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Cost-Effective Supporting Materials for Immobilization of Yeast by Using Sweet Sorghum Juice for Bioethanol Production

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Abstract: **Bioethanol is one of the best alternative fuel due to its ease of production, lack of toxicity and availability from renewable sources like agricultural wastes. In this study, Saccharomyces cerevisiae was used for production of bioethanol with sweet sorghum juice by immobilized-cell system. Adsorption technique was followed for immobilization using low cost supporting materials like Banana pseudo stem, pine apple peel, barks and scrub pad as the carriers. The scanning electron microscopy was carried as further confirmation to reveal efficient adsorption of the immobilized yeast cells. Intially 200g/L of sugar concentration was used to carry out repeated batch fermentation of which all the carriers showed consumption of about 95% total sugars after 48 h. The immobilized cell reactor was operated over a period of 12 days without breakage of the carriers. The overall ethanol yield with immobilized cells were obtained between 0.32-0.34 g/g in repeated batch fermentation. The maximum ethanol productivity and yield were observed with banana pseudo stem carrier for seven sequential batches (408 h) with no loss of ethanol production efficiency, making it a potential application in industries.**

Index Terms: **Sweet sorghum juice, Immobilized yeast, repeated batch fermentation, Bioethanol, Banana Pseudo Stem**

I. INTRODUCTION

An upsurge of active research interest in renewable, non-polluting and non-petroleum fuels has been stimulated in the present energy scenario (Agarwal, 2007). Biofuels would be the best alternative source due to its ease of production, lack of toxicity and rich in various renewable resources like agricultural wastes of the energy crops (Vohra, 2014). Sweet sorghum (*Sorghum bicolor*) the most favorable substitutive energy crops for bioethanol, that can be grown within 3-4 months (Prasad, 2007; Wu, 2010), having good amounts of fermentable sugars glucose, sucrose, fructose, nutrients as well as trace elements which aid in yeast growth during ethanol production in fermentation processes (Laopaiboon, 2009; Walker, 1996). In addition, it has a short growing period of 120-150 days and is cultivated at nearly all temperatures and tropical climate areas (Wu, 2010). Repeated batch fermentation process with the immobilized cell system is one of the promising methods for effective ethanol productivity and higher yield with minimal production costs (Ghorbani., 2011). Amongst various techniques employed for cell immobilization, adsorption is most widely followed as it is highly stable and cost effective. There is a rising necessity for an inexpensive, reusable and readily available material that can be economical to scale up the production (Kumar, 2013). Consequently, low-priced carriers are mandatory for cell immobilization for industrial scaling to reduce substantially reduce the cost by recycling the immobilized matrix (Uroosa, 2018). In the present study, ethanol production by the yeast strains was achieved with the cell immobilization on four different supporting materials, Banana pseudo stem (BPS) (Ajay, 2014), Scrub pad (SP) (Titiporn, 2017, Prosopis juliflora bark (PJB) (Saravanakumar, 2013) and Pine apple peels (PAP) (Aophat, 2014) in the repeated batch fermentation process and obtained ethanol yields between them were compared.

II. MATERIALS AND METHODS

A. Raw material:

Fresh sweet sorghum juice was provided by International Crops Research Institute for the Semi-Arid-Tropics (ICRISAT), Patancheru, Hyderabad, Telangana, India (Latitude: 17.5188557 and Longitude: 78.27771659999996). The juice was extracted by mechanical extractor and immediately filtered the extracted

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juice with the glass wool. Then, the juice was kept at -20°C until use.

B. Microorganisms and inoculum preparation:

The commercial alcoholic brewer's yeast, Saccharomyces cerevisiae, was purchased from Saf Yeast Company Pvt Ltd., Hyderabad and it was used throughout the experiments. The inoculum preparation for *Saccharomyces cerevisiae* contained 10 g/L yeast extract, 20 g/L peptone, and 20 g/L glucose incubated at 30°C for 12 h at 100 rpm.

C. Support materials and Cell immobilization:

The supporting materials used for the immobilization are Pine apple peels, Banana pseudo stem, Prosopis juliflora bark and Scrub pad. These are cut into square pieces with dimensions of 2×2×1 cm^3 in order to make uniform structures and are added into the seeding media and autoclaved for 15 min at 121°C. The incubated yeast cells are centrifuged at 3000 rpm for 10 min at 4°C aseptically and are transferred on to the autoclaved supporting materials that were present in the seeding media for further incubation of 12 hours at 30°C for the immobilization process to take place, followed by the transfer of the immobilized supporting material into the sterile sweet sorghum juice for the production of ethanol.

