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Evaluation of Alkaline Peroxide Pretreatment for Extraction of Cellulose from Selected Plant Biomasses

T.N. Fernando[1*], A.G.B. Aruggoda[1], S.A Ariadurai[2], C. K. Disanayaka[3] and S. Kulathunge[3]

[1] Department of Agricultural and Plantation Engineering, Faculty of Engineering Technology, The Open University of Sri Lanka, Nawala, Nugegoda, Sri Lanka.

[2]Department of Textile and Apparel Technology, Faculty of Engineering Technology, The Open University of Sri Lanka, Nawala, Nugegoda, Sri Lanka.

[3] Sri Lanka Atomic Energy Board, No. 60/460, Baseline road, Orugodawatta, Wellampitiya, Colombo.

Corresponding Author email: nilanthifernando45@yahoo.com, Tel:+94719154065

Abstract - *The compositional characteristics of Corn leaves, Corn Husks, Bagasse, Guinea Grass and Sugarcane leaves were established in terms of lignin, cellulose and hemicellulose contents, to assess the suitability of agricultural wastes for the extraction of cellulose. The objective of the present study was to evaluate the performances of the alkaline hydrogen peroxide method by applying three different concentrations of pretreatment solutions on five selected plant biomasses; Corn leaves, Corn husks, Bagasse, Guinea grass and Sugar cane leaves. In addition the best pretreatment solution was evaluated based on the extraction of maximum cellulose percentages with minimum weight losses of Plant biomaterials. For the experiment, three prepared pretreatment solutions named as S1 (prepared by mixing10ml of 1.25 mol/L and1 ml of 99% hydrogen peroxide), S2 (prepared by mixing10ml of 2.5 mol/L and 0.75 ml of 99% hydrogen peroxide) and S3 (prepared by mixing10ml of 5 mol/L and 0.5 ml of 99% hydrogen peroxide) were prepared. Fifteen treatments with three replicates were applied combined with five Plant biomass and three different concentrations of pretreatment solutions. On visual inspection of extracted fibers, wool type formations were observed. Analysis of results indicated that biomass type was significant for percentage weight losses (weight losses %) (P = 0.012) and not significant for percentage of cellulose extracted (cellulose %). Depending on pretreatment solutions, weight losses % (P=0.045) and cellulose % (p = 0.007) were significant. Increasing of concentration of pretreatment solution resulted in high weight losses in the biomasses. Highest weight losses occurred in pretreated corn leaves and corn husk with all three pretreatment solutions (>70%). All pretreated fibers showed highest extraction of Cellulose % (>80%). Among them Guinea grass showed the highest (87%) and corn husk showed the lowest value (74%). According to the results, S2 solution was the optimum pretreatment solution for extracting maximum cellulose with minimum weight losses of plant biomaterials from selected plant biomasses.*

Key words: *Biomasses, Cellulose, Alkaline hydrogen peroxide, pretreatment*

1 INTRODUCTION

Cellulose probably is the most abundant organic compound in the world which is mostly produced by plants. It is the most structural component in Plant cells and tissues. Cellulose is a natural long chain polymer that plays an important role in human food cycle indirectly. Cellulose based materials are the most commonly available polysaccharide in world which can be used to process of biodegradable polymers, bio ethanol, veterinary foods, wood and appear, fibers and clothes, cosmetic and pharmaceutical industries etc. Most agricultural residues such as corn husks, corn stove, bagasse, sugar cane leave, guinea grass are rich with cellulose fibers. Those materials are known as lignocellulosic materials also. Lignocellulosic plant materials are a matrix of hemicelluloses wrapped around long chains of cellulose encased by lignin. Pretreatment methods are necessary to remove or alter the lignin. Several pretreatment methods such as dilute sulfuric acid; steam explosion, lime and pH controlled hot water (Wyman et al., 2005) are used to remove lignin from lignocellulosic materials. The main disadvantages of these pretreatments are the high temperatures and pressures required, as well as the potential environmental impacts of toxic chemicals. Applying of High temperatures and pressures are costly and difficult to maintain on-farm on a large scale.

