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Modification on the conventional procedure to measure AOC in drinking water

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Abstract: Additional phosphorus will be introduced to water sample if the conventional procedure is used to measure assimilable organic carbon(AOC) in drinking water. It has been shown that there are the cases that phosphorus is the limiting nutrient for microbial growth in drinking water. The measured value of AOC would not be able to indicate appropriately the regrowth potential of bacteria in this case. The conventional procedure used to measure AOC was modified to avoid the introduction of additional phosphorus to water sample in this study. It was shown that it was feasible to measure AOC in water using the modified procedure. Furthermore, the measured value of AOC determined by the modified procedure could indicate appropriately the regrowth potential of bacteria in drinking water despite either organics or phosphorus was the limiting nutrient for bacterial regrowth.

Keywords: drinking water; assimilable organic carbon; bacterial regrowth; phosphorus; organics

Introduction

AOC(assimilable organic carbon) has been used as one of the main parameter to indicate the regrowth potential of heterotrophic bacteria in drinking water for a long time. The measurement of AOC is based on the conventional hypothesis that biodegradable organic carbon is the only limiting nutrient for the regrowth of heterotrophic bacteria in drinking water distribution systems (Bruce, 1984; Peter, 1990; Mark, 1996; Liu, 2000; Van, 1982). The principle to determine AOC is that the reproduction of *Pseudomonads Fluorescent* strain P17 and *Spirllum* strain NOX is in direct proportion to the concentration of assimilable organic carbon in drinking water (Van, 1982; Miettinen, 1996). At present, the procedure advanced by Dr. Liu is widely used in China to measure AOC in drinking water(Liu, 2000).

Recently, the cases that phosphorus is the limiting nutrient for bacterial regrowth in drinking water have been found(Miettinen, 1996; 1997a; Sathasivan, 1999; Minna, 2002; Sang, 2003). This changes the conventional thinking that biologically available organic carbon is the sole limiting nutrient for bacterial regrowth. It has been shown that when phosphorus is the limiting nutrient for bacterial regrowth, the reproduction of P17 will be depressed and in direct proportion to the concentration of phosphorus rather than the concentration of biological available organic carbon in drinking water(Markku, 1999). Then the feasibility to use the measured value of AOC determined by the conventional procedure advanced by Liu to indicate the regrowth potential of heteotrophic bacteria in drinking water should be challenged if phosphorus is the limiting nutrient for bacterial regrowth.

The purpose of this study is to modify the conventional procedure advanced by Liu so that the measured value of AOC can indicate appropriately the regrowth potential of heteotrophic bacteria in drinking water when either organics or phosphorus is the limiting nutrient for bacterial regrowth.

1 Materials and methods

1.1 Water samples

Three different water samples were prepared by adding some nutrients to deionized water. The concentration of organic carbon added to all the water samples was 200 μg/L (CH_3COONa, measured as acetate-C). The concentration of PO_4^{3-}-P(NaH_2PO_4) appended to the three water samples was 0, 1 and 20 μg/L, respectively.

In order to meet the demand of microbes for other nutrients, a mixture of inorganic nutrients including NH_4NO_3, $MgSO_4 \cdot 7H_2O$, $CaCl_2 \cdot 2H_2O$, KCl and NaCl was added to all the water samples. The concentration of each inorganic nutrient added to the water samples were 250 μg/L of N(including NH_4^+-N and NO_3^--N), 10 μg/L of Mg^{2+}, 27 μg/L of Ca^{2+}, 53 μg/L of K^+ and 40 μg/L of Na^+, respectively.

1.2 Analysis

AOC of all water samples was determined using both the conventional and modified procedure. BRP (Bacterial Regrowth Potential) of the two water samples with 1 μg/L and 20 μg/L PO_4^{3-}-P was measured according to a method modified based on the method proposed by Sathasivan (Sathasivan, 1999).

Three basic steps were followed when BRP was determined: (1) Preparation of inoculum: Raw water was treated by biological pretreatment process followed by coagulation, sedimentation and sand filtration. About 200 ml of the effluent was collected, incubated in the dark at 20℃ for 5 d then filtered with a 2 μm polycarbonate membrane filter. The filtrate was used as inoculum for BRP analysis.

