



## Biofilms

The development of biofilms and the role they play in corrosion and deposition processes may be the most misunderstood and underestimated factor in the treatment of cooling water and other industrial water systems. Ask any water treatment professional about calcium carbonate scale or to explain the major attributes of a typical corrosion cell, and you may get a reasonable explanation provided with some confidence. Ask about biofilms, and you will get a different response. This review is designed to provide a basic understanding of what biofilms are, the problems they can cause, and what might be done to deal with them.

### What is a biofilm?

Simply stated, a biofilm consists of microbial cells (algal, fungal, or bacterial) and the extracellular biopolymer they produce. Generally, bacterial biofilms are of most concern in industrial water systems since they are generally responsible for the fouling of heat transfer equipment. This concern is due in part to the minimal nutrient requirements that are required for many species to grow. One should keep in mind that the more nutrients available in the form of useable organic carbon, the greater the diversity and numbers of organisms that can be supported. When dealing with cooling towers and spray ponds, algal biofilms also are a concern. Not only will algal biofilms foul distribution decks and tower fill, but algae will also provide nutrient (organic carbon) that will help support the growth of bacteria and fungi. Algae do not require organic carbon for growth but instead utilize CO<sub>2</sub> and the energy from light to manufacture carbohydrate. Thus, a cooling water system with little organic carbon can generate its own through the growth and dispersal of algal cells.

Microorganisms can be found in both the bulk water and on the surfaces of industrial water systems. Bacteria attach to surfaces by proteinaceous appendages referred to as fimbriae (Fig. 1). Once a number

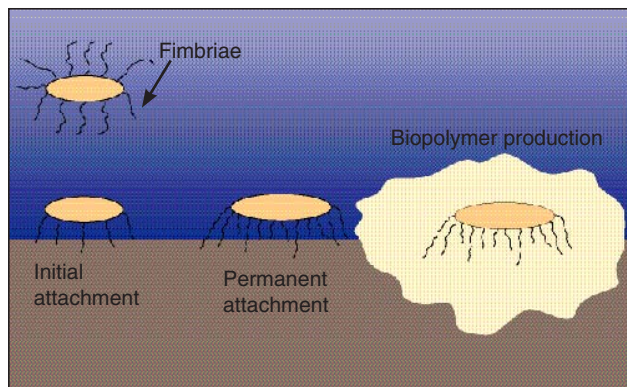


Fig. 1 — Attachment of bacteria to surfaces.

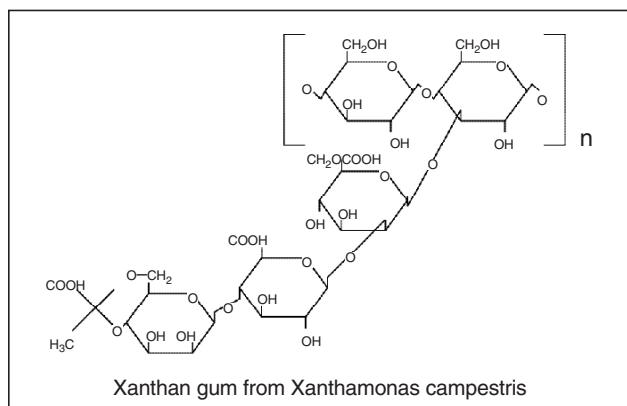


Fig. 2 — Structure of xanthan gum.

of fimbriae have attached the cell to the surface, they make detachment of the organism very difficult. One reason bacteria prefer to attach to surfaces is that the organic molecules adsorbed there provide nutrient. Once attached, the organisms begin to produce material termed extracellular biopolymer or “slime” for short. The amount of biopolymer produced can exceed the mass of the bacterial cell by a factor of 100 or more. The extracellular polymer (EPS) produced may provide a more suitable protective environment for the survival of the organism. The fimbriae, the EPS, and even the flagella may be involved in cell attachment.

