35218,initial concentration 75 thousand / ml, without dye (0), in the presence of methyleneblue (I), eosin (II), fluorescein (III), riboflavin (IV). Concentration photosensitizer 100 mg / l; Irradiation with red light (λ = 620-780 nm), the lamp power is 250 watts. T = 20 ° C, stirring speed 100 rpm.

From the analysis of Fig. 3 it follows that eosin causes the death of Escherichia coli cells of ATCC 35218 only in the first 20 minutes of observations, after an hour and until the end of observations their amount becomes higher than in the control. The reasons for the low efficiency of eosin are explained by the inhibitory effect of the phenolic group inhibiting the development of free radical oxidation [24, 25], and, consequently, the PD photosensitizer.

A study of PD of a number of photosensitizers found that fluorescein for Escherichia coli ATCC 35218 at a concentration of 100 mg / l is significantly less effective than at a concentration of 10 mg / l for Candida albicans ATCC 24433 cells. Thus, for 2 hours of observation, the number of prokaryotic cells decreases in 1.5 times, whereas under the same conditions the level of eukaryotes decreases by 11.0 times. The disadvantage of fluorescein is its low solubility in water and the appearance of the color of the solution even at a low concentration of 10 mg / l. These properties reduce the prospects for its use for water disinfection in swimming pools and for water treatment purposes for food purposes.

With respect to Escherichia coli, ATCC 35218 methylene blue and riboflavin, introduced at a concentration of 100 mg / l, have the most effective lethal effect (Figure 3). These photosensitizers destroy practically 100% of microorganisms introduced in relatively high concentrations of 75,000 / ml for 2 hours of observation (Fig. 3). It should be noted that for natural reservoirs, lower concentrations of sensitizers are usually used, the single application is 1.0-2.5 mg / l [18], significant suppression of the number of pathogenic microorganisms is achieved with repeated application of the photosensitizer for a long time (7-28 days).

The photosensitizers under investigation are expediently used for rapid photodynamic disinfection of water. In the process of water treatment of water used for food purposes in children's sanatoriums, school and preschool institutions, riboflavin (vitamin B2) is the most promising. The compound is an important essential factor, regulates protein, carbohydrate, lipid metabolism, supports the visual function of the eye, is part of flavoproteins, which are an important link in the electron transport chain, regulate redox processes. The daily intake of vitamin B2 is difficult to obtain with food, since in most foods it is present in low amounts. When using water with riboflavin at a concentration of 10 mg / l, the daily norm of a vitamin can be obtained by a child, using 200 ml, and an adult - 300 ml. Taking more riboflavin 5-10 mg / day is used for medicinal purposes for a long time (up to six months), overdoses do not pose a danger, since vitamin B2 refers to water-soluble vitamins and is eliminated from the body during the day. Thus, for complete destruction of microorganisms in drinking water, it is advisable to use riboflavin with stirring for 2 hours and simultaneous irradiation with red light.

III. CONCLUSION

Red light in the absence of photosensitizers by 10-20% reduces the number of museum strains of microorganisms: eukaryotes of Candida albicans ATCC 24433 and prokaryotes of Escherichia coli ATCC 35218.

All studied sensitizers exhibit photodynamic action, which manifests itself after 2 min of observation. For Candida albicans ATCC 24433 riboflavin and fluorescein produce the most lethal effect and reduce the number of colonies in 2 hours of exposure in a red light flux of 3.0 and 11.0 times, respectively.

Eosin against Candida albicans ATCC 24433 and Escherichia coli ATCC 35218 exhibited a low photodynamic effect, which is explained by the peculiarities of its chemical structure.

With respect to Escherichia coli ATCC 35218 riboflavin and methylene blue, introduced at a concentration of 100 mg / L are most effective, almost 100% of microorganisms are destroyed in 2 hours of observation. Riboflavin (vitamin B2) is promising for usage in the technology of special water treatment for preschool and school meals.

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