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(2) Inoculation of water sample: Water sample to be analyzed was poured into prepared 50 ml vials and pasteurized in 65℃ water bath for 30 min. After cooling, the water sample was inoculated with 0.5 ml of the inoculum. Afterwards, the water sample was incubated 7 d at 20℃ in the dark. (3) Enumeration of bacteria: Microbial counts in the water sample after incubation were determined by spread plating on R2A-agar plates (Standard Methods for the Examination of Water and Wastewater, 1995). R2A-agar plates were incubated 7 d at 22—25℃ before the CFU (colony forming unit) were counted.

2 Results and discussion

2.1 Limitation of the conventional procedure

Considerable amount of phosphorus will be introduced from inoculum to water samples during the inoculation of P17 and NOX if the conventional procedure is used to determine AOC.

According to the conventional procedure, the inoculum of P17 (or NOX) to be inoculated to water samples is prepared by inoculating 100 μl of the originally prepared inoculum of P17(or NOX) to 50 ml of culture medium which contains 2000 μg/L of acetate-C (CH_3COONa is used) (Wang, 1999). The detailed composition of the conventional culture medium used to incubate P17 and NOX is listed in Table 1.

It can be seen from Table 1 that the concentration of K_2HPO_4 in the conventional culture medium is 7000 μg/L and the concentration of KH_2PO_4 is 3000 μg/L. Then the phosphorus concentration in the medium is 1931 μg/L.

Table 1 Detailed composition of the conventional culture medium used to incubate P17 and NOX

Compound	Acetate-C	K_2HPO_4	KH_2PO_4	$MgSO_4 \cdot 7H_2O$	$(NH_4)_2SO_4$	NaCl	$FeSO_4$
Concentration, μg/L	2000	7000	3000	100	1000	100	1

In addition, because the concentration of P17(or NOX) in the prepared inoculum is very high, the volume of the inoculum inoculated to water samples is usually very small. In order to avoid operating error as possible during inoculation, the inoculum needs to be diluted before it is inoculated. According to the practice, the concentration of P17(or NOX) in the inoculum should be diluted to 1/2—1/3 of its initial concentration and the actual volume of diluted inoculum inoculated to water samples is usually about 80 μl. The ultra-pure water amended with several inorganic nutrients (Table 2) is used to dilute the inoculum according to the conventional procedure. It can be seen from Table 2 that the concentration of PO_4^{3-}-P in the diluting water is very high and the actual concentration is 3047 μg/L.

Table 2 Concentration of inorganic nutrients in the water used to dilute inoculum

Compound	K_2HPO_4	KNO_3	NH_4Cl
Concentration, mg/L	17.1	144	76.4

Because the actual volume of diluted inoculum inoculated to water samples is about 1/500 (the actual ratio lies on the concentration of P17 or NOX in the inoculum) of the volume of the water samples, the concentration of phosphorus in the water samples will increase by about 4—5 μg/L due to the introduction of phosphorus from the diluted inoculum. Compared with organics, microorganisms require much less phosphorus. Therefore, if phosphorus concentration in the water sample to be analyzed is extremely low and phosphorus is limiting nutrient for bacterial regrowth, the measured value of AOC will be greatly affected due to the introduction of additional phosphorus from inoculum if the conventional procedure is used. Then the effect of phosphorus concentration on the measured value of AOC might not be seen and the measured value of AOC can not indicate properly the potential of bacterial regrowth in the water.

2.2 Modification on the conventional procedure

In order to make the measured value of AOC could be used as a feasible and proper parameter indicating the regrowth potential of bacterial in drinking water even if phosphorus was the limiting nutrient for bacterial regrowth, two modifications were applied to the conventional procedure in this study.

One of the modifications was to reduce the phosphorus concentration in the conventional culture medium used to incubate P17 and NOX. The concentration of K_2HPO_4 in the modified culture medium was reduced to 200 μg/L and the concentration of KH_2PO_4 was reduced to 100 μg/L, concentrations of all the other nutrients remained the same to the conventional culture medium. Then the phosphorus concentration in the modified culture medium was 59 μg/L and the ratio of organic carbon to phosphorus was 100∶3, which could meet the demand of P17 and NOX. The other modification was that K_2HPO_4 was not added to the diluting water used to dilute the inoculum. Since this diluting water was only used to dilute the inoculum during the inoculation of P17 and NOX, this modification could not adversely affect on the growth of P17 and NOX.

