



Comparative Study of the Yield of Reducing Sugar from Hydrolysis of *Ximenia caffra* Seed Coat and *Phoenix dactylifera* Stone Using Bioalkali Extracted from Plantain Peel

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ABSTRACT

Lignocelluloses comprising cellulose, hemicelluloses, lignin among others can be pretreated with cost effective method to yield fermentable sugar, using waste agricultural residues as pretreatment chemical and substrates. This study was aimed at investigating the potential of bioalkali extracted from plantain (*Musa paradisiaca*) peel ash as a viable alternative alkaline reagent for the pretreatment of *Ximenia caffra* seed coat and *Phoenix dactylifera* stone. The ash of burnt plantain peel was characterized using x-ray fluorescence (XRF) spectroscopy with the oxide of potassium as the dominant oxide (54.2 wt%), the concentrations of the potash alkali (BioKOH) extracted were quantified titrimetrically. The Taguchi L9 orthogonal array design was used as the response surface method to design the hydrolysis process. The factors of BioKOH concentrations (0.022–1.950%), Solid to liquid ratio (1:30-1:90 cm³w/v) for varying treatment times (15–60 min) at a temperature of 121°C were optimized for the hydrolysis. The reducing sugar in the hydrolysates obtained was characterized using Fourier Transform Infrared Spectroscopy and measured using 3,5-dinitrosalicylic acid (DNS) method. The pretreatment condition of 0.022% BioKOH, 60 mins and 1:30 cm³w/v was determined to be the most effective as it utilized the least amount of BioKOH while generating 22.56 mg sugar/g of *Phoenix dactylifera* stone. The sour plum seed coat gave a better yield of 30.14 mg sugar/g for factors of BioKOH concentrations (0.022%), Solid to liquid ratio (1:60cm³w/v) for varying treatment times (15 min) at 121°C. Therefore, the plantain ash can serve as bioalkaline and can be scaled up for economic benefits.

Keywords: Date palm stone, Sour plum seed coat, Lignocellulose, Bioalkali (BioKOH), Plantain peel, Reducing sugars.

Introduction

The growing demand for energy, materials and food, depletion of fossil raw material reservoirs and increasing environmental concerns have all increased interest in renewable resources. The quest for sustainable and environmentally friendly energy source has given rise to renewable feedstocks such as biomass which are rich sources of cellulose and hemicellulose.¹

Lignocelluloses mainly consist of cellulose, hemicelluloses, and lignin which are bonded together by covalent bonding, various intermolecular bridges, and van der Waals' forces forming a complex structure, making it resistant to enzymatic hydrolysis and insoluble in water.² Therefore, an efficient, less energy-intensive and cost-effective pretreatment method is a necessity for producing fermentable sugar at economically viable cost. Pretreatment of biomass is a crucial step to overcome lignocelluloses recalcitrance in the conversion to ethanol.

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Pretreatment can be divided into three main categories: (a) physical, (b) chemical, and (c) biological. Physical pretreatment processes have proven to be energetically unviable and biological pretreatment methods can be expensive and time consuming.^{3,4} Chemical pretreatment techniques on the other hand has been the most widely studied and alkaline pretreatment in particular, has seen considerable success.

Potassium hydroxide is a relatively less explored pretreatment agent but could potentially be used for lignocellulose pretreatment due to its reported reactivity with carbon nanofibers and carbon nanostructures⁴ and its ability to deacetylate biomass.⁵⁻⁷

Currently, there is a large scope to investigate and restore the challenges in the pretreatment processes which finally leads to the development of the tailor-made effective pretreatment methods for diverse types of lignocellulosic biomass. Hence, emphases on green industrial principles were capitalized on, using waste agricultural residues as pretreatment chemical and substrates.

Bio-alkali is the alkali derived from the ashes of burnt biomaterials. Agricultural materials contain a good percentage of mineral salts. When these materials are burnt in air, carbohydrates, fats, proteins and vitamins will volatilize. The resulting ashes contain oxides of these minerals. Some of these are basic oxides of potassium and sodium, which when dissolved in water yield their corresponding hydroxides (alkali). Research has shown that alkaline from plantain peel ash has been used to produce soap,⁸ and lubricating grease of good quality. Hence, it is anticipated that alkali produced from the ash of agricultural waste serves as potash biocatalyst for hydrolysis of biomass.