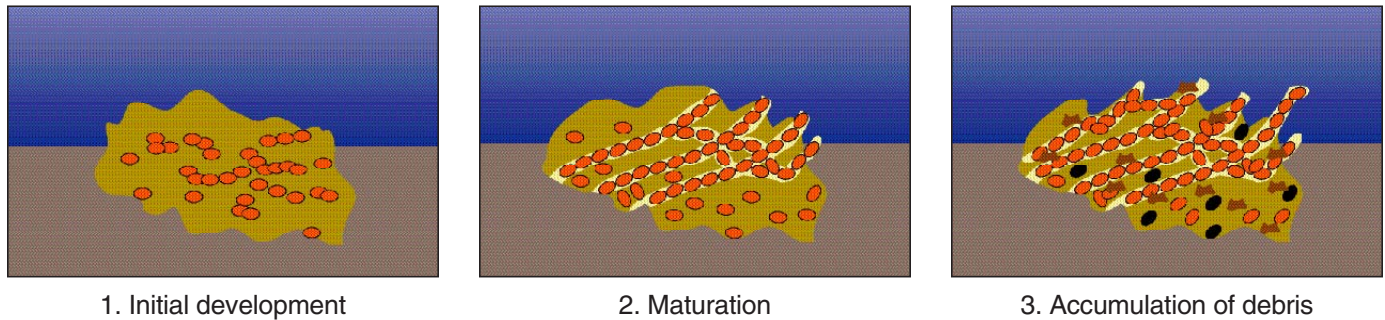
The extracellular biopolymer consists primarily of polysaccharide, protein, and water. In fact, biofilms are generally greater than 90% water. The polysaccharides produced vary depending on the species but are typically made up of repeating oligosaccharides, such as mannose, gulose, glucose, galactose, xylose, and others. An often cited example of a bacterially produced biopolymer is xanthan gum, produced by *Xanthomonas campestris* (Fig. 2). This biopolymer is produced for use as a thickening agent in a variety of food and consumer products. Gellation of some biopolymers can occur upon addition of divalent cations, such as calcium and magnesium. The electrostatic interaction between carboxylate functional groups on the polysaccharide and the divalent cations results in a bridging effect

between polymer chains. Bridging and crosslinking of the polymers help to stabilize the EPS, making the biofilm more resistant to shear.

### Problems associated with biofilms

Once bacteria begin to colonize surfaces and produce biofilms, numerous problems begin to arise, including reduction of heat transfer efficiency, fouling, corrosion, and scale. When biofilms develop in low flow areas, such as cooling tower film fill, they may initially go unnoticed since they will not interfere with flow or evaporative efficiency. After time elapses, the biofilm becomes more complex, often with filamentous development. This matrix will accumulate debris that may impede or completely block flow (Fig. 3).

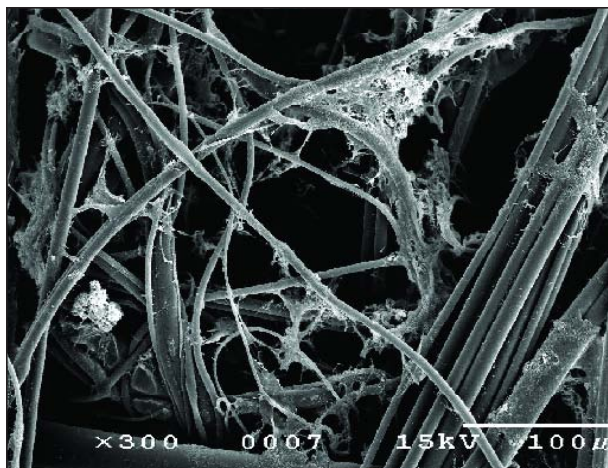
Fig. 3 — Stages of fouling development.



Biofilm structure is often imagined as a coating of microbial cells and biopolymer that is spread evenly across a surface. In reality, biofilm structure is much more complex. Biofilms may be patchy and highly channelized, allowing nutrient-bearing water to flow through and around the matrix.

Algal biofilms may foul cooling tower distribution decks, tower fill, and basins. When excessive algal biofilms develop, portions may break loose and can be transported to other parts of the system, causing blockage as well as providing nutrient for accelerated bacterial and fungal growth. Biofilms can cause fouling of filtration and ion exchange equipment. Fig. 4 shows a 75 micron cartridge filter fouled with biofilm. In photograph A (300X), the filter fibers are shown to be fouled with an unknown material. Closer examination of the foulant exhibited in photograph B (9000X) shows the foulant to be bacterial cells and extracellular biopolymer. Bacterial biofilms may also foul heat exchange equipment. Bacterial fouling of heat exchangers can occur quickly due to a process leak or influx of nutrient. The sudden increase in nutrient in a previously nutrient-limited environment will send bacterial populations into an accelerated logarithmic growth phase which can result in a

Fig. 4 — 75 micron cartridge filter fouled with biofilm.



