The 2019 World Congress on
Advances in Nano, Bio, Robotics and Energy (ANBRE19)
Jeju Island, Korea, September 17 - 21, 2019

Ultra Low Cost Conventional and Single Use Hanging-Bag Photobioreactors from 3D Printed Parts

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ABSTRACT

Microalgae and Cyanobacteria are photoautotrophic aquatic organisms capable of lipid, protein and carbohydrate accumulation. Their relative simplicity, wide range of metabolites, large number of uncharacterized species as well as their growth behavior make them interesting candidates for the production of a wide range of compounds and as a research tool.

Polyethylene (PE) tubing or bags can be used as low cost photobioreactors (PBR’s) for the cultivation of photoautotrophic organisms like cyanobacteria and microalgae. Bubble column based cultivations of these organisms on a liter scale is a valuable tool for the determination of cultivation parameters and small scale biomass production for research purposes as well as mass production after numbering up or adoption to larger diameter reactor systems. Use of innovative manufacturing technologies like 3D printing opens possibilities for the design and low cost manufacturing of new designs, hardware and custom features on PBR’s.

Here we have explored ways and possibilities to further decrease the cost of liter scale PBR’s by the use of 3D printing for fittings and other related hardware as well as the use of PE bags for low cost single use reactors in the liter scale to simplify handling and reduce labor cost which could increase the accessibility of photoautotroph cultivation and research.

1. Introduction

Microalgae and cyanobacteria are interesting organisms to produce lipids, pigments and many other classes of compounds. As phototroph organism they can fixate inorganic carbon from CO_2 with the help of light. Recently the use of these organism to produce long chained carbohydrates, which can be converted into biofuel, has seen much interest. Similarly has their use for flue gas purification and carbon sequestering attracted much research(Bhola et al. 2014). It should be noted that as eukaryotes microalgae are interesting production organism for a wide range of therapeutic proteins and that they as well as cyanobacteria are commercially relevant for the production of pigments(Yaakob et al. 2014). Some of these organisms produce also extracellular polymeric substances – often saccharides - of a wide variety. The purpose of EPS production is not fully understood, they may offer competitive advantages by showing antiviral or anti-microbial properties, facilitate water retention in a biofilm or provide a form of energy storage(Xiao and Zheng 2016).

Photobioreactors are needed for the cultivation of these organism at various scales. For production of biofuel or food protein open systems may suffice. However closed systems are preferred for high value compounds and process development as contamination with fungi, viruses and bacteria as well as losses of supplied CO_2 remain a challenge(Acién et al. 2017). Necessary investment into laboratory and pilot scale reactors of conventional design can be a significant cost factor in a research project. Low cost alternatives like hanging bag reactors offer solutions to this problem. Here we have developed ultra-low-cost reactors of various design, with a focus on ease of manufacture, cost and handling. For this 3D printed fittings and PE tubular film were mostly used. Designs were tested with a cultivation of the cyanobacterium *Chlamydomonas asymmetrica*, which has shown to produce significant amounts of EPS. We hope that the here described reactors reduce the needed initial investment for algae and cyanobacteria research to make this field more accessible.

2. Materials and Methods

ABS and PETG Filaments with a diameter of 1.75mm were purchased from Prusa Research (Prague, Czech Republic). Tubular PE film with 70µm wall thickness was purchased from Ihwa (Busan, Korea). Parts were designed in Fusion360 from Autodesk (Mill Valley, USA), sliced and converted to GCODE with PrusaSlicer 2.0 and fabricated on a Prusa 3D printer mk3 from Prusa Research equipped with a 0.6mm nozzle. A layer height of 0.35mm, extruder temperature of 255℃ and bed temperature of 100℃ was used for ABS, while PETG was extruded at 240℃ with the bed at 85℃.