Date pits have a hard seed coat that makes the seed components difficult to digest. It is necessary to process the seeds before feeding them to livestock.⁹ Alkali treatments increase the digestibility of fibrous materials. Application of a 9.6% sodium hydroxide (NaOH) solution to ground date

pits decreased neutral detergent fibre (NDF) content and increased *in vitro* digestion rates. This treatment was more effective on finely ground date pits (4 mm vs. 8 mm).¹⁰ Nasse *et al.* reported that the lignocellulosic components of date palm seed pit is cellulose 32%, hemicellulose 30% and lignin 37%.¹¹ The Large sour plum, *Ximenia caffra* of the family Olacaceae is widely distributed in sub-Saharan Africa and bears fruit even in drought years.^{12, 13} Fruit ellipsoidal or ovoid drupe, about 3.5 cm long and 2.5 cm in diameter, greenish when young, orange to red flesh when ripe with juicy pulps and a smooth skin. The Seeds are up to 2.5 cm long, 1 cm thick and hard coated. The seed kernels are edible and known for their high oil content (~ 65%), hence their use as a domestic biofuel.¹⁴ The aim of this work is to determine the optimum conditions for the production of reducing sugar from date palm stone (DPS) and sour plum seed coat (SPSC) using BioKOH hydrolysis. Three factors were optimized namely BioKOH concentration, solid to liquid ratio and reaction time. Response Surface Methodology (RSM) using Taguchi L9 orthogonal array Design was applied for optimizing these independent variables.

Materials and Methods

Biomass

Ximenia caffra source and processing

The ripe fruits were handpicked from trees in Kebbi State of Nigeria. The fruit pulp was removed and then the seed was dried under the sun for two days. The dried seed nuts were hand-shelled to extract the inner nut and the seed coat of interest was milled using Christy and Morris laboratory mill (Chelmsford England).

Phoenix dactylifera source and processing

The date palm fruits were purchased from the local market in Zaria, Kaduna State, Nigeria in June, 2017, and was identified at Narict Bioresources Unit, Zaria, Nigeria. The flesh was removed manually by hand and the seeds were soaked in water, washed to free them from any adhering date flesh and then dried under the sun for two days. The seeds were then milled using Christy and Morris Laboratory mill.

Musa paradisiaca source and processing

Plantain peels were collected from eateries in Kaduna State, Nigeria in July, 2017, and was identified at Narict Bioresources Unit, Zaria, Nigeria. They were washed with deionized water and dried by placing them in an oven at a temperature of 60°C until they were dried enough to break with the hand. The dried peels were burnt inside the combustion pan with portable acetylene gas burner (Oxford) and then the ash was sieved through a 0.8 mm sieve and then assayed using XRF. Exactly 0.06 kg of the ashes obtained was boiled in 1 dm³ deionised water for one hour at 100°C in a 2 dm³ beaker using a hot plate. The slurry was kept for 12 h before draining the extract. After draining, the first extract was kept in a separate sealed beaker and equal volume of cold deionised water as the volume of the first extract was poured into the slurry for further extraction. This was repeated the third time, and the extracts kept in different beakers. The specific gravity and pH of the alkali were measured.^{15, 16} The filtrate/extracts obtained were then quantified titrimetrically against 0.1M HCl for concentration of bioKOH.^{17, 18}

Experimental Design

Response surface method (RSM) was used to find the optimum conditions for pretreatment of date palm seed pit. Taguchi L9 Orthogonal array design with three independent variables: (a) reaction time (b) solid to liquid ratio and (c) concentration of KOH/BioKOH as shown in table 1, was applied to maximize reducing sugar yield from lignocellulosic

Table 1: Taguchi L9 Design Matrix for DPS and SPSC Hydrolysis

| Factors | Symbol | Range and levels | | |
|-----------------------------|----------------|------------------|-------|-------|
| | | | | |
| Concentration (% w/v) | X ₁ | 1.950 | 0.157 | 0.022 |
| Solid:liquid ratio (w:v) | X ₂ | 1:30 | 1:60 | 1:90 |
| Reaction time (min) | X ₃ | 15 | 30 | 60 |

biomass (*Ximenia caffra* seed coat and *Phoenix dactylifera* stone). The experimental design created by Minitab 16 software resulted in nine experimental trials.