Photograph A (300X)



Photograph B (9000X)



Table 1  
Thermal conductivity comparison of  
deposit-forming compounds and biofilm.

Substance	Thermal Conductivity (W m <sup>-1</sup> K <sup>-1</sup> )
CaCO <sub>3</sub>	2.6
CaSO <sub>4</sub>	2.3
Ca <sub>3</sub> (PO <sub>4</sub> ) <sub>2</sub>	2.6
Fe <sub>2</sub> O <sub>3</sub>	2.9
Analcite	1.3
Biofilm	0.6

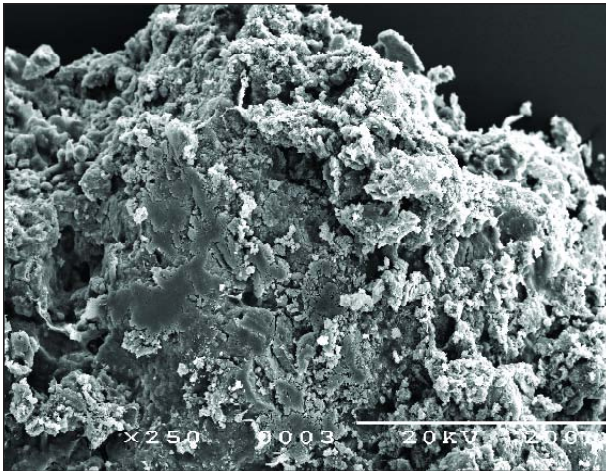


Fig. 5 — Ca<sub>3</sub>(PO<sub>4</sub>)<sub>2</sub> scale from human tooth.



Fig. 6 — Scale magnified 7,000X  
to show bacterial biofilm.

rapid accumulation of biofilm. The biofilms that develop will then interfere with heat transfer efficiency. Table 1 demonstrates the thermal conductivity of a variety of deposit-forming compounds in contrast to biofilm. A lower number indicates a greater resistance to heat transfer.<sup>1</sup>

Deposits in the form of biofilm and biofilm with entrapped suspended debris are generally easy to comprehend, but biofilms may often lead to the formation of mineral scales as well. Calcium ions are fixed into the biofilm by the attraction of carboxylate functional groups on the polysaccharides. In fact, divalent cations, such as calcium and magnesium, are integral in the formation of gels of some extracellular polysaccharides. These calcium ions are fixed in place by the biofilm at the heat transfer surface, thereby making them more readily available to react with carbonate or phosphate anions that are present as well. This would then provide nucleation or crystal growth sites that would not normally be present on a biofilm-free surface. Additionally, biofilms may entrap precipitated calcium salts and corrosion byproducts from the bulk water that will act as crystal growth sites.

A typical biofilm-induced mineral deposit with which we all are familiar is the calcium phosphate scale that the dental hygienist removes from our teeth. When biofilms grow on tooth surfaces, they are referred to as plaques. If these plaques are not continually removed, they accumulate calcium salts, mainly calcium phosphate, to form tartar (scale) (Figs. 5 & 6). One could make a comparison between rinsing your mouth twice daily with an antiseptic mouthwash to control plaque and feeding microbicides and biodispersants to control biofilm-related deposition in heat exchangers. If biofilms in heat exchangers are not controlled, then, like dental plaques, mineral scale may deposit.

The growth of bacteria and formation of biofilms may also result in another problem, that of corrosion. Microbiological corrosion may be defined as corrosion that is influenced by the growth of microorganisms, either directly or indirectly. To understand microbially influenced corrosion, or MIC for short, it helps to have a basic understanding of corrosion chemistry. This document is not intended to provide that information, but some water treatment training manuals will. In essence, corrosion occurs on a metal surface due to some inherent or environmental difference between one area on that surface and another. These differences will create anodic and cathodic areas, setting up a basic corrosion cell (Fig. 7). The anode is the area at which metal is lost. The electrons given