Researchers have shown that alkaline hydrogen peroxide application at atmospheric pressure and room temperature is an effective pretreatment method (Banerjeeet al., 2012 and Gould., 1985). (Banerjeeet al., 2012) noted that alkaline hydrogen peroxide is under studied compared to other methods of delignification. Alkaline peroxide pretreatment is more effective at solubilizing lignin and improving digestibility than alkali treatments, another form of mild pretreatment (Karagoz et al., 2012).

In agricultural field, Superabsorbent polymers (SAPs) are mostly used to increase available water in the soil which enables the plants to survive longer under water stress and enhanced water holding capacity of the plant. Carbohydrate SAPs (polysaccharides) are the cheapest and most abundant and renewable organic materials. Chitin, cellulose, starch, and natural gums (such as xanthan, guar and alginates) are some of the most important polysaccharides. Cellulose is the most preferred polysaccharide for processing of biodegradable SAP in Sri Lanka. Since starch is edible it is not used to produce SAPs. When processing cellulose based SAP, cellulose is first needed to be separated from selected plant biomasses with minimum cost as natural cellulose compounds like cotton are not abundant in Sri Lanka. However alkaline hydrogen peroxide pretreatment is a simple and effective method to remove lignin from lignocellulosic material and there is no ideal pretreatment concentration to separate cellulose from selected plant biomass such as Corn leaves, Corn husks, Bagasse, Guinea grass and Sugar cane leaves.

The objective of the present study is to evaluate the performance of the alkaline hydrogen peroxide method by applying three different concentrations of prepared pretreatment solutions on five selected plant biomasses; Corn leaves, Corn husks, Bagasse, Guinea grass and Sugar cane leaves. In addition the best pretreatment solution was evaluated based on the extraction of maximum cellulose percentages with minimum weight losses of Plant biomaterials to produce Sri Lankan cellulose based Super Absorbent Polymer from selected plant biomasses.

2 LITERATURE REVIEW

2.1 Lignocellulosic plant materials

Lignocellulosic plant materials are a matrix of hemicelluloses wrapped around long chains of cellulose encased by lignin (Figure 1). Cellulose, a glucose polysaccharide, forms highly dense and order group with a degree of polymerization in the 10000s (before any treatment). The degree of polymerization is the number of nonnumeric units in a large molecule, or specifically for cellulose the degree of polymerization is equivalent to the number of glucose molecules. Cellulose forms micro fibrils: these long interwoven chains of cellulose (micro fibrils) order themselves in to a highly dense structure the cellulose is considered to be crystalline. Crystalline cellulose is generally un-reactive and insoluble because the structure is too stable. Hemicellulose is a mixture of hexoses and pentose with a much lower degree of polymerization (100-200) but can physically prohibit enzymatic access to the cellulose. Lignin is the largest non-carbohydrate portion of plant matter. Lignin cannot be broken down enzymatically to its basic components (monomeric alcohol groups) and hence serves to protect and support the plant (Brown et al, 2003).

Pretreatment methods are necessary to remove or alter the lignin in order to increase the accessibility of cellulose and hemicellulose during enzymatic hydrolysis. An ideal pretreatment would optimize the amount of sugars released during hydrolysis and limit sugar losses due to inadvertent conversion to fermentation inhibitors (Modenbach et al, 2012); similarly an ideal pretreatment is effective, simple, inexpensive, non-inhibitory and compatible with high biomass loadings (Banerjee et al, 2011). A pretreatment is considered effective when the physical barrier of the plant cell wall is disrupted (the interconnections between the lignin, cellulose and hemicellulose are loosened) and the cellulose crystallinity is reduced (Wyman et al, 2005). Some pretreatment methods like steam explosion and acid pretreatments required for high temperatures (Modenbach et al, 2012). Alkaline Hydrogen Peroxide has been successful at normal room temperatures (Gould et al, 1985, Yang et al, 2002, Rabelo et al, 2008 Rabelo et al, 2008 and Banerjee et al, 2012) Mild pretreatment conditions provide an important advantage for the large scale on-farm production of biofuels. If little energy is needed to control the temperature during pretreatment, the overall energy balance will be more favorable.