It could be calculated according to the two modifications that phosphorus introduced to water samples during inoculation of P17(or NOX) would be less than 0.1 μg/L, thereby the remarkable increase of phosphorus concentration in water samples could be avoided.

In order to investigate the feasibility to incubate P17 and NOX using the modified medium, P17 and NOX were incubated in both the modified medium and the conventional medium, respectively. The final concentration of P17 and NOX in the modified medium was compared with the final concentration of P17 and NOX in conventional medium after

incubation. The results are shown in Fig.1 and Fig. 2(three independent results were shown in each figure).

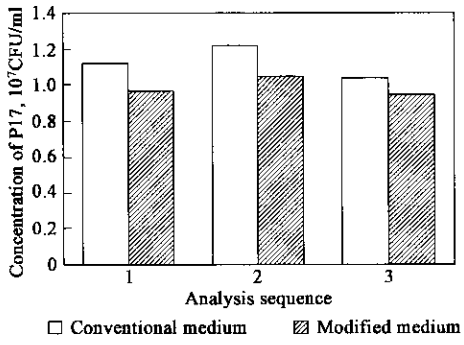

Fig.1 Final concentration of P17 in the modified and conventional medium

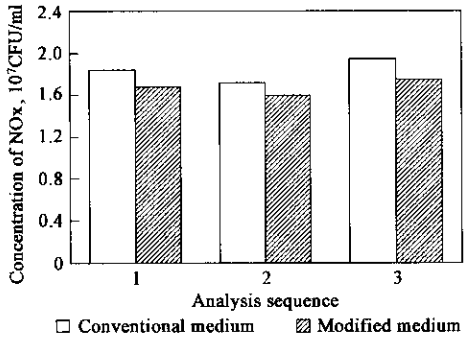

Fig.2 Final concentration of NOX in the modified and conventional medium

It can be seen from Fig.1 that there was little difference between the concentration of P17 in the inoculum prepared by the modified medium and the concentration of P17 in the inoculum prepared by the conventional medium. The similar result was gotten from the incubation of NOX in the two media(Fig. 2). This was to say that this modification on culture medium had little effect on the growth of P17 and NOX. This result indicated that it was feasible to incubate P17 and NOX using the modified medium.

2.3 Comparison between the two procedures for AOC measurement

In order to investigate whether the modified procedure was feasible to determine AOC in water and whether the measured value of AOC could indicate the regrowth potential of bacteria, both the modified procedure and the conventional procedure were used to measure AOC in water samples and the results were compared.

2.3.1 Measured value of AOC in water sample with 20 μg/L PO_4^{3-}-P

Because CH_3COONa is the reference substance for AOC_{P17} and AOC_{NOX} measurement and it can be assimilated completely by both P17 and NOX, only one of P17 and NOX is needed to determine the overall AOC in all the water samples used in this experiment and the measured values of AOC_{P17} and AOC_{NOX} should be around 200 μg/L in theory if organic carbon is the limiting nutrient for bacterial regrowth (Liu, 2000). Since the concentration of organic carbon in the water sample was 200 μg/L and the concentration of phosphorus was 20 μg/L, it could be sure that organics was the limiting nutrient for bacterial regrowth in this water sample and the increase of phosphorus could not change obviously the measured value of AOC_{P17} and AOC_{NOX}.

By the conventional procedure and modified procedure, AOC of this water sample was measured using P17 and NOX, respectively. Three independent results are shown in Fig.3 and Fig.4, respectively.

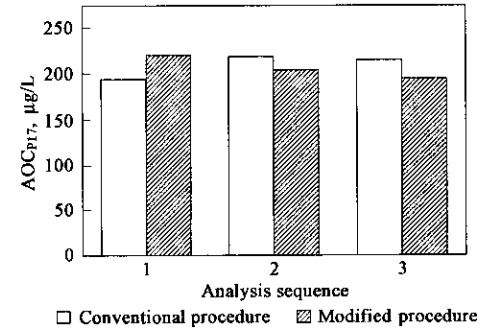

Fig.3 Measured value of AOC_{P17} in the water sample with 20 μg/L PO_4^{3-}-P

It could be seen from Fig.3 that the measured value of AOC_{P17} determined by the modified procedure was similar to the measured value determined by the conventional procedure and was around 200 μg/L. The similar results were gotten from the measurement of AOC_{NOX} in this water sample(Fig. 4). This indicated that if organics was the limiting nutrient for bacterial regrowth in drinking water, the modified procedure was fitting for the measurement of AOC and the measured value was the same as the value determined by the conventional procedure.