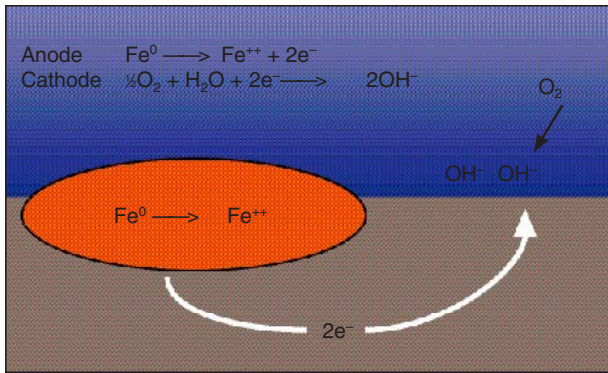


Fig. 7 — Basic corrosion cell.

up by the metal flow to the cathode to be consumed in a reduction reaction. Microbiological corrosion is electrochemical corrosion where in some manner the presence of the microorganisms is having some influence in the creation or acceleration of corrosion processes.

Microorganisms can influence corrosion in a variety of ways. Formation of localized differential cells, the production of mineral and organic acids, ammonia production, and sulfate reduction are just a few of the mechanisms by which bacteria, fungi, and algae can influence corrosion. The formation of localized cells is the primary mechanism of corrosion caused

by iron oxidizing bacteria, such as *Gallionella* sp., *Arthrobacter siderocapsulatus*, and *Pseudomonas putida*.<sup>2</sup> When iron and manganese oxidizing organisms colonize a surface, they begin to oxidize available reduced forms of these elements and produce a deposit. In the case of iron oxidizing organisms, ferrous iron is oxidized to the ferric form ( $\text{Fe}^{2+} \longrightarrow \text{Fe}^{3+} + 1\text{e}^-$ ) with the electron lost in the process being utilized by the bacterium for energy production. As the bacterial colony becomes encrusted with iron (or manganese) oxide, a differential oxygen concentration cell may develop, and the corrosion process will begin. The ferrous iron produced at the anode will then provide even more ferrous iron for the bacteria to oxidize. The porous encrustation (tubercle) may potentially become an autocatalytic corrosion cell or may provide an environment suitable for growth of sulfate-reducing bacteria. Figs. 8, 9, and 10 illustrate the stages of localized corrosion cell development resulting from growth of iron oxidizing bacteria.

Corrosion may also develop when localized cells are formed, due simply to biofilms developing on metal surfaces. The oxidation of iron or manganese is not a requirement for the development of a localized corrosion cell.

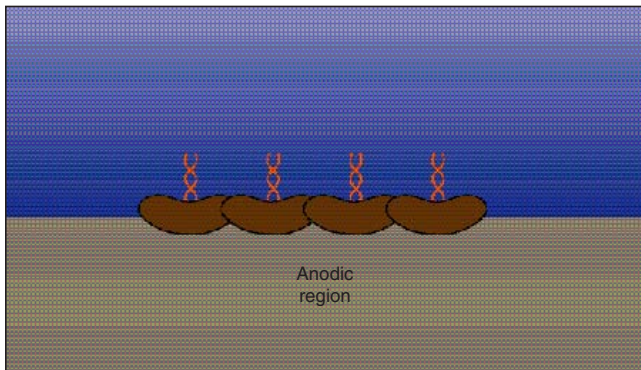


Fig. 8 — Bacterial growth and iron oxidation.

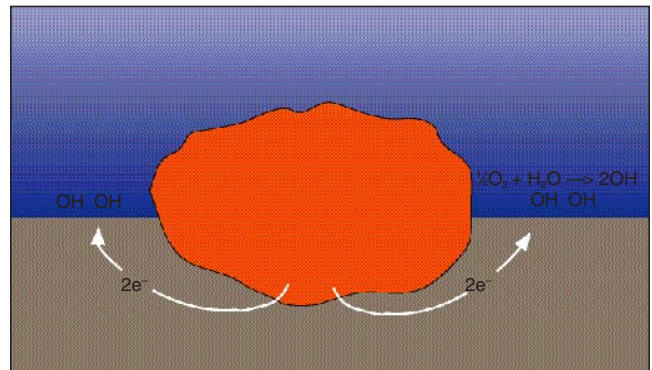


Fig. 9 — Corrosion cell development.