The effectiveness of alkaline hydrogen peroxide pretreatment is affected by a number of environmental conditions; including the temperature, pH, reaction time, hydrogen peroxide loading, solids concentration and particle size of the feedstock (Karagoz et al, 2012 and Gray et al, 2013). Banerjee et al, 2011 was presents mixed results for the effect of temperature on AHP. (Sun et al, 2000 and Selig et al, 2009) show increasing sugar yields with increasing temperatures on maize stems and corn stover respectably, while (Karagoz et al, 2012) attained higher yields with rapeseed straw at 50°C than 70°C due to H2O2 decomposition at higher temperatures. It is unknown whether decreasing the temperature below typical ambient conditions has any effect on the pretreatment effectiveness. As noted previously, hydrogen peroxide requires an alkaline pH to produce the oxidizing radicals necessary to degrade lignin. NaOH has traditionally been used raise the pH up to 11.5(Gould et al, 1985) achieved approximately 50% delignification with an initial pH measurement of 11.5. The question then becomes whether or not it is necessary to maintain the initial pH for the duration of the reaction. In (Banerjee et al, 2012) kilogram scale experiment the pH was adjusted at different times (0, 3, 6, 12, 18, 24 and 36 hours) to compare the effect of pH adjustment throughout the experiment on the efficacy of the pretreatment; a slight increase in glucose yield was found. The pH increases for the duration of alkaline hydrogen peroxide pretreatment; (Sun et al, 2001) found that the increase of pH increases the hemicelluloses solubilized and regular pH adjustment is unnecessary. Further research showed that a constant pH of 11.5 was not necessary. As the reaction becomes more alkaline an increasing amount of lignin and hemicellulose are solubilized (Fang et al, 1999 and Silverstein et al, 2007). When hydrogen peroxide is raised to an alkaline pH (11.5-11.6) it dissociates into hydrogen and the hydroperoxyl anion (HOO-), as seen in Equation 1. The anion then reacts with remaining peroxide to form highly-reactive hydroxyl radicals which attack the lignin structure (Equation 2).

$$E = \quad H_2O_2 \rightleftharpoons H^+ + HOO^- \qquad (1)$$
$$E = \quad H_2O_2 + HOO^- \rightarrow HO\cdot + O_2^-\cdot + H_2O2 \qquad (2)$$

The reaction between lignin and the hydroxyl radical yields low molecular weight water-soluble oxidation products. During hydrogen peroxide application, plant material has been shown to disintegrate into small, highly dispersed fibers (Gould et al, 1985). The cell wall loses most of its rigid structure and becomes less uniform in texture (Martel et al, 1990 and Selig et al, 2009), providing access points for enzymatic hydrolysis. Alkaline peroxide pretreatment expands the lignocellulosic matrix, providing more access points for enzymes between the lignin, cellulose and hemicellulose.

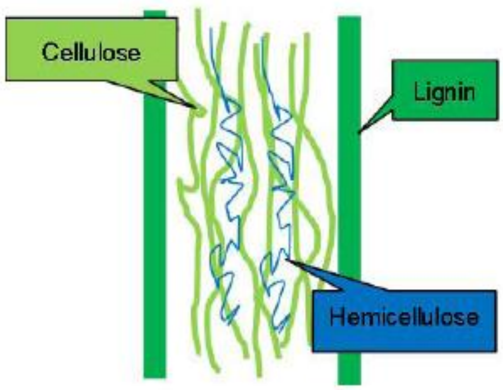

Figure 1 General structure of lignocellulosic plant materials (Wyman et al, 2005**).**

Table 1 Chemical composition of agro-industrial waste available

Agro- Industry waste	Chemical composition (%W/W)					
	Moist.	Total solid	Ash	Cellu.	Hemi-Cellu.	Lignin
Corn straw	1.92	97.78	10.8	61.2	19.3	6.9
Baggases	8.34	91.66	1.9	30.2	56.7	13.4
Sugarcane straw	-	-	5.7	33.6	28.9	31.8

Notes: (%w/w) = percentage based on dry weight.
Source: Corn straw and Baggases (http://www.scielo.br), Sugarcane straw (De silva et al, 2010)

2 METHODOLOGY

2.1 Alkaline Peroxide Pretreatment

This method was performed at room temperature and atmospheric pressure. Three different concentrations of pretreatment solutions and five different biomasses; Corn leaves, Corn Husks, Bagasse, Guinea Grass, Sugar cane leaves were used for the experiment. Pretreatment solutions were prepared by mixing different concentrations of NaOH with equal volume (10 ml) and different volumes of 99% hydrogen peroxide (H_2O_2) as follows:

Pretreatment solutions	NaOH concentration		99% (H_2O_2) volume
S1	10ml of 1.25 mol/L	+	1 ml
S2	10 ml of 2.5 mol/L	+	0.75 ml
S3	10 ml of 5 mol/L	+	0.5ml

Five biomasses used were named as follow for the ease of interpretation.