D. Fermentation process

The concentrated juice was disinfected at 121°C for 15min in shake flask. The pH of the medium was set to 5 by calcium hydroxide. 10% of inoculum was added under sterile conditions and incubated in a rotor shaker at 100 rpm at 30°C with 20% of free headspace volume of the flask. The growth parameters and production of ethanol was monitored for every 12 hours for a period of 0-48hours. All the experiments are conducted in triplicates and the average yields were reported.

E. Analytical methods:

The concentration of different sugars (both reducing and non-reducing sugars) in each fermentation medium the supernatant of the sample was analyzed by a high performance liquid chromatography (HPLC) (Waters, Austria) detected using a RID-10A refractive index detector (Waters,Ireland and carbohydrate High performance 4 µm(Waters) column. Ethanol was measured by the same system using SunFire TM C8 5 µm column (Waters, Ireland) and operating at 45 °C with the mobile phase: water; flow rate = 1 mL/min. Yeast growth (biomass) was assessed by measuring the optical density of aliquots collected, using a UV-Visible spectrophotometer (UV-2450, Shimadzu) at wavelength 660 nm, and the amount of biomass produced ($g.L^{-1}$) was evaluated by a calibration curve of biomass dry weight ($g.L^{-1}$) versus optical density at 660nm.

1) Scanning Electron Microscopic

To assess the morphology of the supporting materials the BPS, SP, PJB, and PAP immobilized cells were soaked in 3.5% glutaraldehyde for 6 hours and dried after treating with 50, 70, 90, 95 and 100% ethanol followed by overnight retention in a desiccator to eliminate the moisture and were analyzed using Scanning Electron Microscopy (Zeiss) at 10.00 kV.

F. Statistical analysis

Each experiment was performed at least three times in triplicates from the beginning and results are reported results were expressed as mean ± standard error using Microsoft Excel.

III. Results and Discussion:

A. Immobilization of yeast cells on different supporting materials:

The yeast cells were grown on different supporting materials and their morphology was detected using scanning electron microscopy to reveal the manner of immobilization of cells in the BPS, SP, PJB, and PAP. Based on the morphology and free cell biomass concentration in media the BPS was found to have a large amount of immobilized cells compared to other supporting materials, which might be due to the highly fibrous, vacuous and porous nature of its stem (Jagdish et al., 2014) that lead to increased surface area with maximum number of cell adsorption (Fig.1a) which was latter followed by SP as it was earlier reported improve the enzyme production via adsorption technique (Ariyajaroenwong et al., 2015). (Fig.1b), PJB (Fig.1c) and PAP (Fig.1d). The PJB and PAP were found to be less efficient in adsorption in contrast to the earlier materials which might be due to the less surface area that is available for the cells to adsorb. These results indicate that the structure of the supporting materials play crucial role in maximum amount of cell adsorption and higher mass transportation.

B. Ethanol fermentation by immobilized yeasts- Repeated batch

S. cerevisiae was immobilized onto 2 x 2 x $1cm^3$ of support materials BPS, SP, PJB and PAP and repeated batch fermentation was used to assess their stability and capacity for cell immobilization and ethanol production. The initial sugar concentrations of fermentation broth in cycles 2–6 (200 g/l) were similar to that of cycle 1. The fermentation time of individual cycle in the frequent batch system was kept persistent at 48 h. The changes in the consumption of total sugars, total ethanol production and dry biomass concentration during repeated batch profile (six cycles) of sweet sorghum juice by the immobilized yeast *S.cerevisiae* on four diverse support materials were shown in Fig.2. At the end of the first cycle of BPS 93% sugars were utilized and the ethanol yield ($Y_{p/s}$ = ethanol (gram) per sugar (gram) utilized) was 0.344 g/g with 67% efficient fermentation whereas the ethanol yield for other supporting materials SPS, PJB and PAP were 0.33,0.325 and 0.322 g/g respectively. The maximum ethanol conc. (g/l), ethanol productivity (Q_p (g/L/ h) = Ethanol conc per fermentation time) and ethanol fermentation efficiency (E_y % = (ethanol yield /0.511) × 100) were shown in