Pretreatment

One gram of milled DPS was separately soaked with 30, 60 and 90 cm³ of 0.022, 0.157 and 1.950% (w/v) bio-alkali that was extracted and previously quantified titrimetrically. Another set (for comparison) of one gram milled SPSC was soaked with bio-alkali solution with equal concentrations, respectively in a 250 cm³ Erlenmeyer flask and then autoclaved at 121°C for 15, 30 and 60 min in accordance with the Taguchi L9 experimental design as shown in Table 1. After hydrolysis, the hydrolysate was separated using filter paper.¹⁹⁻²¹ Fourier transform infrared spectroscopy (FTIR) was carried out to determine the active groups in the hydrolysate and the total reducing sugars in the filtrate were determined by the 3,5-dinitrosalicylic acid (DNS) method described by Miller in 1959.²²

Statistical Analysis

Microsoft Excel 2016 Software was used for data analysis at 95% confidence level. To evaluate the significance and contribution of each parameter to the production of reducing sugar from date palm stone and sour plum seed coat, the student t-test was adopted to determine the probability value (p = 0.05).

Results and Discussion

Physicochemical parameters of the bioalkali from plantain peel

From the present study, ashing 5.0 kg of the plantain peels, 0.47 kg of ash was obtained which is 9.4% of the total plantain peel. This is in agreement with the work of Uyigüe *et al.* where 7.33% ash content was obtained from 3.43 kg of empty-oil-palm-bunch.¹⁷ The pH values for bioalkali are 12.0, 11.2 and 10.2 for the first, second and third extract, respectively which confirms alkali production from plantain peel ash. This agrees with the work of Olabanji *et al.*²³ who reported pH 12.88 for plantain peel ash solution. The effect of increased pH on biomass lies on delignification and high yield of monosaccharide.²⁴ Specific gravity values of bioalkali for the first, second and third extract are 1.050, 1.010 and 0.900 mS/cm, respectively. The actual concentration of potash (KOH) in the plantain peel ash extracts measured using 0.1 M HCl gave mean volume of HCl used as 87.07, 7.03 and 0.97 cm³ for the first, second and third extract, respectively. These are potash-alkali concentrations of 19.5 g/dm³, 1.57g/dm³ and 0.22 g/dm³, respectively for the three extracts (Table 2). A low potash-alkali concentration of these kind produces high yield of reducing sugar as corroborated by Sharma *et al.*²¹ This is more evident in Table 3, showing XRF spectroscopy results of the various oxides contained in the plantain peel ash. The various oxides obtained range from 0.0468 to 54.1881wt%. The percentage weights are approximately 54.19, 11.58, 10.49, 6.96, 5.08, 4.80, 3.30, 2.77, 0.46, 0.17, 0.09, 0.05, 0.0 and 0.0 Wt % for oxides of K > Si > P > Ca > Cl > Mg > S > Al > Fe > Mn > Zn > Ti > Sr > Na and Cr, respectively in above order. With slight disorder, the metal oxide arrangement is in collaboration with the work of Olabanji *et al.*²³ Amongst other impurities, the oxide of potassium has the highest percentage of 54 wt %, hence it is a viable source of potash biocatalyst (bioalkali) for DPS and SPSC hydrolysis.

Information from IR Spectra

As presented in Figure 2, it is evident from the spectra of DPS and SPSC hydrolysates using BioKOH that reducing sugar was synthesized with bands at 3387, 1643, 1388, 1080 cm⁻¹ and 3356, 1635, 1388, 1080 cm⁻¹ respectively, which indicated the presence of -OH stretching vibration in -OH functional groups, C=O stretching was also pronounced which serves as reducing sugar active group, CH₂ bending vibration in alkanes, C-O stretching representing pyranose ring skeletal vibration in ethers as presented in Table 4.