1- Bagasse (B)
2- Sugarcane leaves (SL)
3-Corn leaves (CL)
4- Guinea grass (GU)
5- Corn husk (CH)

Combinations of three pretreatments solutions and five plant biomasses, fifteen treatments with three replicates were applied as follow.

Treatment No	Treatments		
1	B	+	S1
2	SL	+	S1
3	CL	+	S1
4	GU	+	S1
5	CH	+	S1
6	B	+	S2
7	SL	+	S2
8	CL	+	S2
9	GU	+	S2
10	CH	+	S2
11	B	+	S3
12	SL	+	S3
13	CL	+	S3
14	GU	+	S3
15	CH	+	S3

Ten milliliter from each prepared pretreatment solution was added to 1 g of biomass. Total of 45 g of biomass with 450 ml of pretreatment solution was added to 1000 ml beaker shake for 24 hours at 90 rpm. The mixtures were kept at room temperature for another 24 hours for further degradation. The mixtures were immersed in 10% HCl for 4 hours, washed with tap water once and heated at 60 0C for 24 hours and then kept in a desiccator until reach to a constant weight. Percentages of weight losses were evaluated. Two factor factorial design was applied with Minitab version 14 for analysis. Cellulose percentages were determined by using chlorination method as described by the (Silverstein et al, 2007). 1 g of sample from each pretreated fiber biomass was placed in a flask (500 ml) separately and 80 ml of distilled water was added and shake the samples slowly at 150 rpm thermostat in a 1 g of NaClO2 and 0.5 ml of acetic acid was added and the flask was covered with glass top and boiled at 70 0C for 60 min in a thermostat. This procedure was repeated three times. Then the mixtures were kept at 700C while shaking at 150rpm for overnight. Samples were allowed to cool. Then they were filtered using a filter flask and washed with 10% Acetone. Insoluble portions were dried in an oven at 105 0C for 4 h, flowed by cooling at a desiccator until reach to a constant weight. Holocellulose (combination of cellulose and hemicellulos) contents were calculated as follow.

Holocellulose content (%),

$$E = \frac{Final\ weight\ after\ treatment}{Initial\ weight\ of\ sample} x\ 100 \qquad (3)$$

Holocellulose were treated with cold sodium hydroxide (20 ^{0}C) for approximately 4 hours for hydrolysis of hemicelluloses. The final weights were measured to gain the weight of the cellulose in each biomass sample. Laboratory cost was calculated for producing 1 g of cellulose.

3 RESULTS AND DISCUSSION

On visual inspection of extracted fibers, wool type formations were observed (figure 2). Percentage of weight losses of plant biomaterials (weight losses %) and percentage of cellulose (cellulose %) obtained from the two variables; biomass and pretreatment solutions are shown in table 2. According to analysis data, biomasses were significant for weight losses % (P = 0.012) and not significant for cellulose %. Depending on pretreatment solutions, weight losses % (P=0.045) and cellulose% (p = 0.007) were significant. Figure 3 shows the variation of interaction plot of cellulose % on bio masses and pretreatment solutions. According to that, percentages of cellulose extracted fibers from all biomasses were highest at pretreatment of S2. At the same time pretreatment solution S3 showed the lowest cellulose % compared to S2 and S1on cellulose extracted from bagasse, corn leaves, and guinea grass and corn husks. Variation of main effect of cellulose % is shown in figure 4 according to which highest cellulose% was obtained from guinea grass (87%) and lowest obtained from corn husk (74%). Additionally, all pretreated fibers consisted of more than 80% of cellulose. Also S2 pretreatment solution applied media had highest amount of cellulose compared to those with S1 and S3. Fang et al, 1999 has found that the increasing alkaline condition in pretreatment solution resulted in increased in amount of lignin and hemicellulose solubilizing. Figure 5 indicates the interaction plot of weight losses %. These results indicated that S3 pretreated media consisted of highest amount of weight losses of plant biomaterials when compared to those with S1 and S2. Further, variation of main effects is shown in figure 6. The main effects of weight losses have increased as S3>S2>S1. The results of the present study show Sodium hydroxide concentration of used pretreatment solutions increase in solutions as S1<S2<S3. High alkalinity (NaOH concentration) of S3

solution where created may be the reason for the high weight losses% in selected biomasses. Additionally, figure 5 shows that the corn leaves and corn husk had highest amount of weight losses (>70%) compared to other three bio mass. The low content of lignin in corn straw may be the reason for highest amount of biomass degrades (http://www.scielo.br).