Table.1-4 for all four supporting materials. These results show that the supporting material being the key parameter for the enhancement of ethanol production efficiency in immobilization system (Lakkana, 2012). Our results in the present study indicates that the BPS has utilized more sugar thereby producing more amount of ethanol followed by the SP, PJB and PAP at 48 hours of each fermentation cycle in the repeated batch system. There was no transformation or destruction in the physical structure of the supportive materials was witnessed even after six cycles of repeated batch fermentation indicating mechanical strength and durability of the carriers for yeast cell immobilization, whereas the studies done by Phisalaphong, 2007 and Ellaiah, 2004 have reported the breakage and instability in shape of 40% of the calcium alginate beads that have been used as immobilization supports in repeated batch fermentation. Santos. 2008 and Chandel 2009 have used alkaline pretreated sugarcane bagasse and sugarcane stalks as support materials to enhance the affinity of the cells during immobilization. Our study was successful in producing ethanol using low cost carriers without any pretreatments making it more cost effective and ecofriendly.

CONCLUSION

The current study highlights the efficient usage of low cost supporting materials like BPS, SP, PJB and PAP for the production of ethanol from sweet sorghum juice by repeated batch fermentation using immobilization technology with inexpensive, abundant and readily available agro-waste carriers with no pretreatment making it more cost effective in large scale production without compromising the ethanol productivity.

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Table 1: Production of ethanol in 1st batch fermentation (hours) from sweet sorghum juice by *S. cerevisiae* immobilized on the BPS.

Time(h)	Sugars (g/l)	Parameters (mean±SE)				
		P (g/l)	$Y_{p/s}$(g/g)	Q_p(g/(l.h)	X (g/l)	E
0	200±2.31	9.00±0.00	0.0066±0.00	0.004±0.03	0.001±0.0005	0
12	123±2.89	25.11±0.75	0.1212±0.00	2.09±0.04	0.0246±0.0020	23.4±0.85
24	72±2.31	55.75±3.25	0.2618±0.01	4.64±0.14	0.0466±0.0026	51.9±1.21
36	35±0.58	62.43±3.21	0.2907±0.01	5.168±0.02	0.0523±0.0026	57.79±0.83
48	14±2.31	72.43±1.47	0.3363±0.02	6.018±0.01	0.0606±0.0040	67.3±0.32

Table 2: Production of ethanol in 1st batch fermentation (hours) from sweet sorghum juice by *S. cerevisiae* immobilized on the SP

T (h)	Sugars (g/l)	Parameters (mean±SE)				
		P (g/l)	$Y_{p/s}$(g/g)	Q_p(g/(l.h)	X (g/l)	E
0	200±2.309	0.004±0.003	0.0066±0.003	0.0006±0.005	0.001±0.0005	0
12	123±2.886	24.312±1.039	0.115±0.0089	2.02±0.01	0.023±0.002	22.67±1.21
24	74±1.732	54.918±1.328	0.26±0.0115	4.57±0.03	0.045±0.002	51.21±0.95
36	36±1.732	62.006±1.096	0.294±0.012	5.16±0.06	0.05±0.001	57.79±1.09
48	17±1.732	70.718±1.414	0.336±0.011	5.88±0.02	0.0586±0.002	65.84±0.56

Table 3: Production of ethanol in 1st batch fermentation (hours) from sweet sorghum juice by *S. cerevisiae* immobilized on the PJB.

T (h)	Sugars (g/l)	Parameters (mean±SE)				
		P (g/l)	Yp/s(g/g)	Q_p(g/(l.h)	X (g/l)	E
0	200±1.15	0.0036±0.003	0.001±0.0005	0	0.001±0.0005	0
12	143.33±4.33	21.943±1.12	0.105±0.001	1.83±0.02	0.002±0.0002	20.44±0.89
24	96.333±3.17	47.301±1.90	0.224±0.003	3.92±0.03	0.043±0.001	43.89±1.04
36	48.333±1.45	58.198±1.24	0.275±0.014	4.84±0.04	0.048±0.001	54.13±0.99
48	18.333±1.45	68.4±1.58	0.323±0.014	5.69±0.01	0.055±0.002	63.64±0.54

Table 4: Production of ethanol in 1st batch fermentation (hours) from sweet sorghum juice by *S. cerevisiae* immobilized on the PAP.