The strain of *Chlamydomonas asymmetrica* used in this study was previously isolated from fresh waters of South Korea and cultured in modified AF6 medium with the composition 0,4 mg/L MES buffer, 0,01 mg/L $CaCl_2$, 0,02 mg/L Citric acid, 0,02 mg/L Fe-citrate, 0,03 mg/L $MgSO_4$, 0,14 mg/L $NaNO_3$, 0,022 mg/L NH_4NO_3, 0,01 mg/L KH_2PO_4, 5 µg/L Na_2EDTA, 0,98 µg/L $FeCl_3$, 0,18 µg/L $MnCl_2$, 0,11 µg/L $ZnSO_4$, 0,02 µg/L $CoCl_2$, 0,0125 µg/L Na_2MoO_4, 0,01 µg/L Thiamine, 0,02 µg/L Biotin, 0,01 µg/L Cyanocobalamin and 0,01 µg/L Pyridoxine. The medium was regulated to pH 6.6 and autoclaved two times for 20 min at 121℃. Vitamin solution was injected after cooling.

3. Results and Discussion

Material and manufacture

The Pipe PBR body was made from Poly(methyl methacrylate) also known as acrylic glass and PE tubular film. PMMA has offers a wide variety of glass transition temperatures (T_g) from 85℃ to 165℃. Thus, care must be taken when choosing a PMMA pipe for PBRs to make sure that the planed thermal microorganism reduction method does not exceed the T_g of the chose piping material. Compared to silicate glass PMMA has less UV and VIS transmittance, which reduces the overall photosynthetic yield in a PMMA bioreactor compared to one constructed from glass. On the other hand, height savings, ease of processing and shock resistance are far superior in PMMA.

Similarly, to PMMA, the tubular film material PE is available in a wide range of T_g and melting temperatures. However, to the authors knowledge no PE tubular film is available which does not significantly shrink at the standard autoclaving temperature of 121℃. Thus, this material can only be pasteurized at 80℃ instead of sterilized via autoclaving at 121℃. During our work with single and multi-use PE based hanging bag reactors we have not encountered contaminations which might be attributed to incomplete inactivation of possibly present contaminants. This may be related to the use of factory extruded tubular film for single use reactors, where little to no biological materials is found on the films in the first place.

To reduce the overall cost of the reactor setup, rapid manufacturing of fittings is desirable. We tried different materials for fused deposition modeling (aka '3D printing'). As a high melting temperature, high glass transition temperature and low cost are desired for low cost reactors PETG as well as ABS are good candidates. Specialized polymers with higher melting points and glass transition temperature are available but require specialized i.e. expensive 3D printer. PETG offers mostly superior physical properties to ABS, except for impact strength. Additionally, it has better characteristics for 3D printing e.g. better bed and layer adhesion and a lower thermal expansion coefficient which helps with dimensional stability during the cooling of the hot extruded 3D print. Unexpectedly, and to our knowledge unreported, repeated pasteurization cycles at 80℃ shrink PETG very significantly. Thus, fittings were manufacture from ABS.

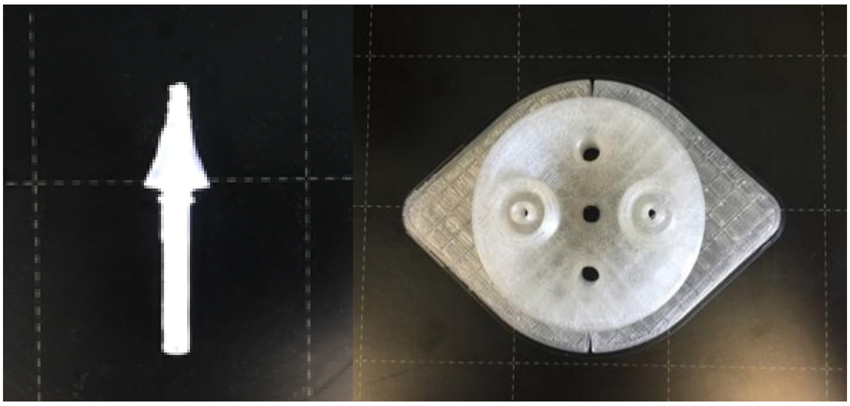

Fig. 1 Single use fitting (left) and multi use fitting (right) 3D printed from ABS.

Common conventional hanging bag reactors offer sampling and aeration ports at the bottom or from the top via tubing weighted with a lead sinker. The design shown in Fig. 1 choses ports at the bottom to emulate the geometry and handling of vertical glass pipe reactor. Here the main advantage is ease of setup and use, cost and comparability as well as short sampling tubes – thus reducing minimum necessary sampling volume.