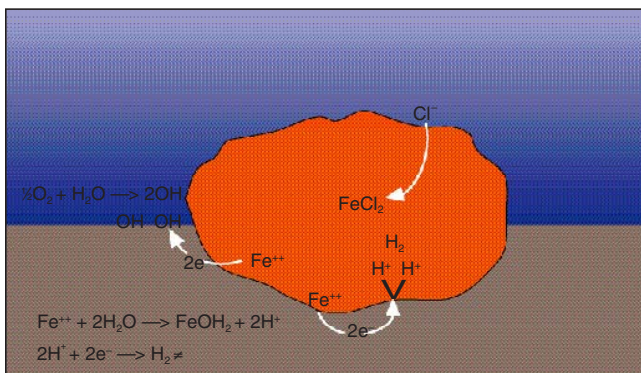


Fig. 10  
Tubercle and autocatalytic cell development.

Numerous factors can contribute to localized corrosion on metal surfaces. The production of ammonia by the reduction of nitrates or nitrites may lead to severe localized loss on copper-based metallurgy. The production of organic acids, such as acetic, butyric, or citric acid, may help solubilize protective metal oxide films. Inorganic acid, such as sulfuric acid produced by *Thiobacillus* sp., can also have detrimental effects. As biofilms develop, they will eventually achieve a thickness at which oxygen concentration is either very low or completely excluded. At a thickness of just 200 microns, the oxygen concentration within the biofilm is reduced to near zero ppm. When this



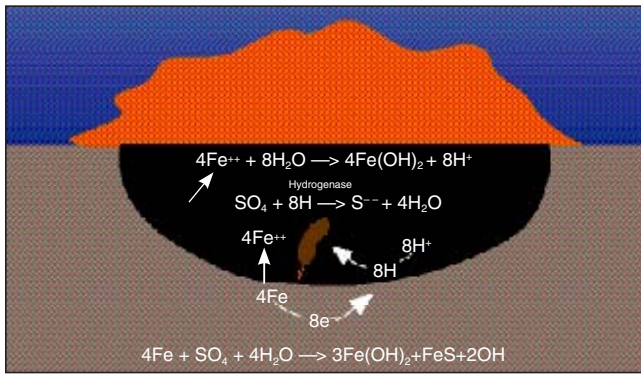


Fig. 11 — Corrosion by SRB.

occurs, facultative and obligate anaerobes (such as *Desulfovibrio* sp. and *Clostridium* sp.) can flourish.

Anaerobic sulfate-reducing bacteria (e.g. *Desulfovibrio*) are the bacteria most often considered when discussing microbial corrosion. These organisms can seek out and colonize areas deficient in oxygen, such as those found within porous corrosion tubercles, within biofilms, and under debris. These bacteria are responsible for rapid and severe metal loss in industrial water systems. This type of corrosion is easily recognizable from the characteristic sulfide byproduct present within the corrosion cell. Sulfate-reducing bacteria primarily cause corrosion by utilizing the molecular hydrogen produced at the

cathode, thereby depolarizing it (Fig. 11). Since the rate of corrosion is under cathodic control, removal of cathodic reduction products will increase the rate of corrosion. Systems that are sulfate-limited will have less of a tendency to be attacked by SRB.

### Biofilm control

It can be seen that the growth of bacteria on surfaces in cooling and process water systems can lead to significant deposit and corrosion problems. Once this is understood, then the importance of controlling biofilms becomes quite clear. Too often microbiological control efforts focus only on planktonic counts, that is, the number of bacteria in the bulk water. While some useful data may be gathered from monitoring daily bacterial counts, monthly or weekly counts are simply time wasted and have little meaningful use. Planktonic counts do not necessarily correlate with the amount of biofilm present. In addition, planktonic organisms are not generally responsible for deposit and corrosion problems. There are a few exceptions, such as a closed loop system, where planktonic organisms may grow, degrade corrosion inhibitors, and excrete acids which depress pH. Systems with low planktonic counts may have a significant biofilm problem and vice versa. Therefore, efforts should focus on general biofilm control.