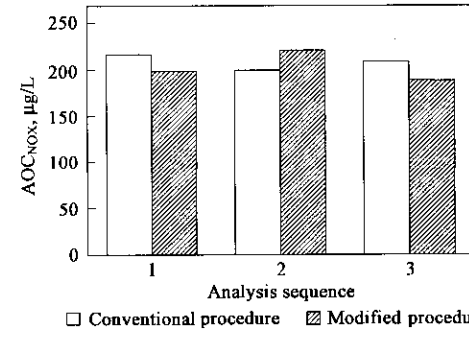

Fig.4 Measured value of AOC_{NOX} in the water samples with 20 μg/L PO_4^{3-}-P

2.3.2 Measured value of AOC in water sample without PO_4^{3-}-P

Since the concentration of organic carbon in this water sample was 200 μg/L and no phosphorus was added, it could be inferred that phosphorus was the limiting nutrient for bacterial regrowth in this water sample and the increase of phosphorus could obviously change the measured value of AOC_{P17} and AOC_{NOX}.

AOC of this water sample without PO_4^{3-}-P addition was measured independently two times using P17 and NOX, respectively, by the conventional procedure and the modified procedure. The results are shown in Fig.5.

It could be seen from Fig.5 that the measured value of AOC in the water without phosphorus addition determined by

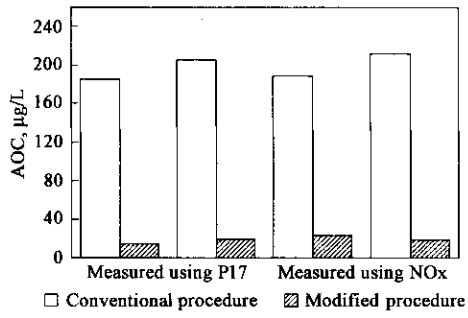

Fig.5 Measured value of AOC_{P17} and AOC_{NOX} in the water sample without PO_4^{3-}-P addition

the modified procedure was much lower than the measured value of AOC determined by the conventional procedure. Because considerable amount of phosphorus was introduced to the water sample during the inoculation of P17 and NOX when the conventional procedure was used to determine AOC, the introduced phosphorus could meet the demand of P17 and NOX for reproduction, which resulted in the measured value of AOC_{P17} and AOC_{NOX} being about 200 μg/L. However, when the modified procedure was used, little phosphorus could be introduced to the water sample, the growth of P17 and NOX would be depressed remarkably because of the deficiency of phosphorus. This made the measured value of AOC_{P17} and AOC_{NOX} be much less than the measured value determined by the conventional procedure.

2.3.3 Relation between the measured value of AOC and BRP

BRP indicates the growth of an assemblage of indigenous heterotrophic bacteria in drinking water. Therefore, BRP shows exactly the regrowth potential of bacteria in drinking water with limited substrates to support their growth. In this experiment, BRP in water samples with 0.5 μg/L and 20 μg/L PO_4^{3-}-P was analyzed and AOC_{P17} was determined by both the conventional procedure and the modified procedure. The results are shown in Fig.6.

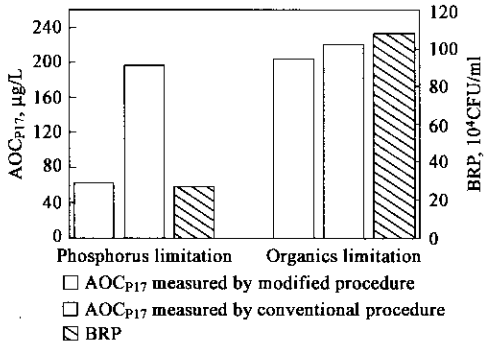

Fig.6 Measured value of AOC_{P17} and BRP in water samples with 0.5 and 20 μg/L PO_4^{3-}-P