Table 2: Physicochemical parameters of bioalkali.

| Parameters | Bioalkali | | |
|----------------------|---------------|----------------|---------------|
| | First Extract | Second Extract | Third Extract |
| pH | 12.0 | 11.2 | 10.2 |
| Specific Gravity | 1.050 | 1.010 | 0.900 |
| Conductivity (mS/cm) | 11.13 | 7.65 | 1.13 |
| KOH (g/L) | 19.50 | 1.57 | 0.216 |
| KOH (%) | 1.950 | 0.157 | 0.022 |

Table 3: X-ray Fluorescence spectroscopy Analysis of burnt Plantain Peel Ash Showing the Analyte Concentration.

| Element | Concentration |
|--------------------------------|---------------|
| Na ₂ O | 0.0000 Wt % |
| MgO | 4.8035 Wt % |
| Al ₂ O ₃ | 2.7684 Wt % |
| SiO ₂ | 11.5773 Wt % |
| P ₂ O ₅ | 10.4901 Wt % |
| SO ₃ | 3.2959 Wt % |
| Cl | 5.0814 Wt % |
| K ₂ O | 54.1881 Wt % |
| CaO | 6.9613 Wt % |
| TiO ₂ | 0.0726 Wt % |
| Cr ₂ O ₃ | 0.0000 Wt % |
| Mn ₂ O ₃ | 0.1689 Wt % |
| Fe ₂ O ₃ | 0.4582 Wt % |
| ZnO | 0.0874 Wt % |
| SrO | 0.0468 Wt % |

Table 4: Infrared spectra analysis of SPSC and DPS Hydrolysate.

| Samples | C-O | CH ₃ C-H | C=O | O-H |
|-------------------|---------|---------------------|---------|---------|
| | m-s | M | m-s | s-broad |
| SPSC (bio-alkali) | 1080.17 | 1388.79 | 1635.69 | 3356.25 |
| DPS (bio-alkali) | 1080.17 | 1388.79 | 1643.41 | 3387.11 |

M- medium, S - strong,

Reducing sugar production by bioalkali hydrolysis

In the present study, Taguchi L9 orthogonal array design was used to investigate the optimal conditions of the reducing sugar production from pretreated DPSP. There were three factors namely concentration of KOH/BioKOH, solid-liquid ratio and reaction time and three levels of each parameter were varied as shown in Table 5.

The nine experiments were designed as shown in Table 5. From the study, the yield of reducing sugar ranges between 9.28 - 22.56 mg/g and 8.62 - 30.14 mg sugar/ g substrate for bioalkali pretreatment of DPS and SPSC respectively. By varying concentration of the alkali, solid:liquid ratio and reaction time, the maximum reducing sugar at optimum conditions as observed at the experimental run number 7 and 8 was 22.56 and 30.14 mg sugar/g substrate for BioKOH pretreatment of DPS and SPSC, respectively. The slight variation in the yield level could be attributed to the different lignocellulose material and the age difference of the biomass. The probability value (p-value) is a tool for evaluating the significance and contribution of each parameter to the production of sugar. As shown in Figure 1, for a one-tail t-test, (p = 0.05), since experimental value (0.196448607) is larger than the p-value, it shows that there is no significant difference between Bio-KOH Hydrolysis of (DPS) and (SPSC).

Conclusion

The estimation of reducing sugar by the pretreatment of lignocellulosic biomass (Sour Plum Seed Coat and Date Palm Stone) using BioKOH was effectively achieved through a statistical Taguchi L9 orthogonal array Design. The result indicated that the optimal conditions for reducing sugar production from pretreated date palm stone and sour plum seed coat by BioKOH hydrolysis are 0.022 % (v/v) of BioKOH, solid to liquid ratio of 1:30 cm³(w/v), reaction time of 60 (mins) and 0.022% v/v of BioKOH, solid to liquid ratio of 1:60 cm³(w/v), reaction time of 15 min which gave the maximum reducing sugar retention of 22.56 and 30.14 mg sugar/g substrate, respectively. There was no significant difference in the hydrolysis of both substrates using Bioalkali. Hence the overall benefit of this study is that the knowledge gap of appropriate stoichiometry for pretreatment of DPS and SPSC from alkali synthesized from ashes of plantain peel prior to the release of reducing sugar has been provided.