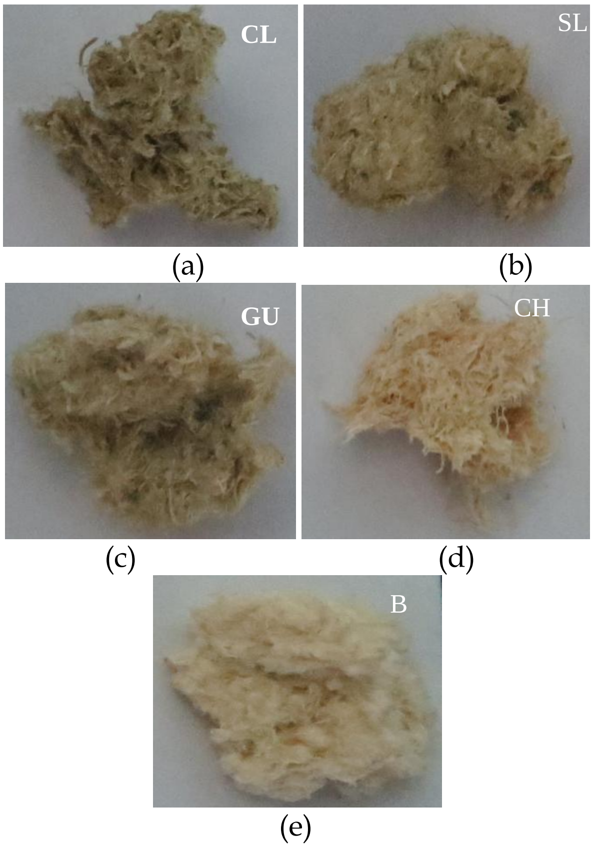

Figure 2(a-e): CL, SL, GU, CH, B. Extracted fibers after S2 pretreatment

Table 2 Probability values for measured parameters

Variables	Weight loss%	Cellulose %
Biomasses	0.012	0.283
Pretreatment solutions	0.045	0.007

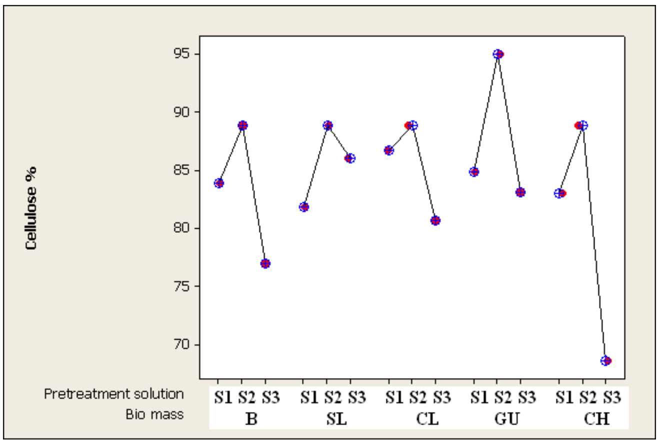

Figure 3 Variation of interaction plot of cellulose %Vs biomass and pretreatment solutions