T (h)	Sugars (g/l)	Parameters (mean±SE)				
		P (g/l)	$Y_{p/s}$(g/g)	Q_p(g/(l.h)	X (g/l)	E
0	200.00±1.15	0.001±0.00	0.001±0.0005	0	0.001±0.0005	0
12	143.66±4.33	19.437±2.44	0.093±0.004	1.70±0.04	0.021±0.001	19.02±1.47
24	97.66±3.48	46.072±1.24	0.224±0.008	3.85±0.1	0.0423±0.006	43.16±1.20
36	49.30±3.09	57.636±1.56	0.269±0.013	4.77±0.02	0.048±0.005	53.4±1.05
48	18.66±2.60	67.171±1.28	0.323±0.003	5.62±0.05	0.0573±0.005	62.91±0.69

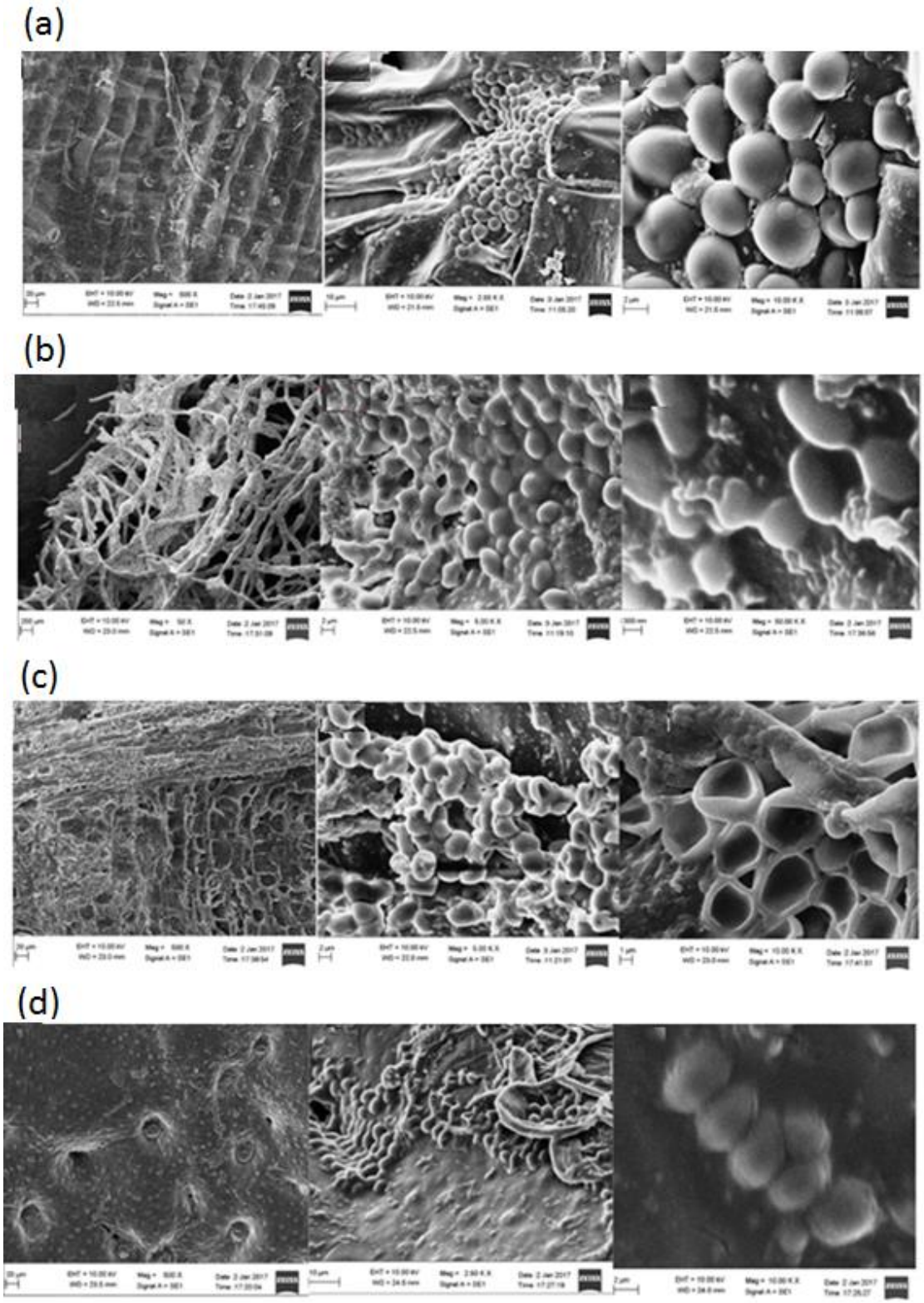

Fig. 1: Scanning microscope of the outer surface of immobilized a) BPS b) SP c) PJB d) PAPwith magnification of 500X, 2.50 K X, 10.00 K X respectively.