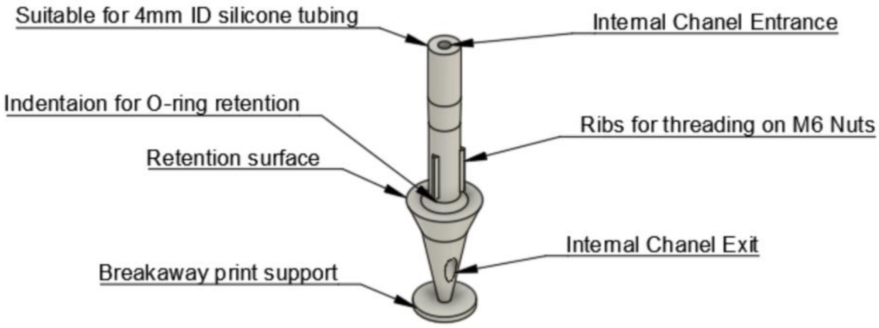

Fig. 2 Single use fitting design.

The PBR-tubing fittings shown in Fig. 2 are inserted through the film of the bag and immediately tightened with a M6 nut, pinching the reactor film between the flared head of the fitting and the nut. The reactor can thus be pasteurized either empty or with the media filled already and either before or after attaching fittings.

Fig. 3 Single use bag reactor with 3D printed fittings.

<u>Design</u>

The multi use bag reactor for a liquid culture was constructed consisted of two identical 3D printed inner fittings with integrated ports for tubing connections. A pair of matching outer fittings locks the tubular PE film in place between the inner and outer fitting. Due to small ridges of triangular integrate into the mating surface of the inner fitting and the slight elastic behavior of the PE tubular film, no additional gasket was needed. Holes in the top and bottom fit rigid air hose, which can be permanently sealed with a drop of cyanoacrylate glue.

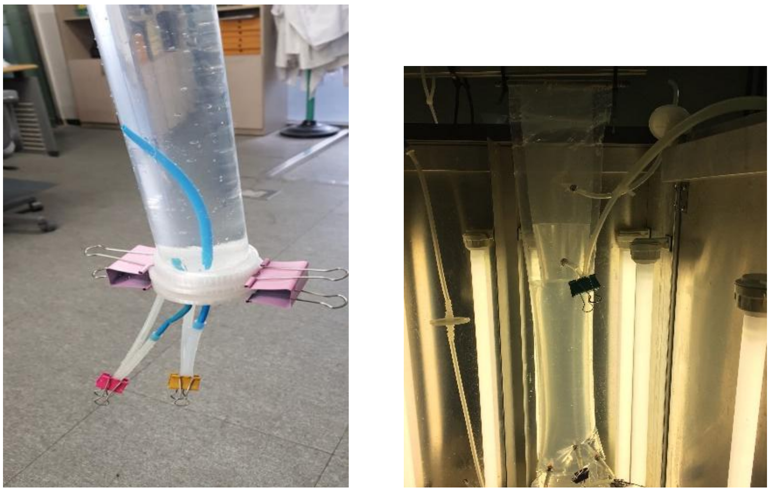

Fig. 4 Fitting clamped fitting for multi use bags (left) and bag in bag reactor with single use fittings (right).

The Bag in Bag reactor is here to the authors knowledge suggested the first time. Conventionally a temperature-controlled fluid is flowing through a metal tube inserted into the reactor and acts as heating and cooling element. This offers very good heat transfer thanks to the high thermal conductivity of around 15W/mK. Silicone tubing is sometimes used as an alternative. Here however the internal bag in the reactor (see in Fig. 4) is connected to a temperature control unit with water as coolant. While the thermal conductivity of the bag material and silicone tubing are similar (around 0.3W/mK) the thinner bag with larger surface offer substantial higher heat transfer rates. But more importantly the internal bag reduces the available volume in the bag reactor for algae cultivation into a hollow cylinder. Therefore, for the same volume a larger surface area and a lower cultivation layer is created. This should reduce shading effects and lead to short light/dark cycles.

A modified version of the multiple use bag reactor used an acrylic pipe instead of film, which necessitated the use of o rings as seals at the bottom and top of the assembly. Fig. 5 shows this assembly modified for the cultivation of Chlamydomonas in a biofilm, where an expanded porous UHMW PE tube serves as the scaffold for the cyanobacterium. The porous tubing includes a hydrophilic surface modification allowing wicking of the cultivation media circulating through tube to the biofilm (Fig. 5). This design allows for rapid change in media nutrient composition and harvest with relatively low water content, reducing energy expenditure for the dewatering of biomass. Low media volume also allows for a faster change in media composition, which can potentially be used to induce desired behavior in the cultivated microorganism.