Since organic carbon in all the water samples was 200 μg/L, if the phosphorus was 0.5 μg/L, phosphorus would be the limiting nutrient for bacterial regrowth. If the phosphorus were 20 μg/L, organic carbon would be the limiting nutrient for bacterial regrowth. It could be seen from Fig.6 that the BRP in the water sample with 0.5 μg/L phosphorus was much less than the BRP in the water sample with 20 μg/L phosphorus. This indicated that although the concentration of organic carbon in the two water samples was the same, the ability of substrates in the two water samples to support the growth of bacteria was different. The regrowth potential of bacteria in the water sample with 20 μg/L phosphorus was higher than in the water sample with 0.5 μg/L phosphorus.

It was shown that the two measured values of AOC_{P17} determined by conventional procedure in the two water samples had little difference. That was to say, the two values could not indicate the actual difference between the regrowth potential of bacteria in the two water samples. On the other hand, it could be seen from Fig.6 that the measured value of AOC_{P17} in the water sample with 0.5 μg/L PO_4^{3-}-P determined by the modified procedure was much less than the measured value of AOC_{P17} in the water sample with 20 μg/L PO_4^{3-}-P determined by the modified procedure. This result was corresponding to the BRP of the two water samples and illuminated that the measured value of AOC determined by the modified procedure could indicate properly the regrowth potential of bacteria in drinking water despite phosphorus or organics was the limiting nutrient for bacterial regrowth.

In addition, it could be seen from Fig. 6 that the measured value of AOC_{P17} in the water sample with 20 μg/L phosphorus determined by modified procedure was similar to the measured value of AOC_{P17} determined by conventional procedure. This indicated that the measured value of AOC determined by the two procedures could indicate properly the regrowth potential of bacteria in drinking water if organics was the limiting nutrient for bacterial regrowth.

2.4 Discussion

It could be seen from this study that if phosphorus was the limiting nutrient for bacterial regrowth in drinking water and the conventional procedure advanced by Liu was used to determine AOC, the measured value of AOC could not show exactly the regrowth potential of bacteria in the water due to the introduction of phosphorus from the inoculum of P17 and NOX. If the modified procedure advanced in this study was used, this limitation could be avoided and the measured value of AOC could indicate exactly the regrowth potential of bacteria in water.

It should be noted that the term AOC has been advanced over ten years before the cases that phosphorus may become the limiting nutrient for bacterial regrowth in drinking water are found. The conventional AOC refers to the portion of BDOC (biodegradable dissolved organic carbon) that is converted to bacterial biomass (Van, 1982; Liu, 2000). Therefore, the actual AOC in drinking water is not related to the phosphorus concentration from this point of view. However, AOC is used as a parameter to index the regrowth

potential of bacteria in drinking water in practice. Its importance for drinking water industry would lose if AOC could not be used as a parameter to index the regrowth potential of bacteria. In addition, as far as the technique to measure AOC is concerned, the measurement of AOC is a bioassay, so the measured value of AOC is related to the growth of P17 and NOX. If phosphorus is the limiting nutrient for bacterial regrowth in the drinking water to be analyzed, the growth of P17 and NOX should also be affected. Then the feasibility to use the measured value of AOC determined by conventional procedure to index the regrowth potential of bacteria in drinking water should be challenged.

It could be seen from this study that if organics was the limiting nutrient for bacterial regrowth in drinking water, it is feasible to use the measured value of AOC determined by either the conventional procedure or the modified procedure as the index of the regrowth potential of bacteria because the two values are the same. The two measured values also represent the conventional AOC that is converted to bacterial biomass from BDOC. However, if phosphorus was the limiting nutrient for bacterial regrowth in drinking water, it is not feasible to use the measured value of AOC determined by the conventional procedure as the index of the regrowth potential of bacteria. It has been shown that the regrowth potential of bacteria in the drinking water of some area is not related to the measured value of AOC (Gibbs, 1993; Miettinen, 1997b). The main reason is that phosphorus is the limiting nutrient for bacterial regrowth in this water (Miettinen, 1996; 1997a).