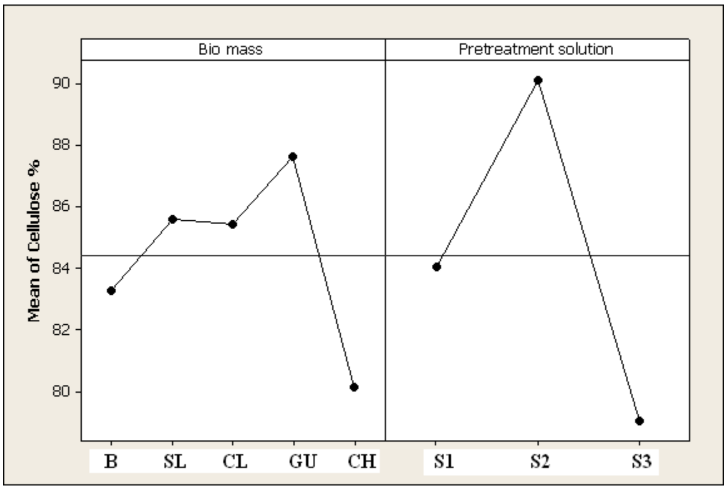

Figure 4 Variation of main effect plot for cellulose%,Vs biomass and pretreatment solutions separately

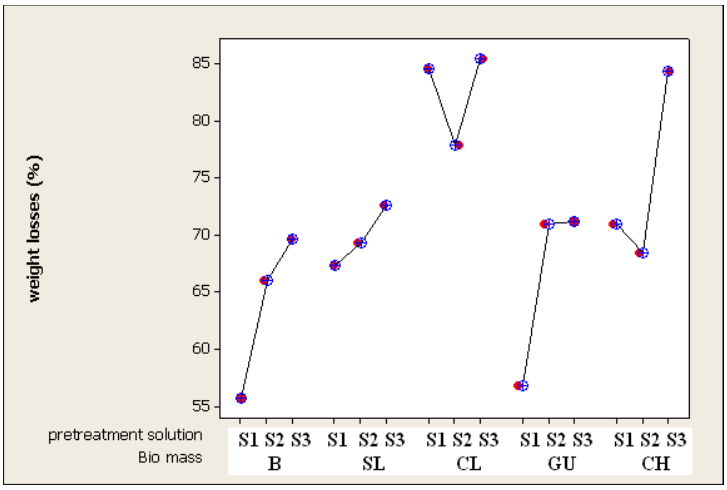

Figure 5 Variation of interaction plot of weight losses% Vs bio mass and pretreatment solution

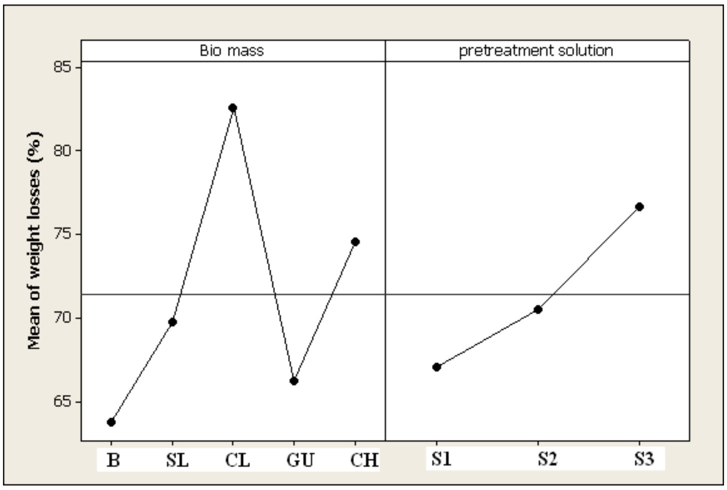

Figure 6 Variation of main effect for weight losses % Vs biomass and pretreatment solutions separately

Table 3 Results of cost analysis

Materials used	Price for S1 pretreatment(Rs)	Price for S1 pretreatment(Rs)	Price for S1 pretreatment(Rs)
NaOH	0.375	0.75	1.5
H_2O_2	1.1	0.825	0.555
Total price for materials	1.475	1.575	2.005
Electricity	43	43	43

(5g of raw materials was needed to obtain 1g of cellulose. 50W shaker was operating 24h).

4 CONCLUSIONS

Increasing of concentration of pretreatment solution resulted in high weight losses in the bio masses. Highest weight losses of plant biomaterials occurred in pretreated corn leaves and corn husk with all three pretreatment solutions (>70%). All pretreated fibers showed highest extraction of Cellulose % (>80%). Among them Guinea grass shows the highest (87%) and corn husk (74%) shows the lowest values. According to the results, S2 solution was the optimum pretreatment solution for extracting cellulose with minimum weight losses of plant biomaterials from selected biomasses. Cellulose processed using S2 will be considered in the production of Sri Lankan Super Absorbent Polymer.

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