Fig. 5 from left to right: Biofilm reactor from acrylic pipe, porous tubes of expanded UHMW PE and 3D printed fittings, Biofilm at day 1, Biofilm at day 36 and close up of cultivated biofilm of *Chlamydomonas asymmetrica* after cultivation for 36 days at 100x magnification.

We found that the use of silicone O-rings is highly recommended as different brands of nitrile rubber O-rings seem exhibit aquatic toxicity and inhibit the growth of spirulina platensis and the here used *Chlamydomonas asymmetrica*.

Cost savings

An in-depth cost analysis for raceway, tubular and flat panel plants of 1ha and 100ha size was done by Norsker et al.(Norsker et al. 2011) For these plants, biomass costs of around 0.5USD to 1.5USD per KG of dry biomass were estimated in temperate regions. Of that approximately one third was allocated to the plant infrastructure, installation, instrumentation etc. Smaller scale, benchtop / lab scale reactors have generally a much higher initial investment into instrumentation. Prices for simple PBR vessel range approximately from 50USD to 500USD in the small liter scale. Hanging bag reactors can be fund at the lower end of this price range, on the condition that fittings are manufactured and not purchased.

Table 1. Estimated cost for 2L single use bag, multi use bag and Acrylic pipe PBS

Item	Single use bag-in-bag		Multi use bag		Acrylic pipe	
		Cost		Cost per unit		Cost
PE tubular film	2m	0.08 USD	1m	0.08 USD		
Pin connectors	6ea	0.03 USD				
O-rings	12ea	0.05 USD				
M6 Nuts	6ea	0.1 USD				
Inner bag holder			2ea	1.1 USD		
Outer bag holder			2ea	0.7 USD		
clamps			4ea	0.3 USD		
Tube (Acrylic - 70cm)					1ea	25 USD
Tube fitting					2ea	1.1 USD
Air filter	1ea	0.4 USD	1ea	0.4 USD	1ea	0.4 USD
Sum		1.94 USD		5.26 USD		27.6 USD

Single use connectors (Fig. 1 left) use 1.5g of ABS filament which cost approximately 0.03USD, 1m PE tubular film with a ID of 65mm was purchased for 0.08USD, a M6 stainless steel nut for 0.1USD while the O-rings were purchased for approx. 0.05USD. The single use bag design shown in Fig. 3 uses a minimum of 3 fittings, each with two o rings and a M6 nut. With a combined cost of approximately 1.2USD. A liter of modified AF6 medium, as used for the cultivation of *Chlamydomonas asymmetrica*, costs approximately 0.89USD (see Table 2). Therefore, for single use bioreactors the cost of the reactors is substantial in comparison to the media cost, and a reduction in reactor cost affects the over all cost of cultivation significantly.

Table 2. Cost of cultivation media per liter in USD – estimated from prices at Sigma Aldrich for commonly purchased quantities.

Component	Amount in g/L	Approx. price in USD
$NaNO_3$	0.14	0.02092
NH_4NO_3	0.022	0.00343
$MgSO_4 \cdot 7H_2O$	0.03	0.00584
K_2HPO_4	0.05	0.01610
KH_2PO_4	0.01	0.00223
$CaCl_2 \cdot 2H_2O$	0.01	0.00203
Fe-citrate	0.02	0.00433
Citric acid	0.02	0.00357
MES monohydrate	0.4	0.82734
$Na_2EDTA \cdot 2H_2O$	0.005	0.00192
$FeCl_3 \cdot 6H_2O$	0.00098	0.00022
$MnCl_2 \cdot 4H_2O$	0.00018	0.00005
$ZnSO_4 \cdot 7H_2O$	0.00011	0.00003
$CoCl_2 \cdot 6H_2O$	0.00002	0.00001
$Na_2MoO_4 \cdot 2H_2O$	0.0000125	0.00001
Thiamine	0.00001	0.00001
Biotin	0.00002	0.00289
Cyanocobalamin	0.00001	0.00000
Pyridoxine	0.00001	0.00002
Sum in USD / L of media:		0.89