Conflict of interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

Table 5: Design Matrix of Taguchi L9 Array and Experimental Results Obtained for Bio Alkali Hydrolysis of (DPS) and (SPSC).

| Runs | Factor Settings | | | | Reducing Sugar | | Yield (mg/g) | |
|------|-------------------|--------------------|-------|---------------------|----------------|--|--------------|--|
| | Concentration (%) | Solid:liquid (w/v) | Ratio | Reaction Time (min) | DPS | | SPSC | |
| | | | | | | | | |
| 1 | 1.950 | 30 | | 15 | 11.25 ±0.61 | | 8.62 ±1.73 | |
| 2 | 1.950 | 60 | | 30 | 9.28 ±3.82 | | 10.68 ±1.17 | |
| 3 | 1.950 | 90 | | 60 | 12.36 ±2.46 | | 10.05 ±5.70 | |
| 4 | 0.157 | 30 | | 30 | 21.95 ±0.82 | | 21.64 ±0.37 | |
| 5 | 0.157 | 60 | | 60 | 11.95 ±0.35 | | 12.85 ±1.20 | |
| 6 | 0.157 | 90 | | 15 | 10.59 ±3.93 | | 9.84 ±3.09 | |
| 7 | 0.022 | 30 | | 60 | 22.56 ±0.83 | | 25.71 ±0.37 | |
| 8 | 0.022 | 60 | | 15 | 16.34 ±2.15 | | 30.14 ±1.23 | |
| 9 | 0.022 | 90 | | 30 | 16.09 ±5.08 | | 30.01 ±4.05 | |

*Applying statistical software MINITAB release 16, each value for Red. Sugar yield using BioKOH and InorganicKOH is an average of 3 trials and the standard deviations given.

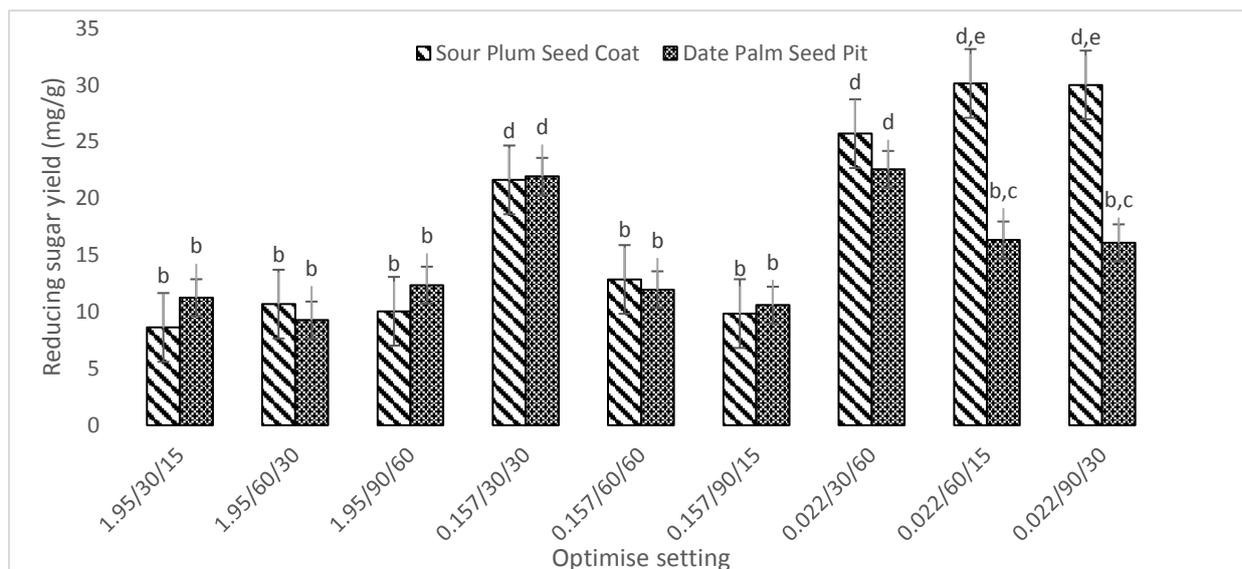


Figure 1: Reducing sugar yield for SPSC and DPS Using Bioalkali Hydrolysis

*The Mean values (experimental runs for pretreatment of SPSC and DPS using BioKOH) sharing the same letters are non-significantly different at 95% confidence interval.