As shown in Fig. 6, if phosphorus is the limiting nutrient for bacterial regrowth, the measured value of AOC determined by the modified procedure was less than the measured value determined by the conventional procedure, but the measured value determined by the modified procedure showed exactly the regrowth potential of bacteria in drinking water. Therefore, despite organics or phosphorus was the limiting nutrient for bacterial regrowth in drinking water, the measured value of AOC determined by the modified procedure can be used as the proper parameter to index the regrowth potential of bacteria in drinking water although the measured value of AOC can not represent all the portion of BDOC that is converted to bacterial biomass if phosphorus is the limiting nutrient for bacterial growth.

3 Conclusions

In this study, the conventional procedure advanced by Liu used to measure AOC was modified to avoid the introduction of additional phosphorus to water samples to be analyzed so that the measured value of AOC can indicate properly the regrowth potential of bacteria despite organics or phosphorus was the limiting nutrient for bacterial regrowth in drinking water.

It was shown that if organics was the limiting nutrient for bacterial regrowth in water, the measured value of AOC_{P17} and AOC_{NOX} determined by the modified procedure was similar to the corresponding measured value determined by the conventional procedure. This indicated that it was feasible to measure AOC in drinking water using the modified procedure. If phosphorus was the limiting nutrient for bacterial regrowth in drinking water, the measured value of AOC determined by the modified procedure was corresponding to the BRP of the drinking water and was less than the measured value determined by conventional procedure. This illuminated that the measured value of AOC determined by the modified procedure could indicate properly the regrowth potential of bacteria in drinking water if phosphorus was the limiting nutrient for bacterial regrowth.

The results indicated that the measured value of AOC determined by the modified procedure could indicate appropriately the regrowth potential of bacteria in drinking water despite organics or phosphorus was the limiting nutrient for bacterial regrowth.

References:

Bruce E R, Vernon L S, 1984. Achieving biologically stable drinking water[J]. J AWWA, 76(10): 106—114.

Gibbs R A, Scutt J E, Croll B T, 1993. Assimilable organic carbon concentrations and bacterial numbers in a water distribution system[J]. Water Sci Technol, 27: 156—166.

Liu W J, Wang Y J, Zhang L P *et al*., 2000. Determination of assimilable organic carbon in drinking water[J]. Jishui Paishui, 26(11): 1—5.

Mark W L, Nancy J W, Darrell B S, 1996. Full-scale studies of factors related to coliform regrowth in drinking water[J]. Appl Environ Microbiol, 62(7): 2201—2211.

Markku J L, Miettinen I T, Vartiainen T *et al*., 1999. A new sensitive bioassay for determination of microbially available phosphorus in water[J]. Appl Environ Microbiol, 65(5): 2032—2034.

Miettinen I T, Vartiainen T, Martikainen P J, 1996. Contamination of drinking water[J]. Nature, 381: 654—655.

Miettinen I T, Vartiainen T, Martikainen P J, 1997a. Phosphorus and bacterial growth in drinking water[J]. Appl Environ Microbiol, 63(8): 3242—3245.

Miettinen I T, Vartiainen T, Martikainen P J, 1997b. Microbial growth and assimilable organic carbon in Finnish drinking waters[J]. Water Sci Technol, 35: 301—306.

Minna M K, Leena K K, Markku J L *et al*., 2002. The microbial community structure of drinking water biofilms can be affected by phosphorus availability [J]. Appl Environ Microbiol, 68(1): 434—439.

Peter M H, 1990. Measurement of biodegradable organic matter and bacterial growth potential in drinking water—a review[J]. J AWWA, 82(7): 78—86.

Sathasivan A, Ohgaki S, 1999. Application of new bacterial regrowth potential method for water distribution system—a clear evidence of phosphorus limitation[J]. Water Res, 33(1): 137—144.

Sang J Q, Yu G Z, Zhang X H *et al*., 2003. Phosphorus limitation on bacterial regrowth in drinking water[J]. Journal of Environmental Sciences, 15(6): 773—778.

Standard methods for the examination of water and wastewater[M], 1995. 19th ed. Washington DC: American Public Health Association.

Van D K, Visser A, Hijnen W, 1982. Determining the concentration of easily assimilable organic carbon in drinking water[J]. J AWWA, 74(10): 540—545.

Wang Z S, Liu W J, 1999. Treatment of slightly polluted raw water[M]. Beijing: Construction Industry Press of China.

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