<u>Cultivation of *Chlamydomonas asymmetrica*</u>

For the evaluation of the here shown low cost PBRs a comparison with a conventional glass tube PBR was performed. Media, aeration rate, volume, and strain were identical, however due to the different shape of the bioreactor attachments, the installation differed and the incoming light intensity was 13.7 µmol/m^{2}s for the glass tube reactor and 12.7 µmol/m^{2}s for the hanging bag reactor respectively. The resulting growth curves are shown in Fig. 6. Growth rate in the exponential phase was 0.0316 h^{-1} and 0.0423 h^{-1} for the glass and hanging bag reactor respectively. Subsequently linear growth due to substrate limitation was observed, faster in the glass than in the hanging bag reactor. The glass reactor had a slightly smaller inner diameter of

61mm, compared to 65mm for the hanging bag. This reduced the mean path length of light, reduces self-shading at higher optical densities and effectively increases the irradiated area and thus the total light energy input when identical lighting conditions are provided. It should also be noted that hanging bag reactors made from PE tubular films show generally a lower UV/Vis light transmittance than glass reactors. Reflectance on the other hand tends to be high in glass reactors, unless anti reflective coating is applied.

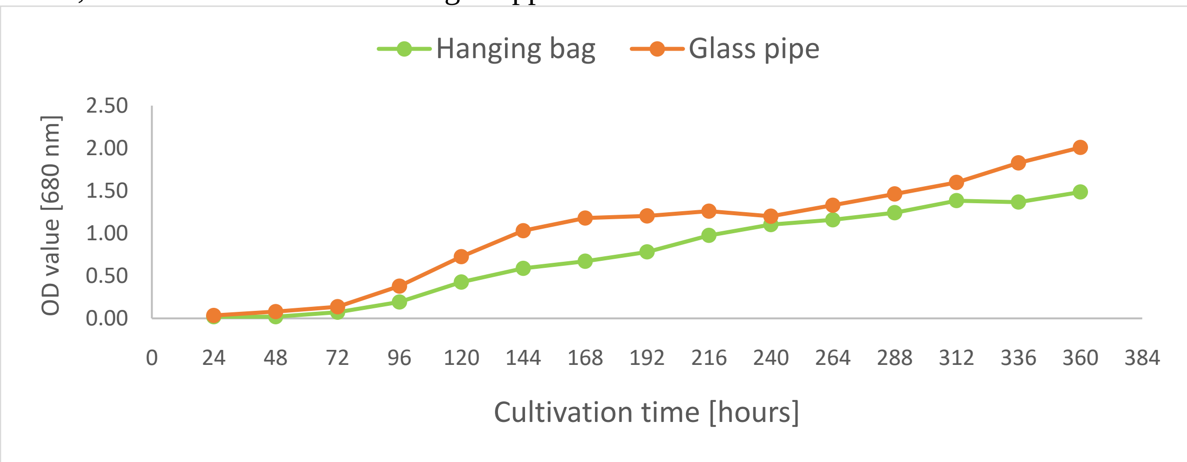

Fig. 6 Comparison of growth curves of *Chlamydomonas asymmetrica* in hanging bag reactor and glass tube reactor as plot of the optical density (OD 680 nm) depending on cultivation time in range of 24 to 360 hours. The samples are measured in triplicates and showed standard deviations below 2.3% from day 3 onwards.

The reactor tests were performed with the chosen organism due its capacity to produce large quantities of EPS. To demonstrate this, a culture was grown until an OD of 1.2 and EPS concentration in the cultivation media determined daily. Fig. 7 shows a nonlinear increase in EPS at higher OD, suggesting that EPS production is not negatively regulated with cell density. Further studies with developed hanging bag reactors and biofilm reactors for the optimization of EPS production in the growth and stationary phase are planned.