Biofilms can be controlled through the use of microbicides, biodispersants, and by limiting nutrients. Microbicides, both oxidizing and nonoxidizing, can be effective in overall biofilm control when applied properly. The oxidizing microbicides, such as chlorine, bromine, chlorine dioxide, peracetic acid, and ozone, can be extremely effective in destroying both the EPS and the bacterial cells. When using oxidizing microbicides, one must be sure to obtain a sufficient residual long enough to effectively oxidize the biofilm. Unfortunately, there are those who are overly concerned with the corrosive nature of the oxidizing microbicides and fail to apply the needed residual oxidant required to control biofilm. Low residual oxidant levels may significantly reduce planktonic counts but may not be sufficient to control biofilm. The level of oxidant and duration required will vary from system to system. Low level free residual oxidant (FRO) will kill planktonic bacteria, yeast, mold and protozoa. However, medium to high dosages of FRO are needed to oxidize biofilms. Algal mats particularly require high dosages of FRO. It is generally more effective to maintain a higher residual for a duration of several hours than it is to continuously maintain a low residual. Continuous low level feed may not achieve an oxidant concentration sufficient to oxidize the polysaccharide and expose the bacteria to the oxidant. Another misconception is with the use of chlorine at alkaline pH (> 8.0). It is often thought that chlorine is ineffective for controlling microorganisms at elevated pH. This is only half true. Certainly, the hypochlorous acid form of chlorine (HOCl) is more effective at killing cells than the hypochlorite form (OCl<sup>-</sup>). However, the hypochlorite is actually very effective at oxidizing the extracellular polysaccharide and the proteinaceous attachment structures. Therefore, the use of chlorine in alkaline cooling waters can still be extremely effective when applied properly. This is especially true when combining chlorine with bromine or with a compatible nonoxidizing microbicide such as a polyquat. When this is done, one achieves both oxidation of the extracellular material and sufficient kill of the microorganisms.

Bromine compounds, such as NaBr used to generate free residual HOBr and organobromine biocides, are very effective oxidizing biocides. Bromine is extremely deadly to microbes, and the kinetics of the kill reactions is very quick.

Certain nonoxidizing microbicides are also effective in controlling biofilm. Effective control is greatly dependent on the concentration of the product feed, frequency of addition, dosage fed, and resistance of the incumbent population to the product fed. Control cannot generally be achieved by once-a-week additions as is common in "full service" applications. Typical application for effective control may include a slug addition of product 2 to 5 times a week. As with oxidizing microbicides, frequency and dosage will depend on the system conditions. It is generally most effective to alternate nonoxidizing microbicides at every addition to ensure broad spectrum control. Most nonoxidizing microbicides will have little effect in destroying the extracellular polysaccharide found in the biofilm. However, many of these microbicides may be able to penetrate and kill bacteria found within the biofilm, resulting in decreases in the population and weaknesses in the biofilm structure. Thus, combining the use of nonoxidizing and oxidizing microbicides is a very effective means of controlling biofilm. When using a nonoxidizing microbicide in conjunction with an oxidizing agent, there should be a slight to no residual oxidant concentration present in the system at the time of addition. Sufficient time should be allowed for the nonoxidizing microbicide to work before resuming oxidant feed unless an oxidant compatible microbicide is being used (i.e., polyquat). Using combination biocides has proven very effective in killing unwanted bacterial species.<sup>3</sup> Application of synergistic biocides, in particular, can give improved biocide performance against harmful bacteria.<sup>4</sup>

Biofilm control programs can be made more effective through the use of biopenetrant/dispersant products. Products that penetrate and loosen the biopolymer matrix will not only aid in sloughing the biofilm but will also expose the microorganisms to the microbicides. Biodispersants are especially effective when dealing with systems that have a high TOC loading and a tendency to foul. These products are typically fed in slug additions prior to microbicide feed. Low level continuous feed may also be effective. However, in some cases low level continuous feed may not be as effective since it may not reach a certain threshold amount required to produce the desired effect. Recent developments in biodispersant technology are making this approach more effective and popular than ever before. Enzyme technologies that will break down the extracellular polysaccharides and degrade the bacterial attachment structures (fimbriae) are currently being developed and patented. These technologies provide biofilm control where microbicide use is environmentally limited or restricted, or provide a means of quickly restoring fouled cooling water systems to a clean, efficient operable state. Recent applications in the field proved that enzyme technologies improve the efficiency of biocontrol programs.

The importance of biofilm control must not be taken lightly. It is the fundamental basis for controlling a high percentage of deposition and corrosion problems in process and cooling water systems. Once these fundamentals are understood, effective treatment strategies can be developed.

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