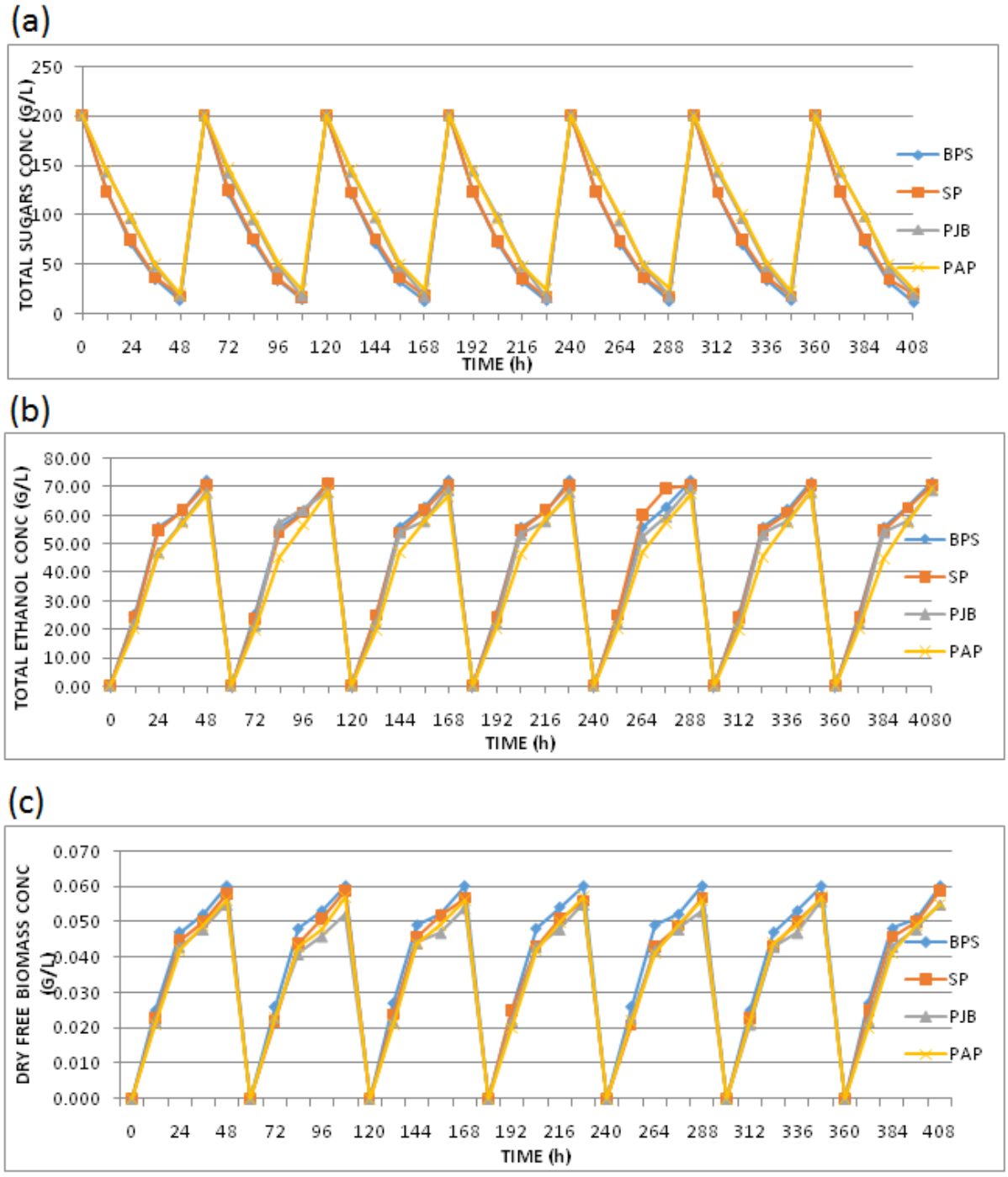

Fig. 2: Comparison of repeated batch profile (six cycles) of ethanol production from sweet sorghum juice with S.cerevisiae by four different supporting material of BPS, SP, PJB and PAP on the $2x2x1cm^3$: a)consumption of total sugars, b) ethanol production, c) dry free biomass concentration.

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