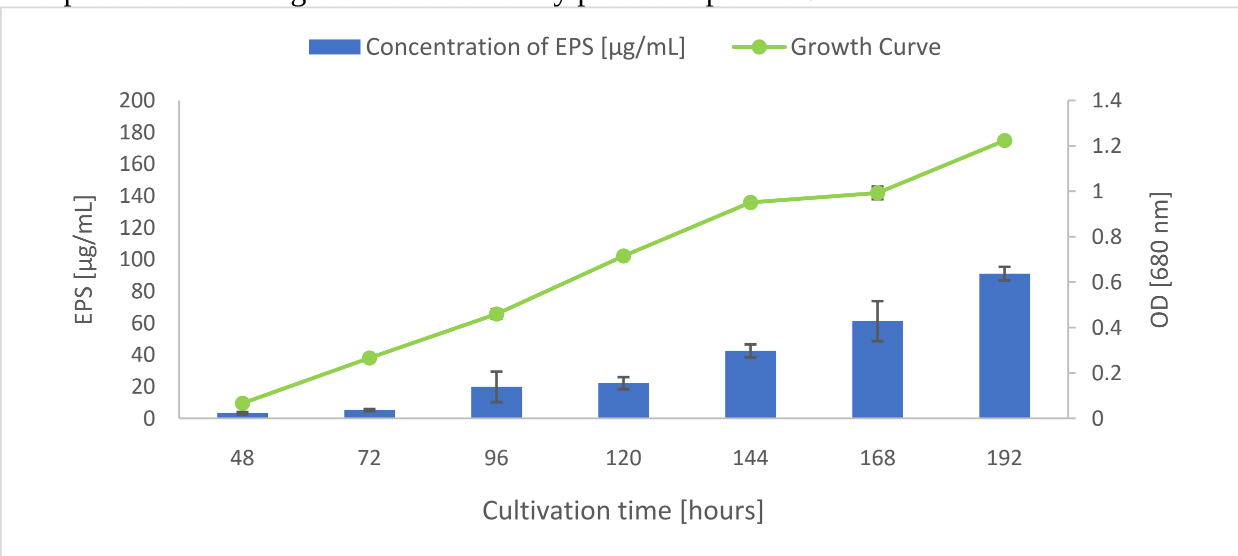

Fig. 7 Growth and EPS concentration curves for *Chlamydomonas asymmetrica* in a 2 Liter hanging bag reactor over 192 hours. Samples were measured in triplicates.

Limitations

The used PE tubular films of 65mm ID and 70micrometer limits the maximal height of vertically installed hanging bag PBR due to its relatively low yield strength. Bags and pin fittings were tested until destruction. Briefly, the single use fitting pin were starting to leak at a pressure of 0.067 bar or 70cm of water column. This limits the volumes which can be safely handled for this type of fitting to about 2L. This is only the case when the fitting is installed at the lowest point of the reactor where hydrostatic pressure is the highest. The bag started plastic deformation and subsequent failure from a water column height of 250cm or 0.245bar. Welds on the other hand could not be tested until destruction as surrounding film failed before the welds.
Top mounted fitting with tubing inside the bag is therefore an option if larger cultivation volumes are desired. The downside of additional tubing inside the bag and thus added shading as well as inferior mixing might be offset by the larger culture volume of up to 7L.

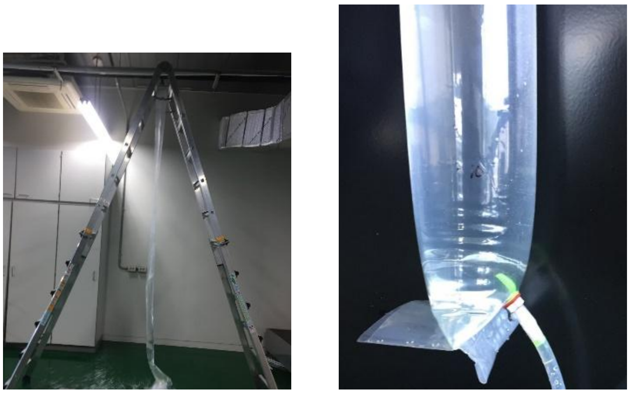

Fig. 8 Leak test for 65mm ID, 70µm wall thickness hanging bag (left) and leak test for 3D printed single use fitting inserted into 70µm wall thickness PE bag (right).

4. Conclusion

Several different PBR designs were developed and tested. An ultra low cost variant was developed, manufactured, tested, compared with a glass reactor and used for the cultivation of *Chlamydomonas asymmetrica* to produce EPS. Single use reactors were found to offer significant reduction in the cost of a cultivation as the reactor cost is a major contributor.

5. References

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