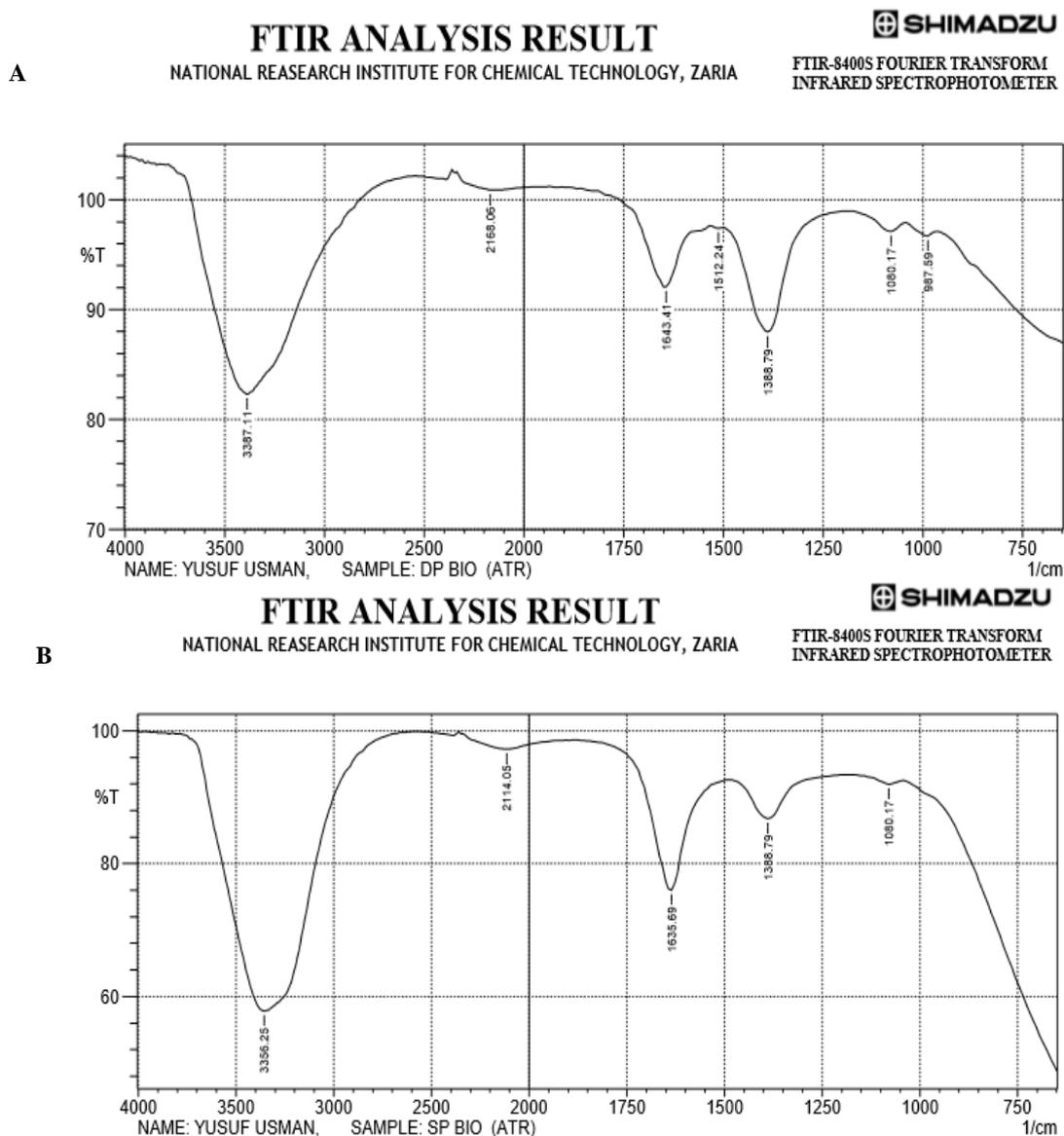


Figure 2: FTIR spectrogram of (A) DPS and (B) SPSC hydrolysate.

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